Lipoprotein (a), oxidised LDL and carotid atherosclerosis in patients with systemic lupus erythematosus

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PL1   Cutting Edge of Lupus Research Plenary

PL1.01 & PO1.E.1

Retroelements as trigger of lupus
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Objectives: We tested the hypothesis that lupus disease is caused by, or contributed to, so-called endogenous retroelements—genomic segments of 'selfish' DNA. Forty percent of our genome consist of such retroelements, many of them active. As an indication that the retroelements may cause lupus disease, patients that lack the enzyme Trex1 suffer from systemic lupus erythematosus, chilblain lupus or Aicardi-Goutières syndrome—an inflammation of the brain, with lupus disease developing in the course of the disease. Trex1 is a DNA exonuclease that degrades retroelement cDNA. **Results and Conclusions:** To show that unrestricted retroelements contribute to autoimmune disease, we increased the concentration of endogenous retroviral cDNA in B/W mice and, thereby, exacerbated lupus disease. Conversely, to ameliorate autoimmune disease, we introduced human APOBEC3 (abbreviated A3) enzymes into mice that lack Trex1. The lack in Trex1 makes the mice inefficient in retroelement restriction; the single mouse Apobec3 enzyme does not cover a wide range of retroelements, whereas the seven human A3 enzymes do. We will present data to show that human A3 enzymes are advantageous in Trex1-deficient mice with autoimmune disease. This indicates that retroelements are indeed a cause for the disease.

PL1.02 & PO1.E.2

Long-lived plasma cells adoptively transferred from NZB/W mice cause immune complex nephritis in immunodeficient Rag1-/- mice
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Previously, we showed that long-lived plasma cells refractory to immunosuppression significantly contribute to autoantibody production in NZB/W mice used as a model of lupus nephritis. Since immunosuppressive and B cell depletion therapie affecting only short-lived plasmablasts and plasma cells can induce remission of the disease, the aim of this study was to elucidate the role of autoreactive long-lived plasma cells in the pathogenesis of lupus nephritis. For this purpose, CD138+ plasmablasts and plasma cells lacking B and T cells were isolated from spleens of >6 month-old NZB/W mice with high levels of anti-dsDNA antibodies and adoptively transferred to immunodeficient Rag1-/- mice (3 000 000 cells/mouse). Shortly after transfer, total IgG and IgM as well as IgG and IgM anti-dsDNA antibodies were detected in the recipient mice. The levels of these antibodies remained constant for the whole observation period of 21 weeks. Mature non-dividing plasma cells but not plasmablasts were detectable in bone marrow, spleen, and kidneys of the recipient mice. This demonstrates that these persistent antibody levels were exclusively secreted by long-lived plasma cells since no B cells were transferred, excluding them as a source of the newly generated short-lived plasma cells. The mice developed proteinuria (maximum of 300 mg/dl 21 weeks after plasma cell transfer). Renal immunohistology showed immune complex nephritis with depositions of IgG, IgM and C3. The findings show that autoantibodies secreted by long-lived plasma cells can cause nephritis and suggest that long-lived plasma cells refractory to conventional immunosuppression and B cell depletion should be considered as candidate targets for future therapeutic strategies.

PL1.03 & PO1.E.3

**Defective migration of tissue-resident dendritic cells:
a novel pathogenesis of cutaneous lupus**
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Objectives: Tissue-resident dendritic cells (DC) are believed to carry antigens from tissues to tissue-draining lymph nodes to induce immunity. We posit that this important function of DC plays a role in the pathogenesis of lupus. Using skin-resident DC as a model of tissue DC, we investigated the role of DC migration, and underlying mechanisms, in cutaneous lupus. **Methods:** We assessed skin-DC migration using ex vivo and in vivo assays in MRL/MpJ-Faslpr/lpr (MRL-lpr) and MRL/MpJ-Fas+/+ mice that develop lupus dermatitis. Skin harbors DCs that express langerin (Lang) and reside in epidermis and dermis, hereafter called Langerhans cells (LC), and Lang-negative DCs in dermis, called dermal DC. Subsequently, we generated MRL-lpr mice that express enhanced green-fluorescent-protein (EGFP) driven by a Lang promoter by introgressing the Lang-EGFP knock-in mutation from B6 background, which allows visualization of LC in vivo. We then tested whether modulating skin-DC migration affects lupus dermatitis. **Results:** LCs from MRL mice exhibited a profound defect in their ability to emigrate from the epidermis, to migrate through the dermal lymphatics, and to immigrate into skin-draining lymph-nodes (Eriksson and Singh, J Immunol Cutting Edge, 2008). Dermal DCs (Lang-negative), but not blood-derived DCs, also exhibited a similar defect in MRL strains. Since conventional DC migration assays require their activation, we subsequently used Lang-EGFP knock-in mice to assess LCs at steady-state. The proportion of LC (EGFP+ cells) was markedly lower in skin-draining lymph-nodes of MRL-lpr mice than in B6 mice, thus confirming what we found using inbred mice. Importantly, skin-DC migration defect precedes the onset of inflammation and correlates with the severity of dermatitis. Conversely, treatment with glycolipid α-galactosylceramide that binds CD1d restores skin-DC migration and ameliorates dermatitis, whereas deficiency of CD1d in MRL-lpr mice worsens dermatitis. Surprisingly, α-galactosylceramide-mediated increase in skin-DC migration does not require NKT-cells, but is associated with an expansion of epidermal γδ T-cells in a CD1d-dependent manner in MRL-lpr mice. Furthermore, CD1d and epidermal γδ T-cells play a physiological role in skin-DC migration, since the genetic deficiency of CD1d or γδ T-cells, but not of NKT-cells, in normal backgrounds reduces skin-DC migration. Finally, epidermal γδ T-cells are reduced in MRL mice as compared to control mice. **Conclusions:** We elucidate a novel mechanism, whereby CD1d-dependent epidermal γδ T-cells normally facilitate skin-DC migration from skin to skin-draining lymph nodes. This regulatory mechanism is disrupted in lupus dermatitis-prone mice, providing evidence for a novel pathogenetic mechanism of cutaneous lupus.

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PL1.04 & PO1.E.4

Endothelial progenitor cell dysfunction in SLE

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Background & Objectives: SLE is associated with increased risk of coronary heart disease (CHD) and is itself an independent risk factor for clinical and subclinical atherosclerosis. Dysfunctional circulating endothelial progenitor cells (EPCs) may contribute to abnormal endothelial repair and subsequent atherogenesis. Our aims were to determine whether EPC number and/or function are impaired in SLE patients and to examine potential mechanisms for abnormalities. ***Methods:*** Patients with SLE (ACR 1997 criteria) had a clinical assessment of disease activity (SLEDAI-2k), SLICC damage index and cardiovascular risk factors. Healthy controls were recruited from the local community. Aortic pulse wave velocity (PVW) was measured using a standard protocol (Micromedical Pulse Trace) as a measure of early atherosclerosis. Peripheral blood mononuclear cells were isolated from patients and healthy controls. Cells were labelled with fluorescently-conjugated CD133 and CD34 antibodies and corresponding isotype controls (FITC or PE) to identify EPCs using flow cytometry. Formation of colony-forming units (CFU) following 7 days in culture was utilised to measure EPC function. Ageing phenotype of EPC-CFU was determined by extracting DNA and measuring relative telomere length using real-time quantitative PCR. ***Results:*** We studied 56 patients and 48 controls; median(IQR) age was 54(47, 58) and 46(30, 58) years respectively. The mean(SD) percentage of CD34/CD133+ EPCs in SLE was not different to controls [0.03(0.02)% vs. 0.02(0.02)%, P=NS]. We demonstrated CFU were of endothelial origin with immunohistochemistry using Di-acetylated LDL and CD31. 31 SLE patients had reduced mean(SD) number of CFU [7(6) vs. 12(12), P=0.05] compared with 22 controls and formed fewer large CFU, defined as a cluster of >100 cells, (16% vs 65%, P<0.0001). Within the patients group, there was no association between EPC function and age, disease activity, traditional risk factors or levels of C3, C4 complement or antibodies to dsDNA. Patients with low numbers of CFUs had higher aortic PWV [9.2(5.3) vs. 5.7(2.3), P = 0.06]. Of note, mean (SD) EPC-CFU telomere length was reduced in 5 SLE patients [0.64 (0.57)] compared with 5 age matched controls [2.7 (4.04)]. ***Conclusions:*** We have noted reduced regenerative capacity of and a premature senescent phenotype of the endothelium in SLE patients. Impaired vascular repair mechanisms may particularly affect the early stages of atherogenesis such as vascular stiffness. Improving the reparative capacity of EPCs may represent a novel therapeutic target to attenuate CHD risk in this population.

PL1.05 & PO1.E.5

Trait-stratified genome-wide association study identifies novel and diverse genetic associations with serologic and cytokine phenotypes in systemic lupus erythematosus

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Objectives: Systemic lupus erythematosus (SLE) is a highly heterogeneous disorder, characterized by differences in autoantibody profile, serum cytokines, and clinical manifestations. SLE-associated autoantibodies and high serum interferon alpha (IFN-α) are important heritable phenotypes in SLE which are correlated with each other, and play a role in disease pathogenesis. These two heritable risk factors are shared between ancestral backgrounds. The aim of the study was to detect genetic factors associated with autoantibody profiles and serum IFN-α in SLE. ***Methods:*** We undertook a case-case genome-wide association study of SLE patients stratified by ancestry and extremes of phenotype in serology and serum IFN-α. Single nucleotide polymorphisms (SNPs) in seven loci were selected for follow up in a large independent cohort of 450 SLE patients and 522 controls using a multi-step screening approach based on novel metrics and expert database review. The seven loci were: leucine-rich repeat containing 20 (LRRC20); protein phosphatase 1H (PPM1H); lysophosphatidic acid receptor 1 (LPAR1); ankyrin repeat and sterile alpha motif domain 1A (ANKS1A); protein tyrosine phosphatase, receptor type M (PTPRM); ephrin A5 (EFNA5); and V-set and immunoglobulin domain containing 2 (VSIG2). ***Results:*** SNPs in the LRRC20, PPM1H, LPAR1, ANKS1A, and VSIG2 loci each demonstrated strong association with a particular serologic profile (all OR>2.2 and p<8x10-4). Each of these serologic profiles was associated with increased serum IFN-α. SNPs in both PTPRM and LRRC20 were associated with increased serum IFN-α independent of serologic profile (p=3.2 x 10-6 and p= 5.0 x 10-3 respectively). None of the SNPs were strongly associated with SLE in case-control analysis, suggesting that the major impact of these variants will be upon subphenotypes in SLE. ***Conclusions:*** This study demonstrates the power of using serologic and cytokine subphenotypes to elucidate genetic factors involved in complex autoimmune disease. The distinct associations observed emphasize the heterogeneity of molecular pathogenesis in SLE, and the need for stratification by subphenotypes in genetic studies. We hypothesize that these genetic variants play a role in disease manifestations and severity in SLE.

PL1.06 & PO1.E.6

A genetic model for IRF5, autoantibodies, and interferon alpha in systemic lupus erythematosus

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Objective: High interferon alpha (IFN-α) is implicated as a heritable risk factor for systemic lupus erythematosus (SLE). Interferon regulatory factor 5 (IRF5) variants are associated with susceptibility to SLE, and SLE-specific autoantibodies may stimulate IFN-α production through the Toll-like receptor/IRF5 pathway. We investigated the associations between IRF5 variants, autoantibodies, IFN-α, and SLE susceptibility in a large multi-ancestral cohort. ***Methods:*** Haplotype-tagging SNPs in IRF5 and serum autoantibodies were determined in 1034 SLE patients and 989 controls of European ancestry, and 555 SLE patients and 680 controls of African-American ancestry. The IRF5 promoter insertion/deletion (indel) was sequenced in 262 SLE cases

and 386 controls. Serum IFN-α activity was measured using a functional assay. *Results:* Logistic regression of phenotypic variation in European-derived subjects resolved the IRF5 SLE-risk haplotype into allelic associations with particular autoantibodies [rs2004640T with anti-dsDNA (OR=2.41, p=3.3x10-14), rs10488631C with anti-Ro (OR=2.46, p=2.8x10-13), and a minor haplotype with anti-La (OR=1.96, p=0.035)]. Greater than 70% of the attributable risk of SLE due to IRF5 alleles was found in subjects who had these particular antibodies (40% of the European cohort). Anti-nRNP and anti-Sm autoantibodies were not associated with IRF5 alleles in European ancestry. Meanwhile, in African-Americans the European-derived SLE-risk haplotype was present due to admixture and demonstrated a similar association with both SLE susceptibility and autoantibodies. Interestingly, the IRF5 promoter indel was present on African chromosomes and not in LD with other haplotype tagging SNPs. This promoter indel was a strong SLE risk factor in African-Americans (OR=2.16, p value=7.2x10-7), and was associated with anti-nRNP antibodies in African-Americans. In SLE patients of both ancestral backgrounds, elevations in IFN-α related to IRF5 risk alleles only occurred in patients who had the particular autoantibody associated with that allele. This supports a model as follows: IRF5 gene variant + specific autoantibody = high IFN and risk of SLE. *Conclusions:* These data suggest that specific SLE-associated autoantibodies cooperate with IRF5 variants to dysregulate IFN-α production, and consequently increase risk of SLE. These results demonstrate the power of phenotypic variability when informed by genomic origin to disentangle complex genetic relationships.

PL1.07 & PO1.E.7

**Proinflammatory SLE neutrophils induce
vascular damage and synthesize IFNs**

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Objectives: While a potential role for neutrophils in lupus pathogenesis and organ damage was described decades ago, the exact role that this cell subset plays in SLE has not been well characterized. Neutrophil-specific genes are abundant in PBMC microarrays from lupus patients due to presence of low density granulocytes (LDGs) in mononuclear cell fractions. However, the functionality and pathogenicity of these LDGs have not been characterized. We developed a technique to purify LDGs from lupus PBMCs and assessed their phenotype, function and potential role in disease pathogenesis and vascular damage. *Methods:* LDGs, their autologous lupus neutrophils and healthy control neutrophils were compared in their microbicidal and phagocytic capacities, generation of reactive oxygen species, activation status, L-selectin shedding, inflammatory cytokine profile and type I IFN expression and signatures. Since SLE patients display evidence of accelerated endothelial cell apoptosis not coupled by proper vascular repair, we also assessed the capacity of LDGs to kill endothelial cells and their antiangiogenic potential. *Results:* LDGs were present in all SLE samples studied and high number of these cells in peripheral blood correlated with the presence of vasculitis, skin involvement and arthritis. When compared to control neutrophils and their autologous normal-density lupus neutrophils, LDGs display an activated phenotype, secrete increased levels of type I IFNs, TNF-α and IFN-γ, but show impaired phagocytic potential. Importantly, LDGs induce significant endothelial cell cytotoxicity and synthesize sufficient levels of type I IFNs to disrupt the capacity of endothelial progenitor cells to differentiate into mature endothelial cells. Further, LDG depletion restores the functional capacity of endothelial progenitor cells and abolishes type I IFN synthesis. There was no significant correlation between age, disease duration and/or use of immunosuppressive drugs or corticosteroid dose and the presence of LDGs. *Conclusion:* We have characterized in detail the phenotype of a low-density neutrophil subset which appears to be present in higher numbers in SLE patients with distinct clinical manifestations. LDGs have preserved neutrophil function overall but display impairments in phagocytic potential, have a proinflammatory phenotype and display pathogenic features, including the capacity to synthesize type I IFNs and induce vascular damage. As such, LDGs may play an important dual role

in premature cardiovascular disease development in SLE by simultaneously mediating enhanced vascular damage while inhibiting vascular repair. The potential role of these cells in lupus pathogenesis (in part mediated by enhanced type I IFN synthesis) and on organ damage warrants further investigation.

PL1.08 & PO1.E.8

**Chromatin-activated neutrophils represent
a major source of interferon-α**

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Objectives: Chromatin fragments, especially oligo-nucleosomes, represent major autoantigens SLE. Nucleosomes (DNA-histones complexes) are present in the circulation of patients due to an impaired chromatin clearance. Interferon-α (IFN-α) plays an important role in SLE development (known as the IFN-α signature). IFN-α concentrations are increased in SLE patients and favor the differentiation of monocytes in dendritic cells (DC). Although plasmacytoid DC (pDC) are known to secrete IFN-α, this cell type represents a minor cell population. Moreover, only a few lupus stimuli have been reported to induce IFN-α secretion by pDC. We have therefore investigated whether other stimuli and cell types may be responsible for the strong IFN-α secretion observed in SLE patients. *Methods:* Chromatin was purified from calf thymus. Peripheral blood mononuclear cells (PBMC) and neutrophils (polymorphonuclear leukocytes, PMN) were freshly isolated from the blood of healthy individuals. The different cell types were characterized by flow cytometry. Cells were activated with different stimuli and IFN-α production was estimated by flow cytometry upon intracellular staining. IFN-α secretion was confirmed by ELISA. PMN activation was verified by measuring CD66b up-regulation (flow cytometry) and IL-8 secretion (ELISA). *Results:* We show for the first time that isolated PMN secrete IFN-α upon activation. Using both flow cytometry and ELISA, nucleosomes and CpG-oligonucleotides (CpG-ODN, a Toll-like receptor (TLR) 9 ligand) were the best IFN-α inducers, although R848 (a TLR8 ligand) triggered also IFN-α secretion. On the contrary, lipopolysaccharides (TLR4 ligand) usually did not induce IFN-α. When autologous PMN and PBMC were mixed, PMN-derived IFN-α production was often more pronounced, suggesting that several cell types interplay. In co-cultures, pDC also produced IFN-α upon activation with CpG-ODN and nucleosomes but to a lower extent. Even monocytes produced IFN-α in response to some stimuli. Nucleosome-induced IFN-α production by PMN correlated to IL-8 secretion and CD66b up-regulation. PMN isolated from healthy donors and SLE patients are currently being compared. *Conclusions:* This is the first report showing both that activated PMN can secrete IFN-α and identifying the stimuli involved. PMN are known to play a major role in inflammation and IFN-α-producing PMN may participate in SLE pathogenesis, especially upon migration into tissues, such as kidneys. Since PMN are 200 times more frequent than pDC in the blood, they may represent a major source of IFN-α in SLE. The increased concentrations of circulating chromatin in combination with the capacity of PMN to secrete IFN-α may partly favor the break of the peripheral tolerance in patients.

PL2   Lupus 2010 - The State of the Art

PL2.1

What have we learned from clinical trials?
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Hydroxychloroquine, corticosteroids (CS) , and aspirin are the only approved drugs in lupus and no new drug has been added during the last 30 years. Off-label use of azathioprine (AZA), mycophenolate mofetil (MMF),

cyclophosphamide (CY), methotrexate, leflunomide, and cyclosporine is common either as steroid-sparing agents or for severe lupus. Efforts during the past decade to introduce novel targeted drugs such as the "toleragen" were met with equivocal results. MMF also had its own difficulties, with RCT failing to support claims of superiority in earlier studies. Biologic therapies of proven efficacy in rheumatoid arthritis such as abatacept (CTLA-4Ig) and rituximab (anti-CD20 mAb) have failed to demonstrate efficacy in RCTs. Only recently a new biologic agent, belimumab, has managed to surpass this perceived "curse" in lupus trials demonstrating efficacy compared to placebo in a composite responder index, albeit with a small treatment effect. While such difficulties are common in trials for other diseases, a feeling of disappointment is widespread in some circles. Is this feeling justified? Why approving new drugs has turned out to be so hard for a disease where major therapeutic successes were previously first achieved in rheumatology? *In a way, lupus is the victim of its own success.* The introduction of CY with major effectiveness for severe lupus has created a standard of care difficult to surpass. Earlier studies fell into the "trap" of trying to demonstrate superiority against a drug with major efficacy but with major complications such as sterility. Eventually, non inferiority trials and comparators of lesser efficacy such as CS, AZA or MMF became acceptable. While the field still suffers from the lack of the ideal outcome measure, several trials have demonstrated a lack of understanding of basic principles of the management in lupus. Thus, trials do not always distinguish whether they are targeting mild or more severe disease, fresh patients or patients with long-standing disease with potential significant irreversible damage. Trials in severe lupus do not make the distinction between induction vs maintenance therapy. More importantly, new major events- not present at the beginning of the disease- or flares may appear at any time during its course. The understandable pressure to complete trials in a short period of time is a major problem in view of the fact that the very first RCT at NIH demonstrated that for certain manifestations (eg nephritis), at least 5 years of follow-up are needed to demonstrate efficacy. Short-term outcomes also suffer from the effect of background therapy that does not allow the drug under examination to demonstrate its efficacy or the loose crossover criteria that lead to withdrawal of treatment early on. Drugs anticipated to have major treatment effects may need different designs form those with smaller treatment effects. Equally important, drugs with slower mode of action (for instance CY) may not be compared with drugs of rapid action (eg CS or MMF) in trials of short duration. Some drugs (eg AZA) may not be good as induction treatment but may be better as maintenance. Notwithstanding the inherent difficulties in trial design, we remain optimistic that existing therapies with proven efficacy in other diseases or novel therapies will reach their way in lupus.

PL2.2

Lessons from genetics

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Systemic lupus erythematosus (SLE) is the prototypic autoimmune disease, and like most human diseases SLE is genetically complex. This means that multiple genes, as well as non-genetic factors, contribute to disease risk and outcome. Until recently, the genetic complexity of SLE has been a major obstacle to the identification of genetic risk factors. However, recent developments in molecular genetic and statistical methodology have led to the discovery of a large number of genes that influence SLE risk. In particular, the successful completion of several genome wide association studies (GWAS) has led to the identification of over 20 SLE risk genes, many of which overlap with recent findings in other autoimmune diseases. Many of the recently identified SLE risk genes function in the toll-like receptor and type I interferon signaling pathways, supporting the fundamental importance of these pathways in disease. One recently identified risk gene, tumor necrosis factor alpha-induced protein 3 (TNFAIP3), which encodes the A20 protein, nicely illustrates several features of work in this area. TNFAIP3 is a negative regulator of NF-κB, a key transcription factor in inflammatory responses. Multiple genetic variants have been associated with SLE risk, including a nonsynonymous SNP that results in an amino acid substitution at amino acid 127 in the OTU domain. This variant has been shown to decrease the ability of the A20 protein to inhibit

TNF-induced NF-κB activity in an *in vitro* system, thus suggesting functional significance of this variant. Of interest, multiple other autoimmune diseases, including rheumatoid arthritis, Crohn's disease, type 1 diabetes, psoriasis and Celiac sprue have now been associated with genetic variation in this region, however the pattern of genetic association across these diseases is quite complex. As with most of the other recently identified risk genes, much additional work, including DNA sequencing to identify potential causal variants and functional studies will be required to more fully elucidate the nature of the recently identified genetic associations. Given the phenotypic heterogeneity of SLE, additional work will also be required to refine genotype-phenotype associations, as genetic associations are likely to differ across clinical and serologic SLE subgroups. Extension of this work to other ethnic populations is also likely to be particularly informative. Finally, future work in this field will focus on other potential sources of heritability, including rare and structural variants that have not been adequately examined in recent GWAS and epigenetic modifications such as DNA methylation.

PL2.3

Where are we in SLE? Landmarks and futurescapes.

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Recent years have shown progress in treatment and understanding pathogenesis. Direct comparisons between the highly targeted IMP inhibitor, mycophenolate mofetil (MMF) and the multi-targeted cyclophosphamide (Cyt) show these treatments are at least equivalent, with similar rates of serious adverse events. Cyt has significantly lower response rates in African Americans. Pathogenesis studies show an broad array of genes, epigenetic changes, cells, activation pathways, cytokines and molecular targets. Beyond the roles of autoantibodies, adaptive T cells and B cells in causing SLE, we have identified importance of other cell systems. Thirty-to 40 genes increase risk for SLE or clinical subsets ; newer work studies gene combinations and epigenetic changes that alter gene transcription, including DNA hypomethylation and the influence of miRNAs. The innate immune system expands autoimmune response to RNA- and DNA-containing lupus antigens. Cytokines made by innate immune cells , such as IFN -alpha and TNFalpha , are targets of novel therapeutic trials. Initial work confirming the upregulation of interferon-induced genes in some patients also showed signals for upregulated neutrophil genes; recent data suggests low density granulocytes (increased in SLE) may be the source and are toxic to endothelium, perhaps facilitating vascular damage. There is interest in targeting cytokines that drive B cell maturation into plasmablasts, such as BLyS and IL-6. Among the many pathways inappropriately activated or inactivated in T and B cells of SLE patients, some can be targeted by small molecules, including various kinases. Moving beyond searches for new, safer, more effective treatments are strategies to prevent damage. Can we change progression to scarring and atherosclerosis by altering proteins that cause scarring (hepatic growth factor, TGFbeta) or by interfering with oxidative damage to tissues, cells and lipids? There is an intense search for identifying new biomarkers in urine or in serum/plasma that can identify lupus flares when they first begin, such as monocyte chemotactic protein (MCP-1) or mRNA for Foxp3. Two additional questions are of interest – 1) will most patients respond to targeting a single molecule – or will they require general immunosuppression followed by highly targeted therapies to maintain improvement? 2) can we prevent SLE? Would vaccines against viruses such as EBV prevent one of the environmental triggers, that in a genetically and hormonally susceptible person, result in SLE? At our next meeting, we are likely to have some answers.

CS1   Cardiovascular Disease in Lupus

CS1.1

Type I interferons and premature cardiovascular disease in SLE
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SLE is characterized by strikingly higher rates of premature cardiovascular disease, with up to a 50-fold increase over matched controls. While immune dysregulation observed in SLE may play the dominant role in atherogenesis, the exact mechanisms leading to enhanced cardiovascular risk in lupus remain to be determined. Our group previously reported that individuals with SLE and no traditional cardiovascular risk factors display a striking imbalance between endothelial cell damage and repair manifested by an increase in the levels of circulating apoptotic endothelial cells which is not coupled by proper endothelial repair, as shown by a significant decrease in the numbers and function of bone marrow derived endothelial progenitor cells (EPCs) and circulating myeloid angiogenic cells (CACs). High levels of circulating apoptotic endothelial cells in SLE strongly correlate with endothelial dysfunction, a surrogate marker of future atherosclerosis development. Our group previously reported that IFN-α induces EPC/CAC apoptosis and skews myeloid cells away towards nonangiogenic phenotypes including dendritic cells (DCs). Importantly, neutralization of type I IFN pathways restores a normal EPC/CAC phenotype and function in SLE. We have also shown that the New Zealand Black/New Zealand White F_1 murine model of lupus, where type I IFNs are considered to play a prominent role in pathogenesis, is also characterized by endothelial dysfunction, decreased EPC numbers and aberrant function of phenotype and function of EPCs. All these observations support a potential role for type I IFNs in the development of premature atherosclerosis and altered vasculogenesis in SLE. Our group has recently described a neutrophil subset in SLE which is proinflammatory and has the capacity to synthesize type I interferons. These cells are toxic to the endothelium and interfere with proper vasculogenesis through type I IFN activity. Ongoing studies are determining the role that type I IFNs have in progression of atherosclerosis and vascular damage in human and murine lupus.

CS1.2 & PO1.E.14

Monocytes from SLE patients with carotid artery plaque and dysfunctional HDL contain upregulated PDGFRbeta that enhances monocyte chemotaxis
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Objectives: Accelerated atherosclerosis is a major co-morbid condition of women with SLE. Monocytes are the main immune cell involved in atherosclerosis initiation and progression. The presence of dysfunctional, pro-inflammatory HDL (piHDL), which occur in approximately half of SLE patients but only 4% of healthy controls, correlates with carotid artery plaque with an OR of 16. We hypothesized that piHDL, in addition to being an SLE biomarker for accelerated atherosclerosis, might directly influence monocyte gene expression and function. ***Methods:*** Peripheral blood monocytes were isolated from 54 SLE patients. Subjects were stratified into three groups: 1) carotid artery plaque+piHDL+ 2) plaque-piHDL+ and 3) plaque-piHDL- (n=18/group). Transcript levels of 84 atherosclerosis-specific genes were examined by real-time PCR-based gene arrays. RNA from the human monocyte cell line THP-1 was isolated and examined by real-time PCR after direct, acute treatment with normal, anti-inflammatory HDL or piHDL that was isolated from SLE patients. Monocyte chemotaxis and TNFα/monocyte chemoattractant protein-1 (MCP-1) secretion were measured in THP-1 cells after piHDL/HDL treatment and inhibitors of piHDL and platelet-derived growth factor receptor beta (PDGFRbeta). ***Results:*** PDGFRbeta was upregulated in both primary monocytes from patients with plaque plus piHDL and in THP-1 monocytes

acutely treated in vitro with piHDL compared to normal HDL. MCP-1 transcript levels were significantly upregulated in plaque-piHDL+ subjects versus the other two groups, suggesting the presence of piHDL might couse monocytes to migrate into the subendothelial space and initiate atherosclerosis. Monocyte chemotaxis was enhanced after treatment with piHDL versus normal HDL. Abnormal migration of piHDL-treated monocytes was restored to levels observed in cells treated with normal HDL after in vitro treatment with the PDGFR inhibitor imatinib or an apoJ-mimetic peptide (which is known to reverse piHDL function). Increased piHDL-mediated TNFalpha protein levels in treated monocytes were also reduced with both inhibitors. ***Conclusions:*** Dysfunctional piHDL directly influences expression of a small number of transcripts and proteins in primary monocytes and a human monocyte cell line. Inhibition of piHDL through reducing piHDL oxidation using an apoJ-mimetic peptide or blocking PDGFRbeta kinase activity with imatinib restored monocyte migration to normal levels. These experiments suggest piHDL influences monocyte biology and could directly promote accelerated atherosclerosis in SLE patients.

CS1.3 & PO2.E.16

Effect of rosuvastatin on homocysteine, hsCRP and endothelial markers in systemic lupus erythematosus (SLE): a randomized controlled trial
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Objectives: To study the effect of rosuvastatin (crestor) therapy on homocysteine, hsCRP and biomarkers of endothelial activation / injury in patients with stable SLE with subclinical atherosclerosis ***Methods:*** Asymptomatic SLE patients who had abnormal coronary calcification (Agatston score ≥1) or abnormal carotid intima-media thickness (IMT) (≥0.8mm at any site) by Doppler ultrasound were randomized in a double-blinded manner into 2 arms: (1) rosuvastatin (10mg/day); or (2) placebo (one tab/day) for 12 months. Levels of homocysteine, hsCRP, sVCAM-1, P-selectin and thrombomodulin were measured at baseline, month 6 and month 12. SLEDAI scores were assessed at 2-month intervals. ***Results:*** 138 SLE patients were invited for screening of atherosclerosis but 6 declined. Fifty patients did not have atherosclerosis, 2 were not eligible and 8 declined to participate. Finally 72 patients were enrolled(97% women). The mean age was 50.8±9.7 years and SLE duration was 11.8±7.1 years. At baseline, their mean Agatston score was 30.9±68 and mean carotid IMT was 0.67±0.13mm. The mean SLEDAI score was 1.6±1.7 and the SDI damage score was 1.3±1.4. The prevalence of traditional risk factors is as follows: smoking(10%), menopause(57%), diabetes mellitus(3%), hypertension(33%), LDL-cholesterol level >2.6mmol/L(44%) and waist-hip ratio >0.85(50%). Antiphospholipid antibodies were present in 35% of patients. 36 patients were randomized to each of the rosuvastatin and placebo arm of treatment. No statistically significant differences in clinical characteristics were evident at baseline. At month 12, a significant drop in total and LDL-cholesterol level was observed in the rosuvastatin group. A significant reduction in hsCRP level was observed in the rosuvastatin arm (p=0.02) but not in the placebo arm. The difference in hsCRP level between the rosuvastatin and placebo arms was significant at month 12 (p=0.03) after adjustment for baseline hsCRP values, risk factors and the mean SLEDAI score (AUC). A greater reduction in hsCRP level was observed in patients with baseline LDL of >2.6mmol/L treated with rosuvastatin. The drop in thrombomodulin level from baseline to month 12 was also significant in rosuvastatin-treated patients (p=0.04). However, serial changes in homocysteine, sVCAM-1 and P-selectin levels were not statistically significant. As the levels of thrombomodulin and sVCAM-1 correlated with disease activity, a subgroup analysis of those patients who had SLEDAI scores of <=2 at month 0, 6 and 12 revealed a significant drop in both thrombomodulin (p=0.001) and hsCRP (p=0.04) levels at month 12 in patients treated with rosuvastatin only. Rosuvastatin was well tolerated. Numerically more patients treated with rosuvastatin suffered from gastrointestinal upset and neurological symptoms. ***Conclusions:*** In SLE patients with subclinical atherosclerosis, low-dose rosuvastatin leads to a significant reduction in hsCRP level after 12 months' therapy. Rosuvastatin also significantly reduces the level of thrombomodulin in patients with very low disease.

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CS1.4 & PO2.A.1

Antibodies towards high density lipoproteins inhibit the anti-inflammatory properties of the lipoproteins

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Anti-aterogenic properties of high density lipoproteins (HDL) are well recognised: they prevent the oxidative modification of LDL and its consequent uptake by monocytes and inhibit cytokine-induced adhesion molecule production. Anti-HDL (aHDL) antibodies have been reported in patients with systemic lupus erythematosus (SLE). Our group has previously showed the presence of these antibodies in two different cohorts as well as an association with decreased paraoxonase activity, increased biomarkers of endothelial dysfunction (nitric oxide, adhesion molecules: VCAM-1 and ICAM-1), reduced total plasma anti-oxidant capacity and an increase in disease-related damage and activity indices. This study aimed at confirming the capacity of aHDL antibodies, purified from SLE patients, to block the anti-inflammatory properties of HDL in vitro. aHDL antibodies were purified from serum of patients with SLE, by affinity chromatography. Human HDL were covalently coupled to CNBr-activated Sepharose 4B and assembled in column C10/10. Patients sera, selected according to the highest immunoreactivies observed in previous studies were injected through the column, and the antibodies directed towards HDL were retained. After elution with 0.1 M Glycine pH 2.4 and neutralization fractions collected were analyzed at 280 and confirmed by ELISA their capacity to bind to the HDL. Fractions containing immunoglobulins with binding activity were pooled and concentrate under vacuum. The production of vascular adhesion molecules (VCAM-1) by human umbilical vein endothelial cells (HUVECs) can be induced with tumour necrosis factor- alfa (TNF-alfa) and this can be prevented by pre-incubation with HDL. We investigated the effect of the purified antibodies on the expression of VCAM-1. Confluent monolayers of HUVECs were incubated with culture medium with 1% of serum (basal), HDL at a concentration 1.6mg/mL or HDL+aHDL antibody (10μg/mL or 50μg/mL) for 16 h at 37°C in 5% CO2. After wash, cells were incubated for a further 4 h in the basal or stimulated state following the addition of TNF-alfa, 10ng/mL. Expression of VCAM-1 was assessed by flow cytometry using a monoclonal anti-human VCAM-1- Fluorescein antibody. Values are expressed relative to the samples that were incubated with 1% of serum. aHDL antibodies purified from SLE patients abrogate the inhibitory effect of HDL on VCAM-1 expression, in more than 90%, when compared with the control (non-specif human IgG) (p=0.0026, and p=0.0081 respectively). This study shows that aHDL antibodies isolated from patients with SLE can inhibit HDL-associated anti-inflammatory properties in vitro, and may contribute to the pathogenesis of atherosclerosis.

CS1.5 & PO1.E.15

High oxidative stress is associated with a 15.8 fold increased odds for the progression of subclinical atherosclerosis

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Objective: Increased oxidative stress is a major contributor to the pathogenesis of atherosclerosis (ATH). Patients with SLE also demonstrate high oxidative stress and an unexplained increase in ATH. Our group and others have previously reported that several biomarkers and demographic variables associated with increased oxidative stress, including pro-inflammatory HDL (piHDL), elevated leptin, homocysteine, and increased age, are individually associated

with subclinical ATH in SLE. It is unknown, however, whether these bio-markers of oxidative stress can be combined into an oxidative risk profile that better predicts future progression of atherosclerosis. Here we hypothesize that baseline presence of high oxidative stress is associated with longitudinal accumulation of subclinical atherosclerosis. **Methods:** Female SLE subjects not taking statins were studied. B-mode and Doppler scanning of carotid arteries was performed at baseline and at 24-36 months. Antioxidant function of HDL was measured as the change in fluorescence intensity caused by oxidation of DCFH by LDL in the presence or absence of test HDL. Fluorescence in the absence of HDL was normalized to 1.0. Values greater than 1.0 after the addition of HDL indicated piHDL. Plasma leptin was measured by ELISA, and homocysteine was determined by HPLC in the UCLA clinical lab. **Results:** Follow-up ultrasounds were completed on 129 SLE women. Overall, 28.9% (37) of SLE patients had new plaques. Factors associated with plaque progression on bivariate analysis included the baseline presence of plaque (p<0.001), increased age (p<0.001), piHDL (p<0.001), high leptin levels (p=0.001), and increased homocysteine (p=0.05). Although piHDL was the strongest predictor for plaque progression on multivariate analysis (OR 14.2 (95% C.I. 2.2 - 161.3,), with a negative predictive value of 93.3%, the positive predictive value was only 48%. We next used a random forests model to determine which variables were most predictive of plaque progression, and also the most significant cutpoints to dichotomize each variable. We determined the most significant predictors were age >48, piHDL, high leptin values ≥ 34ng/dL, and high homocysteine (≥12). We then created an "oxidative stress" variable, with low oxidative stress defined as 0-1 predictors, and high stress defined as 2-4 predictors. The "high" oxidative stress variable still had a negative predictive value for plaque progression of 91%, but the positive predictive value was 61%. In multivariate analysis controlling for other cardiac risk factors and disease factors, patients with high oxidative stress had a 15.8 fold increased odds for plaque progression (95% CI 3.8-65.7), and 10.8 fold increased odds for IMT progression (95% CI 3.3-38.6). **Conclusions:** Formation of an oxidative stress profile that incorporates several biomarkers and demographic factors that increase oxidative stress may provide a more complete means to identify patients at risk for progression of atherosclerosis

CS2   T Cells and Lupus

CS2.1

TH17 in lupus nephritis

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In patients with systemic lupus erythematosus (SLE), both CD4 positive and the expanded CD3+CD4-CD8- (double negative cells) produce the proinflammattory interleukin 17 (IL17). IL17-producing T cells infiltrate the kidneys and may contribute to tissue inflammation and injury. Il-23-treated lymph node cells from MRL/lpr mice injected to RaG1-/- mice caused the appearance of autoimmunity and lupus nephritis. B6.lpr mice made deficient in IL23 receptor failed to develop autoimmunity and lupus nephritis. The above studies in humans and mice strongly suggest a role for IL-17 in the expression of lupus nephritis and make it a rational target for treatment.

CS2.2 & PO1.K.1

Frequency and functional activity of Th17, Tc17 and other T cell subsets in systemic lupus erythematosus

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Objectives: To compare frequency and functional activity of peripheral blood (PB) T cell subsets, namely Th(c)17, Th(c)1 and Treg cells and the amount of type 2 cytokines mRNA in active and inactive Systemic Lupus Erythematosus (SLE) and healthy controls (NC). ***Methods:*** We recruited SLE patients with active (SLEDAI≥5) and inactive disease (SLEDAI<5) and healthy age- and gender-matched controls. One blood sample was collected from each subject. We performed a flow cytometry quantification of Th17, Tc17, Th1, Tc1 and Treg cells and of intracellular expression of IL-2, TNF-alpha, IFN-gamma and IL-17 at single cell level in Th(c)17 and Th(c)1 cell subsets. Quantification of TGF-beta1 and FoxP3 mRNA in peripheral Treg and whole blood IL-4 and IL-10 mRNA expression was performed by real-time PCR. ***Results:*** This study included 47 subjects (34 SLE patients, 15 with active and 19 with inactive disease and 13 NC). Compared to NC, SLE patients presented a trend for increased proportion of Th(c)17 cells, but with lower amounts of IL-17 per cell and also a decreased frequency of Treg, but with increased production of TGF-beta and FoxP3 mRNA. In active compared to inactive SLE, there was a marked decrease in the frequency of Th(c)1 cells, an increased production of type 2 cytokines mRNA and a distinct functional profile of Th(c)17 cells, consisting of a higher proportion of these cells producing TNF-alpha and IL-2 with a minor proportion secreting IFN-gamma. ***Conclusions:*** Our findings suggest a functional disequilibrium of T cell subsets in SLE that may contribute to the inflammatory process and disease pathogenesis.

CS2.3 & PO1.K.2

Normally occurring NKG2D+ CD4 T cells are immunosuppressive and inversely correlated with disease activity in juvenile-onset lupus
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Rationale: The NKG2D receptor stimulates natural killer cell and T cell responses upon engagement of ligands associated with malignancies and certain autoimmune diseases. However, conditions of persistent NKG2D ligand expression can lead to immunosuppression. In cancer patients, tumor expression and shedding of the MHC class I-related chain A (MICA) ligand of NKG2D drives proliferative expansions of immunosuppressive, interleukin-10 (IL-10) and transforming growth factor-β producing NKG2D+CD4+ T cells that also have the ability to cause Fas ligand-mediated inhibition of bystander T cell proliferation. These T cells are autoreactive and functionally reminiscent of regulatory T cells. ***Methods:*** Serial peripheral blood samples collected at times of high and low disease activity from 19 SLE patients diagnosed before (n=18) or at (n=1) age 18, and age-matched control individuals, were examined for frequency, antigen-specificity, cytokine profile and regulatory activity of NKG2D+CD4+ T cells by polychromatic flow cytometry and cellular assays. Patient and control plasma samples were tested for concentrations of soluble MICA and MICA autoantibodies by ELISA and LAB-Screen MICA (One Lamda) and Luminex technology. ***Results and Conclusions:*** Comparisons of mean frequencies of NKG2D+CD4+ T cells in patients with active and inactive disease and controls showed significant correlations between the disease in general and inverse correlations with disease activity in particular. The immunosuppressive phenotype of these T cells corresponded to that described in tumor patients and to the functional signature of normally occurring NKG2D+CD4+ T cells. Importantly, the NKG2D+CD4+ T cells appeared functionally uncompromised in all juvenile SLE patient samples studied. Soluble MICA was detected in all patient plasma samples but an accurate

assessment of concentrations was impaired by the presence of autoantibodies. Thus, correlations between frequencies of NKG2D+CD4+ T cells and soluble MICA ligand could not be made. As with other autoimmune diseases, there is considerable interest in restoring immune tolerance by reconstitution of functionally active regulatory T cells in SLE. However, adoptive transfer approaches are hampered by difficulties including the absence of surface markers for unequivocal identification and isolation. In contrast, the NKG2D+CD4+ T cells can be readily identified and in vitro expanded, and may thus offer new treatment modalities.

CS2.4 & PO1.K.3

Increased frequency of circulating CXCR5+CD57+CD4+ follicular T helper cells in patients with systemic lupus erythematosus
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Objectives: Production of high-affinity auto-antibodies is central to the pathogenesis of systemic lupus erythematosus (SLE). CXCR5+CD57+CD4+ follicular T helper cells (TFH) play an important role in the generation of high affinity autoantibody secreting, long-lived plasma cells in part through their production of IL-21. The TFH subset is expanded in sanroque mice exhibiting excess germinal center formation along with additional features of SLE, such as autoantibody production and renal disease. In humans, CXCR5+ T cells are found in the peripheral blood of healthy donors; however, these cells are in a resting state and lack the expression of costimulatory molecules. In this paper, we compared the frequency of CXCR5+CD57+CD4+ follicular T helper cells in the peripheral blood of SLE patients with that of healthy donors along with their expression of IL-21. We also compared IgG secretion and B cell proliferative responses from B cells cultured with and without IL-21 in order to confirm the important role of this cytokine in inducing B cell proliferation and antibody production. ***Methods:*** Peripheral blood was collected from five patients with SLE defined by ACR criteria and five healthy controls. The distribution of T cell subsets was defined by the expression of surface markers (CD3, CD4, CD8, CD57, CXCR5) determined through flow cytometry. IL-21 expression on the T cell subsets was determined by intracellular staining and detected by four color flow cytometry. B cells were purified by a MACS column and cultured with IL-21 in the presence of CD40L or anti-Ig. B cell proliferation was determined by 3H-TdR uptake and the IgG secretion in the culture supernatant was examined by ELISA. ***Results:*** Our study demonstrated a significantly increased population of CXCR5+CD57+CD4+ T cells in the peripheral blood of SLE patients compared to healthy controls (P<0.01). These CXCR5+CD57+CD4+ T cells also expressed the costimulatory molecule ICOS. Intracellular staining showed that the CXCR5+ CD57+ cells expressed significant higher IL-21 than CXCR5-CD57-CD4+ T cells and CD8+ T cells. Recombinant IL-21 potently induced B cell proliferation and IgG secretion in vitro. ***Conclusion:*** This is the first study that demonstrates circulating CXCR5+CD57+CD4+ T cells in SLE patients. This could reflect an excessive TFH response in SLE that would suggest increased circulating IL-21 secreting TFH are central to the B cell hyper-reactivity underlying the pathogenesis of SLE.

CS2.5 & PO1.K.4

Lack of PKC δ kinase activity in T cells induces a lupus-like disease
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Objective: Systemic lupus erythematosus (SLE) is an autoimmune disease caused by a mechanism not yet well understood. Our group has reported that T cell DNA hypomethylation causes gene dysregulation by overexpressing immune genes leading to autoreactivity and autoimmunity. T cells from lupus patients display impaired ERK phosphorylation that is proportional to disease activity and also show hypomethylation of the same promoter sequences

demethylated by ERK inhibitors and Dnmt inhibitors. These results correlate with the findings that impaired ERK pathway signaling may contribute to human lupus by decreasing DNA methylation in lupus. We characterized the signaling defect in idiopathic and drug-induced lupus T cells, showing that impaired protein kinase C (PKC) δ is the molecule responsible for the decreased ERK pathway signaling. More recent work suggests that oxidative damage to PKC δ is causing the ERK pathway signaling defect. In this work we investigated whether a lack of PKC δ activity in T cells is sufficient to cause a lupus-like disease. *Methods:* A dominant negative PKCδ (generous gift from Dr. Yuspa, NIH) was introduced into pTRE2 vector. The transgenic mice were bred with a transgenic SJL strain containing a reverse tetracycline transactivator under the control of a CD2 promoter. With this tet-on system, is possible to generate an inducible PKC δ defect in T cells. Gene expression was measured by RT-PCR, anti-dsDNA antibodies by ELISA, and signal transduction activation by Western Blot. *Results:* The dominant negative PKC δ was measured in the spleen of double transgenic animals with and without doxycycline in the drinking water to verify inducibility. Animals with significant amounts of dnPKCδ only in spleen, lymph nodes and thymus after doxy treatment were used. T cells from mice treated with doxy overexpressed the dnPKC δ and had decreased ERK phosphorylation, The effects of decreased PKCδ activity on Dnmt 1 expression were also studied. The number of Dnmt1 transcripts was reduced compared with animals without Doxy (Mean±SE: 0.51±0.11 vs 4.31±0.93, n=3) and it was inversely related to the expression of methylation sensitive gene CD70 (2.33±0.4 vs 0.05±0.03, mean±SE, n=4, p≤0.05), reproducing the abnormalities observed in lupus T cells. The doxy-treated animals also produced significant amounts of anti dsDNA compared to untreated mice (p= 0.028). *Conclusions:* The lack of PKCδ activity in T cells induces a lupus-like disease by modifying gene expression most likely through DNA demethylation, resulting in the production of anti-dsDNA antibodies, a hallmark in lupus disease.

CS3   Epigenetics and Lupus

CS3.1

MicroRNA – mediated immune dysregulation in human lupus
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Systemic Lupus Erythematosus is a complex autoimmune disease caused by genetic and epigenetic alterations. DNA methylation abnormalities play an important role in SLE disease processes. MicroRNAs (miRNAs) have been implicated as fine-tuning regulators controlling diverse biological processes at the level of posttranscriptional repression. Dysregulation of miRNAs has been described in various disease states, including human lupus. Whereas previous studies have shown miRNAs can regulate DNA methylation by targeting the DNA methylation machinery, the role of miRNAs in aberrant CD4+ T cell DNA hypomethylation of lupus is unclear. Here, by using high-throughput microRNA profiling , we identified that two miRNAs (miR-21 andmiR-148a) overexpressed in CD4+ T cells from both lupus patients and lupus-prone MRL/ lpr mice, which promote cell hypomethylation by repressing DNA methyltransferase 1 (DNMT1) expression. This in turn leads to the overexpression of autoimmune-associated methylation-sensitive genes such as CD70 and LFA-1 via promoter demethylation. Further experiments revealed that miR-21 indirectly down-regulated DNMT1 expression by targeting an important autoimmune gene RAS guanyl nucleotide-releasing protein 1 (RASGRP1), which mediated the Ras-MAPK pathway upstream of DNMT1; miR-148a directly down-regulated DNMT1 expression by targeting the protein coding region of its transcript. Additionally, inhibition of miR-21 and miR-148a expression in CD4+ T cells from lupus patients could increase DNMT1 expression and attenuate DNA hypomethylation. Together, our data demonstrated a critical functional link between miRNAs and the aberrant DNA hypomethylation in lupus CD4+ T cells and could help us to develop new therapeutic approaches for SLE in future.

CS3.2 & PO1.G.31

Lupus, DNA methylation and the environment
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Environmental agents interact with the immune system to cause lupus-like autoimmunity in genetically predisposed people. How the environment modifies the immune system to cause lupus though, is incompletely understood. A growing body of evidence indicates that one mechanism by which the environment can alter T cell gene expression and break tolerance is by inhibiting DNA methylation, an epigenetic mechanism regulating gene expression. Early work demonstrated that the lupus-inducing drugs procainamide and hydralazine induce autoimmunity by inhibiting replication of CD4+ T cell DNA methylation patterns during mitosis, causing aberrant overexpression of genes that convert normal T cells into autoreactive, pro-inflammatory, cytotoxic cells, and that normal CD4+ T cells treated with these drugs or other DNA methylation inhibitors cause a lupus-like disease in mice. Identical changes in CD4+ T cell DNA methylation and gene expression were found in T cells from lupus patients with active disease, suggesting that environmental agents could also contribute to the development of lupus-like diseases by inhibiting CD4+ T cell DNA methylation. Replication of DNA methylation patterns depends on DNA methyltransferase 1 (Dnmt1) enzyme activity and the methyl donor S-adenosylmethionine (SAM), and is inhibited by S-adenosylmethionine (SAH), indicating that exogenous agents decreasing Dnmt1 activity and SAM levels, or increasing SAH, will synergize to cause DNA demethylation. The incidence of lupus also increases with age, while T cell Dnmt1 levels decrease with age, and recent studies demonstrate that age-dependent decreases in Dnmt1 levels synergize with low folate or methionine levels, and increased homocysteine levels, to demethylate T cell DNA and cause overexpression of genes contributing to lupus. Dnmt1 levels are also regulated by the ERK pathway, and exogenous ERK pathway inhibitors including xenobiotics, UV light and oxidative stress, can all demethylate T cell DNA. Together, these studies suggest that age, diet, xenobiotic Dnmt inhibitors, UV light and oxidative stress can interact to inhibit DNA methylation in CD4+ T cells, altering gene expression and promoting the development of lupus-like autoimmunity in genetically predisposed people.

CS3.3 & PO1.G.1

Whole genome methylation scan reveals both hypomethylated and hypermethylated genes in lupus CD4+ T cells
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Objectives: Inducing a T cell DNA methylation defect causes lupus-like autoimmunity in animal models. T cell DNA methylation defect correlates with disease activity in lupus patients. Herein, we perform a whole-genome methylation scan in CD4+ T cells to identify differentially methylated genes in lupus patients and determine the functional implication of differentially methylated loci upon gene expression. *Methods:* We used DNA immuno-precipitation with anti-5-methylcytidine antibody coupled with microarray hybridization to determine genome-wide DNA methylation patterns in CD4+ T cells from lupus patients and controls matched for age, sex and race. Input and immunoprecipitated DNA from each sample were differentially labeled and hybridized to arrays with ~ 385,000 probes, covering all UCSC-annotated CpG islands and promoter regions for all RefSeq genes. CD4+ T cell gene expression data from Gene Expression Omnibus (GEO) were utilized and normalized signal intensity expression values were analyzed to detect differences between lupus patients and controls. Pathway analysis and literature mining analysis were performed using Ingenuity and IRIDESCENT soft-

ware. *Results:* We identified genes differentially methylated within the 5kb upstream to 5kb downstream of the transcription start site between CD4+ T cells from lupus patients and controls. We found 624 hypermethylated and 661 hypomethylated genes in lupus patients. Functional analysis revealed that top canonical pathways shared among hypermethylated genes include Cell Cycle Regulation, Wnt/β-catenin Signaling, Dendritic Cell Maturation, Graft-versus-Host Disease Signaling, IL-10 Signaling, and p38 MAPK Signaling. Shared canonical pathways among hypomethylated genes include IL-15 Production, Dendritic Cell Maturation, p38 MAPK Signaling, Graft-versus-Host Disease Signaling, FLT3 Signaling in Hematopoietic Progenitor Cells, and Interferon Signaling. Furthermore, many differentially methylated genes in lupus CD4+ T cells were also variably expressed, suggesting that the methylation changes observed, at least in a subset of genes, might be functionally relevant. Of the hypomethylated genes, 112 were found to be overexpressed, while 99 hypermethylated genes were underexpressed in lupus CD4+ T cells. Some disease-relevant hypermethylated genes in lupus CD4+ T cells include CASP3, BCL6, ICAM1, and GZMB. On the other hand, the genes CASP2, FCRL3, STAT1, STAT2, TYK2, IL-18, and TNFSF13 were hypomethylated. *Conclusions:* We performed the first whole-genome methylation analysis in lupus CD4+ T cells and mapped differentially methylated regions across the genome. In addition to global T cell DNA hypomethylation that has been previously described in lupus, our data suggest that promoter and CpG Island hypermethylation in CD4+ T cells might also plays a role in the pathogenesis of lupus.

CS3.4 & PO1.G.2

X chromosome signatures associated with the microRNome of human peripheral blood CD4+ T cells; integrating the epi-microRNome into the molecular basis of human lupus
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Objectives: Hypomethylation of regulatory sequences correlates with active transcription. Sequences on the inactive X may cause gene overexpression upon demethylation. X chromosome inactivation has been implicated in female predisposition to autoimmunity. Here we have investigated the epigenetic regulation of X chromosome miRNAs in CD4+ T cells to identify the effect of miRNA on gender bias in autoimmunity. *Method:* Methylation sensitive mRNA and miRNA from CD4+ T cells were identified by using experimentally demethylated in vitro system using DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (5-azaC). Transcript profiling for isolated RNA was performed by using Affymetrix GeneChip Human Genome U133 Plus 2.0 Array. Genome-wide qPCR miRNA expression profiling was performed using System Biosciences (SBI) QuantiMir™ RT Kit. Results from hypomethylated experiments were compared to expression profiles from active lupus patients to identify methylation sensitive genes differentially expressed in lupus patients. Most likely oppositely-correlated miRNA: RNA interactions were identified by RNA22 tool from IBM research. *Results:* When the differential expression between women and men were compared without T cell stimulation, 5-AzaC caused a total of 97 genes to differentially express between women and men of which 78 were female biased genes. However, PMA-ionomycin stimulation caused downregulation of 157 genes out of 170 differentially expressed genes in women. Using a factor of 2 fold as a cut-off, we identified a total of 104 miRNAs up-regulated in 5AzaC treated CD4+ T cells, of which 27 were female biased and 11 were male biased. The X chromosome encoded miRNAs; hsa-let-7f-2*, hsa-miR-503, hsa-miR-421, hsa-miR-188-3p, hsa-miR-513-a-3p and hsa-miR-374a were found to be expressed higher in women compared to men in when the DNA was hypomethylated. Furthermore hsa-let-7f-2*, hsa-miR-503 and hsa-miR-188-3p were highly expressed in active female lupus patients tested (n =4) compared to the healthy controls. Functional Annotation clustering of these miRNA targets revealed that the genes belonging to "response to stress" category being most affected. *Conclusion:* These results suggest that the miRNA overexpression due to DNA demethylation may contribute to the downregulation of genes observed in hypomethylated CD4+T cells. Furthermore, the demethylation of X chromosome resulting in

elevated levels of X chromosome miRNAs, may play a major role in the decreased mRNA levels in stimulated CD4+ T cells in women. This in turn may contribute to the gender differences of gene expression levels. Better understanding of the intertwined connection between epigenetics to miRNAs and gene expression could add a new dimension to the understanding of molecular mechanisms leading to gender bias in autoimmune diseases.

CS4   Outcomes in Lupus

CS4.1

Biomarkers for diagnosis and disease activity in SLE: are clinical associations in early translational studies valid?
Ward, Michael M.; Tektonidou, Maria G.
National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, US Department of Health and Human Services, Bethesda, MD, USA

Objective: Biomarkers can aid diagnosis and assessment of disease activity, yet early translational studies of new biomarkers may be misleading if not designed to address information bias. We evaluated if translational research studies of potential biomarkers in systemic lupus erythematosus (SLE) incorporated design features important for valid clinical associations. *Methods:* We searched ten journals for translational research studies published in 2005-2009 that reported associations between new laboratory markers and the presence of disease (SLE versus controls) or measures of disease activity. We examined studies for the following design features: age-, sex- and race-matching between groups; control for the effects of treatment on the expression of the biomarker; inclusion of both patients with early and late disease, or both active and inactive disease; longitudinal or cross-sectional design; and use of validated measures. *Results:* Forty-eight studies examined potential biomarkers for diagnosis and 27 studies examined biomarkers for disease activity assessment. Lymphocyte markers, other cell surface markers, and gene expression profiles were the most commonly examined biomarkers. Age-matched and sex-matched groups were examined in 62% and 58% of studies of diagnosis, respectively. Although 70% of studies reported information on treatment, only 52% examined if treatment affected the biomarker. Only 33% of studies included both patients with early and late SLE. Among studies of biomarkers of disease activity, age-adjusted or sex-adjusted comparisons were included in 37% and 40% of studies, 59% examined if treatment affected the biomarker, and only 40% of studies included a longitudinal component. The proportion of studies including important study design features tended to be higher than comparable studies in rheumatoid arthritis (N = 67 for diagnosis; N = 9 for disease activity). *Conclusion:* Early translational studies of potential biomarkers in SLE often do not include study design features needed for proper interpretation of clinical associations. Attention to these features could reduce false-positive and false-negative associations.

CS4.2

Malignancy in SLE
Bernatsky, Sasha
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Persons with systemic lupus erythematosus (SLE) have an increased risk of certain types of malignancies. Of particular concern are hematologic cancers, especially non-Hodgkin lymphoma, where a three- to four-fold increased incidence is seen in SLE, compared to the general population. There is some evidence that immunosuppressive medications play a role in mediating this risk, although there appear to be other factors driving the association as well. There is some evidence that lupus disease activity may itself be a mediator of the association between SLE and lymphoma. In addition to hematologic cancer risk, lung cancer also is increased in SLE compared to the general population, and smoking may drive this risk in large part. Last but not least,

cervical dysplasia has been flagged as a concern for women with SLE. Some of these issues will be highlighted in Dr. Bernatsky's presentation. The discussion will feature some novel initiatives of the Systemic Lupus International Collaborating Clinics (SLICC).

CS4.3 & PO2.K.1

Statins reduce disease activity scores (SLAM-R) and cumulative organ damage (SLICC) in SLE patients from a multi-center, multi-ethnic, US multi-institutional cohort.

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Purpose: Statins, in addition to lower cholesterol levels, have are cholesterol anti-thrombotic, anti-inflammatory and cardiovascular protective effects. Cardiovascular morbidity and accelerated atherosclerosis are important features of SLE. However, It is uncertain whether statins may have beneficial effects in patients with SLE. *Objectives:* To examine longitudinally the impact of statins on levels of biomarkers of disease (cytokines/chemokines) and disease activity (SLAM-R) and with damage accrual (SLICC-ACR Damage Index, SDI).on in patients with SLE *Methods:* Sera from 21 SLE patients (ACR criteria) from a multi-ethnic, multi-center cohort (LUMINA) were assessed at baseline and at a subsequent visit after treatment with statins was started. Patients that were on more than 10 mg prednisone/day, or on other immunosuppressive therapy were excluded (age range: 16-67; mean age: 44.6): 38% were African American, 9% were Caucasians, and 4% were Puerto Rican Hispanics , 86% were females and 14% were males. .Levels of IL1b, IL-6, IL-8, IFN-α, IP-10, MCP-1. VEGF, sCD40L and TNF-α , were measured in serum using a Millipore Millliplex™ Multiplex Assay and titers of soluble (s) E-selectin (E-sel), intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), IgG and IgM anticardiolipin antibodies (aCL) were detected by ELISA. High sensitive C reactive protein (hsCRP) was determined by nephelometry. Changes in the levels of the biomarkers after treatment were calculated and the effects of statins were tested using a signed rank test. Spearman correlations were used to compare changes in levels of biomarkers with changes in SLAM-R or SLICC scores. *Results:* SLAM-R and SLICC scores were significantly affected by statins (p values 0.0199 and 0.039, respectively). IL1b, IL6, IL8, TNF-α VEGF, IP-10, sCD40L and sVCAM-1 levels were reduced after treatment with statins by: 28%, 28%, 52%. 43%, 57%, 42%, 52% and 62% respectively but the reduction was not statistically significant. Importantly, the changes in IL6, after treatment with statins correlated with changes in SLICC scores (p value: 0.0362). hsCRP was increased in 50% of the subjects at baseline and reduced by 37% after treatment with statins. *Conclusions:* Significant number of SLE patients' samples showed a decrease in levels of some cytokines and chemokines and of hsCRP after treatment with statins. Importantly, SLAM-R and SLICC scores were significantly reduced by statins and the changes in IL6 correlated with a significant decrease in organ damage accrual (SLICC).

CS4.4 & PO1.F.1

The Georgia Lupus Registry: a population-based estimate of the incidence of SLE in patients with chronic cutaneous lupus

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Emory University, Atlanta, GA, USA

Objectives: It is deemed that a small proportion of patients with chronic cutaneous lupus (CCLE) develop SLE. One population-based study found that 12% patients with different forms of cutaneous lupus progressed to SLE. We studied the incidence of SLE and demographic risk factors in patients with CCLE from a large population-based registry of lupus in Georgia. *Methods:*

The Georgia Lupus Registry is a population-based registry aimed to estimate the incidence and prevalence of SLE in Atlanta, GA. Multiple sources are used to find potential cases of SLE and cutaneous lupus. Nearly 250 demographic and clinical elements were abstracted from medical records. Sources included: 96% hospitals, 100% high yield rheumatologists, 36% high yield dermatology practices, and the major dermatopathology laboratory in the target area. CCLE was defined as lupus profundus, discoid, chilblain, or mucosus lupus, and the outcome as SLE (≥4 ACR criteria or 3 ACR criteria and diagnosis of SLE by rheumatologist). The Kaplan-Meier method was used for the analysis. The risk-time was from the date of CCLE to the date of event, or to the censored end-point (12/ 31/2004). *Results:* Among 708 CCLE cases, 190 were prevalent and 24 had unknown dates of CCLE. 494 CCLE cases were analyzed (79% female, 73% blacks, 17% whites).

Table 1. Cumulative Incidence of SLE after CCLE

Year after diagnosis of CCLE	Cumulative Incidence (%)	95% CI	n SLE	n at Risk
Year 1	3.0	1.5-4.5	12	435
Year 2	4.7	3.1-7.1	21	375
Year 5	15.2	12.3-19.8	58	238
Year 10	30.0	24.7-36.3	88	109
Year 15	44.0	36.9-51.8	106	54
Year 20	60.0	51.2-69.7	119	26
Year 25	66.9	57.1-79.9	123	11

When compared by gender, incidence estimates at 2, 5 and 10 years were higher in females than males (F: 5.6, 17.1 and 33.7%; M: 1.2, 8.1, and 14.2%, respectively, p=0.01). No differences were found by race or age at CCLE. *Conclusions:* This population-based study shows that there is considerable progression to SLE among patients with CCLE-onset. The cumulative incidence of SLE at 10 years was 30%. The incidence estimates were significantly higher among females, particularly since the few years after the diagnosis of CCLE. Prospective studies assessing disease-related risk factors and potential triggers, principally in females with CCLE, are needed to improve early recognition of patients at higher risk of SLE.

CS4.5 & PO2.D.36

An introduction on Chinese registry of systemic lupus erythematosus: preliminary data from CSTAR (Chinese SLE Treatment and Research group)

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Introduction: CSTAR is the first Chinese on-line registry of lupus, which is supported by Chinese National Key Technology R&D Program. The data was collected from 106 centers, which covered 30 provinces in China. The aim is to clarify several epidemiologic aspects of SLE in China and establish a platform to provide resource for future studies . *Methods:* CSTAR started with a multicentre, consecutive and prospective design. The cohort that is developing and studying a large and growing cohort of uniformly evaluated individuals from Chinese populations to achieve the goals previously mentioned. The registered patients must fulfill four or more of the American College of Rheumatology criteria for the classification of SLE. All CSTAR centers use the same protocol-directed methods to provide uniform evaluations, which include demographic data, clinical history, laboratory and radiological examinations, disease activity evaluations (BILAG2004, SLEDAI and PGA). All data are collected by e-CRF combined with biospecimen collections. *Results:* Preliminary analyses of CSTAR data from 1344 baseline evaluations were available. The demographic data showed the mean age of this cohort was 32.7±12.3 yrs(5-77), which consisted of 1242(92.4%) female and 102(7.6%) male patients (female to male ratio, 12.2). The mean age at onset was 29.4yrs(1.4-76.1) with diagnosis at the age of 30.6yrs(6-77). 78(5.8%) patients had family history of rheumatic diseases including 20(1.5%) cases with SLE and other 58(4.3%) ones with rheumatoid arthritis, primary Sjogrens' syndrome or systemic sclerosis. 66(4.8%) abnormal pregnancies were recorded among 1375 experiences. The characteristics of the CSTAR cohort were

summarized with comparison of others in Table 1. The leading manifestations at onset were hematological disorders 772(57.4%), arthritis 748(55.7%), malar rash 630(46.9%), nephropathy 616(45.8%) and fever 516(38.4%), but rare ones might be neurological 60(4.5%), cardiac 43(3.2%) and gastrointestinal involvements 48(3.6%). The profiles of major autoantibodies included 1310(97.5%) antinuclear antibodies, 903(67.2%) anti-dsDNA, 139(10.3%) anti-Sm, 143(10.6%) anti-RNP, 226(16.8%) anti-SSA, 124(9.2%) anti-SSB and 99(7.4%) anti-rRNP. The cohorts were stratified by SLEDAI scores to stable group(<5) with 349(46%), mild active(5-9) with 361(26.9%), moderate active(10-14) with 395(29.4%) and sever active with 239(17.8%). ***Conclusion:*** CSTAR cohort provided epidemiological data and phenotypes of Chinese patients with SLE, which would be a resource to depict morbidity and mortality with a long-term followup. Biospecimen also made further basic studies possible in the future with international collaborations.

Author	Petri, et al.	Wang, et al.	Euro-lupus project	CSTAR
Number of patients	574	539	1000	1344
Geographical area	America	Asia, Malaysia	Europe	Asia, China
Malar rash	331 (57.7)	410 (76.1)	311 (31.1)	630(46.9)
Discoid lesions	162 (28.2)	30 (5.6)	78 (7.8)	86(6.4)
Photosensitivity	335 (58.4)	222 (41.2)	229 (22.9)	370(27.5)
Oral ulcers	219 (38.2)	185 (34.3)	125 (12.5)	288(21.4)
Arthritis	NR	272 (50.5)	481 (48.1)	748(55.7)
Serositis	NR	NR	NR	205(15.3)
Hematological involvement	NR	263 (48.8)	182 (18.2)	772(57.4)
Nephropathy	319 (55.6)	399 (74)	279 (27.9)	616(45.8)
Neurological involvement	NR	123 (22.8)	194 (19.4)	60(4.5)

CS5   B Cells in Lupus

CS5.1

Differential therapeutic impact on B cell subsets in SLE
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B cell directed therapies have gained interest in the treatment of SLE within the last decade. The mechanism of action by anti-CD20 and anti-CD22 therapy provide some interesting insights. While anti-CD20 therapy using rituximab leads to a normalization of a number of disturbances of peripheral blood subsets after their substantial, recent evidence indicates that plasmablasts with a steady state phenotype are not fully depleted by this monoclonal antibody therapy. These remaining cells carry characteristic of mucosal origin with mainly IgA production being IgA+/β7 integrin$^+$/CCR10$^+$/HLA-DR^{high} and may potentially arise from either B1 B cells of the peritoneal cavity or B2 B cells continuously generated in bone marrow. They secrete IgA *in vitro*, that bound to bacterial antigens as did IgA+ peripheral B cells under healthy conditions which all expressed mutated V_H genes of IgA$_1$ isotype. The humanized monoclonal anti-CD22 antibody epratuzumab which has also been studied in patients with SLE is able to inhibit B cell proliferation and can influence the expression of the adhesion molecules, CD62L and β7 integrin. In in vitro studies, a substantially higher CD22 binding by epratuzumab was found on peripheral B cells, while there was no binding on T cells and low binding on macrophages/monocytes. Surprisingly, naïve B cells had significantly higher epratuzumab binding than memory B cells. The down-modulation of the surface expression of CD62L and β7 integrin preferentially by naïve B cells may indicate that epratuzumab has a potential to impair trafficking of peripheral B cells which could explain a preferential reduction of peripheral B cells as observed in a phase II a study in SLE.

CS5.2

Autoimmunity and tolerance: tracking self-reactivity to its source
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Accumulating evidence indicates that B-lymphocytes are key players in innate and adaptive branches of immunity, and that impairment of some of their functions can lead to a variety of disorders in humans. In SLE, a number of B lymphocyte alterations have been recognized, leading to novel immuno-intervention strategies based on specific B lymphocyte targeting. Despite this therapeutic progress, the precise mechanisms that underlie loss of B cell tolerance to self-antigens in autoimmune disease remain under scrutiny. During early B cell development, the pre-B cell receptor, formed by the assembly of newly formed immunoglobulin heavy chains with the B lymphocyte-restricted lambda 5 and Vpre-B chains, plays a role in negative selection of self-reactive B cells. Reduced expression of pre-B receptors may underlie an altered pre-BCR-mediated negative selection checkpoint of autoantibody-producing B cells, leading to deficient B lymphocyte signaling and abnormal expression of B cell subsets. At later B cell developmental stages, clonal deletion or clonal anergy may be disturbed so as to contribute to autoreactivity formation. Additionally, we have found that alterations in receptor editing, a chief mechanism of B cell tolerance to self, can contribute to SLE through at least two different mechanisms. Whereas impaired secondary rearrangements may result in ineffective silencing of B cells, exacerbation of receptor editing can give rise to the emergence of autoreactive receptors from clones that were initially devoid of autoreactivity. Both alterations can promote the pathogenesis of autoimmune diseases by favoring the uncontrolled emergence and/or persistence of autoreactive B cell clones. In addition to predisposing genes, we have found that epigenetic factors can have an impact on lymphocyte tolerance to self-antigens. Since epigenetic mechanisms can affect various biological properties — from the eye color of fruit flies to the morphology of flowers — further investigation into the impact of epigenetics might provide us with unexpected clues that will help elucidating more fully the basis of deregulated tolerance in autoimmune disease.

CS5.3 & PO1.I.1

The plasma cell compartment in NZB/W mice is not responsive to IVIG-treatment
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Introduction: Systemic lupus erythematosus (SLE) is the prototype of a systemic autoimmune disease characterized by the formation of autoantibodies directed i.e. against double stranded DNA. Intravenous immunoglobulins are one of the therapeutic options in severe organ involvement. The treatment with specific natural polyclonal anti-dsDNA anti-idiotypic antibodies (IVIG-ID) resulted in reduction of anti-dsDNA antibody titers, prolongation of survival and and improvement of renal pathology in NZB/W F1 mice, the mouse model for SLE. Recently, it has been shown that long-lived plasma cells resistant to immunosuppressive drugs contribute to maintenance of autoimmunity. ***Objective:*** to study the effect of IVIG-ID on both short-lived and long-lived plasma cells in NZB/W mice with full-blown disease as well as in young mice in the phase of the establishment of the long-lived plasma cell compartment. ***Methods:*** 6 weeks, clinical healthy and 7 months old NZB/W mice with full-blown disease were treated 1x/week for 3 weeks i.v. with 2 mg/kg bw/injection of IVIG-ID. BrdU-feeding was started 2 weeks prior to treatment. Mice were sacrificed at the end of week 3 of treatment. BrdU-incorporation was measured by flow cytometry. Antibody-secreting cells from spleen, bone marrow and kidneys were quantified according to their isotype and specificity using Elispot. ***Results:*** Absolute viable lymphocyte count was not affected by the treatment neither in spleen nor bone marrow in both mouse groups. Numbers of total IgG- and IgM-secreting plasma cells as well were not changed by

the treatment regimen in the organs investigated. DNA-specific plasma cells in the spleen as well as in bone marrow were not reduced independent of their isotype. The frequency of long- and short-lived plasma cells was not changed either. In the spleen of old mice a mean of 46 ±9 % of the plasma cells of controls was long-lived compared to 48 ± 21% in treated animals. In young mice 33±4 % of the plasma cells in the spleen were long-lived in treated mice compared to 37±5 % in untreated mice. In bone marrow no changes could be found either. *Conclusion:* Both preventive and therapeutic administration of IVIG-ID does not show a significant effect on plasma cells originating from spleen, inflamed kidney or bone marrow in NZB/W mice. Therefore, other mechanisms leading to reduction of serum anti-dsDNA antibody titers should be taken into consideration. This suggests that temporary reduction of anti-dsDNA antibody titers by IVIG-ID is not due to plasma cells depletion.

CS5.4 & PO1.I.2

The effects of the anti-CD22 monoclonal antibody epratuzumab on peripheral blood B cells and immune responses *in vivo* and immunoglobulin production *in vitro*

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UCB, Slough, UK

Objectives: Epratuzumab is a monoclonal antibody against CD22 currently being evaluated clinically in patients with Systemic Lupus Erythematosus (SLE). The aim of the current study in Cynomolgus monkeys was to investigate the effects of epratuzumab on circulating B cell numbers and on the immune response to the challenge antigens keyhole limpet hemocyanin (KLH) and tetanus toxoid (TT). Additionally, the effect of epratuzumab on immunoglobulin production from human B cells in culture was assessed. *Methods:* In one study, Cynomolgus monkeys received 4 weekly doses of epratuzumab at 10, 60 or 160 mg/kg and peripheral blood CD20+ B cell numbers were enumerated by flow cytometry. In a second study, Cynomolgus monkeys received epratuzumab at 3 different dose levels (1X 60mg/kg, 1X 10mg/kg or 4X 60mg/kg), or saline. The primary immune response to administered KLH and the secondary immune response to TT were then monitored over time using ELISAs to measure anti-TT and anti-KLH titres in serum. Human peripheral blood mononuclear cells (PBMC) or purified B cells from human tonsils were cultured *in vitro* with a range of stimuli and the effect of epratuzumab on IgG and IgM production, assessed by ELISA, was monitored after 5 days in culture. *Results:* There was a 40-50% reduction in the numbers of circulating B cells in Cynomolgus monkeys after treatment with epratuzumab at all doses tested, which occurred within 24 hours of dosing. Animals treated with saline showed a primary anti-KLH response, with an increase in both IgG and IgM antibody levels. Epratuzumab did not inhibit this response at any dose tested, and there was no significant difference between the groups when area under the curve of the response over time for each animal was assessed. A robust IgG anti-TT was demonstrated but, again, no significant difference was observed between the differently dosed groups and saline controls. The production of IgG and IgM from human PBMC or tonsil B cells in culture was unaffected by incubation with a range of concentrations of epratuzumab. *Conclusions:* Epratuzumab treatment caused a reduction in B cells but had no effect on the capacity to raise an antibody response to challenge antigens in Cynomolgus monkeys *in vivo*. The production of immunoglobulin by B cells in culture was also unaffected by epratuzumab. This might indicate that the efficacy of epratuzumab in SLE patients is unlikely to be accompanied by a gross effect on the capacity to generate an adaptive immune response.

CS6 New Drugs in Development

CS6.1 & PO2.E.1

Belimumab, a BLyS-specific inhibitor, reduced disease activity, flares, and steroid use in patients with seropositive systemic lupus erythematosus (SLE): BLISS-52 study

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Objective: To assess the efficacy and safety of belimumab, a BLyS-specific inhibitor, in seropositive patients with moderate to severe SLE. *Methods:* BLISS-52 was a phase 3, 52-wk double-blind study including 865 seropositive (ANA≥1:80 and/or anti-dsDNA ≥30 IU/mL) SLE patients with SELENA-SLEDAI (SS) ≥6 (stable standard of care [SOC] therapy ≥30 d) from Latin America, Asia-Pacific, and Eastern Europe. Patients were randomized to receive belimumab (1 or 10 mg/kg) or placebo in addition to SOC with dosing on day(s) 0, 14, 28, then q28d for 48 wk. Disease activity was assessed using SS, BILAG, and SS Flare Index (SFI). The primary endpoint was the SLE Responder Index (SRI) at wk 52, including improvement in SS (≥4-point decrease), no new BILAG 1 A/2 B flares, and no >0.3-point worsening in Physician's Global Assessment (PGA). *Results:* Baseline disease characteristics were similar across treatment groups, with mean values as follows: disease duration 5.3 y; SS 9.8; BILAG 1 A/2 B 58%; ANA+ 95%; anti-dsDNA+ 75%; low C4 59%; proteinuria (>0.5 g/24 h) 25%; antimalarials 67%; prednisone-equivalent dose of steroid ≥7.5 mg/d 69%; immunosuppressants 42%. Significant improvements were observed in SRI response rates for the 1-mg/kg and 10-mg/kg belimumab dose groups at 51% (p=0.0129) and 58% (p=0.0006), respectively, vs placebo at 44%. Significant improvement was also observed in at least 1 belimumab treatment group in the following measurements: SS ≥4-point reduction; improvement or no >0.3-point worsening in PGA; reduction in steroid use; reduction in flare rates, and increase in time-to-first flare (Table 1). Rates of overall AEs, deaths, serious AEs, infections, and lab abnormalities were comparable between belimumab and placebo groups. Serious or severe infusion reactions were slightly higher with belimumab than with placebo. No malignancies were reported. *Conclusions:* Belimumab significantly reduced SLE disease activity, flare rates, and steroid use, and increased time-to-first SLE flare in patients with seropositive SLE. The overall rate of AEs, including serious AEs and infections, were comparable in the belimumab and placebo groups. (NCT00424476)

	Placebo n=287	Belimumab (1 mg/kg) n=288	Belimumab (10 mg/kg) n=290
Efficacy			
SRI at wk 52*, n (%)	125 (44)	148 (51) *p=0.013*	167 (58) *p= 0.0006*
• SS ≥4-point reduction	132 (46)	153 (53) *p=0.019*	169 (58) *p=0.0024*
• No PGA >0.3-point worsening	199 (69	227 (79) *p=0.0078*	231 (80) *p=0.0048*
• No new BILAG 1 A / 2 B scores	210 (73)	227 (79) *p=0.086*	236 (81) *p=0.018*
% PGA improvement at wk 24, mean (SE)	22 (2.6)	30 (2.2) *p=0.034*	37 (2.4) *p< 0.0001*
Steroid reduction from >7.5 mg/d by 25% from baseline or to ≤ 7.5 mg/d during wk 40-52, n (%)	23 (12)	42 (21) *p=0.025*	38 (19) *p=0.053*
Steroid increase from ≤ 7.5 mg/d to >7.5 mg/d at wk 52, n (%)	34 (36)	25 (30) *p=0.56*	17 (20) *p=0.020*
SFI flare, % (hazard ratio [HR]) / Median time to first flare, day	80 / 84	71 (0.75) / 126 *p=0.0026 †*	71 (0.76) / 119 *p=0.0036 †*
• Severe SFI flare	23	18 (0.76) *p=0.13*	14 (0.57) *p=0.0055*
New BILAG 1 A / 2 B flare, % (HR)	30	27 (0.89) *p=0.48*	19 (0.58) *p=0.0016*
Safety, n (%)			
AEs	263 (92)	264 (92)	266 (92)
Serious AEs	*36 (13)*	*47 (16)*	*41 (14)*
Infections	183 (64)	197 (68)	194 (67)
Serious infections	*17 (6)*	*22 (8)*	*13 (5)*
Infusion reactions	49 (17)	47 (16)	48 (17)
Hypersensitivity	*1 (<1)*	*4 (1)*	*2 (1)*
Discontinuations	61 (21)	48 (17)	49 (17)
Due to AEs	*19 (7)*	*16 (6)*	*15 (5)*

*Patients who withdrew from the study prior to wk 52 visit or who used protocol-prohibited medications were considered treatment failures
†P-values were obtained from Cox proportional hazard model for time to first flare.

CS6.2 & PO2.E.2

Belimumab, a BLyS-specific inhibitor, reduced disease activity and severe flares in patients with seropositive SLE: BLISS-76 study

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Background: To assess the efficacy and safety of belimumab in patients with seropositive SLE. **Methods:** 819 seropositive (ANA ≥1:80 and/or anti-dsDNA ≥30 IU/mL) SLE patients with SELENA-SLEDAI (SS) ≥6 (on stable standard of care [SOC] therapy ≥30 d) were treated in this phase 3 76-wk double-blind international study of belimumab (1 or 10 mg/kg) plus SOC or placebo plus SOC on days 0, 14, 28, then q28 d for 72 wks. Efficacy analyses included SS, BILAG, and SS Flare Index (SFI). Primary endpoint was the wk 52 SLE Responder Index (SRI) response rate: improvement in SS (≥4-point decrease), no new BILAG A or 2 B flares, and no >0.3-point worsening in Physician's

Global Assessment (PGA) vs baseline. **Results:** Mean baseline disease characteristics across treatment groups were generally similar: SLE disease duration 7.5 y; antimalarial use 63%; steroid use 76%; prednisone-equivalent dose of steroid ≥7.5 mg/d 46%; immunosuppressant use 56%; proteinuria (>0.5 g/24 h) 16%; low C4 53%; SS 9.7; BILAG 1 A/2 B 64%; ANA+ 92%; anti-dsDNA+ 64%. SRI response rates were 41% (p=0.1041) in the 1-mg/kg and 43% (p=0.0207) in the 10-mg/kg belimumab dose groups (vs placebo 34%). Significant improvement was observed in at least 1 of the belimumab treatment groups for SS ≥4-point reduction; no >0.3-point worsening in PGA; reduction in severe flares; improvement in SF-36 Physical Component Score (PCS) and FACIT-Fatigue (Table 1). Statistically significant improvement in PGA or reduction in steroid use was not seen with belimumab treatment. Overall AEs, deaths, serious AEs, infections, and lab abnormalities were comparable in the belimumab and placebo groups. Serious or severe infusion reactions were modestly increased with belimumab compared with placebo. A total of 6 malignancies occurred, with similar proportions across all study groups. **Conclusion:** In the BLISS-76 study, belimumab significantly improved the SRI response rate as well as reduced SLE disease activity and severe SLE flare rates in patients with seropositive SLE. The overall rates of AEs, including serious AEs and infections, were comparable in the belimumab and placebo groups. (NCT00410384)

	Placebo n=275	Belimumab (1 mg/kg) n=271	Belimumab (10 mg/kg) n=273
Efficacy			
SRI at wk 52*, n (%)	93 (34)	110 (41) *p=0.1041*	118 (43) *p=0.0207*
• SS ≥ 4-point reduction	98 (36)	116 (43) *p=0.0869*	128 (47) *p=0.0062*
• No PGA >0.3-point worsening	173 (63)	197 (73) *p=0.0120*	189 (69) *p=0.1258*
• No new BILAG 1 A / 2 B scores	179 (65)	203 (75) *p=0.0108*	189 (69) *p=0.3193*
% Change in PGA at wk 24, mean (SE)	+26.18 (4.21)	+28.14 (3.62) *p=0.5149*	+27.57 (3.37) *p=0.4682*
Prednisone reduction from >7.5 mg/d by 25% from baseline or to ≤ 7.5 mg/d during wks 40-52, n (%)	16 (13)	25 (19) *p=0.2034*	20 (17) *p=0.5323*
Prednisone increase from ≤ 7.5 mg/d to >7.5 mg/d at wk 52, n (%)	48 (32)	33 (23) *p=0.1092*	45 (29) *p=0.6088*
SFI flare, % (hazard ratio [HR]) / Median time to first flare, day	83 / 82	79 (0.89) / 85 *p=0.2324†*	79 (0.93) / 84 *p=0.4796†*
• Severe SFI flare % (HR)	24	16 (0.64) *p=0.023*	18 (0.72) *p=0.0867*
New BILAG 1 A / 2 B flare, % (HR)	34	28 (0.78) *p=0.1191*	32 (0.93) *p=0.6135*
Change in SF-36 PCS from baseline at wk 52, mean (SE)	+2.85 (0.52)	+4.37 (0.51) *p=0.012*	+3.41 (0.47) *p=0.51*
Change in FACIT-Fatigue score from baseline at wk 52, mean (SE)	+2.86 (0.66)	+5.73 (0.66) *p=0.0023*	+4.63 (0.63) *p=0.1376*
Safety, n (%)			
AEs/ Serious AEs	251 (91) / 49 (18)	251 (93) / 57 (21)	251 (92) / 57 (21)
Infections/ Serious	185 (67) / 14 (5)	196 (72) / 17 (6)	196 (72) / 19 (7)
Infusion reactions/ Serious/ Severe	27 (10) / 1 (0.4) / 1 (0.4)	41 (15) / 2 (0.7) / 1 (0.4)	36 (13) / 3 (1.1) / 3 (1.1)
Hypersensitivity	0 (0)	4 (2)	0 (0)
Malignancies	1 (<1)	3 (1)	2 (1)
Discontinuations/ Due to AEs	70 (26) / 21 (8)	55 (20) / 17 (6)	64 (23) / 22 (8)

*Patients who withdrew from the study prior to wk 52 visit or who used protocol-prohibited medications were considered treatment failures.
†P-values were obtained by the Cox proportional hazard model for time to first flare.

CS6.3 & PO2.E.4

Epratuzumab demonstrates clinically meaningful improvements in patients with moderate to severe systemic lupus erythematosus (SLE): results from EMBLEM™, *a phase IIb study*

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Objectives: This 12-week, multicenter, phase IIb, randomized, double-blind, placebo-controlled study was designed to assess the efficacy and safety of epratuzumab in SLE and select a dose and regimen (NCT00624351). ***Methods:*** Patients with moderate/severe SLE (≥ 1 BILAG 2004 A or ≥ 2 Bs) were randomized to 1 of 6 intravenous regimens: placebo (standard of care), cumulative doses (cd): 200, 800, 2400, or 3600mg of epratuzumab in equal, divided doses using 2 every other week (EOW) infusions or 2400mg cd as 4 equal infusions 1 week apart. A combined group of 2400mg cd (1200mg EOW plus 600mg weekly) was analyzed. Concomitant oral corticosteroids/immunosuppressives were to be stable before first infusion and during the study. Primary endpoint was a combined responder index of clinical disease activity at Week 12, defined as reduction of all baseline BILAG A to B/C/D and BILAG B to C/D in all body systems, no BILAG worsening in other organ systems, and no deterioration in SLEDAI or PGA, with no increase in corticosteroids/immunosuppressives over baseline. ***Results:*** At baseline, in the entire population (N=227) mean age was 38.8 years, 94% were female, 78% Caucasian; with high disease activity (70% with ≥ 1 BILAG A, mean total scores: BILAG 15.2, SLEDAI 14.8). Compared with placebo responder rate (21.1%), responder rates were statistically greater in epratuzumab 2400mg (600mg weekly) (45.9%; p=0.03) and 2400mg combined groups (40.5%; p=0.02); and showed clinically meaningful improvement in the 2400mg (1200mg EOW) group (Table). At Week 12, 18.9% of patients in both 2400mg cd groups achieved enhanced BILAG improvement (improvement of all body systems to BILAG C or better, with no worsening) versus 13.2% in placebo. Epratuzumab was well tolerated with incidence of SAEs and infusion reactions similar to placebo.

Table: Combined responder index, Week 12 (ITT population)

Dose regimen	Placebo	Epratuzumab					
		cd 200mg	cd 800mg	cd 2400mg	cd 2400mg	cd 2400mg	cd 3600mg
	(n=38)	100mg EOW (n=39)	400mg EOW (n=38)	600mg weekly (n=37a)	1200mg EOW (n=37)	Combined group (n=74)	1800mg EOW (n=38)
Responders, n (%)	8 (21.1)	12 (30.8)	10 (26.3)	17 (45.9)	15 (40.5)	32 (43.2)	9 (23.7)
Odds ratio (95% CI) vs placebo		1.7 (0.6-4.7)	1.3 (0.5-3.9)	3.2 (1.1-8.8) p=0.03b	2.6 (0.9-7.1) p=0.07b	2.9 (1.2-7.1) p=0.02b	1.2 (0.4-3.4)

a2 patients randomized but never received drug
bp values were not adjusted for multiple comparisons

Conclusions: Epratuzumab cd 2400mg demonstrated clinically meaningful improvements in disease activity in patients with moderately to severely active SLE at 12 weeks, with responder rates twice those of placebo. Results validate the combined index emphasizing BILAG and support phase III trials of epratuzumab in SLE.

CS6.4 & PO2.E.3

BILAG-measured improvement in moderately and severely affected body systems in patients with systemic lupus erythematosus (SLE) by epratuzumab: results from EMBLEM™, a phase IIb study

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Objectives: Efficacy of 2 epratuzumab doses was demonstrated in a 12-week, multicenter, phase IIb, randomized, double-blind, dose and regimen-ranging, placebo-controlled study in SLE (NCT00624351). Statistically or clinically significant efficacy was seen in epratuzumab 600mg every week for 4 weeks and 1200mg every other week (EOW) for 2 weeks, respectively. Here we report improvement in BILAG 2004 index by body system in these 2 dose groups. ***Methods:*** A combined index of disease activity was used to assess efficacy (defined as BILAG improvement: reduction in all body systems of baseline BILAG A to B/C/D and BILAG B to C/D; no BILAG worsening in other organ systems; no deterioration in SLEDAI or PGA). Independent central readers determined BILAG grades for all 9 systems. Results for the BILAG improvement component of the combined index in body systems for which a sufficient number of patients per treatment group (≥ 5) had baseline disease activity to assess response is reported: musculoskeletal, mucocutaneous, cardiorespiratory, neuropsychiatric, constitutional, and renal. ***Results:*** BILAG status at baseline and Week 12 is reported in the table. Similar numbers of patients had BILAG A/B at baseline in all treatment arms in each system, apart from cardiorespiratory (where the placebo arm contained more patients). By Week 12, a higher percentage receiving epratuzumab 600mg weekly, compared with placebo, improved baseline BILAG A/B scores among these 6 systems to BILAG D, indicating no active disease. A higher percentage receiving epratuzumab 1200mg EOW, compared with placebo, improved baseline BILAG A/B scores to BILAG C or D in musculoskeletal, neuropsychiatric, and renal systems.

Table. Change in BILAG grade among subjects with flares at baseline

Body System	Treatment group	BILAG grade at baseline A/B (severe/ moderate disease) n	BILAG grade (shift) at Week 12		
			A/B (severe/ moderate disease) n (%)	C (stable disease) n (%)	D (inactive disease) n (%)
Musculoskeletal	Placebo	31	18 (58)	7 (23)	6 (19)
	Emab 600mg weekly	35	13 (37)	12 (34)	10 (29)
	Emab 1200mg EOW	34	13 (38)	10 (29)	11 (32)
Mucocutaneous	Placebo	31	18 (58)	9 (29)	4 (13)
	Emab 600mg weekly	32	19 (59)	4 (13)	9 (28)
	Emab 1200mg EOW	32	15 (47)	10 (31)	7 (22)
Cardiorespiratory	Placebo	17	10 (59)	2 (12)	5 (29)
	Emab 600mg weekly	7	0 (0)	0 (0)	7 (100)
	Emab 1200mg EOW	9	8 (89)	0 (0)	1 (11)
Neuropsychiatric	Placebo	11	6 (55)	3 (27)	2 (18)
	Emab 600mg weekly	6	1 (17)	0 (0)	5 (83)
	Emab 1200mg EOW	8	3 (38)	0 (0)	5 (63)
Constitutional	Placebo	9	1 (11)	1 (11)	7 (78)
	Emab 600mg weekly	7	0 (0)	0 (0)	7 (100)
	Emab 1200mg EOW	10	2 (20)	2 (20)	6 (60)
Renal	Placebo	5	3 (60)	2 (40)	0 (0)
	Emab 600mg weekly	6	3 (50)	1 (17)	2 (33)
	Emab 1200mg EOW	6	3 (50)	3 (50)	0 (0)

Emab, Epratuzumab. Placebo, N=38.
Emab 600 mg weekly, N=37. Emab 1200 mg EOW, N=37.

Conclusions: Treatment with epratuzumab 600mg weekly during a 12-week cycle provided greater BILAG improvement over placebo in disease activity in all affected body systems. Efficacy was particularly prominent in cardio-respiratory and neuropsychiatric systems in which symptom improvements are often difficult to achieve. Within specific body systems, the majority had symptom reduction or absence of active disease after treatment. This analysis supports that epratuzumab may be an effective treatment for SLE.

CS6.5 & PO2.E.5

Safety and pharmadynamic response with administration of single and repeat doses of rontalizumab in a phase I, placebo controlled, double blind, dose escalation study in SLE

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Objectives: The expression of interferon regulated genes is elevated in patients with SLE and may be involved in the disease pathogenesis. The main objectives of this study were to assess the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of rontalizumab, a humanized IgG1 monoclonal antibody that neutralizes human interferon alpha. **Methods:** This was a Phase I dose escalation study of single and repeat doses of rontalizumab or placebo in adults with mild-moderate SLE. Dose levels ranged from 0.3 mg/kg to 10 mg/kg administered by the intravenous (IV) and subcutaneous (SC) routes. Expression levels of seven IFN-regulated genes (IRGs) were measured by quantitative RT-PCR throughout the dosing and follow up periods for all patients. **Results:** Sixty patients (pts) were enrolled into 6 dose cohorts of 8 rontalizumab/2 placebo pts each. The mean (±SD) age was 47.4±10, and 95% of pts were female. 70% were Caucasian, and 27% African-American. Median time from SLE diagnosis was 6.7 yrs, and the mean SELENA SLE-DAI baseline score was 3.4±2.7. Predefined criteria for dose limiting toxicity were not met at any dose level. The incidence of adverse events (AE) and serious AE was comparable between active and placebo groups, and there was no apparent relationship between dose and the incidence of AE. The incidence of infections was similar across dose cohorts. One serious infection and one case of malignancy were reported. The PK profiles of rontalizumab at all dose levels exhibited apparent biphasic distribution and appeared dose proportional following both single dose IV and SC administrations. The clearance, volume of distribution and terminal half-life were similar to other humanized monoclonal IgG1 antibodies that bind soluble ligands. Interim analysis identified elevated IRGs in approximately 50% of patients as compared to normal healthy donors. There was a rapid decline in expression of all seven IRGs in the majority of patients after dosing with 3 mg/kg and 10 mg/kg IV cohorts. In general, the reduction was sustained for one month following a single dose; a similar trend was observed during the repeat dose phase. The integrated gene expression reduction over time (AUEC) started to deviate from placebo at 1-3 mg/kg and appeared to reach maximum at 10 mg/kg. **Conclusion:** The safety profile and PK properties of rontalizumab support further investigation in larger trials. The PD results demonstrate a dose-dependent decline in the expression of IRGs consistent with the expected down-modulation of the IFN signaling pathway, suggesting the possibility of clinical benefit in SLE.

CS6.6 & PO2.E.6

Efficacy and safety of rituximab (RTX) in patients (Pts) with proliferative lupus nephritis (LN): results from randomized, double-blind phase III LUNAR study at week 52

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Objectives: Small, uncontrolled studies suggested that RTX may be beneficial in LN. The efficacy and safety of RTX compared to placebo (Pbo) added to background mycophenolate mofetil (MMF) and corticosteroids in pts with proliferative LN were evaluated. **Methods:** Pts with class III/IV LN and UPCR >1 were randomized 1:1 to receive RTX (1000mg) or Pbo on days 1, 15, 168, and 182. The primary endpoint (EP), analyzed by a stratified Wilcoxon rank sum test, was the % of pts with complete (CRR) or partial renal responses (PRR). **Results:** The 72 pts randomized to each arm had similar baseline (BL) characteristics. Mean age at entry was ~30yrs; ~90% were female; 28% were Black, 36% Hispanic, 31% White; and 67% had class IV LN. BL mean UPCR was 4.0 ±2.8, and serum creatinine was 1.0 ±0.5mg/dL. Mean daily MMF dose was 2.4+0.63g in Pbo and 2.7+0.41g in RTX. There were no statistically significant differences in the primary or clinical secondary EPs. Blacks and Hispanics randomized to RTX had greater responses compared to Pbo than Whites, but statistical significance was not reached. RTX had a greater effect on levels of anti-dsDNA and complement at Wk52 compared to Pbo. Peripheral CD19+ B-cells were depleted in all RTX pts. Serious adverse events (SAEs) and infectious SAEs were similar between groups. Neutropenia (4 vs 1), leukopenia (9 vs 3), and hypotension (9 vs 3) occurred more frequently in RTX. Two deaths (sepsis and pneumonitis) occurred in the RTX group. **Conclusion:** To date, LUNAR is the largest randomized, placebo-controlled trial to evaluate RTX as an intervention in LN. Although there were numerically more responders in the RTX group (57% vs 46%), the study did not show a statistically significant difference in primary or clinical secondary EPs. RTX had a significant effect on serologic markers, although the clinical significance of this is unclear. AEs and SAEs were similar in frequency between groups, with no new or unexpected safety signals.

Table: Efficacy EPs and Safety

	Pbo (N=72)	RTX (N=72)	p-value*
Primary	N (%)	N (%)	
CRR	22 (30.6)	19 (26.4)	0.55
PRR	11 (15.3)	22 (30.6)	0.55
Key Secondary			
Pts with BL UPCR>3 reduced to UPCR<1 at Wk 52	53.7	47.4	0.51
Change from BL in anti-dsDNA Ab (%)	-50	-69	<0.01
Change from BL in C3 (mg/dL)	+25.9	+37.5	<0.03
Exploratory			
Pts with BILAG Renal Domain Score C at Wk 52	28 (38.9)	39 (54.2)	0.07
Overall response (CRR+PRR)	33 (45.8)	41 (56.9)	0.18
Black	9/20 (45)	14/20 (70)	0.20
Hispanic	11/23 (48)	16/29 (55)	0.78
White	13/26 (50)	10/19 (53)	>1.00
Pts who started cyclo-phosphamide prior to Wk 52	8 (11.1)	0	0.006
Safety	**(N=71)**	**(N=73)**	
SAE	25 (35.2)	22 (30.1)	
Infusion-related SAE	2 (2.8)	1 (1.4)	
Infection AE	61 (85.9)	61 (83.6)	
Infection SAE	12 (16.9)	12 (16.4)	
HACA+	4 (5.6)	8 (11.1)	
Deaths	0 (0)	2 (2.7)	

*All p-values are 2-sided and not adjusted for multiplicity

CS6.7 & PO2.E.7

Repletion of vitamin D deficiency is safe and well-tolerated among African Americans (AAs) with SLE

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Background/Purpose: A high prevalence of vitamin D(vitD) deficiency has been previously described in patients with SLE, particularly AAs, with important implications for bone and immune system health. To test whether oral vitD repletion in patients with SLE is safe and well-tolerated, and determine the effective dose for replacement therapy, an open-label interventional study of vitD3 (cholecalciferol) was conducted. **Methods:** This was a 12 week open-label interventional study with 3 doses of vitD3: 800IU/day, 2000IU/day, or 4000IU/day (ClinicalTrials.gov: NCT00418587). Participants were recruited from the SLE in Gullah Health (SLEIGH) study of AAs from the Sea Islands of SC and GA, a population previously reported to have a 95% prevalence of low 25(OH)D<30ng/ml. Inclusion criteria included: SLE (≥4 ACR Criteria) with stable disease (no BILAG A or B in prior 4weeks), stable prednisone ≤20mg/day in prior 4weeks, baseline 25(OH)D of <30ng/ml, must be willing to discontinue other vitD supplements, have no hypercalcemia or hypercalcuria at screening, and no nephrolithiasis history. Total daily calcium and vitD intake was determined at baseline using the Block Food Frequency Questionnaire and serum 25(OH)D levels were determined by RAI (DiaSorin). Results were analyzed using descriptive statistics. **Results:** Out of 28 screened, 18 patients qualified and were enrolled into the study. At baseline, mean age was 43.7±12.2years, BMI was 30.9±8.2 kg/m2, mean vitD intake was 133±121 IU/day, mean calcium intake was 635±351mg/day, mean prednisone dose was 4.3±5.7mg/day, and 93% were women. Six patients completed 12 weeks of 800IU/day, 6 completed 12 weeks of 2000IU/day, and 6 completed 12 weeks of 4000IU/day of vitD3. Compliance by pill counts was 98.5±12.7%, there were no dropouts and no treatment-related AEs. There were no disease flares or new anti-dsDNA antibody positivity. After 12 weeks, 67% of those taking 800IU/day, 83% of those taking 2000IU/day, and 67% of those taking 4000IU/day repleted to ≥30ng/ml. Even at 4000IU/day, only 33% reached a threshold of 40ng/ml after 12 weeks. Dose-dependent reductions in PTH were seen in all 3 dose groups (mean reduction 43.6pg/ml to 36.6pg/ml). Taking prednisone (50%) or hydroxychloroquine (64%) did not significantly influence 25(OH)D response, but hydroxychloroquine use did reduce the 1,25(OH)2D to 25(OH)D ratio from 2.0 to 1.4. **Conclusions:** Repletion of 25(OH)D levels with daily vitD3 at commonly used doses of 800-2000 IU daily as well as higher doses of 4000 IU daily was safe and well-tolerated among patients with SLE. Despite the observation that sunlight exposure can trigger disease flares, oral vitamin D repletion does not appear to cause activation of autoantibody production. At least 2000 IU/day of vitD3 is required to replete 25(OH)D to >30ng/ml and higher thresholds may be required for immune health.

CS6.8 & PO2.F.8

Tacrolimus (Tac) versus mycophenolate mofetil (MMF) for the treatment of membranous lupus nephritis: a randomized controlled trial

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Objectives: To compare the efficacy of tacrolimus (Tac) and mycophenolate mofetil (MMF) for induction treatment of membranous lupus nephritis. **Study design:** Randomized controlled trial **Methods:** Data from a subgroup of patients with membranous lupus nephritis (ISN/RPS class V or mixed V+III/IV) were extracted from a randomized comparative trial of the efficacy of Tac and MMF for the induction treatment of 128 patients with active lupus nephritis. Patients were randomized to receive prednisolone (0.6mg/kg/day for 6 weeks and tapered by 5mg/week till <10mg/day) with either (1) Tacrolimus (0.1mg/kg/day, taper to 0.06mg/kg/day if response satisfactory at month 3); or (2) MMF (2gm/day, augmented to 3gm/day if response suboptimal at month 3). Clinical response and adverse events were compared at 6 months.

Results: 46 patients (42 women, age 40.0±13.5 years, SLE duration 69.9±76 months) with membranous lupus nephritis (V in 19 patients and V+III/IV in 27 patients) were studied (25 treated with Tac and 21 treated with MMF) – 28(61%) had creatinine clearance (CrCl) <90ml/min, 25(54%) had nephrotic syndrome and 23(50%) received anti-hypertensive treatment at baseline. The median histologic activity and chronicity scores were 5 (IQR 4.25) and 3 (IQR 2.25), respectively. Baseline renal parameters and levels of anti-dsDNA / complements were similar between the two groups of patients. At the end of 6 months, significant improvement in C3, C4, anti-dsDNA levels, serum albumin, proteinuria, urine protein-to-creatinine ratio (UP/Cr) renal and extra-renal SLEDAI scores were observed in both groups (p<0.001). Improvement in CrCl was observed in the MMF-treated patients but not in Tac-treated patients, but the differences were not statistically significant in both groups. The magnitude of proteinuria and UP/Cr improvement was similar in both arms of patients and the inter-group difference in proteinuria and UP/Cr at month 6 was not statistically significant after adjustment for baseline values. Complete or partial response occurred in 23 (92%) patients treated with Tac and in 15 (71%) of patients who received MMF. Infections and gastrointestinal upset was more common in MMF-treated patients but tremor, headache, alopecia and transient increase in serum creatinine were more frequently encountered in Tac-treated patients. **Conclusions:** Both MMF and tacrolimus, when combined with high-dose prednisolone, is effective in ameliorating proteiuria and preserving renal function as initial treatment of membranous lupus nephritis. Tacrolimus tends to be more effective than MMF in inducing a partial or good clinical response, and is associated with a lower frequency of infections in this subgroup of patients with lupus nephritis.

CS7 Pathogenic Mechanisms in Lupus

CS7.1

Follicular helper T cells in immunity and autoimmunity

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Follicular helper T cells (T_{FH}), defined by expression of the surface markers CXCR5, PD-1 and ICOS, are located in germinal centers where they provide help for B cell maturation through the secretion of IL-4 and IL-21. We have defined the transcriptional regulation of these cells, and the role of B cells in their induction in normal and autoimmune responses in mice, with ongoing studies devoted to understanding the control of their migration and development, as well as their characteristics and function in humans. These studies will be presented.

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CS7.2

Neutrophil gelatinase associated lipocalin (NGAL) and its role in the pathogenesis of antibody mediated nephritis

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Background: Anti-double stranded (ds)DNA antibodies are instrumental in the pathogenesis of lupus nephritis (LN), although the mechanism by which these antibodies contribute to kidney damage has yet to be determined conclusively. We had found that treatment of mesangial cells with pathogenic anti-DNA antibodies induced the expression of multiple proinflammatory mediators known to be involved in LN. One of the most highly induced genes was Neutrophil Gelatinase Associated Lipocalin (NGAL; AKA Lipocalin-2). NGAL has recently been shown to be upregulated in response to a variety of inflammatory, ischemic, and other insults to the kidney, and in fact is a sensitive and early urine biomarker in several types of human disease. *Purpose:* To determine whether NGAL is expressed in the context of lupus nephritis, and its role in the pathogenesis of renal injury induced by nephritogenic antibodies. *Methods:* NGAL expression in serum, urine, and kidneys was analyzed in two murine lupus models (MRL/lpr and NZB x NZW F1 mice). Nephrotoxic serum nephritis (NTS) was induced by passive transfer of preformed rabbit anti-mouse glomerular antibodies to C57Bl/6 wild type and NGAL knockout mice. *Results:* MRL/lpr and NZB x NZW F1 lupus mice displayed higher kidney mRNA expression of NGAL than age-matched non-autoimmune BALB/c mice. In addition, serum NGAL was elevated in old as compared to young MRL/lpr (p<0.01), old MRL/+ (p<0.05), and old BALB/c mice (p<0.05). Importantly, NGAL kidney expression significantly correlated with anti-dsDNA antibodies titers (p<0.05). NGAL specific immunostaining was detected in kidneys of nephritic MRL/lpr and NZB x NZW F1 mice, but not in BALB/c mice. Similar elevations of serum and kidney NGAL were observed in non-autoimmune mice with induced nephrotoxic serum nephritis. To determine if NGAL upregulation is material in the pathways leading to renal injury, we compared the severity of renal damage in NGAL wild type and deficient mice following induction of NTS. We found that NGAL deficient mice had significantly attenuated proteinuria on days 7, 14, and 21 as compared to NGAL sufficient mice. In vitro, NGAL induced significant apoptosis and decreased proliferation of kidney cells. *Conclusions:* In vivo, nephritogenic antibodies upregulate NGAL, which then appears to be instrumental in the pathogenesis of antibody mediated nephritis. Whether the protection from nephritis observed in NGAL deficient mice is related to the proapoptotic effect of NGAL observed in vitro remains to be determined.

CS7.3

Anti-DNA/anti-NMDAR cross-reactive antibodies contribute to NPSLE

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A subset of anti-DNA antibodies cross-reacts with the NR2A and NR2B subunits of the N-methyl-D-aspartate receptor (NMDAR). These antibodies are found in serum, cerebrospinal fluid and brain tissue in lupus patients. They cause neuronal dysfunction and neuronal apoptosis only if they penetrate brain parenchyma; thus, only cerebrospinal fluid titers of these antibodies correlate with symptoms. Moreover, when these antibodies are present in the serum of a pregnant mouse they cause altered fetal brain development with subsequent cognitive impairments. Most recently, we have found that these antibodies preferentially bind the open receptor and that antibody concentration determines whether exposure leads to enhanced excitatory post-synaptic potentials or to neuron death. These studies provide a paradigm for investigating the role of antibody in neuropsychiatric lupus.

CS7.4

The evil trio in lupus pathogenesis

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Background: Systemic lupus erythematosus is a polygenic disease. The past year has been a very important year in lupus genetics, with several reports uncovering potential candidate genes in human lupus. The challenge ahead is to confirm these associations, and to dissect out the molecular pathways and mechanisms that lead to disease. *Results:* In this respect, the laboratory mouse has been very useful. Murine lupus is phenotypically similar to human SLE. Genome scans have been reported in almost all commonly studied murine models of lupus. Collectively, many of these studies highlight the presence of strong disease loci on murine chromosomes 1, 4, 7 and 17. Studies in mice have allowed researchers to engineer mouse models harboring individual genetic loci, also called congenic strains, to understand how each locus operates. Our work has focused on defining the steps leading to lupus, using C57BL/6-based B6.Sle1, B6.Sle2, and B6.Sle3 congenic strains. Collectively, these studies have uncovered at least 3 different types of mechanisms that can contribute to lupus pathogenesis: (a) breach in B and T cell tolerance, leading to anti-self reactivity; (b) amplification of the autoimmune response by the innate immune system; (c) local processes in the end-organ that facilitate nephritis. Collectively, the above studies indicate that violating checkpoints in the adaptive immune system and also the innate immune system, coupled with enhanced inflammatory processes in the end organs constitute key events in lupus pathogenesis. The confluence of these three forces leads to full-blown lupus nephritis, similar to the disease seen in patients. *Conclusion:* Autoreactive lymphocytes, pro-inflammatory innate immunity and disease-promoting events in the end-organs are three forces that drive lupus nephritis. Understanding each of these mechanisms at the molecular level is essential for effective disease management in SLE.

CS7.5 & PO1.M.1

Pristane-induced lupus (PIL) in BALB/c mice: linking severe organ involvement with T-cell response

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Objective: PIL is a murine model of experimental lupus with antibodies (Abs) against nuclear antigens and various affections of internal organs. PIL can be observed in different mouse strains: We here characterize organ involvement in BALB/c, identify mice with severe renal lupus (nephritis-PIL) and try to link renal SLE with the T- and B-cell response in the respectice animals. *Methods:* Mice were injected i.p. with either 0.5ml of pristane or with PBS as control and sacrificed after 8 months. Histology was obtained from various tissues: kidney, lung, CNS, heart, liver, spleen, stomach and intestines (staining with HE and additional PAS stainings for kidney samples). Lymphocytes were isolated from intraperitoneal granulomas that represent the major site of inflammation and analyzed separately for each mouse (by FACS) to allow correlation with (renal) pathology. Spleenic lymphocytes served as control population. We assessed frequencies of lymphocyte subtypes (CD4+, CD8+ and CD19+) and the percentages of CD4+ regulatory T cells (Treg) and CD4+ activated T effector cells (Teff) as well as their Th1, Th2 and Th17 phenotype (intracellular staining for FoxP3 and IFNγ, IL-4, IL-17, respectively). In addition, anti-chromatine and anti-histone serum-Abs were determined. *Results:* All PIL, but no control developed lupus pneumonitis (pulmonary capillaritis with pulmonary haemorrhage). 28% of PIL mice developed proliferative lupus glomerulonephritis (corresponding to GN WHO III and IV in humans). Less prominent lymphocytic infiltration was also found in PIL spleens, livers and large intestines. The skin, heart and small intestines did not show signs of inflammation. We compared the T cell response in severely sick nephritis-PIL

with PIL mice without renal involvement (moderate–PIL). In both groups, we found a higher frequency of activated Teff within the intraperitoneal granulomas (in both PIL groups >15% vs. <5% of Teff in healthy or PIL spleens, p<0.01%) and an increased Teff/Treg ratio. Granuloma lymphocytes of both PIL groups had a similarly prominent Th1 response (28.3 and 25.5%, respectively, p=n.s.). Nephritis-PIL had an increased frequency of Th2 (42.4±24.7% vs. 22.3±11.6%, p=0.004) and, more prominent, of Th17 (35.1±27.2% vs. 15.3±8.6%, p=0.002) when compared to moderate-PIL. Both groups of PIL mice developed anti-chromatine- and anti-histone-Abs, but without difference in serum levels or temporal occurence. ***Conclusion:*** PIL in BALB/c is characterized by pneumonits, increased lymphocytic infiltrates in spleen, liver, large intestine, and, less frequent, by lupus glomerulonephritis. While, in line with the literature, PIL mice overall show an upregulated Th1 response, severely ill mice with lupus nephritis also exhibit an increased frequency of Th2 and Th17 cells. Thus, Th2 and particularly Th17 at the primary site of inflammation may be important in driving lupus GN in PIL mice.

CS8 Apoptosis and Lupus

CS8.1

The role of cell death in the generation of extracellular DNA
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DNA is a large polymeric macromolecule that plays a key role in the pathogenesis of systemic lupus erythematosus (SLE), serving as an autoantigen and autoadjuvant. This role reflects the intrinsic immunological activity of DNA which, depending on context and its participation in immune complexes with anti-DNA autoantibodies, can lead to the stimulation of nucleic acid sensor sensors. These sensors are located in the cytoplasm of cells and include both toll-like receptor (TLR) 9 as well as various non-TLR receptors. Stimulation of these sensors can lead to the activation of the innate immune system, including production of type 1 interferon, as well as B cell activation. Furthermore, immune complexes with DNA can deposit in the kidney to incite nephritis. For DNA to form immune complexes, it must leave its usual intracellular location, trafficking from the nucleus to the extracellular space. This process can occur during cell death, both apoptosis and necrosis, as shown in *in vitro* and *in vivo* models. In animal models, the expression of extracellular DNA occurs with the administration of apoptotic or necrotic cells or the treatment of mice with agents such as anti-Fas or hepatotoxins that can cause liver cell death. With the administration of dead cells, the extent of extracellular DNA is influenced by the presence of macrophages, prior inflammation as well as animal sex. Interestingly, in these models, the amount of extracellular DNA is greater in female mice than male mice, a finding perhaps related to the increased propensity of females for autoimmunity. In the extracellular space, DNA can exist in a free form as well as a particulate form called microparticles. Microparticles are small membrane-bound vesicles that exit from cells during activation or apoptosis and contain a variety of nuclear and cellular components. DNA, in the form of microparticles, is antigenically active, with particles capable of forming immune complexes. Together, these findings suggest that the extracellular release of DNA can be an important step in the pathogenesis of SLE, providing a source of antigen to stimulate autoantibody production as well as form immune complexes.

CS8.2

After apoptosis: inflammation and autoimmunity
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It is now generally accepted that defective clearance of dying cells predisposes to SLE and, perhaps other systemic autoimmune disorders. One example of this association is the lupus like disease that develops in the absence of the apoptotic cell opsonin, MFGE-8. Precisely how an increase in dying cells leads to a break in tolerance is poorly understood. The identification of specific death associated molecular patterns (DAMPS) or 'alarmins' released from necrotic cells helps to explain induction of inflammation. Furthermore, intracellular sensors that recognize self RNA and DNA are increasingly been characterized. To evaluate how apoptotic cells are processed in the absence of opsonins, we examined intracellular trafficking of an apoptosis-associated antigen (OVA) by dendritic cells in mice deficient for MFGE-8. We observed that, in the absence of MFGE-8, apoptotic cell debris persisted longer in endosomes whereas in the presence of MFGE-8, apoptotic material trafficked to lysomes. The change in intracellular trafficking was associated with an increased T cell response to apoptosis associated OVA by CD8 T cells indicating enhanced cross presentation. Furthermore, MFGE-8 deficient mice spontaneously developed a skin rash associated with infiltration of CD8+ T cells. MFGE-8 deficient RIP-OVA transgenic mice developed diabetes with higher frequency and greater rapidity at an earlier age following adoptive transfer of OT-1 T cells. These findings indicate that, in addition to the known role of MFGE-8 in promoting uptake of apoptotic cells by tingible body macrophages in germinal centers, this protein also influences the route of antigen processing in dendritic cells. In the absence of MFGE-8, antigen specific CD8T cells are excessively stimulated leading to loss of tolerance. As SLE patients have evidence of activated CD8 T cells, opsonin deficiency may set up a positive feedback loop where autoreactive CD8+ T cells contribute to further generation of apoptotic antigens.

CS8.3

Clearance deficiency is involved in the etiology and pathogenesis of lupus
Herrmann, Martin

The inefficient clearance of dying cells can result in the accumulation of apoptotic cell remnants. This occurrence is considered an intrinsic defect that can cause the permanent presence of cellular debris responsible for the initiation of systemic autoimmunity in diseases such as systemic lupus erythematosus (SLE). If postapoptotic debris accumulates in germinal centers, activates complement and functions as a survival signal for B cells that accidentally had become autoreactive by somatic hypermutation, autoimmunity could arise (etiology). The accumulation of postapoptotic remnants and fragments derived from secondary necrotic cells remnants (SNEC) in the presence of autoantibodies against apoptotic cells or adaptor molecules obliges its pathological elimination and maintains autoinflammation. The autoimmunity occurring in SLE patients involves complex antigens that contain nucleic acids (virus mimetic). Complexes of autoantibodies, proteins and nucleic acids are likely to be mistaken by the immune system for opsonized viruses, resulting in the production of type I interferons, a hallmark of SLE (pathogenesis). The pathogenicity of autoantibodies is thought to strongly increase if autoantigens are accessible for immune-complex formation. The latter may be considered a binary pyrogen formed from less pro-inflammatory components. The accessibility of cognate autoantigens, in turn, is likely to be related to impaired or delayed clearance of apoptotic cells.

CS8.4

The TAM kinases in lupus and inflammation

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The TAM kinases (tyro 3, axl, and mer) are key regulators of innate immunity and serve as important receptors for binding and phagocytosis of apoptotic cells. Mice lacking one or more of these receptors develop lupus-like auto-immunity. We have investigated the role of these receptor tyrosine kinases and their ligands (Gas 6 and protein S) in autoimmunity in mice and in humans. It is primarily tingible body macrophages that express mer in tissues and in inflammatory sites, and Gas 6 that serves as the principal ligand for macrophages. Macrophages from mer-deficient mice have enhanced expression of TNFα and IL-12, and are resistant to downregulation of cytokines by complexes of apoptotic cells and antinuclear antibodies. Mer also is important in downregulating glomerular inflammation through its expression on mesangial cells. B cells activated through MHC II show increased mer expression, which is apparently involved in T-B interactions. Dendritic cell mer expression is crucial in determining their cytokine profile in the presence of apoptotic cells. Human expression of mer is minimal on resting mononuclear cells, yet these cells express axl constitutively. Tyro 3 expression increases upon B-cell activation in vitro. Gas 6 levels in lupus patients are similar to those seen in normal controls, while Protein S levels are reduced, particularly in patients with anti-phospholipid syndrome. These studies highlight the importance of the TAM ligands in experimental autoimmunity and in regulation of the immune response. The TAM ligands are potential targets for therapeutic intervention in immune andi nflammatory diseases. Supported by grants from NIAID and NIDCR.

CS8.5 & PO1.A.1

DNase1 activity and circulating nucleosomes in healthy relatives of systemic lupus erythematosus patients

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Objective: To determine DNase1 activity levels in sera from healthy first-degree relatives of patients with systemic lupus erythematosus (SLE). ***Methods:*** We evaluated 18 families with two or three members affected with SLE. All the SLE patients fulfilled at least four of the American College of Rheumatology criteria. In 13 families there were two SLE patients in each one, whereas 4 families included 3 SLE affected individuals in each and one family had 6 members affected. Overall, we collected 44 SLE patients, 168 healthy relatives and 106 were first-degree relatives (parents, brothers/sisters, and sons/daughters). An ethnically matched random healthy control population (102 blood donors) was also included in the study. The ratios men/women were similar in both groups (controls and healthy relatives) (1.07 versus 1.12). DNase1 activity was measured by an enzyme radial diffusion assay based on the DNA hydrolysis in an agar gel, and nucleosome levels were measured by a commercial ELISA kit. Data were analyzed by the statistical program SPSS 12.0. The distribution of neither Dnase1 activity values nor nucleosome levels followed a non-normal distribution as observed by using the Kolmogorov-Smirnov test; therefore, non-parametric statistical tests such as Mann-Whitney U test, and the Spearman test were used. ***Results:*** DNase1 activity was higher in the healthy relatives group than in the control population (41.70 ± 20.86 µg/ml versus 24.75 ± 12.3 µg/ml) this difference being statistically significant ($p<0.005$). The statistical differences were maintained in each relationship's first-degree group: parents, 46.98 ± 23.36 µg/ml, ($p<0.005$), brothers/sisters 41.55 ± 19.21 µg/ml ($p<0.005$), and sons/daughters, 39.17 ± 15.95 µg/ml ($p<0.005$). With regard to the circulating nucleosomes, levels were higher in the healthy relatives group (0.759 ± 0.71) than in control blood donors (0.554 ± 0.67) but statistic-

ally differences were not reached ($p=0,056$). Statistically significant differences in nucleosomes levels were seen between healthy relatives and patients (0.759 ± 0.71 vs 0.508 ± 0.68) ($p=0.011$). Interestingly, a negative correlation was established between the levels of nucleosomes and Dnase1 activity in healthy relatives ($r=-0.37$; $p=0.006$). ***Conclusions:*** The high DNase1 activity found in healthy first-degree relatives of SLE patients may represent a feedback regulation system. This mechanism would counteract the increased ratio of apoptosis that is probably intrinsic to these families.

CS9   Genetics of Lupus

CS9.1

Genes that make lupus possible

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he predictions made from the observations of familial aggregation ($\lambda_s=8$ to 29) and the identical twin concordance rate of ~25% that systemic lupus erythematosus would be a strong genetic phenotype for genetic analysis have been validated. Recent experimental data have increased the number of genetic effects convincingly established to contribute to human lupus by five-fold, relative to what was known before 2007. The ~35 genes associated with a lupus phenotype have either been replicated in multiple samples and are near or exceed the threshold for genome wide significance ($p>5X10^{-8}$) or are rare variants that convincingly contribute to lupus in specific situations. Lupus is second, among all inflammatory conditions, to only inflammatory bowel disease in the number and diversity of genes now implicated in causation of the phenotype. Examples of newly discovered genes include ITGAM, STAT4 and MECP2/IRAK1. ITCAM is also known as CD11b or CR3 and has both integrin cell adhesion and complement component C3bi binding activities. ITGAM appears to be very important in lupus renal disease. The responsible polymorphism appears to be a single amino acid change (R77H) in ITGAM. STAT4 is expressed in a number of cell types. After IL-12 binds its receptor STAT4 is phosphorylated and then forms a dimer that migrates to the nucleus to induce the transcription of interferon γ. The responsible polymorphism is not yet known. MECP2 and IRAK1 share a haplotype block on the X chromosome. One or both of these genes is probably responsible for the observed haplotypic association, either the intracellular signaling of IRAK1 or the consequences of methylated cytosines in gene expression by MECP2. Both are very attractive candidate genes. The DNA shared by these two genes is the first gene on the X chromosome established to contribute to lupus, a disease found in women ten-times more frequently than in men. The application of new genomic technologies to lupus will provide many new, previously unsuspected insights into the pathophysiology of this enigmatic, potentially deadly, and, as yet, unsolved disease and will provide us with many new molecular targets for the development of new therapeutics.

CS9.2 & PO1.G.19

Gender specific association of X-linked TLR7 with male SLE

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Objective: Duplicated Tlr7 promotes lupus-like disease in male BXSB-Yaa mice, prompting us to evaluate TLR7 in SLE patients especially in males. Methods SNP genotyping was conducted using Illumina Infinium platform in the discovery panel, and Taqman in replication panels. Relative expression of TLR7 mRNA in PBMCs was measured by RT-qPCR. Allelic specific transcription analysis was performed by pyrosequencing. **Results:** Fine-mapping the 23-kb TLR7 region using 11 SNPs in 1,434 SLE cases of Eastern Asian descent vs. 1,591 EA controls showed association of 2 TLR7 SNPs with SLE (rs5935436 in the promoter, p = 1.8×10-3; rs3853839 in the 3'UTR, p = 6.7×10-4). In this discovery panel, the association signal of rs3853839 was mainly found in Chinese (cases/controls; 563/522, p = 6.3×10-6) with a higher OR in males than females (OR=5.6 vs. 1.5), but not detected in Koreans (845/1,022, p = 0.32). This association with SLE with a stronger male effect was replicated in both independent panels of Chinese (2,340/2,436, p = OR = 2.7 vs. 1.2, 9.0×10-4) and Japanese (560/913, OR = 3.5 vs. 1.2, p = 0.007). Rs 5935436 was not associated with SLE in replication panels. In the combined analysis of 4,334 cases and 4,940 controls, the G allele of rs3853839 was associated with SLE (p = 6.5 x10-10), exhibiting a higher OR in males than females (OR = 2.3[1.6-3.3]) vs. 1.2[1.1-1.3], p = 4×10-4). Allelic expression analysis revealed that healthy Chinese controls of either gender carrying the G risk allele had significantly increased TLR7 transcript in PBMCs than those carrying C allele only, which was similarly found in female Chinese SLE patients; female G/C heterozygotes express 2.7-fold higher G allele-containing TLR7 transcript than C allele-containing transcript. Furthermore, clinically inactive female SLE patients carrying "GG" genotype showed a more pronounced IFN signature in PBMCs compared to those carrying "GC" and "CC" genotypes (p = 0.02), indicating rs3853839 might regulate the expression of TLR7, affecting IFN response in vivo. **Conclusion:** Association between the X-linked TLR7 SNP and SLE with a stronger male effect was identified in Eastern Asians and replicated in independent Chinese and Japanese panels. This risk allele may confer elevated expression of the RNA-binding TLR7, predisposing to the development of SLE, especially in male Chinese.

Ethnicity	Panels	Sex	Case/Control	G Risk Allele Frequency		P	Odds Ratio (95%CI)
				Case	Control		
Eastern Asian	Discovery	M	126/229	0.83	0.73	0.038	1.79 [1.03-3.13]
		F	1308/1362	0.79	0.76	3.00E-03	1.22 [1.06-1.39]
		All	1434/1591	0.80	0.76	6.70E-04	1.24 [1.1-1.41]
Chinese	Replication 1	M	196/931	0.92	0.81	2.31E-04	2.73 [1.57-4.74]
		F	2144/1505	0.83	0.80	4.24E-03	1.19 [1.06-1.34]
		All	2340/2436	0.83	0.80	9.02E-04	1.21 [1.08-1.35]
Japanese	Replication 2	M	36/390	0.89	0.69	0.014	3.51 [1.22-10.2]
		F	524/523	0.75	0.71	0.037	1.23 [1.01-1.49]
		All	560/913	0.75	0.70	7.00E-03	1.28 [1.07-1.53]
Eastern Asian	Combined	M	358/1550	0.89	0.77	1.33E-06	2.33 [1.64-3.30]
		F	3976/3390	0.80	0.77	1.19E-07	1.24 [1.14-1.34]
		All	4334/4940	0.81	0.77	6.50E-10	1.27 [1.17-1.36]

CS9.3

Genetics of lupus: what did we learn from the mouse?

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Great progress has been made in the field of SLE genetics in the past few years. Genome-wide association studies conducted on human SLE patients have provided over 20 validated susceptibility genes. In murine models of SLE, such as the NZM2410, MRL/lpr and BXSB.Yaa strains, studies that have been initiated over a decade ago as linkage analyses have started to identify susceptibility genes. In this context, this presentation will review the current status of genetic research in the NZM2410 model. So far, the susceptibility genes that have been identified affect B cells, T cells, and renal functions, and they correspond to known genes whose function had not been previously implicated in SLE or autoimmune pathogenesis. In addition, this presentation will show that spontaneous models of murine lupus constitute excellent models of the genetic architecture of human SLE. This notion has been greatly strengthened by the convergence of the functional pathways that are defective in both human and murine lupus. Within these pathways, variants in a number of genes have now been shown to be directly associated with lupus in both species. Consequently, murine models will continue to serve a pre-eminent role in lupus genetics research.

CS9.4

Trans-ethnic association studies and the SLE-susceptibility locus *TNFSF4*

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Objectives: We have established the TNF superfamily member *TNFSF4*, as a SLE susceptibility gene in Northern Europeans. This gene operates at the T-cell-APC interface as a late-stage lymphocyte costimulator. Several genetic polymorphisms in the 5' region of the gene predispose to SLE and form an overtransmitted 98kb haplotype ($P_p < 10^{-5}$, after permutation, OR=1.6, 95% CI=1.27-1.89). Strong LD between SNPs in this region in Europeans has prevented further resolution of the association signal however the associated risk haplotype correlates with a 6-fold increase in OX40L transcript and OX40L protein levels. **Methods:** We adopted a trans-racial fine mapping approach complemented by deep sequencing; 50 SNPs were employed in a SE Asian population (1119 cases, 1347 controls) and in a Hispanic population (1531 cases, 796 controls). SNPs identified from Yoruba Hapmap or which were previously associated were typed in an African-American cohort (1414 cases, 1765 controls) and 67 SNPs in a Northern European Cohort (3783 cases, 8145 controls). We examined key SNPs in an additional cohort of Afro-Caribbean's, recruited in the UK, to further resolve the association signal in West Africans. In a complimentary strategy to define functionally relevant polymorphisms, the OX40L locus (120kb encompassing the gene and 5' region) was deep sequenced in *TNFSF4*hi and *TNFSF4*lo individuals (n=100) from our UK cohort using the Next Generation 454 Titanium platform. **Results:** The association is strongly replicated in the Asian (P_p=2 x 10^{-11}, OR=1.74, CI=1.49-2.04) and Hispanic population (P_p=1.5 x 10^{-5}, OR= 1.60, CI=1.4-1.6) with multiple SNPs in the 5' region of *TNFSF4* associated with SLE. The risk haplotype in these populations is structured similarly to that in Europeans (P_p=1.5 x 10^{-16}, OR=1.59, 95% CI=1.29-1.96). At this locus, recombination patterns differ most markedly between European and West African individuals, seen as greater haplotypic diversity in the African-American cohort after correction for admixture using global major ancestry informative markers. In this population the *TNFSF4* association with SLE is replicated (P_p=4.8 x 10^{-6}, OR=1.50, CI-1.1.38-1.70) and haplotype structure delineates the association signal to focus it on two shorter regions within the 5' upstream region which may represent multiple gene effects. The UK Afro-Caribbean data and our 454 sequencing results are currently in analysis. **Conclusions:** We have replicated and refined the *TNFSF4* association with SLE in multiple populations to establish it as a global lupus susceptibility gene. The validity of trans-ethnic studies to map association signals in complex traits has been confirmed.

CS9.5 & PO1.G.3

Genome-wide association study of cardiac manifestations of neonatal lupus identifies risk variants in the ERG, TCF19,C6orf10 and MICB-TNF-AIF1 region

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Objectives: Isolated congenital heart block (CHB) is highly associated with maternal anti-Ro/SSA antibodies. CHB carries a substantial mortality, approaching 30%, and morbidity, with over 60% of surviving affected children requiring lifelong pacemakers. To date, complete atrioventricular (AV) block is irreversible. This is a rare disease and factors beyond requisite maternal autoantibody are unknown. Data from twin studies and the 10-fold increased recurrence rate of cardiac neonatal lupus (NL) in subsequent pregnancies indicate a strong genetic contribution to risk. We posit that fetal genes influence the response to maternal autoantibodies. **Methods:** Children of European an-

cestry (n=116) with cardiac NL were identified from the U.S. Research Registry for Neonatal Lupus. Cases were genotyped using the Illumina 370K SNP platform and merged with 3351 controls from the International Consortium on Systemic Lupus Erythematosus Genetics (SLEGEN). Standard quality control and admixture-adjusted tests of association were computed. **Results:** Outside the HLA region, a strong association was detected at 21q22, upstream from the transcriptional regulator ERG (rs743446, p=5.45E-06, OR = 2.40). Within the HLA, associated regions include PSORS1C1 (rs3130544, p = 1.94E-07, OR = 2.77) and a missense mutation (proline to serine) at TCF19 (rs7750641, p = 1.58E-07, OR = 2.79), at Class I; several variants in the MICB-NFKBIL1-LTA-TNF-LTB-AIF1 region at Class III (rs2230365, p= 1.00E-03, OR=0.46; rs2857595, p= 1.96E-09, OR = 2.37; rs3128982, p= 6.40E-06, OR=1.86; and rs3099844, p= 4.52E-10, OR= 3.34; and the C6orf10 locus at class II (rs3115553, p=2.69E-05, OR=1.81; rs6457536, pa=1.74E-05, OR=1.84; and rs7775397 (p = 1.35E-09, OR=3.30). These are consistent with our previous results (Clancy 2002). With the exception of the HLA region, no loci previously implicated in autoimmune diseases achieved genome-wide significance in the CHB children. **Conclusion:** These results suggest that genetic variation near ERG, PSORS1C1, LTA/TNF/LTB and C6orf10 in the fetus may promote an abnormal tissue response initiated by exposure to maternal autoantibodies. Identification of risk loci is an incremental step towards discovery of a fetal genetic component that contributes to the anti-SSA/Ro associated development of life-long cardiac damage.

CS10 T Regs in Lupus

CS10.1

Characterization of regulatory T cells (Treg) and Treg subsets in patients with systemic lupus erythematodes (SLE)

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CD4$^+$CD25$^+$ Treg that specialize in the suppression of immune responses play a critical role in the regulation of peripheral immune tolerance. This has led to the hypothesis that either quantitative and/or qualitative deficiencies of Treg might be involved in the outbreak of overt autoimmune disease.
However, as for SLE, but similar for other autoimmune diseases, data concerning Treg numbers and function are often conflicting. One of the reasons for controversial observations is certainly, at least in humans, the lack of a specific Treg marker molecule. Expression of CD25, but also of Foxp3, can be induced upon T cell activation and in principle this holds true for all Treg associated marker molecules such as CTLA-4, GITR, LAG-3 or CD127. In line with this, our own studies demonstrated decreased proportions of CD4$^+$CD25^{high} T cells but increased proportions of CD4$^+$Foxp3$^+$ T cells in SLE patients with high disease activity, suggesting that the expression of Foxp3 on CD4$^+$ T cells in SLE patients at least to some extent, reflects the activation of CD4$^+$ T cells (1). In addition we observed a distinct CD4$^+$Foxp3$^+$ T cell population in SLE patients that lacked CD25 expression (CD4$^+$CD25$^-$Foxp3$^+$) (2). CD4$^+$CD25$^-$Foxp3$^+$ T cells phenotypically resembled CD4$^+$CD25$^+$Foxp3$^+$ Treg but were partially deficient in regard to their suppressive capacity in vitro. In summary analysis of Treg in patients with autoimmune diseases, such as in SLE, must be made with caution. Chronic inflammatory stimuli might create an environment that substantially affects Treg in regard to their developmental and regenerative potential.

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2. Bonelli M, Savitskaya A, Steiner CW, Rath E, Smolen JS, Scheinecker C. Phenotypic and functional analysis of CD4+ CD25- Foxp3+ T cells in patients with systemic lupus erythematosus. *J Immunol*. 2009;182(3):1689-95.

CS10.2

Targeting effector and regulatory T cells in patients with SLE

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T cells from patients with the autoimmune disease systemic lupus erythematosus (SLE) display a characteristic hyperactive phenotype. Furthermore, Foxp3 expression is increased within the CD4 population, which could also reflect increased T cell activation. Although CTLA-4 expression was increased in responder T cells, its expression was normal on the Foxp3+ population. However, CTLA-4 was unable to regulate responder T cell proliferation, lipid microdomain formation and phosphorylation of TCR-z following CD3/CD28 co-stimulation, in contrast to healthy responder T cells. Although lupus T cells responded in vitro to CD3/CD28 co-stimulation, there was no parallel increase in CTLA-4 expression, which would normally provide a break on T cell proliferation. These defects were associated with exclusion of CTLA-4 from lipid microdomains providing an anatomical basis for its loss of function. Collectively our data identify CTLA-4 dysfunction as a potential cause for abnormal T cell activation in patients with SLE, which could be targeted for therapy.

CS10.3 & PO1.L.1

Homeostatic imbalance of regulatory and effector T cells due to IL-2 deprivation amplifies murine lupus

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The origins and consequences of a regulatory T cell (Treg) disorder in systemic lupus erythematosus (SLE) are poorly understood. In the (NZBxNZW) F1 mouse model of lupus, we found that CD4+Foxp3+ Treg failed to maintain a competitive pool size in the peripheral lymphoid organs resulting in a progressive homeostatic imbalance of CD4+Foxp3+ Treg and CD4+Foxp3− conventional T cells (Tcon). In addition, Treg acquired phenotypic changes that are reminiscent of IL-2 deficiency concomitantly to a progressive decline in IL-2-producing Tcon and an increase in activated, IFN-gamma-producing effector Tcon. Nonetheless, Treg from lupus-prone mice were functionally intact and capable to influence the course of disease as shown by adoptive transfer of Treg into mice with already established disease. Systemic reduction of IL-2 levels early in disease promoted Tcon hyperactivity, induced the imbalance of Treg and effector Tcon, and strongly accelerated disease progression. In contrast, administration of IL-2 partially restored the balance of Treg and effector Tcon by promoting the homeostatic proliferation of endogenous Treg. IL-2 treatment of diseased mice also strongly impeded disease progression that was most efficient by application of a repetitive regimen. In summary, an acquired and self-amplifying disruption of the Treg-IL-2 axis contributed essentially to Tcon hyperactivity and the development of murine lupus. The reversibility of this homeostatic Treg disorder provides novel and promising approaches for the selective treatment of SLE (Humrich et al., Proc Natl Acad Sci USA: Vol. 107, 2010)

CS10.4 & PO1.L.2

Spontaneous dicer deficiency and altered miRNA in lupus-prone MRL/lpr mice correlates with loss of Treg function

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Objective: To study the mechanism of reduced suppressive function in MRL/fas[lpr/lpr] Treg cells. ***Methods:*** Spleens were collected from four week (pre disease) and 16 week (diseased) old lupus prone MRL/fas[lpr/lpr] (MRL) and control C3H/HeOuj (C3H) mice. Following RBC lysis, Treg cells were enriched and used for phenotype analysis, functional assays and RNA extraction. CFSE labeled splenocytes were cultured with Treg cells from MRL and C3H mice. Three days later cells were harvested, gated on CD4+ T cells and dead cells excluded based on size and 7-AAD and analyzed for proliferation. Seven-color flow cytometry was used to analyze the phenotype of Treg cells from MRL and C3H mice. RNA was extracted for analysis of *dicer* expression and miRNA analysis. Quantitative real time PCR analysis was performed for assessing *dicer* expression. miRNA expression was compared in the Treg cells from MRL and C3H mice using the SABiosciences miFinder PCR array. ***Results:*** CD4+ CD25+ foxp3+ Treg cell frequencies and numbers were increased in lupus prone MRL mice compared to non-autoimmune C3H mice. Flow cytometric analysis revealed an increase in CD69+ CD62L− Treg cells in lupus prone MRL mice compared to C3H control mice. Functional analysis showed that Treg cells from MRL mice were less suppressive compared to Treg cells from control C3H mice and higher frequencies of CD69+ CD62L− Treg cells were detected in MRL mice that showed reduced suppressive function. The phenotype of MRL Treg cells with reduced suppressive function is similar to that observed in Treg cells from *dicer* knock-out mice. Therefore we asked if Treg cells from MRL mice show reduced dicer expression compared to C3H mice. Real time PCR analysis confirmed that MRL Treg cells indeed expressed lower dicer message compared to Treg cells from control C3H mice. Further miRNA analysis showed increased expression of miRNA's 16, 21, 23a, 27a, 155 and let7g in Treg cells from MRL mice compared to C3H mice. ***Conclusions:*** Treg cells from MRL mice exhibit an altered phenotype (CD69+ CD62L−) and are functionally defective. Reduced *dicer* expression was observed in Treg cells with reduced suppressive capacity. Target prediction analysis of miRNA showed that *dicer* and CD62L are targets for miR-23a and 27a. Therefore spontaneous lymphoproliferative disease in MRL mice is a result of reduced Treg function and associates with dicer deficiency and possibly increased miR-23a and 27a expression.

CS10.5 & PO1.L.3

Increased proportions of CD4+CD25-Foxp3+ T cells in patients with systemic lupus erythematosus (SLE) correlate with the development of glomerulonephritis

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Objectives: CD4+CD25+Foxp3+regulatory T cells (Treg) that specialize in the suppression of immune responses might be critically involved in the pathogenesis of autoimmune diseases. Little, however, is known about the role of CD4+CD25-Foxp3+ T cells. We have recently described increased proportions and functional properties of this Treg subpopulation in patients with SLE. Here we analyzed proportions of CD4+CD25-Foxp3+ T cells in SLE patients and their correlation with different organ manifestations. ***Methods:*** Phenotypic analysis of peripheral blood CD4+CD25-Foxp3+ T cells was performed by flow cytometry (FACS) in SLE patients and healthy controls (HC). The percentage of CD4+CD25-Foxp3+ T cells was analyzed in SLE patients with different organ manifestations and correlated with clinical data, the daily cortisone dose and the SLE disease activity index (SLEDAI). ***Results:*** Proportions of CD4+CD25-Foxp3+ T cells are increased in patients with SLE as compared to HC. In addition we found a significant correlation with the SLEDAI score. Moreover, a significant correlation of proportions of CD4+CD25-Foxp3+ T cells with the presence of glomerulonephritis in SLE

patients in contrast to other organ manifestations was observed. In line with this, proportions of CD4+CD25-Foxp3+ T cells correlated with the extent of proteinuria. *Conclusions:* In summary we found a significant increase of CD4+CD25-Foxp3+ T cells in patients with SLE who suffer from glomerulonephritis suggesting their involvement in kidney pathology. Ongoing analysis of kidney biopsies have been designed to unravel their role in the development of glomerulonephritis in SLE patients.

CS11 Pediatric: Special Needs and Challenges for Children and adolescents with SLE: A Global Perspective

CS11.1

SLE in Children and Youth: The Global Challenge
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Systemic Lupus Erythematosus in children and youth poses unique challenges. First the diagnosis is often overlooked in this age group. Second, the manifestations differ from those in the adult population. Third, the management requires the expertise of physicians trained in both pediatrics and rheumatology. The problem of under-recognition of childhood SLE is probably worldwide, and requires attention to the problem in medical school curricula, and in the training programs in pediatrics, rheumatology and family medicine. Early exposure of medical students and pediatric residents to pediatric rheumatology clinics is needed. Even organizations which purport to represent patients with SLE sometimes neglect the child and youth with this disease. The clinical manifestations of SLE are often more severe in children and adolescents than in adults. This fact frequently influences the therapy recommended. Therapy including corticosteroids and cytotoxic drugs may have side-effect of particular importance to the child such as limitation of growth, and the accumulation of risk for long-term complications such as atherosclerosis and malignancy. Evidence principally from North America and Europe indicates that some ethnic and racial groups are at much higher risk for SLE than others. Unfortunately, similar data from Asia and Africa are lacking, and the world-wide burden of SLE can only be roughly estimated. The absence of such data limits the ability of physicians in the developing world to demonstrate the need for better education and access to newer, albeit more expensive treatment. A global collaborative effort to determine the burden of disease caused by SLE in children and adolescents could be an initial step leading to better care and healthier lives.

CS11.2

Clinical research in pediatric SLE: the APPLE experience
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There have been few clinical trials in pediatric SLE and treatment is largely based on research in adults. Through the Childhood Arthritis and Rheumatology Research Alliance (CARRA) and the Atherosclerosis Prevention in Pediatric Lupus Erythematosus (APPLE) trial, the pediatric rheumatology community in the United States and Canada has collaborated on a complex multicenter prospective randomized controlled trial, which serves as a model for future clinical and translational research studies. Although it took three years, the APPLE study fully enrolled in 2006 with 221 children and adolescents with SLE. Follow up was completed in 2009, and the database was locked May 2010 with results available later this year. This flagship CARRA trial faced issues unique to the complex study design of APPLE (use of shared ultrasound machines), problems common to studies in SLE (how to capture

SAE's and successfully enroll minority subjects), as well as the more general difficulties performing trials in small, academic sites. There is large variability in the enrollment rate at different sites dependent on principal and site investigator involvement, adequate training of all research personnel, and site specific issues. Importantly, aggressive interventions initiated centrally either by networks such as CARRA or study principal investigators are successful in improving site performance. Experience and data gained from the APPLE trial has successfully been used to leverage funding for larger clinical research efforts moving forward, particularly the establishment of a CARRA wide registry for children and adolescents with SLE as well as other defined rheumatic diseases.

CS11.3

The UK pediatric lupus group
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Background: Progress in understanding rare pediatric autoimmune diseases such as juvenile-onset SLE (JSLE) are hampered by small patient numbers cared for in any one centre. To address this, a collaborative national cohort of patients was developed to include a comprehensive collection of clinical phenotypic data, related biobank and investigation of immune function and biomarkers in JSLE *Aims:* To determine the demographics and longitudinal clinical characteristics of JSLE across the UK; develop a related biobank; investigate the immunopathogenesis of JSLE; facilitate international comparative studies and trials. *Methods:* Through the establishment of the UK JSLE Study Group, using multi-disciplinary consensus methodology, a comprehensive, prospective portfolio of clinical data collection was developed assessing: demographics, disease presentation, activity, damage and response to medication. Following full research ethical approval, the UK JSLE Cohort Study & Repository was established in August 2006 and is currently recruiting patients from 16 centres across the UK (all of the main JSLE centres). All patients with two or more of the ACR diagnostic criteria for SLE in patients 16 years or younger at the time of diagnosis are eligible for recruitment to this study, following informed consent. *Results:* To date, over 240 patients have been recruited. Clinical characteristics of this cohort will be presented in detail. All patients have been consented for collection of a related biobank for genetic and autoantibody profiling which is now being established. Initial investigation has explored the role of neutrophil apoptosis in the development of JSLE, demonstrating significant dysregulation of both pro- and anti-apoptotic mediators. Further work is focussing on surface expression of nuclear autoantigens and the role of toll-like-receptors in triggering the adaptive immune system leading to loss of tolerance and development of autoantibody production and the longitudinal investigation of renal biomarkers in lupus nephritis. This programme of translational research is integrated into a comprehensive portfolio of clinical studies / trials being developed by the UK Paediatric Rheumatology Clinical Studies Group. *Conclusions:* Through multi-centre, multi-disciplinary collaborative and consensus research methodology, a national Cohort Study & Repository of patients with JSLE has been is established with a comprehensive research agenda exploring the clinical characteristics, immunopathology and genetics of JSLE in the UK.

CS11.4

Special needs and challenges for children and adolescents with SLE: a global perspective
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The Indian Perspective – Introduction: The child with SLE in India has to contend with many serious issues that are unique to our part of the world. The needs and challenges exist in many areas and span a whole gamut of issues ranging from awareness of this condition, infectious complications that arise in a tropical climate, resources available to treat, patient retention and also

social taboos about disease and disability. I will discuss each of them briefly. **The challenges:** (1) Physician and Public awareness. *(i) Physician awareness.* SLE in children is seen in about 1: 10000. It is thus a relatively rare disease and the prevalence is one tenth of juvenile arthritis. There are two reasons why this condition is not well recognized by physicians at an early stage of the disease. One: In India there still exists a huge burden of infectious diseases which is for several reasons: A tropical climate, poor preventive health and hygiene especially availability of safe drinking water and waste disposal, inequitable distribution of health care infrastructure , lack of awareness in the population and late self referral to available health care facilities. It is also an endemic area for tuberculosis and several vector borne diseases such as malaria and dengue. As a result, the burden of infectious diseases is high and so common place that SLE unless not an obvious presentation with a malar rash and nephritis is often overlooked and mistaken for an infectious disease. Two: There is a lack of post graduate training in Paediatric Rheumatology in India and there are only a handful of centres in metropolitan cities that cater exclusively to this population. Thus children with rheumatologic diseases are not recognized early and interestingly when they have joint pains are traditionally referred to the orthopaedic surgeons who are not tuned to recognizing these conditions. The child often goes from one medical care facility to the next seeking attention until the correct diagnosis is reached. The child with an explosive onset of disease who is very unwell and seeks care at a tertiary level centre is far more likely to be appropriately diagnosed and managed than the patient with indolent disease who has low grade fever and fatigue with mild cytopenia. *(ii) Public awareness.* Is virtually nonexistent, especially about complex immunoinflammatory conditions such as SLE. This is probably for two reasons: the adult literacy rate in India is about 61% and less in women and in rural areas. It is with literacy that one develops awareness and knowledge and can self refer and seek medical advice from an appropriate resource. Secondly; illness in the family especially in the girl child is kept a secret from the extended family and friends. The secrecy shrouding any illness especially a chronic one is an impediment to the patient with SLE as appropriate advice is not sought from elders or other knowledgeable persons in the family. (2) Social Taboos. Rudyard Kipling once famously remarked "East is East, and West is West, and never the twain shall meet". This holds true for all the social taboos that surround illness in India and impact on the care given to the girl child. This gender inequality is slowly fading and not very prevalent in large metropolitan cities in India, but is certainly seen in the tier II cities and in the rural India. The girl child is not usually given the same educational and social opportunities as her male counterpart, and may indeed be denied expensive and prolonged care that is to be provided at a centre far removed from the home –town that can offer care and support but not a cure. (3) Patient Retention and family fatigue. This is an area of concern both in India and the developed world. As the disease is a chronic one where the child or adolescent patient tends to have repeated flares, it is challenging to keep the child and the family engaged with the medical team. We are handicapped in that we do not have a trained pool of nurse specialists and social workers who can be useful team members and follow up and council the families who do not attend follow up appointments. (4) Alternative systems of medical care. There are several systems of medical care followed in India: Ayurvedic, Homeopathic, Unani, Yoga, Spiritual healing, etc. Several of these systems promise magical cures and patients who have persistent active disease are lured into these therapies often with disastrous results. Unfortunately there is no appropriate legislation that can prevent these practices. (5) The resources available to treat. *(i) Financial.* The disparities across the world are stark. The gross national income per capita in the USA is $ 44070, in Australia is $33940, and in India is $2460. The per capita government expenditure on health is also very low in India: a mere $ 21, in the USA is $3074 and in Australia is $2097. India's total expenditure on health amounts to 5.1% of the gross domestic product (GDP), while its per capita total expenditure on health is $80 compared to an average of over $220 spent by many other developing countries. This expenditure far lags behind the industrialized world. Private expenditure on health as percentage of total expenditure on health is 80% in India, 55% in USA and 33% in Australia. Thus in India , there is certainly a major resource crunch, suboptimal money is spent by the government on health care , a fraction of the population is covered by the health insurance which in India does not support out patient care. This country is still grappling with polio eradication and tuberculosis therapy such that diagnosis and therapy of SLE is not on the radar as yet. *(ii) The health care delivery system.* India has a population of

1.2 billion people. To organize and deliver an affordable health care system that is equitable, efficient, progressive and user friendly is indeed a daunting task that we have not been able to achieve to date. The delivery of health care is fragmented: It is organized by both the public (Government) and the private sectors. Centres of excellence exist in both the private and public sector but there is no efficient system of referral or a defined catchment area for each centre. There are over 250 medical colleges in India but hardly any that have rheumatology services/care. Thus, it is the patient who has to make a tremendous effort to reach the correct health care provider. *(iii) Medical personnel.* As mentioned earlier there is a shortage of trained personnel in Paediatric Rheumatology in India and the child with SLE may get medical care from several different sources: An internist, a paediatrician , an adult rheumatologist or infrequently from a paediatric rheumatologist. The care given to the child is thus very variable and when the family are faced with a child who is persistently unwell they begin to "doctor shop" and move from one centre to the next. The long term care invariably suffers as different doctors only see one facet of the child's illness and do not come to terms with the patient specific issues nor do they develop a bond and a trusting relationship with the child and family. *(iv) Medications and regular investigations.* All medications and the investigations needed to follow children with SLE are available in India; the main difficulty faced by the patient is the cost: Drugs such as mycophenolate and Intravenous Immunoglobulin and investigations such as C3 /C4 and DsDNA are costly and unaffordable by a vast majority of patients. A fraction of the patients are covered by medical insurance which is not well developed in India; most patients have to self pay. **The Needs:** To meet the above detailed challenges the lupus patient in India has several needs: (i) There should be an efficient well trained pool of Paediatric Rheumatologists available in nodal medical centres distributed across the country. To provide this service the undergraduate and post graduate training in paediatric rheumatology should be robust. (ii) The medical insurance facilities should be available for all children with chronic diseases and must include outpatient care. (iii) A support structure such as an India Lupus Group and a well trained net worked pool of nurse specialists and social workers should be involved in the care of these children. They should be advocates of these young girls and should discuss social taboos, alternative systems of care and the need for regular follow up. (iv) There should be vocational and educational guidance along with a smooth transition to adult rheumatology care. (v) Many if not all our newly diagnosed patients should be recruited into international drug trials. (vi) The data base of these children should be maintained such that "India specific issues" can be well identified. **Conclusion:** It is with international collaborative efforts and advocacy for these children at home that we may aspire to fulfil the needs of our SLE patients and give them the best quality life that they deserve.

KS1   Lessons Learned from Animal Models

KS1.1

Deep sequencing reveals extensive functional variations in SLE susceptibility genes
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Genetic predisposition is a potent element in susceptibility to SLE. Previous studies by the International Consortium for Systemic Lupus Erythematosus (SLEGEN) have associated more than 20 genomic segments with susceptibility. These studies have localized causative genes into small genomic segments, but have not identified the precise genetic variations responsible for the functional changes that cause the disease. Towards elucidating the genetic lesions that are causative for SLE susceptibility, we have initiated deep sequencing studies of all of the genomic segments exhibiting suggestive or significant association with susceptibility to SLE. These studies are being performed using a targeted sequence enrichment strategy and the Illumina GAIIx next generation sequencer. We have completed sequencing on more than 95 individuals and our initial analysis of data from 32 Caucasian samples has

revealed more than 2000 novel single nucleotide polymorphisms (SNP) or deletion/insertion variations (DIP) in 2.9 megabases derived from 25 genomic segments showing significant associations with SLE. Our ongoing analysis of the organization of these polymorphisms, utilizing median joining algorithms to network the SNP haplotypes formed in strong linkage disequilibrium with SNPs associated with SLE, has delineated multiple allele lineages with extensive functional variations in haplotypes that are associated with susceptibility to SLE. These results indicate that many of the SNPs currently used to identify susceptibility genes actually mark multiple lineages of alleles. Further, significant functional variations still exist among these alleles, which all carry markers that are strongly associated with SLE. As a result, we believe that our ongoing delineation of functional lesions in SLE-associated alleles will significantly improve our understanding of the functional changes that actually mediate disease susceptibility and ultimately increase the accuracy of relative risk estimates. The overall characteristics of the susceptibility alleles identified to date support the hypothesis that dysregulations of the adaptive and innate immune systems interact to mediate susceptibility to SLE in humans.

PL3 Lupus Epidemiology and Pathogenesis

PL3.1

Blood microarray analysis in SLE

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The past decade has seen an explosion in the use of DNA-based microarrays. These techniques permit to assess RNA abundance on a genome-wide scale and have been used to analyze the blood transcriptome in a wide range of human autoimmune diseases, including SLE. Microarray-based research is facing significant challenges with the analysis of datasets which contain noise, are difficult to interpret, and do not compare well across laboratories and platforms. We recently proposed a novel module-level microarray data mining strategy emphasizing the selection of coordinately expressed genes or transcriptional modules. Once these transcriptional determinants have been characterized, changes in gene expression between study groups can then be assessed on a module-by-module basis. This strategy allowed the identification of disease-specific leukocyte transcriptional fingerprints in patients with SLE. Importantly, we demonstrate that modular transcriptional data can be reproduced across microarray platforms and laboratories and is widely applicable to generate robust and interpretable module-level disease activity biomarkers. More quantitative and sensitive high throughput RNA profiling methods are starting to be available and will be discussed. These assays will make it possible in the foreseeable future for transcriptome analyses to become a routine test in the clinical setting.

PL3.2

What do studies of gene-environment interaction tell us?

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The explosion of genetic data and discoveries provides researchers with a myriad of data to be considered in complex diseases long believed to have genetic components interacting in some way with underlying non-genetic factors. As the list of genes potentially associated with systemic lupus erythematosus grows, understanding how to incorporate these results into epidemiologic studies is critical. In addition to discussing some recent gene-environment interaction (GxE) studies, we will consider GxE study design and the associated strengths and limitations. Two frequent approaches include case-control and case-only studies. In the former, control selection is important and carries a number of important assumptions. We will consider the trade-offs of general population controls, unaffected siblings, spouses, and other groups and

how interpretation of results changes. Case-control designs are often chosen because they may require less resources. However, control selection is complicated and some may want to choose another design. The case-only design may be an efficient solution to estimate the association between exposure and genotype among cases, and removes the issues surrounding control selection. However, although we can estimate the relative interaction, we cannot assess the main effect of either the gene or environmental factors. Additionally we will discuss concerns of bias related to confounding and misclassification, which are sometimes dismissed in genetic studies and should not be downplayed in GxE studies. Lastly we will consider measures of multiplicative and additive interaction including the relative excess risk due to interaction, the synergy index, and the attributable proportion due to interaction.

PL3.3

Interferon and cytokines

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Most immune cells are involved in the pathogenesis of lupus, which is considered as the prototype autoimmune disease. A number of cytokine pathways are important in the disease process, and recent data suggest that there are, at least partially, genetic explanations for the increased cytokine production and response in lupus patients. Thus, polymorphisms in the cytokine gene themselves as well as variation in genes regulating cytokine expression are of importance for disease susceptibility. Among lupus risk genes involved in cytokine pathways are *IRF5, STAT4, TYK2, TNFAIP3* and *IRAK1*. Important cytokines in the pathogenesis are interferon- (IFN-) that acts as an immune adjuvant, the B Lymphocyte Stimulator (BLyS) that promotes autoantibody production, and tumor necrosis factor- (TNF-) that contributes to organ inflammation. The precise role of each cytokine in the pathogenesis is, however difficult to determine, but a direct causative role for the type I IFN system is suggested by the observation that long term IFN- treatment can induce a lupus syndrome indistinguishable from spontaneously occurring lupus. In addition, most lupus patients have a prominent type I IFN signature early in the disease. The reason for the ongoing type I IFN production in lupus seems to be the presence of endogenous IFN inducers consisting of immune complexes (IC) containing nucleic acid. Such interferogenic ICs are internalized in plasmacytoid dendritic cells (pDC) via the FcRIIa, reach the endosome and activate Toll-like receptors (TLR) with subsequent induction of type I IFN gene transcription. Produced IFN- will cause activation of both the innate and adaptive immune system, but also increased expression of some autoantigens. The interplay between immune cells, the type I IFN system and key cytokines in the pathogenesis of lupus will be discussed during the session.

CS12 Late Breaking Abstracts

CS12.1 & PO2.G.10

IRF5 is required for disease development in the FcgRIIB-/-Yaa mouse models of SLE

Yasuda, Kei; Richez, Christophe; Bonegio, Ramon G.; Watkins, Amanda A.; Aprahamian, Tamar; Busto, Patricia; Richards, Rocco J.; Liu, Chih Long ; Cheung, Regina; Utz, Paul J.; Marshak-Rothstein, Ann; Rifkin, Ian R.
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Objectives: Polymorphisms in the transcription factor interferon regulatory factor 5 (IRF5) are strongly associated in human genetic studies with an increased risk of developing the autoimmune disease systemic lupus erythematosus (SLE). However, the biological role of IRF5 in lupus pathogenesis has

not previously been tested in an animal model. *Methods:* We crossed IRF5-/- mice with the Fc gamma receptor IIB-/- (FcgRIIB-/-)Yaa and FcgRIIB-/- lupus models to examine whether IRF5 is required for disease development in the models. One effect of IRF5 is to induce the production of the type I interferon (IFN), IFN-alpha, a cytokine implicated in lupus pathogenesis. To address the mechanism by which IRF5 promotes disease, we evaluated FcgRIIB-/-Yaa mice lacking the type I IFN receptor subunit 1 and therefore unable to respond to any type I IFN. We examined disease manifestations. *Results:* We show that IRF5 is absolutely required for disease development in the FcgRIIB-/-Yaa and FcgRIIB-/- lupus models. In contrast to IRF5-sufficient FcgRIIB-/-Yaa mice that developed severe disease, IRF5-deficient FcgRIIB-/-Yaa mice do not develop lupus manifestations and have a phenotype comparable to non-autoimmune wild type C57BL/6 mice. Strikingly, full expression of IRF5 is required for the development of autoimmunity, as IRF5 heterozygotes had dramatically reduced disease. Unlike the IRF5-deficient and IRF5-heterozygous FcgRIIB-/-Yaa mice, type I IFN receptor subunit 1-deficient FcgRIIB-/-Yaa mice maintained a substantial level of residual disease, demonstrating that the pathogenic effects of IRF5 are not primarily mediated through effects on type I IFN production. Furthermore, in FcgRIIB-/- mice lacking Yaa, IRF5-deficiency also markedly reduced disease manifestations, indicating that the beneficial effects of IRF5 deficiency in FcgRIIB-/- mice are not due only to inhibition of the enhanced TLR7 signaling associated with the Yaa mutation. *Conclusions:* We demonstrate that IRF5 plays an essential role in lupus pathogenesis in the FcgRIIB-/-Yaa and FcgRIIB-/- mouse models of SLE and that this is mediated through pathways beyond that of type I IFN production.

CS12.2 & PO1.I.9

WASp deficient B cells play a critical, cell intrinsic role in triggering autoimmunity

Rawlings, David J.; Meyer-Bahlburg, Almut; Schwartz, Marc A.; Panigrahi, Anil K.; Hudkins, Kelly L.; Liu, Chaohong; Sather, Blythe D.; Khim, Socheath; Liggitt, Denny; Song, Wenxia; Silverman, Gregg J.; Luning Prak, Eline T.; Alpers, Charles E.; Becker-Herman, Shirly

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Patients with the immunodeficiency, Wiskott-Aldrich syndrome (WAS), frequently develop systemic autoimmunity. The current study demonstrates that mutation of the WAS gene results in a B cell intrinsic break in tolerance. Whereas this defect leads to autoantibody production in WAS protein deficient (WASp-/-) mice without overt disease, chimeric mice in which only the B cell lineage lacks WASp exhibit severe systemic-lupus-(SLE)-like autoimmunity characterized by high affinity, class-switched autoantibodies, severe renal histopathology, and early mortality. While B cell negative selection was intact, WASp-/- mice exhibited evidence for altered peripheral tolerance beginning within the transitional compartment. In accord with this idea, WASp-/- B cells are hyper-responsive to B cell antigen receptor (BCR) engagement and exhibit impaired BCR internalization. Further, lambda-light chain usage is enriched in WASp-/- B cells beginning at a late transitional B cell stage and this cell population proliferates spontaneously in vivo, suggesting that WASp deficiency directly impacts B cell positive selection. Finally, BCRs specific for apoptotic cell determinants are enriched in WASp-/- mice and, in WASp-/- B cell chimera mice, B cells specific for a subset of such antigens are expanded and undergo T cell-dependent, class switch recombination. Our combined data provide a compelling explanation as to why a large proportion of WAS patients with mixed chimerism following stem cell transplantation develop severe, humoral autoimmunity. Our findings also highlight the primary role for altered peripheral B cell selection in initiation of SLE-like autoimmunity and provide insight into how B cell depletion therapies may operate under such conditions.

CS12.3 & PO2.G.11

Interferon alpha (IFNα) inhibits C-reactive protein synthesis in human hepatocytes. Is the mechanism a decreased STAT3 phosphorylation?

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Objectives: C-reactive protein (CRP) is an acute phase protein mainly produced by hepatocytes in response to interleukin (IL)-6 and IL-1β-triggered signalling via the transcription factors STAT3, C/EBPβ and NFκB. Because of its rapid increase during inflammation it is widely used to monitor inflammatory activity in chronic diseases such as rheumatoid arthritis. CRP has immunomodulating properties with disease-modifying effects demonstrated in lupus-prone mice. However, despite extensive inflammation the serum levels of CRP typically remain low in disease flares of systemic lupus erythematosus (SLE) as well as in viral infections. Since SLE flares are characterized by an elevation of IFNα and/or upregulation of IFNα-regulated genes (the IFN-signature) we have hypothesized that the lack of an adequate CRP response is due to IFNα, and we previously reported that the CRP-promoter activity in a transfected human hepatoma cell line is inhibited by IFNα. In the present study we investigated CRP synthesis in primary human hepatocytes and potential changes in intracellular protein phosphorylation to reveal the mechanisms that yields the inhibition. *Methods:* CRP secretion from freshly isolated primary human hepatocytes was measured by ELISA. Hepatoma G2 (HepG2) cells stably transfected with the 1-kb CRP-promoter and a luciferase reporter gene (generously provided by Dr. Jan Torzewski, Ulm, Germany) were used to study intracellular signalling proteins and transcription factors. The protein phosphorylation was quantified by a multiplex phosphoprotein detection assay. *Results:* IL-6-induced or IL-1β-induced CRP secretion from primary human hepatocytes was inhibited by 46-71% in IFNα treated cells (100 or 1000 IU/mL). CRP secretion induced by combined IL-1β and IL-6 stimulation was inhibited by 19.8-27.7%. A marked increase in STAT1 phosphorylation (122-281%) and a reduced STAT3 phosphorylation (17-35%) was observed in IFNα stimulated HepG2 cells after 5, 15, 30 and 360 minutes. No apparent change in phosphorylation of p38 MAPK or IκB was seen in IFNα treated cells. *Conclusions:* IFNα inhibits CRP secretion in primary human hepatocytes which may explain the modest CRP-response in disease flares of SLE but also in viral infections. We found that STAT1 phosphorylation increased, whereas STAT3 phosphorylation deceased, in IFNα treated cells. STAT1 and STAT3 are known to have counteracting effects and we therefore suggest a mechanism where IFNα-induced STAT1 phosphorylation reduces STAT3 phosphorylation and in this manner CRP-synthesis.

CS12.4 & PO1.B.32

A gene expression score derived from interferon, plasma cell and neutrophil gene clusters is an informative biomarker of lupus flare

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Objectives: Treatment of patients with systemic lupus erythematosus (SLE) aims to achieve clinical remission and avoid severe flare. Several indices have been designed to distinguish disease flare from remission but are not routinely used in clinical practice. Anti-dsDNA antibody titers are frequently used to assess flare in the course of patient management, but those antibodies are not present in all patients and often do not reflect disease activity. A biomarker that predicts or identifies lupus flare might provide a tool for more effective and timely medical intervention. *Methods:* Longitudinal PBMC and plasma samples were obtained over an average 6 visits (2-12) from 23 SLE patients and 5 healthy donors (HD). The duration of the study for individual patients varied from 197 to 812 days. Plasma levels of autoantibodies were evaluated using Multi-Analyte Profiling (MAP) technology (Rules-Based Medicine, Austin, TX). PBMC transcriptional profiles for each visit were established using Human Genome U133 Plus 2.0 Arrays. *Results:* Autoantibody profiling

detected increased plasma levels of 14 specific autoantibodies, including anti-dsDNA, anti-Ro, anti-La, anti-RNP and anti-Smith in SLE patients compared to HD ($p<0.05$; fold change, FC, $= >1.5$). The level of anti-dsDNA antibody paralleled the changes in the SLEDAI score in 22% of patients. One hundred sixty-nine microarray profiles were obtained from PBMC RNA isolated from the patients and control subjects. Data were subjected to K-mean clustering analysis (k=50, I=100). Among others, clusters characterized by plasma cell transcripts, type I interferon-inducible genes, and neutrophil transcripts were observed. Statistical analysis confirmed that those 3 gene clusters distinguished SLE patients from HD. A representative gene from each of the three clusters (CD38, IFIT3 and MMP8) was selected for further analysis as a flare score and reflected the mean of the relative expression values. The score distinguished SLE patients from HD ($p<0.001$; FC=5.6) and was significantly higher during severe lupus flares compare to remissions ($p=0.002$; FC=2.0). Analysis of individual patients showed that the flare score paralleled the SLEDAI score in 51% of patients. **Conclusion:** A score derived from expression levels of genes representing three important pathogenic mediators, plasma cells, interferon and neutrophils, was superior to anti-dsDNA titer as a biomarker of increased SLEDAI score in our study. Validation of this score as a marker of lupus flare may provide an informative tool for improved management of lupus patients and will stimulate an examination of the neutrophil subpopulation associated with disease activity.

CS12.5 & PO2.D.46

The long-term outcomes of leflunomide in patients with lupus nephritis

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Objective: To investigate the long-term outcomes of leflunomide in the treatment of lupus nephritis (LN) beyond six-month induction therapy in a multi-centre, open-label extension of one phase III clinical trial. **Methods:** 108 patients with LN received leflunomide with a loading dose of 50mg/day for 3 days, followed by 30mg/day for six months in one phase III study. 56 patients voluntarily enrolled an open-label extension study and were maintained on leflunomide at dose of 20mg/day, and the dose of prednisone was < 10mg/day. The primary endpoint was a composite of patient survival and renal survival. Renal parameters (24h-proteinuria and serum creatinine) were assessed. **Results:** A total of 56 patients (mean age 27.7±8.8 years) were treated with leflunomide for >6 months; 83.9% (47) of the patients were female. By conclusion of 6 months induction therapy, 85.4% reached clinical remission (complete remission rate was 34.5%, partial remission rate was 50.9%). The mean duration of leflunomide treatment was 47.0±26.7 months (range 9–88 months). During maintenance therapy, one patient died (of lupus relapse) and one patient developed chronic renal failure. The 84-month patient cumulative survival rate was 98%. The rate of proteinuria relapse-free survival was 94.1%, sustained doubling of serum creatinine rate was 2.5%. At the end of follow-up, 88.2% of the 56 patients achieved clinical remission (complete remission rate was 52.9%, partial remission rate was 35.3%), and the cumulative mortality is 0.004/patient-year (1/234.7 patient-year). **Conclusions:** Our data confirm that a maintenance regimen of leflunomide followed by induction therapy with leflunomide achieves good long-term clinical results in control of LN.

CS12.6 & PO2.E.23

Aspreva Lupus Management Study maintenance results

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Objectives: The Aspreva Lupus Management Study (ALMS; protocol number: WX17801) was a large, multinational, multicenter, Phase III trial. The induction phase (data reported previously) compared the efficacy and safety of mycophenolate mofetil (MMF) with cyclophosphamide (IVC), both with corticosteroids, as treatment for active class III–V lupus nephritis (LN).This abstract focuses on the maintenance phase in which the efficacy and safety of MMF was compared with azathioprine in patients who had achieved a partial or complete response in the induction phase. **Methods:** In the maintenance phase of this prospective, double-blind study, patients were re-randomized (Week 24/Month 0) 1:1 to receive either oral MMF (2 g/day) plus placebo or oral azathioprine (2 mg/kg/day) plus placebo. All patients received corticosteroids (maximum dose: 10 mg/day), with dose reduction per investigator judgment. Patients returned for assessment at Week 24/Month 0 plus 2 weeks, Month 1, Month 2, and every 3 calendar months thereafter until Month 36 or study termination. The primary efficacy outcome measure was treatment failure defined as any of the following: death, end-stage renal disease, sustained doubling of serum creatinine, or renal flare [proteinuric or nephritic]. Key secondary parameters included: time to event for each individual component of treatment failure; complete renal remission; combined renal and extra-renal remission; and comparisons of maintenance subgroups. Initial results and updates to this preliminary data will be presented. **Results:** Of 227 patients randomized (safety population) (North America, n=47; Europe, n=48; Asia, n=72; Latin America, n=60; 99 white; 23 black; 76 Asian; 29 'other'; 150 classified as non-Hispanic), 101 withdrew from the study and 126 completed. At baseline, mean [SD] age was 32.0 [10.71] years; 195 (85.9%) were female. Mean [SD] duration of LN was 3.4 [4.44] years. More patients had class IV (n=147) LN than class III (n=22), III/V (n=7), IV/V (n=16), or V (n=35) disease. Measurement of laboratory parameters at baseline revealed the following: serum creatinine (low or normal, n=212; high, n=15); serum albumin (low, n=11; normal, n=212; high, n=4); urine protein (≤1 g/24 hrs, n=156; ≥1 g/24 hrs, n=71); urine protein:creatinine ratio (normal, n=35; high, n=177); serum C3 (low, n=98; normal, n=127; high, n=2) and C4 (low, n=60; normal, n=162; high, n=5); antibodies to double-stranded DNA (absent, n=64; present, n=163). **Conclusions:** ALMS, one of the largest studies conducted in LN to date, will provide evidence on the efficacy and safety of MMF compared to azathioprine as maintenance therapy.

CS12.7 & PO2.E.24

A randomized, double-blind, placebo-controlled study of spliceosomal peptide rigerimod in patients with systemic lupus erythematosus (SLE)

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Objectives: Rigerimod is a novel peptide medication that has putative immunomodulatory actions for patients with SLE. The effect of subcutaneous (sc) rigerimod on SLE disease activity was evaluated. **Methods:** Adults meeting ≥ 4 of the ACR criteria for SLE diagnosis, a clinical score ≥ 6 on the SLEDAI-2K, and no A score on the BILAG index were enrolled. Patients were randomized to receive sc rigerimod 200 µg/4 wk, 200 µg/2 wk, or placebo/2 wk, plus standard of care (SoC) for 12 wks. SoC included antimalarials and oral corticosteroids (up to 80 mg/wk prednisone equivalent) at stable doses for ≥ 4 wks before study treatment. The primary efficacy measure was the SLE Responder Index (SRI) at wk 12 compared with baseline. Adverse events (AEs) were recorded throughout the study. Statistical significance was set at alpha = 0.025. **Results:** A total of 150 patients completed 12 wks of treatment and either the 24-wk follow-up visit or discontinued the study. 150 patients (female, 96%; mean age, 37.6 yr) received study medication (rigerimod 200 µg/4 wk, n=49; 200 µg/2 wk, n= 52; placebo/2 wk, n=49). At wk 12, more patients achieved an SRI response with rigerimod 200 µg/4 wk (53.1%) vs the placebo group (36.2%; $P=0.048$). Similarly, 53.1% of the rigerimod 200 µg/4 wk group achieved a SLEDAI-2K response at wk 12 vs the placebo group (38.3%; $P=0.0734$). The rigerimod 200 µg/2 wk group showed a response rate of 45.1% on both measures vs the placebo group (36.2% and 38.3%, respectively; $P>0.025$). The most frequently reported AEs ($\geq 5\%$ patients) were urinary tract infection and injection site erythema. Most AEs were mild or moderate in intensity. Serious AEs (SAEs; n=7) included gastritis, soft tissue infection, herpes viral pneumonia, diverticulitis (n=1 each), and pneumonia (n=3); the investigator considered soft tissue infection and herpes viral pneu-

monia to be related to rigerimod 200 μg/4 wk and 200 μg/2 wk, respectively. One SAE, pneumonia, resulted in the death of 1 patient and was considered to be unrelated to rigerimod 200 μg/4 wk. **Conclusions:** Following 3 injections of rigerimod 200 μg/4 wk, patients with SLE showed reduced disease activity vs placebo as assessed by SRI and SLEDAI-2K. Rigerimod was generally well tolerated. Further evaluation of this agent is warranted in patients with SLE.

CS12.8 & PO2.E.25

Long-term outcome of autologous hematopoietic stem cell transplantation (autoHSCT) using lymphoablative conditioning in recalcitrant systemic lupus erythematosus patients

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Objectives: Despite recent improvements in mortality and morbidity of SLE patients with major organ involvement, treatment failure and relapse continue to affect significant majority of patients. We conducted a pilot study to test if intensive lymphoablation followed by autoHSCT can result in sustained, complete, treatment-free remission in severe, recalcitrant SLE and to determine whether this approach fundamentally changes abnormal immune response. **Methods:** Patients were enrolled based on active SLE despite prior treatment with IV cyclophosphamide (CYC). Of the 8 patients treated, 2 had transverse myelitis, 1 retinal vasculitis and 5 WHO Class IV nephritis. Stem cell mobilization regimen consisted of 2,000 mg/m2 CYC, 750 mg/m2 rituximab (RTX) and G-CSF. Conditioning regimen consisted of 750 mg/m2 RTX, 4.8 g/m2 CYC and 120 mg/m2 fludarabine, followed by CD34+ selected stem cell infusion and G-CSF. All immunosuppressive medications and hydroxychloroquine were discontinued at the start of mobilization and steroids were rapidly tapered off after the transplant. Clinical response was evaluated by organ specific response criteria. Disease activity indices (SLEDAI and SLAM) were used to assess overall lupus activity. The primary endpoint was complete response (CR) at 24 months defined as no lupus activity and no treatment for lupus (including HCQ and steroids). **Results:** Among the 8 patients, there were 2 early deaths (one from diffuse alveolar damage, one from mycobacterial meningoencephalitis). One patient had lupus flare (retinal vasculitis responding to corticosteroids) 6 months post-transplant. Five patients were successfully tapered off corticosteroids, achieved CR criteria within 6 months of transplant and SLEDAI scores of zero. One of these patients flared 18 months post-transplant, whereas 4 continue to be in CR for 4 (n=2) to 5 years (n=2). All four of these patients continue to have negative ds-DNA antibody and normal complement levels since 6 months after HSCT. The reconstituted immune system showed a significant shift from a phenotype dominated by memory and activated effector T and B cells at baseline to a predominantly naïve phenotype post-transplant. **Conclusions:** Our data indicate that lymphoablative autoHSCT leads to sustained (up to 5 years) clinical and serologic remission, without the use of any maintenance therapy in a subset of otherwise recalcitrant SLE patients. This clinical benefit is associated with marked normalization of the immune repertoire. Reducing to the intensity of conditioning and/or exclusion of patients with multiple organ dysfunction may decrease short term toxicity and would make this approach an acceptable alternative for the treatment of severe SLE.

CS13 Lupus and the Environment

CS13.1

Environmental risk factors for lupus in post-menopausal women: results from the women's health initiative

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Purpose: Previous studies suggest a possible association of farming occupation and agricultural pesticide mixing with systemic lupus erythematosus (SLE), but such exposures are uncommon in the population and risk associated with personal and residential insecticide use is unknown. **Methods:** Using data from the Women's Health Initiative Observational Study cohort (n=76,861, post-menopausal, age 50-79 years), we examined self-reported lifetime personal insecticide use, residential or workplace application by others, and farm history in relation to risk of incident SLE, confirmed by use of disease modifying anti-rheumatic drugs at year 3 of follow-up. Newly reported SLE cases (n=35) were compared to women in the cohort who did not develop SLE or rheumatoid arthritis. Hazard ratios (adj.HR) and 95% confidence intervals (CI) were estimated in multivariable models adjusting for age, race, region, education, occupation, smoking, reproductive factors, asthma, other autoimmune diseases, co-morbidities and farm history. **Results:** SLE risk was associated with more frequent and longer duration of personal insecticide use, and was highest in those reporting very high or high cumulative personal use (0.12% of exposed developed SLE) compared to those reporting never or minimal use (0.02%; adj. HR= 2.96, 95%CI 0.76, 11.51; p for trend=0.046). Increased SLE risk was also seen for women reporting longer term application by others (p for trend=0.016). Longer duration farm history was associated with SLE risk after adjusting for age, but the association was diminished after adjusting for covariates. **Conclusions:** These findings suggest direct personal and indirect residential insecticide exposures may be related to SLE risk in post-menopausal women, and provide rationale for replication studies in other populations and investigation of specific insecticides.

CS13.2

Epstein-Barr Virus and early events in lupus autoimmunity
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Epstein-Barr Virus (EBV) has emerged as a compelling potential trigger of human SLE autoimmunity in susceptible hosts. However, little is known about the involvement of EBV at early, even pre-clinical, time points. For EBV to play important roles in initiating molecular mimicry with autoantigens or instigating interferon-induced pathways in susceptible hosts, then EBV infection should precede clinical SLE and lupus autoimmunity. Utilizing the United States Department of Defense Serum Repository, 130 cases with stored samples from before SLE diagnosis and 520 matched controls were identified and tested for seroconversion against herpesviruses and for standard lupus autoantibodies. EBV-VCA seropositivity was strongly associated with SLE before diagnosis (p=0.0019) as was the presence of antibodies against EBNA-1 (p= 0.0017), whereas no relationship was seen with other Herpes viruses. Antibodies to EBNA-1 preceded autoantibody development in 35 patients, while autoantibodies preceded anti-EBNA-1 in only 1 case. Univariate analysis showed an increase in EBV antibody titers in patients compared to controls using all available samples (mean 3.47 for patients, 2.73 for controls, p<0.0001). The significant increase in titers was most prominent and statistically significant in the two years prior to diagnosis with the

patient mean ISR 3.89 and the control mean ISR 2.89 (P<0.0001). CMV titers were also significantly increased in patients overall (p=0.0025) and in the two years before diagnosis (p=0.0115). When all herpes viruses were included in the conditional logistic regression model for titers 2 years prior to diagnosis, only anti-EBV-VCA titers were significant. Antibodies against EBV-VCA and EBNA-1 are associated with SLE prior to disease onset. SLE patients in this cohort had significantly higher titers of anti-EBV-VCA and anti-CMV than controls, but this trend was not observed with HSV1 or HSV2. These data support the hypothesis that EBV plays a role in the early pathogenesis of human SLE. During this lecture we will also discuss data regarding the temporal evolution of the anti-EBNA-1 response in relation to onset and progression of lupus autoimmunity, as well as data showing unique EBV responses in unaffected blood relatives of lupus patients. We will also present and discuss data regarding the influence of EBV infection on lupus patient B cells compared to matched controls.

CS13.3

**The US Africa lupus gradient hypothesis
revisited: genetics versus environment**
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According to the gradient hypothesis, lupus is rare in West Africa, while being common in African Americans. Potential genetic versus environmental causes for this proposed discrepancy include genetic admixture, infection exposures and vitamin D. One problem with the gradient hypothesis is that the assessments of disease prevalence in Africa were performed in African countries that were not major participants in the slave trade. Health care systems in the West African countries that were part of the slave trade are inadequate to assess lupus prevalence. The Gullah people of the Sea Islands of South Carolina and Georgia are unique in their genetic homogeneity and lack of genetic admixture. Ancestors of the Gullah are known to have originated primarily from Sierra Leone. As it is still impossible to perform an adequate epidemiologic study in Sierra Leone, we assessed serum autoantibody profiles, EBV serologies and 25OH vitamin D levels on over 185 sera from unaffected Gullah females and 71 sera from age matched assymptomatic females from Bo, Sierra Leone. ANA positivity was significantly more common in the Gullah sera compared to Sierra Leone sera, while vitamin D levels were significantly higher in the Sierra Leone sera. These data suggest autoimmunity is more common in African American Gullah than young women in Sierra Leone. Autoantibody spectra, EBV serologies and GWAS studies are in progress to further delineate autoimmunity and genetic similarities/differences between women in Sierra Leone and the Sea Islands of South Carolina.

CS13.4 & PO2.B.1

**Seasonal variation in the incidence of disease flares in
systemic lupus erythematosus (SLE): relationship with
weather parameters and ultraviolet light intensity**
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Objectives: To examine the seasonal variation in disease flares of SLE with regard to individual organ systems and their relationship with weather parameters and environmental ultraviolet light intensity. *Methods:* SLE patients who were followed up in our clinics between 2000 and 2008 were studied. Details of disease flares, defined by the SELENA-SLE flare instrument, were retrieved from review of the electronic medical records. Disease activity scores during the flare episodes were measured by the SELENA-SLEDAI. The monthly rates of disease flares (mild / moderate and severe) and of individual organ systems were calculated. Flares in five organ systems (cutaneous and musculoskeletal, serositis, hematologic flare, renal and neuropsychiatric) were defined by using the individual components of SLEDAI and the SELENA-SLE flare instrument. The rate of SLE flares was correlated by

Pearson's correlation with a number of weather parameters which included mean and maximum temperature, relative humidity, total rainfall, duration of sunshine and mean ultraviolet light intensity index each month as released by the Royal Observatory of Hong Kong. *Results:* 452 SLE patients were studied. There were a total of 425 mild / moderate SLE flares (0.87/100 patient-months) and 314 severe flares (0.64/100 patient-months) recorded. There were a total of 300 cutaneous and musculoskeletal flares (0.61/100 patient-months), 51 serositis flares (0.11/100 patient-months), 196 hematologic flares (0.41/100 patient-months), 196 renal flares (0.41/100 patient-months) and 49 neuropsychiatric flares (0.10/100 patient-months). The monthly rate of severe SLE flare was lowest in June and highest in January and the difference was statistically significant (p=0.042). Renal flare was significantly more frequent in the months January to March compared to June (p=0.041, 0.048, 0.043, respectively). The monthly rates of severe lupus flare and renal flare were negatively associated with the mean daily temperature (r=-0.73, p<0.01; r=-0.68, p=0.015, respectively), mean daily maximum temperature (r=-0.72, p<0.01; r=-0.67, p<0.016), total monthly rainfall (r=-0.73, p<0.01; r=-0.75, p<0.01) and mean ultraviolet light intensity index (r=-0.63, p=0.03; r=-0.69, p=0.012). The monthly total duration of sunshine was associated positively with cutaneous and musculoskeletal flare (r=0.62, p=0.03), but negatively with neuropsychiatric flare (r=-0.65, p=0.021). *Conclusions:* Seasonal variation in lupus flares in different organ system exists. Skin and joint lupus flares were more frequent in periods of more prolonged sunshine but were not associated with environmental ultraviolet light intensity. Severe lupus flare and renal lupus more commonly occurred during the winter months which were associated with lower temperature, humidity and ultraviolet light intensity.

CS14 Clinical Experience

CS14.1 & PO2.D.5

**An analysis of the metabolic syndrome (MetS)
phenotype in UK patients with SLE**
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Objectives: The metabolic syndrome (MetS) is a clustering of metabolic abnormalities reflecting increased adiposity and insulin resistance and is associated with an increased risk of CHD in the general population. Whilst studies show that MetS is more prevalent in SLE, it may not be as strongly associated with measures of adiposity than in population studies. We aimed to assess whether patients with SLE have a different phenotype of the MetS by investigating the prevalence of MetS and each of its criteria in a large lupus cohort. *Methods:* 200 Caucasian women with SLE and 100 healthy controls from the North West of England were studied. MetS was defined using the 2009 Consensus Statement from the International Diabetes Federation. Adiposity, assessed using both waist circumference (WC) and body mass index (BMI), was compared using Wilcoxon's rank sum test. Age-adjusted odds ratios (OR) were generated for the prevalence of both MetS and each criterion in cases and controls. *Results:* Cases were older than controls (median (IQR) age 53 (46-59) years vs. 48 (42-56) years p<0.05). The overall prevalence of MetS in SLE was 29% compared to 18% in controls (Adjusted Odds Ratio = 2.17, 95% CI; 1.13, 4.19). In an age-adjusted analysis, measures of adiposity did not differ between groups. When examining individual criteria for MetS, SLE patients were significantly more likely to have hypertension and low HDL than controls (see Table).

MetS Criteria IDF 2009	SLE cases n = 200	Controls n = 100	Age-adjusted OR (95% CI)	P Value
BMI; median (IQR)	25.9 (23-30)	25.5 (23-29)	-	0.65
WC; median (IQR)	84 (77-94)	82 (76-89)	-	0.35
% meet WC threshold	64%	40%	1.3 (0.8, 2.2)	-
% meet triglyceride threshold	27%	18%	1.9 (1.0, 3.5)	-
% meet HDL threshold	29.5%	14%	2.7 (1.4, 5.1)	-
% meet hypertension theshold	57%	40%	3.4 (1.9, 6.0)	-
% meet fasting glucose threshold	6.5%	5%	1.3 (0.4, 3.7)	-

Conclusions: In our cohort MetS is more prevalent in SLE than controls. This difference is not related to differences in adiposity. This suggests a different phenotype of MetS in SLE which may be related to chronic inflammation rather than to obesity and/or the metabolic effects of corticosteroids. Attention to the individual criteria as well as the whole syndrome may be needed to fully understand the impact of MetS to prognosis in SLE.

CS14.2 & PO2.J.1

Plasma exchange as rescue therapy for critical systemic lupus erythematosus: one center experience

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Objectives: To analyze the role of plasma exchange as a rescuing therapy for critical systemic lupus erythematosus (SLE) patients. *Methods:* A retrospective review was conducted to evaluate the patients with SLE undergoing rescuing plasma exchange (RPE) due to critical manifestations such as diffuse alveolar hemorrhage (DAH), neurolupus, catatrophic antiphopholipid syndrome (CAPS), thrombotic thrombocytopenic purpura (TTP), and cryoglobulinemia between January 1988 and December 2009. The primary outcome detection contained all cause mortality and SLE disease activity index (SLEDAI) scores one month after RPE. The secondary outcome measurement included complications such as infection and hemolysis, cost effectiveness of plasma exchange and the change of autoantibody titers one month after RPE. *Results:* The study population comprised twenty-nine patients with SLE. The mean time for the duration of the disease was eighty-two months, from the diagnosis of SLE to the first RPE (range from one week to twenty-two years). The mean age at first RPE was thirty-eight-year-old. The nineteen events of DAH attacked in seventeen patients, while nine patients for neurolupus, two patients for cryoglobulinemia, one patient for CAPS, and one patient for TTP were evaluated. The overall sessions of PE performed were 196 times, with a median for each patient of six sessions per course (range 1-33). There were four patients died due to septic shock, two patients for PH, one patient for neurolupus, and one patient for TTP. The overall survival rate of all patients was 87.1 percents, while 89.5 percents in PH, 88.8 percents in neurolupus. One case (3.2 percents) had experienced anaphylaxis, while cytomegalovirus viremia occurred following PE in five patients (16.1 percents). There were 61.3 percents of patients receiving low dose prednisolone therapy before the events of PE. The patients received pulse methylprednisolone therapy simultaneously or separately with RPE (750-3000 mg). In total courses, the mean SLEDAI scores were twenty-six and nine before and three weeks after the RPE, respectively. Nevertheless, only one patient received cyclophosphamide pulse therapy. The health care system disbursed all expenses for PE. *Conclusions:* Transient RPE combine with following methylprednisolone pulse therapy instead of pulse cyclophosphamide therapy successfully saved the patients with life threatening complications. Clinicians could cautiously prescribe early RPE and pulse steroid therapy in critical circumstance to prevent laborious complications.

CS14.3 &PO2.K.2

Efficacy and safety of long-term use of thalidomide for refractory cutaneous lupus

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Objectives: Cutaneous manifestations of lupus erythematosus are characterized by a great heterogeneity of clinical manifestations that usually have a chronic and relapsing course. Although is not a life-threatening condition, the lack of a rapid improvement can lead to a significant permanent scarring and disfiguring lesions. Thalidomide is increasingly being shown to be effective for the treatment cutaneous disease refractory to conventional management. The aim is to prospectively evaluate the clinical efficacy and safety of long-term treatment with low-dose of thalidomide in a cohort of 50 patients with refractory cutaneous lupus disease. *Methods:* From 1992 to 2009, fifty consecutive patients with refractory disease (23 with discoid lupus erythematosus (DLE), 12 with subacute cutaneous lupus (SCLE), 4 with profundus lupus, 7 with acute cutaneous lupus, 1 with lupus tumidus, and 3 with a non-specific rash) were treated with thalidomide. Initial treatment was started at 100 mg daily. If the cutaneous lesions vanished, the dose was lowered to 50-25 mg daily as maintenance therapy. Patients were follow-up for a mean of 82±63 months (12-204). Complete response was defined as a total resolution of the cutaneous lesions. Partial response was considered when at least >50% of the improvement was achieved. Patients were followed up periodically and were assessed for the development of neuropathy and other adverse effects. Contraception was initiated in women of childbearing age. *Results:* Forty-seven patients (94%) achieved complete or partial response with thalidomide therapy. Complete response occurred in forty-one patients (82%). Time to remission was as quick as to 10.7±5.30 weeks (4-28). There was an elevated rate of relapses (65.8%), usually 8-16 weeks after thalidomide's withdrawal or reduction. All patients achieved complete response after the drug was reintroduced. No response occurred in 3 patients (6%). The duration of thalidomide therapy was of 21±19.74 months (2-76). The most common adverse effects were sedation, constipation and weight gain. Five women developed amenorrhea during the treatment, but menses returned after its withdrawal. Seven patients reported symptoms of paresthesia, but only in three of them polyneuropathy was confirmed by EMG. One patient, heavy smoker and without antiphospholipid antibodies, had a stroke. None of the three patients with antiphospholipid antibodies developed a thrombotic event. Thalidomide did not improve the systemic disease or the scarring alopecia. *Conclusions:* Low dose thalidomide is a safe and an effective treatment for the different manifestations of cutaneous lupus refractory to conventional therapy. In view of the high rate of relapses after treatment discontinuation, a long-term maintenance dose might be required. The rapid response achieved and the safe profile support that thalidomide, alone or in combination, might be used as initial therapy to avoid sequelae.

CS14.4 & PO2.E.1 - PO2.D.1

Perceived stress in female patients with systemic lupus erythematosus (SLE)

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Objectives: To determine in women with Systemic Lupus Erythematosus (SLE) whether there is an association between patients' perceived stress level and: a) disease activity; b) quality of life; and c) self-reported comor-

bid conditions. ***Method:*** Patients meeting four or more American College of Rheumatology classification criteria for SLE were enrolled in Canada. Disease activity was assessed using the SLE-Disease Activity Index (SLE-DAI), with SLEDAI ≥7 considered active disease. The 10-point Perceived Stress Scale (PSS) was used to assess stress levels, where PSS >20 (population mean + 1 standard deviation) was considered high. Quality of life was assessed using the mental (MCS) and physical (PCS) component scores of the Medical Outcomes Short Form (SF-36). Cormorbid conditions were reported on the Self-Administered Comorbidity Questionnaire and a standardized medical history form. ***Results:*** 156 female patients with SLE, aged [mean (SD)] 39.4 (14.7) years with SLE duration of 11.2 (9.6) years were included in the analysis. The majority of patients were Caucasian (44.9%), followed by Asian (24.4%) and African American (19.2%), and 44.9% had active disease at enrolment. Mean PSS for the group was 17.3 (6.8), which was higher than the female population norm of 13.7 (6.6); 29.4% of patients had PSS >20. Total PSS was higher for those with active disease compared to those with inactive disease [18.7(6.7) vs 16.2(6.6), p=0.023]. There was also a significant inverse correlation between PSS and: disease duration (r = -0.17, p=0.03), MCS (r = –0.50, p<0.0001) and PCS (r = –0.67, p<0.0001). There was no association between PSS and: age, renal status, education level, marital status or having supplementary insurance coverage. Among many comorbidities reported, those with depression [16.7(6.3) vs 19.7 (8.0), p=0.045] and diabetes mellitus [17.0 (6.8) vs 21.3 (4.5), p=0.031] had significantly higher PSS than those without these conditions. There was also a trend towards higher PSS in those with heart disease [17.0 (6.7) vs 19.9 (6.8), p=0.16] and arthritis [16.6 (6.7) vs 18.3 (6.8), p=0.13] compared to those without. ***Conclusions:*** Female patients with active SLE have higher perceived stress regardless of age, education, private insurance coverage, marital status or renal status. Presence of comorbid illnesses contributes to the higher PSS in these patients. Management of active SLE and other comorbid conditions may decrease stress level in this patient population.

CS14.5 & PO2.J.2

Antimalarials have a protective effect against the development of renal disease in Latin American SLE patients

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Background and ***Purpose:*** Antimalarials (AM) have been shown to have numerous beneficial effects in lupus [diminished probability of flares, protective survival effect, longer time-to-damage accrual (specifically renal damage) and an increased probability of remission in mycophenolate mofetil-treated membranous nephritis]. The aim of this study was to determine if they also have a protective effect on the occurrence of renal disease (RD). ***Methods:*** SLE patients from GLADEL, a multi-ethnic, multinational Latin American cohort with a recent SLE diagnosis (≤2 years) have been recruited and followed-up longitudinally. For these analyses, characteristics of those patients with and without RD [persistent proteinuria and/or cellular casts (ACR criterion)] in a 1:2 proportion (nested case-control study design) were compared. Variables significant in these univariable analyses and other relevant variables were then entered in multivariable conditional logistic regression analyses; AM were found to have a protective effect in

this analysis (OR: 0.37; 95%CI 0.23-0.60). Then to adjust for confounding a new model was performed with variables selected (p ≤0.10) from the comparison between AM-takers and non-takers among cases and controls combined (Table 1). ***Results:*** Of the 795 GLADEL cohort patients included in this study, 265 (33.3%) developed RD and 425 (53.5 %) were AM users. Multivariable analyses' results are presented in Table 2. ***Conclusion:*** After adjusting for possible confounding factors, we have demonstrated for the first time a clear protective effect of AM in the development of RD occurrence in SLE patients from this Latin American cohort.

Table 1. Socioeconomic-demographic, cumulative clinical, serologic and treatment characteristics in SLE patients with and without renal disease (combined) as a function of antimalarial use.

Features	Antimalarial Use		
	Yes (n= 425)	No (n= 370)	P value
Gender, Female, %	90.8	91.4	0.7946
Age at disease onset, years*	27.9	29.1	0.0919
Age at diagnosis, years*	29.4	30.6	0.1179
Delay in diagnosis, months*	19.0	18.2	0.7931
Ethnic group, %			
Caucasian (183/158)	43.1	42.7	
Mestizo (187/162)	44.0	43.8	0.9719
African-Latin American (55/50)	12.9	13.5	
Residence, rural, %	7.6	6.6	0.5772
Socioeconomic Status, %			
Upper/upper-middle	12.2	9.7	
Middle	28.0	30.8	0.4365
Lower-middle/lower	59.8	59.5	
Education, years, %			
0-7	30.1	31.4	
8-12	45.9	40.5	0.2609
More than 12	24.0	28.1	
Medical insurance, %			
Without coverage	16.6	18.5	
Partial coverage	21.6	19.6	0.6800
Full coverage	61.9	62.0	
Diabetes, %	0.0	1.4	0.0215
Hypertension, %	12.7	8.7	0.0662
ACR Criterion, %			
Malar rash	65.7	28.7	<0.0001
Discoid rash	16.9	4.6	<0.0001
Photosensitivity	64.9	26.8	<0.0001
Oral Ulcers	43.5	18.9	<0.0001
Arthritis	85.9	41.1	<0.0001
Serositis	22.4	14.9	0.0071
Neurologic disorder	10.8	3.8	0.0002
Hematologic disorder	68.5	35.7	<0.0001
Immunologic disorder	65.7	26.5	<0.0001
Antinuclear antibodies	92.0	38.7	<0.0001
Medications, %			
NSAIDs	47.3	12.4	<0.0001
Azathioprine use	15.3	4.6	<0.0001
Glucocorticoid dose, pulse	12.7	6.5	
Glucocorticoid dose, oral †			
Low (≤ 20mg)	30.8	8.4	
Medium (>20 to <60 mg)	33.4	12.2	<0.0001
High (≥ 60mg)	22.6	10.5	
Cyclophosphamide use	7.1	3.8	0.0440
Death during follow-up, %	5.4	6.0	0.7451

* Mean values for each group; † weighted dose

Table 2. Protective effect of antimalarials in RD among patients with SLE by multivariable analyses adjusting for confounders related to their use (dependent variable: RD).

Features	OR	95% CI	P value
Ethnic group,			
Caucasian		Reference group	
Mestizo	1.35	0.87-2.11	0.1829
African-Latin American	0.74	0.38-1.45	0.3849
Age at disease onset, years	0.98	0.97-1.00	0.0448
Socioeconomic Status			
Upper/upper	0.46	0.22-0.95	0.0346
Middle	0.93	0.58-1.49	0.7618
Lower middle/lower		Reference group	
Diabetes	2.64	0.31-22.78	0.3774
Hypertension	3.01	1.59-5.69	0.0007
ACR Criterion			
Malar rash	1.55	0.95-2.53	0.0771
Discoid rash	0.66	0.34-1.26	0.2028
Photosensitivity	0.75	0.46-1.21	0.2314
Oral Ulcers	2.18	1.42-3.34	0.0004
Arthritis	4.77	2.68-8.51	<0.0001
Serositis	2.42	1.42-4.11	0.0011
Neurologic disorder	0.51	0.24-1.12	0.0943
Hematologic disorder	2.21	1.38-3.52	0.0009
Immunologic disorder	2.16	1.38-3.40	0.0008
Medications			
NSAIDs	0.60	0.38-0.96	0.0337
Antimalarials	0.42	0.25-0.70	0.0008
Azathioprine use	1.59	0.84-3-00	0.1535
Glucocorticoid, oral dose			
Low (≤ 20mg)	1.59	0.81-3.13	0.1745
Medium (>20 to <60 mg)	2.35	1.22-4.50	0.0102
High (≥ 60mg)	1.57	0.76-3.25	0.2228

CS14.6 & PO2.D.2

Prolonged serologically active clinically quiescent (SACQ) systemic lupus erythematosus (SLE): clinical and serologic features

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Objectives: Some patients with SLE are clinically quiescent despite persistent serologic activity, and thus present a clinical dilemma. We aimed to determine the frequency of SACQ and its outcome in a large cohort of SLE patients followed prospectively at a single centre. **Methods:** Patients followed in the Lupus Clinic between July 1970 and April 2008 with visits no more than 18 months apart were identified. SACQ was defined as at least a two year sustained period without clinical activity and with persistent serologic activity (increased anti-dsDNA antibody by Farr assay and/or hypocomplementemia at each clinic visit), during which patients could be taking antimalarials, but not steroids or immunosuppressives. The characteristics of patients with a SACQ period and its features were analyzed. Anti-dsDNA levels were categorized as normal (≤7), low (8-20), moderately (21-50), or highly (>50) elevated. Results are presented using descriptive statistics. Comparisons were made using t-tests and chi-squared tests. **Results:** 56/924 (6.1%) patients had SACQ periods (median 158 weeks, mean 204 weeks). These patients differed demographically from the remainder of the SLE population in terms of presenting SLEDAI-2K (7.34 vs. 10.1, p=0.01), and frequency of use of steroid (33.9% vs. 60.8%, p<0.0001) and immunosuppressive (3.6% vs. 19.4%, p=0.0004) at first clinic visit. Median disease duration at the beginning of the SACQ period was 8.6 years. Thirteen patients had two or more SACQ periods. Median duration of the first SACQ period was 158 weeks. During this period, 35 patients (62.5%) had both elevated anti-dsDNA antibody and hypocomplementemia; thirteen (23.2%) and eight (14.3%) patients had isolated hypocomplementemia or elevated anti-dsDNA antibody, respectively. Among the 43 patients with elevated anti-dsDNA antibody at some point during the SACQ period, the median anti-dsDNA level was normal in five (11.6%), low in 25 (58.1%), moderate in nine (20.9%), and high in four (9.3%). Thirty-three patients (58.9%) flared (median 155 weeks); six (10.7%) became clinically and serologically inactive (median 236 weeks); 17 (30.4%) continued to be SACQ at their most recent visit (median 159 weeks). In patients who flared, the most common manifestations were arthritis (24.2%), mucous membrane involvement (18.2%) and sterile pyuria (18.2%). **Conclusions:** SACQ patients represent a small, clinically important group. Although 59% of SACQ patients flare they do so after median 3 years. Thus prudent therapy would be close observation to discern which SACQ patients will ultimately flare.

CS15 Pregnancy and Lupus

CS15.1

Mediators and mechanisms of pregnancy complications in patients with anti-phospholipid antibodies and patients with lupus: the PROMISSE study

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Pregnancy complications in women with the antiphospholipid syndrome (APS) and/or SLE include recurrent miscarriage, preeclampsia, placental insufficiency, and intrauterine growth restriction (IUGR). The mechanisms leading to placental and fetal injury in vivo are incompletely understood and treatment remains sub-optimal. We have identified complement as an early effector in pregnancy loss and/or IUGR associated with placental inflammation in a mouse model of APS and shown that complement activation causes the release of anti-angiogenic factors and abnormal placental development. **The PROMISSE Study** (**P**redictors of p**R**egnancy **O**utcome: bio**M**arkers **I**n antiphospholipid antibody **S**yndrome and **S**ystemic lupus **E**rythematosus) is a first-time effort to translate our novel findings in mice to humans and determine if alterations in complement regulation of angiogenic factors products predict pregnancy complications in patients with antiphospholipid (aPL) antibodies and/or SLE. In the first 7 years of this prospective, observational study of pregnant patients grouped and analyzed according to the presence or absence of aPL antibodies and preexisting SLE, we have enrolled over 550 pregnant patients in 8 centers, obtained detailed medical and obstetrical information monthly, and serially collected plasma, serum, DNA, RNA, and urine. Preliminary data from this study will be presented. Identification of biomarkers that predict poor pregnancy outcome in these patients will elucidate mechanisms of disease, define targets for treating patients, and generate clinically applicable indicators to permit initiation of interventional trials in patients at greatest risk for pregnancy complications.

CS15.2

Neonatal lupus: updates on pathogenesis, risk and reward

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One of the strongest clinical associations with autoantibodies directed to components of the SSA/Ro-SSB/La ribonucleoprotein complex is the development of congenital heart block (CHB) in an offspring, an alarming prospect facing 2% of primigravid mothers with these reactivities. Other abnormalities affecting the skin, liver, and blood elements are associated with anti-Ro/La antibodies in the maternal and fetal circulation and are now grouped under the overall heading of Neonatal Lupus Syndromes (NLS), Neonatal

Lupus Erythematosus (NLE) or simply Neonatal Lupus (NL). NL was so termed because the cutaneous lesions of the neonate resembled those seen in SLE. The name is misleading and often a cause of undue concern because the neonate does not have SLE and often neither does the mother. Accumulated evidence suggests that anti-Ro/La antibodies are necessary but insufficient for fetal disease. Basic and clinical research is heavily vested in identifying fetal and environmental factors which convert disease susceptibility to overt expression. The pathogenesis of disease is likely complex and several models have been proposed. One focuses on a pathogenic antibody recognizing Ro52p200 and another posits autoantibody perturbation of calcium channel electrogenesis via reactivity of anti-Ro with L type calcium channels. A third considers apoptosis as a means by which the normally inaccessible Ro/La antigens can be trafficked to the cell membrane. Apoptosis is a selective process of physiological cell deletion in embryogenesis and normal tissue turnover and plays an important role in shaping morphological and functional maturity. It is generally accepted that apoptotic cells are rapidly removed to obviate any inflammatory sequelae. Compatible with the need for efficient clearance, human fetal cardiocytes are capable of engulfing apoptotic cardiocytes. This novel physiologic function may account for the general absence of apoptosis noted on evaluation of hearts from electively terminated fetuses. However, histological studies of hearts from fetuses dying with CHB have identified exaggerated apoptosis, suggesting a potential defect in clearance. *In vitro* experiments reveal that antibodies to Ro/La inhibit cardiac uptake of apoptotic cardiocytes, thus explaining the histological findings. The consequence of persistent "opsonized cardiocytes" is to divert uptake to infiltrating macrophages which results in release of proinflammatory and profibrosing cytokines culminating in transdifferentiation of cardiac fibroblasts and subsequent replacement of healthy conducting tissue with scar. The ssRNA component of the immune complex on apoptotic cardiocytes may stimulate macrophage TLR7/8 receptors. Data from the Research Registry for Neonatal Lupus provides practical information regarding counseling of mothers. Half of the women who are asymptomatic at the birth of an affected child will experience progression to clinical autoimmunity, ranging from minor rheumatic symptoms to overt SS or SLE. For women who have had a previous child with CHB, the risk of recurrence in a subsequent pregnancy is 18%. For those who have had a child with rash, the risk of CHB in a subsequent pregnancy is 13%. With regard to fetal monitoring a disturbing observation which has emerged from current research efforts is the rapidity of disease progression with advanced heart block and life threatening cardiomyopathy observed less than 2 weeks from normal sinus rhythm. Once third degree block is unequivocally identified, sustained reversal has never been achieved, despite dexamethasone. Accordingly, strategies aimed at preventing disease before irrevocable scarring ensues, assume high priority. Two studies have concluded that the use of IVIG at replacement doses (400mg/kg given at 12,15,18,21, and 24 weeks of gestation) does not prevent the recurrence of CHB. Consistent with experimental evidence implicating TLR in the pathogenesis, a retrospective study regarding the use of hydroxychloroquine in preventing CHB has provided encouraging results. The clinical significance and treatment of PR prolongation in utero (first degree heart block) is still unclear but most investigators favor close echocardiographic surveillance. Although significant advances have been made, continued studies both at the bench and bedside are needed, including an understanding of the fetal and maternal genetic contributions.

CS15.3 & PO2.O.1

Placental C4d as an indicator of antiphospholipid antibody mediated fetal loss

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Objectives: Recurrent miscarriage and intrauterine fetal death occur 20 to 40 times more often in patients with systemic lupus erythematosus (SLE) and antiphospholipid syndrome than in healthy pregnant women. During trophoblast differentiation, direct binding of antiphospholipid antibodies to tropho-blast cells activates the complement cascade and interferes with trophoblast cell invasion and maturation. We investigated whether deposition of C4d, a marker of classical complement activation, is related to fetal outcome in placentas of patients with SLE and/or antiphospholipid syndrome. **Methods:** We studied 86 placentas of 83 patients by staining them with BI-RC4d polyclonal anti-C4d antibody, and scoring them semiquantitatively (no deposition, focal deposition, diffuse deposition). The patients were subdivided into a case group of 21 patients with SLE and/or antiphospholipid syndrome, a first control group of 40 patients with pregnancies that resulted in live births, and a second control group consisting of 22 patients with pregnancies that resulted in intrauterine fetal death caused by chromosome abnormalities. **Results:** There was a strong association between diffuse perivillous placental C4d deposition and antiphospholipid syndrome (p < 0•001), as well as between diffuse C4d positivity and intrauterine fetal death (p < 0•005). C4d staining was never positive in placentas from patients with normal live births. **Conclusions:** C4d is present in placentas of patients with SLE and/or antiphospholipid syndrome, and is strongly associated with negative fetal outcome. The excessive deposition in some of our cases may be regarded as witness of a very strong antibody-mediated immune response from which inhibitory mechanisms failed, resulting in intra-uterine fetal death. Further prospective studies need to confirm if C4d in a previous miscarriage can be considered as a biomarker of a future complicated pregnancy.

CS15.4 & PO2.O.2

Markers of cardiovascular function in women with systemic lupus erythematosus (SLE) in pregnancy

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Objectives: SLE is associated with significant pregnancy complications, in particular pre-eclampsia, and pregnant women with SLE experience a 20-fold higher maternal mortality. Both SLE and pre-eclampsia significantly increase the risks of subsequent cardiovascular disease (CVD). Transforming Growth Factor beta1 (TGFβ1) is an immunosuppressive growth factor involved in the maintenance of normal blood vessel structure. It is known to be reduced in women with SLE. We hypothesise that TGFβ1 dysregulation accelerates arterial stiffness in women with SLE and thus may predispose them to pregnancy complications. **Methods:** TGFβ1 activation index (AI) and arterial stiffness index (SI) were measured in pregnant women with SLE and in healthy pregnant women at 12, 20, 28 and 36 weeks and at a single time point in the non-pregnancy state. TGFβ1 was assessed by activational immunoassay and SI by digital pulse wave analysis; a measure of systemic arterial stiffness. **Results:** There was no difference in TGFβ1 AI in healthy pregnant women compared with the non-pregnant state (Table). In contrast, TGFβ1 AI was significantly lower in both pregnant and non pregnant women with SLE (table, n=4, p=0.05; and, n=8, p=0.01 respectively). There was a corresponding increase in SI in non pregnant women with SLE (table, n=9, p=0.06) and in late pregnancy (table n=3, p=0.04) compared with healthy controls. **Discussion:** Women with SLE have a lower TGFβ1 activation index and raised arterial stiffness. SLE patients may therefore be intrinsically prone to vascular complications of pregnancy by having early vascular stiffness and low TGFβ1 may contribute to the risk of pre-eclampsia and poor pregnancy outcomes seen in SLE.

	TGFβ1AI	SI
	Median (IQR)	Median (IQR)
Healthy NP	1.67 (1.56-1.94)	7.0 (6.4-7.8)
Healthy P	1.85 (1.56-2.56)	7.2 (6.6-8.3)*
SLE NP	1.20 (0.97-1.59)	8.4 (7.0-11.29)
SLE P	1.25 (0.75-1.67)	8.9*

NP, non-pregnant; P, 12 weeks pregnant, * 36 weeks

CS16 Pediatric: Outcomes that Matter for Children and Youth with SLE: Bone Health, Development and Quality of Life

CS16.1

Outcomes that matter for children and youth with SLE: bone health, development and quality of life. An overview

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Outcomes for pediatric systemic lupus erythematosus (SLE) patients have continued to improve. As access to pediatric rheumatologists improves, earlier diagnosis and more aggressive treatment has meant continuing improvement in the 5 year survival rates. Unfortunately mortality in the pediatric age group from SLE is still much higher in comparison to adults with SLE. In addition the medications themselves, especially steroid therapy continue to contribute to morbidity. Some of these issues will be discussed in greater detail during this session. Over the past few years care of the sick SLE patient has changed. Aggressive treatment or an induction phase consisting of high dose steroids and an immune suppressant has become the norm. This is then followed by a maintenance phase where provided the SLE is controlled, steroid therapy can be minimized and immune suppression adjusted. The aim being a long and lasting remission. It may even be possible to tailor therapy. Studies have shown that certain medications are better in particular subsets of SLE. Cyclophosphamide (CTX) for example seems less effective in black patients with SLE nephritis, and overall it has been reported that over 1/3 of patients fail to achieve a remission on CTX. In comparison it would appear that response to MMF is better in Asian patients with SLE than in other ethnic groups. Unfortunately there are few large scale studies in the pediatric patient with SLE. The APPLE (Atherosclerosis Prevention in Pediatric Lupus Erythematosus) study designed to assess atherosclerosis in pediatric SLE is the first multicenter North American trial to follow a cohort of selected pediatric SLE patients over a defined time period. Pediatric patients are still, unlikely to be included in any trials regarding SLE therapy. So treatment protocols are extracted and modified from adult data. However the ASPREVA Lupus Management Study (ALMS), a randomized controlled trial, included 24 pediatric SLE patients. The trial was designed to study the efficacy of CTX vs mycophenolate mofetil (MMF) as treatment for SLE nephritis. MMF failed to show superiority over CTX in this trial with serious adverse events being similar between the 2 groups. The time has come for pediatric SLE patients to be included in ongoing trials. Now rheumatologists have an increasing number of newer treatment options available in treating SLE. It is important not only to have evidenced based pediatric protocols for treatment of this chronic illness but proven treatment strategies for pediatric SLE as pediatric patients continue to be the sickest and most challenging to treat.

CS16.2

Bone health for children with SLE

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Children with SLE are at increased risk for the development of skeletal deficits including short stature, delayed skeletal maturation and reduced bone mineral density (BMD). Although fractures often do not occur until later in life, the potential effect of childhood and adolescent chronic illness one bone mineral accrual may prevent patients from reaching normal peak bone mass. SLE and its associated therapy may have numerous negative bone effects. These include chronic exposure to glucocorticoids, renal insufficiency, circulating inflammatory cytokines, vitamin D insufficiency, and reduced physical activity. We conducted a longitudinal cohort study to better characterize these risk factors. We aimed to measure bone density at multiple skeletal sites using several radiologic methods, and to characterize the contribution

of patient-, disease- and host-related variables to BMD. We also were interested in characterizing the bone turnover balance and in exploring the role of inflammation in the development of bone deficits in children with SLE. 93 pediatric SLE subjects (mean age 15.5±3.2 yrs), 87% female, of mixed ethnicity (31% Asian, 11% African American, 26% Caucasian, 17% Hispanic, 15% other) underwent densitometry and clinical assessment. SLE disease duration ranged from 1.2-15.3 years (mean 7.5). Mean prednisone dose was 0.2 mg/kg/d (range 0.009 to 6.6 mg/kg/d) and average cumulative lifetime exposure was 72 mg/kg (range 3.4 to 2431 mg/kg). The SLE Disease Activity Index (SLEDAI) ranged from 0-24. When compared to 169 healthy controls, 23% of SLE subjects demonstrated spine BMD Z-scores below -1.5 SD, with a mean (SD) spine BMD Z-score of -0.69 ± 1.4 compared to 0.13 ± 1.06 for controls ($p < 0.0001$). As expected, SLEDAI correlated with prednisone dose (mg/kg/d) ($R = 0.47$, $p < 0.0001$). Vitamin D deficiency was observed in approximately 30% of patients. Both disease- and treatment- related factors may contribute to reduced BMD in children with inflammatory disease, such as SLE. Understanding these factors is important for risk assessment and the development of treatment strategies for children with SLE.

CS16.4

Outcomes that matter for children and youth with SLE: bone health, development and quality of life

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Systemic lupus erythematosus (SLE) in children is a chronic multi-system disease with wide ranging effects on their health-related quality of life (HRQOL). Psychosocial implications of SLE in children are evident in the life-disruptive responsibilities that patients and their families must assume including hospitalizations, multiple sub-specialty visits, frequent laboratory monitoring, and health care costs. SLE and activities related to caring for the disease impose a burden on children's school attendance and performance. Pediatric studies have explored the ideal method of measuring the widespread impact of SLE on HRQOL and the ideal methods of measuring the same. The multidimensional aspect of HRQOL, heterogeneous nature of SLE, and the changing growth and development of children need to be taken into account while measuring HRQOL. A novel HRQOL measure, Simple Measure of the Impact of Lupus Erythematosus in Youngsters© (SMILEY©) has been developed and validated for use in children with SLE and parents. SMILEY© is currently undergoing cross-cultural validation in several countries across the world, and will be a useful adjunct to clinical trials and outcomes research.

CS17 The Interferon Pathway in Lupus

CS17.1

Interferon-regulated biomarkers of disease activity in SLE

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by unpredictable flares of disease activity and irreversible damage to multiple organ systems. Our earlier study showed that SLE patients carrying an interferon (IFN) gene expression signature in blood have elevated serum levels of IFN-regulated chemokines. These chemokines were associated with more severe and active disease and showed promise as SLE disease activity biomarkers. To validate the potential utility of serum chemokine levels as biomarkers for disease activity, we measured serum chemokine levels – CXCL10 (IP-10), CCL2 (MCP-1), and CCL19 (MIP-3B) – in an independent cohort of 267 SLE patients followed longitudinally over one year (1166 total visits). Serum chemokine levels correlated with current visit

lupus activity (p=2x10^{-10}), rising at flare (p=1x10^{-3}) and decreasing as disease remitted (p=1x10^{-3}), and performed better than currently available laboratory tests. Chemokine levels measured at a single baseline visit in patients with mild or inactive disease (SLEDAI ≤4) were predictive of lupus flare (in any organ system) over the ensuing year (p=6x10^{-4}). When we limited the analysis to flares affecting the kidneys, we found that serum chemokine levels had significant ability to predict renal flare (p=2x10^{-3}). We next validated these results in a replication cohort of 257 SLE patients, in which chemokine levels were again elevated in active vs. inactive SLE (p=2x10^{-10}) and predictive of future lupus flare (p=0.001). Monitoring serum chemokine levels in SLE may thus improve assessment of current disease activity, the prediction of future flare, and overall clinical decision-making.

CS17.2

Molecular pathways associated with lupus flare

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Altered function of many components of the immune system underlies the diverse clinical manifestations and variable course of disease in patients with SLE. While anti-double stranded DNA antibodies and complement levels have been used to monitor disease activity and guide therapy, these serologic markers are not universally present or informative. In order to gain insight into lupus pathogenesis and identify biomarkers of flare and targets for therapy, our laboratory has characterized the molecular pathways that contribute to the immunologic dysfunction, autoimmunity and inflammation that result in organ damage and disease in SLE. We have identified interferon-α (IFN) as an important trigger for a broad molecular pathway that is associated with increased disease activity and severity. To define the relationship of IFN pathway activation to disease flare and identify additional molecular pathways associated with flare, we and our collaborators have established and studied a lupus cohort followed longitudinally, approximately every 3 months, over 1 ½ to 2 years. Based on microarray data we have identified multiple gene clusters that define molecular pathways activated in lupus peripheral blood. In addition to the genes activated by IFNα, plasma cell and neutrophil signatures are differentially expressed in patients compared to healthy controls and fluctuate over time in relation to disease flares. We have designed a composite score derived from these gene clusters. Our studies in progress are investigating the hypothesis that quantification of plasma cell, neutrophil and IFN signatures is superior to the IFN signature or anti-dsDNA antibody alone as a marker of future or current flare.

CS17.3 & PO2.G.5

Cytokine attribution of gene expression and histone H4 acetylation changes in SLE monocytes

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Objectives: Monocytes in SLE have been described as having aberrant behavior in a number of assays. Epigenetic changes could contribute to molding of aberrant behaviors by modulating gene expression. Examining the epigenome in SLE is desirable because epigenetic changes can be durable and can dictate responses to subsequent stimuli. To understand the role of cytokines in driving changes to gene expression and the epigenetic landscape we utilized an unbiased approach. **Methods:** Gene expression and the post-translational histone mark, H4 acetylation, were examined using arrays. The U133A 2.0 platform was used for the expression analyses and the GeneChip Human Promoter 1.0R array was used to define H4acetylation (Affymetrix). We compared SLE monocyte gene expression and H4 acetylation with α-interferon, γ-interferon or IL-4-treated monocytes to understand which cytokine effects predominated in SLE monocytes. Transcription factor binding sites were identified and clustering analysis was used to understand the driving forces of

the changes. DAVID was used to relate the findings to biological processes. **Results:** We found that γ-interferon and α-interferon both replicated a broad range of the gene expression changes seen in SLE monocytes. There was less evidence of interferon effects on H4 acetylation patterns. H4 acetylation in SLE monocytes was overall higher than in controls and there was less correlation of H4ac with cytokine-treated cells than when gene expression was compared. The H4 acetylation changes in SLE monocytes mapped in large part to transcription factor binding sites. When DAVID was used to compare biological processes induced by the cytokines with those induced by SLE, we found little overlap, suggesting that monocytes have been impacted by a complex interplay of cues. A set of chemokine genes had down-regulated expression and H4ac. **Conclusions:** There are several lines of evidence suggesting that monocytes have been molded by a complex set of exposures. Our cytokine attribution found that the interferon-responsive genes cluster was up-regulated 36.3% in SLE monocytes, thus leaving a significant gene set unexplained by interferon exposure. The association was even less robust for H4ac. The finding that H4 acetylation was globally increased and this increase appeared to map to TFBSs suggest a globally altered epigenome with a complex etiology. Therefore, monocytes are significantly impacted by both αIFN and γIFN exposure, however, our data suggest that additional cytokines and other exposures contribute to the aberrant monocyte behavior observed in SLE patients.

CS17.4 & PO2.G.1

What are the pathways by which IFN-α decreases vasculogenesis in SLE?

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Objectives: SLE is characterized by accelerated vascular risk due to premature atherosclerosis which is not explained by traditional risk factors. Our group previously proposed that type I Interferons (IFNs) play a crucial role in premature vascular damage by altering the balance between endothelial cell apoptosis and vascular repair mediated by endothelial progenitor cells (EPCs) and myeloid circulating angiogenic cells (CACs). We have now characterized the putative pathways by which type I IFNs interfere with proper vascular repair in SLE. **Methods:** EPC/CACs from control and SLE patients were treated with IFN-α for 6 hours and corresponding changes in gene expression were analyzed with Affymetrix Human U133 Plus 2.0 Genechips. Expression levels of genes of interest were validated with real time PCR. Effects on EPC/CAC function were tested utilizing functional in vitro assays. Proliferation and apoptosis were determined by XTT assay and caspase3/7 activation, respectively. DC phenotype was assessed by FACS. In vivo angiogenesis and validation of putative markers at the protein level was assessed by immunohistochemistry staining of kidney sections from lupus nephritis biopsies and controls. **Results:** Microarray data analysis revealed alterations in various molecules associated to IL-1 mediated signaling and of vascular endothelial growth factor-A (VEGF-A). Downregulation of IL-1β, IL-1 receptor-1 (IL-1R1) and VEGF-A and upregulation of IL-1RN (IL-1 receptor antagonist) and IL-1R2 (decoy receptor) were observed. This indicates a global downregulation of IL-1β function induced by type I IFNs. These abnormalities were more marked in the IFN-treated lupus EPCs/CACs than in the IFN-treated control cells, suggesting that lupus cells were more sensitive to IFN-α effects. Results were confirmed at mRNA and protein level. Treating lupus EPCs/CACs with IL-1β resulted in a significant improvement in their capacity to differentiate into a mature endothelium, therefore abrogating the deleterious effects of IFN-α. These beneficial effects were mediated, at least in part, by an increase in lupus EPC/CAC proliferation, decrease in EPC/CAC apoptosis, and by preventing skewing of lupus EPC/CACs towards non-angiogenic pathways. Confirming that decreased angiogenesis was occurring in vivo in SLE through IL-1 pathway dysregulation, the glomerular and vascular compartments of biopsies from patients with lupus nephritis also showed increased IL-1RN, decreases in VEGF and overall de-

creased endothelial density, as assessed by CD31 expression, while control kidneys as well as kidneys from other immune-mediated diseases with similar amount of renal tissue damage (ANCA+-vasculitis) did not exhibit these abnormalities. *Conclusions:* These results indicate that type I IFNs mediate their antiangiogenic effects by downmodulating IL-1 mediated pathways and further suggest that strategies aimed at blocking type I IFN effects or its downstream pathways may abrogate premature vascular damage in SLE.

CS17.5 7 PO2.G.2

A genetic variant of TLR9 may impact interferon alpha levels in lupus
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Objectives: Genetic variants in proteins relevant to type I interferons (INF) are associated with risk for SLE. Toll-Like Receptors (TLR) 7 and 9 may regulate INF activation by DNA and RNA-associated autoantigens, and in some murine models obstructing TLR9 enhances the autoimmune potential of TLR7. We tested TLR polymorphisms in 221 lupus patients: (TLR7: rs179008, rs5935436, and rs3853839. TLR9: rs187084, rs352139, and rs352140) hypothesizing that inherited variants in TLR might influence levels of interferon alpha in lupus patients. *Methods:* TLR polymorphisms were determined using real time PCR and published primers obtained from Applied Biosystems. An ELISA system for interferon alpha quantification was purchased from Bender Med Systems, and data calculated using their standards after confirmation of the dilution curve. Autoantibodies and other clinical information were obtained from medical records. *Results:* The frequency of each allele at these six common variant sites was similar in Caucasian, African, and Asian patients to previously published reports on these ethnic populations. In an exploratory multivariate model, two TLR9 polymorphisms (rs352139 and rs352140) were associated with anti-RNP antibody (p=0.031 and p=0.040 respectively) and rs352139 was marginally associated with anti-DNA antibody (p=0.067). These two polymorphisms tend to sort together, and further analysis suggested that the potential association with RNP antibodies was due primarily to the rs352140 polymorphism (CT). Positive RNP antibody impacted interferon alpha levels only in those patients homozygous for CC at this position (n=74) (median INF: 0.0 vs 46.2 pg/ml in RNP neg vs RNP+ pts with CC genotype, p<0.001). This difference was not seen in other genotypes. This same genotype also showed a marginal trend to increasing inf alpha levels in anti-dsDNA positive vs negative patients (median INF 0.0 vs 53.6, p=0.145). Interestingly, when interferon levels were compared in genotype subgroups of patients positive for anti-dsDNA the same rs352140 CC genotype was associated with significantly higher interferon alpha levels than was found in heterozygotes or those with two T alleles (median 53.6 vs 0, p=0.045). Further analyses of medications (including separate comparative analysis of patients on no meds, hydroxychloroquine and/or steroids) did not suggest major confounding effects on interferon levels. *Conclusions:* A polymorphism in TLR9 (rs352140 CC) may affect interferon alpha levels in those lupus patients with characteristic autoantibodies associated with TLR activation of the type I interferon pathway. These analyses were exploratory and would need to be confirmed in additional populations and prospective studies.

CS17.6 & PO2.G.3

Inhibition of the lymphotoxin-beta receptor pathway has unexpected effects on kidney pathology in the adenoviral-IFNα BWF1 accelerated model of SLE
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Objective: The lymphotoxin-beta receptor (LTBR) pathway contributes to many important functions in the immune system, such as organization of lymphoid architecture and tertiary lymphoid structures, cellular positioning

and trafficking through high endothelial venules (HEVs). Inhibiting the LTBR system with LTBR-Ig (baminercept) has been efficacious in established animal models of various autoimmune diseases and has undergone extensive clinical testing in rheumatoid arthritis. Here we questioned whether LTβR-Ig is efficacious in a murine model of lupus. *Methods:* Female (NZBxNZW) F1 (BWF1) mice were injected with adenovirus-IFNα at 9 weeks of age and LTβR-Ig was administered (10 mg/kg, twice a week) starting 3 weeks after the viral injection. *Results:* LTBR-Ig blockade had significant efficacy as evidenced by normalization of the serum and urine chemistry and the kidney histology showed reduced glomerular and tubular nephritis. Treatment led to a reduction of lymph node HEV, lymphadenopathy, numbers of splenic activated/memory T cells and plasmacytoid DC. Serum titers of anti-DNA antibodies were not reduced by treatment, there was no shift to less pathogenic isotypes and no obvious loss of immune complex deposition in the glomeruli. For this reason, we hypothesized that downstream effector functions in the kidney were altered, however, such a dampening of local activity by LTBR-Ig was not foreseen by any of the known mechanisms of action. Quantitation of kidney RNA levels of many genes showed notably that macrophage markers were decreased (CD11b, CD14 and CD169) and, while the kidney levels of several chemokines were reduced (CXCL13, CCL2, CXCL9-10 and CCL17), CCL20 expression was dramatically eliminated. CCL20 is produced by epithelial cells and plays roles especially in the gut via CCR6 signaling in the attraction/retention of some DC subsets, Th17 cells and some B cells. *Conclusions:* Administration of LTBR-Ig was efficacious in this IFN accelerated BWF1 model. While many immunological parameters in the secondary lymphoid organs were affected, decreased kidney pathology may rely on local effects on monocytic/DC involvement that is sustained by locally generated CCL20. We suspect that reduced chemokine production by LTBR-Ig may underlie some of this efficacy. CCL20 expression by epithelia in the gut has been previously linked to LTBR signaling. LTBR-Ig is an attractive agent for the treatment of SLE since it has actions at the immunological level as well as potentially on local monocyte/DC events in the kidney.

CS17.7 & PO2.G.4

Increased interferon-alpha activity is associated with increased autoantibody specificities and poor antigen-specific humoral immunity in systemic lupus erythematosus
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Background: Interferon-α (IFNα) has been identified as a key mediator in systemic lupus erythematosus (SLE) pathogenesis. IFNα is a type I IFN that has the ability to disrupt self tolerance by activating antigen-presenting cells containing self-antigen. Elevated IFNα activity is detected in many SLE patient samples, and these elevations have been shown to correlate with disease activity as well as multiple organ involvement. Our goal is to further understand the pathogenic role of IFNα in SLE humoral autoimmunity, as well as to investigate the effect of an antigen-specific challenge in SLE and whether increased IFNα activity in SLE impairs antigen specific responses. *Methods:* This study enrolled 72 SLE patients who met ACR criteria and matched controls. Detailed clinical and therapeutic information, as well as disease activity measures were obtained at baseline and 2, 6, and 12 weeks after influenza vaccination. Influenza humoral immune responses (native/denatured ELISA responses, relative affinities and hemagglutination inhibition) were measured. Serial samples were tested for interferon activity through a reporter cell assay which measures serum's ability to upregulate three interferon inducible genes, MX1, PKR, and IFIT1. Lupus-associated autoantibodies (aAbs) (Ro, La, Sm, nRNP, ribosomal P, dsDNA, ANAs and phospholipid antibodies) were measured by ELISA and immunofluorescence. *Results Summary:* IFNα activity decreased in SLE patients 2 weeks after influenza vaccination compared to baseline levels (baseline mean = 6.4, 2 weeks post-vaccination mean = 3.9, p=0.0195, paired t-test). However, in the subset of patients whose disease activity scores increased after vaccination, this decrease in IFNα activity was

not seen. A correlation was seen between elevated baseline IFNα activity and poor humoral immune response to the influenza vaccine (p=0.0126, r2=0.22). Additionally, a significant association was seen between increased IFNα and total number of lupus-associated autoantibodies (IFNα < 1.0 [n=45], mean aAbs = 2.1, IFNα > 1.0 [n=27], mean aAbs = 3.4, p=0.0003, unpaired t-test). Consistent with previous reports, we also saw a significant association between increased IFNα and disease activity (IFNα < 1.0 [n=45], mean SLEDAI = 7.6, IFNα > 1.0 [n=27], mean SLEDAI = 10.2, p=0.0078, unpaired t-test). *Conclusions:* A unique finding in this study was that IFNα decreases in SLE patients post-influenza vaccination. Increased baseline IFNα activity correlated with a poor humoral response to the influenza vaccine. Increased IFNα activity was also associated with an increased number of lupus-associated autoantibodies, as well as with increased disease activity.

CS18 Autoantibodies and Tissue Pathology

CS18.1

Innate immune mechanisms in experimental lupus

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Objectives: Chronic inflammation and the formation of ectopic lymphoid tissue are associated with many forms of autoimmunity. We have found that chronic inflammation in non-autoimmune prone mice following peritoneal injection of tetramethylpentadecane (TMPD) leads to the overproduction of Type I interferon (IFN-I), resulting in increased expression of IFN-I inducible genes as seen in many SLE patients. TMPD-treated mice develop lupus autoantibodies (anti-DNA, Sm, RNP, and others), inflammatory arthritis, and glomerulonephritis. The objective of these studies was to better understand how chronic inflammation precipitates lupus in mice. *Methods:* Lupus was induced in BALB/c or C57BL/6 mice using TMPD and the critical immune pathways were identified using gene-targeted mice. Myeloid cells responsible for IFN-I production were purified and characterized by flow cytometry. Anti-RNP antibody producing cells were identified using anti-U1A ELISPOT assays and serum autoantibodies were detected by ELISA. The role of ectopic lymphoid tissue in autoantibody production was investigated after transplantation into naïve recipients. *Results:* The induction of autoantibodies and nephritis by TMPD was abolished in mice deficient in the Type I interferon receptor. IFN-I in the TMPD-lupus model was derived largely from a population of myeloid cells with the phenotype Ly6C^{hi}, Ly6G$^-$, CD11b$^+$, CD11c$^-$, B220$^-$, CD4$^-$, Sca1$^+$, most consistent with immature monocytes rather than plasmacytoid dendritic cells. IFN-I production and the development of lupus nephritis and autoantibodies in TMPD-treated mice was found to be mediated exclusively by the TLR7-MyD88 signaling pathway, and was independent of TLR9 and other pathways of IFN-I production mediated by TRIF, IPS-1, or TBK-1. We found that inflammation-induced chronic IFN-I production caused ectopic lymphoid tissue to become a site where antigen-specific B and T cells are activated and proliferate. In addition, large numbers of anti-RNP autoantibody producing plasma cells were found in the ectopic lymphoid tissue and were attracted/retained there by the chemokine SDF-1. *Conclusions:* These studies suggest that chronic IFN-I production plays a direct role in the pathogenesis of experimental lupus and define TLR7-MyD88 signaling as the critical pathway leading to autoantibody production and renal disease in this mouse model. Aberrant B cell homeostasis is a prominent feature of TMPD (inflammation)-induced lupus. The data suggest that autoreactive B cells arise in sites of chronic inflammation, followed by further development in other locations. We now are examining how chronic inflammation promotes the generation of autoreactive anti-RNP B cells and their development into autoantibody-secreting cells.

CS18.2

Cell death in SLE: insights into pathogenesis

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Autoimmune rheumatic diseases like SLE belong to a diverse group of processes in which multiple tissues are actively injured through the activity of various immune effector pathways. Once initiated, the processes amplify over time and are self-sustaining, and the amplitude of the disease process typically varies over time. Such periodic amplification of an immune process (which has characteristic features of an antigen-driven response) suggests that the relevant antigens which drive the disease are periodically exposed. Identifying both the source and pathways underlying such antigen exposure is important for the understanding of disease mechanisms and causes. The specific targets of the autoimmune response in systemic autoimmunity provide particularly helpful insights in this regard. Several principles are apparent: (i) ubiquitously expressed autoantigens (expressed in all nucleated cells) are targeted, and (ii) specific subsets of autoantigens are preferentially targeted in the different diseases. Data suggests that distinct subsets of autoantigens targeted in different disease phenotypes share properties during specific physiologic processes (e.g. programmed cell death), and that the targeted antigens share properties that render them more likely to initiate an immune response (e.g. changes in structure or adjuvant properties). The role of cytotoxic lymphocyte granule-induced structural changes and unique adjuvant properties of autoantigens in this form of cell death have important implications for the initiation and propagation of systemic autoimmunity, and will be reviewed.

CS18.3

Antiphospholipid antibodies and thrombophilia in the antiphospholipid syndrome

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The antiphospholipid syndrome (APS) is characterized by thrombosis in the presence of antiphospholipid antibodies (aPL). Tissue factor (TF), the major initiator of the coagulation system, is induced on monocytes activated by aPL, explaining in part the pathophysiology of this syndrome. Phosphatidylserine-dependent antiprothrombin antibodies (aPS/PT) are as prevalent as beta2-glycorprotein I dependent anticardiolipin antibodies (aCL/b2GPI) in patients with APS, and there were no difference in the clinical manifestations between patients with aCL/b2GPI and those with aPS/PT. Therefore, beta2-glycorprotein I and prothrombin are two major antigenic targets of aPL, and it is highly likely that aCL/b2GPI and aPS/PT shares the pathophysiological properties for thrombophilia. One of the enzymes responsible for this plasma membrane PL asymmetry is lipid scramblase 1 (LSCR1) which plays a major role in the transport of PS from the inner leaflet of cell membrane to the cell surface. The mechanisms how aCL/b2GPI and aPS/PT interact to procoagulant cells will be discussed. 231D was monoclonal aPS/PT, having a strong lupus anticoagulant activity (Arthritis Rheum 60, 2457-67, 2009). The binding of 231D to monocyte cell line, RAW231.7, was investigated by flow-cytometry. 231D bound to RAW234.7 in the presence of prothrombin. RAW264.7 was treated with the monoclonal in the presence of prothrombin. The kinetics of several molecules after 231D exposure was investigated. TF mRNA expression was increased on RAW264.7 treated with 231D. The proteome profiler showed phosphorylation of p38 MAPK, and confirmed by cell ELISA. Pre-treatment with INFa induced lipid scramblase-1 (LSCR1) and significantly enhanced TF mRNA induction by 231D. Those data showed that aPS/PT activates procoagulant cells, similar to aCL/b2GPI induced procoagulant cell activation. The effect of INFa presumably leads to phosphatidylserine expression on cell surface and facilitate the accessibility of phospholipid-binding proteins, followed by further aPL interaction. Those phenomena would be relevant in vivo in APS patients.

CS18.4

B cell apotopes of Ro60 in lupus

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Ro60 is a major target of autoimmunity in SLE, neonatal lupus syndrome and primary Sjögren's syndrome. The human B cell response to Ro60 targets multiple epitopes mapped traditionally by immunochemical techniques. A new approach has been to map apotopes (epitopes expressed on the surface of apoptotic cells) and intracellular epitopes by flow cytometry using apoptotic cells as a natural source of immunogen. This has proved superior to standard epitope mapping by identifying determinants on different configurations of native Ro60, leading to the discovery of new diagnostic markers and providing insight into the mechanisms by which different immunogenic forms of a systemic autoantigen may break immune tolerance. A pivotal immunodominant region of Ro60 spanning amino acids (aa) 193-236 has been identified which harbours either 1) an apotope that is associated with SLE with isolated anti-Ro60 responses, or 2) an intracellular epitope that is linked to SLE and primary SS with linked anti-Ro/La autoantibody sets. These determinants are virtually mutually exclusive, signifying the presence of two immunogenic forms of Ro60 autoantigen: a membrane-bound form on the surface of apoptotic cells, and a cytoplasmic form on the Ro/La RNP complex. The plasma protein beta-2-glycoprotein I (β_2-GPI) binds to Ro60 on the surface of apoptotic cells and blocks opsonisation by anti-Ro60 autoantibodies. Plasmin cleavage of the hydrophobic loop of β_2-GPI abrogates binding to apoptotic cells and reverses the protective effect of β_2-GPI on anti-Ro60 IgG-apoptotic cell immune complex formation. Thus stimulation of plasmin production may eliminate the protective effect of β_2-GPI in maternal anti-Ro60 mediated congenital heart block. Studies are underway to map T-cell determinants in the immunodominant domain of Ro60; determine the in vivo role of the β_2-GPI/Ro60 interaction; and investigate the clonality of the various anti-Ro60 B cell responses.

CS18.5 & PO2.A.2

Anti-heat shock protein 60 autoantibodies are associated with arterial vascular events in patients with anti-phospholipid antibodies

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Objectives: Anti-heat shock protein 60 autoantibodies (anti-HSP60) are associated with coronary artery disease and atherosclerosis, and are known to affect endothelial cells in vitro. However, their association with other vascular events (VE) remains unclear. In patients with systemic lupus erythematosus (SLE), elevated titers of anti-HSP60 have been associated with an increased risk of thrombosis when present with lupus anticoagulant antibodies. We have recently shown that anti-HSP60 promote thrombosis in a murine model of arterial injury. Based on these findings, we hypothesized that the presence of anti-HSP60 autoantibodies, alone or in combination with other thrombogenic risk factors (e.g., anti-phospholipid antibodies [aPL]), would be associated with an elevated risk of VE, in particular in patients with SLE. **Methods:** The study population was derived from the databases of three ongoing cohort studies: two SLE registries and one cohort of individuals with aPL. Only individuals with aPL testing performed on at least two occasions were included. aPL positivity was defined as: anti-cardiolipin (aCL) IgG/IgM >40 APL units, and/or lupus anticoagulant (LA) positive, and/or anti-β2-glycoprotein I (aβ2GPI) IgG/IgM positive, each on ≥2 occasions ≥ 12 weeks apart. Data from a total of 406 participants was captured and four groups were identified: (1) aPL-positive with VE (n=85); (2) aPL-positive without VE (n=83); (3) aPL-negative with VE (n=119); and (4) aPL-negative without VE (n=119). Arterial VE (VE-A) (n=123) or venous VE (VE-V]) (n=97) were confirmed from medical records. Serum anti-HSP60 were determined by enzyme-linked immunoassay and values exceeding the 75th percentile of the healthy controls (n=25) were considered to be high-titer positive. Clinical and demographic variables captured included age, race, gender, family history of cardiovascular disease, smoking, SLE, hypertension, and diabetes mellitus. **Results:** Multivariate analyses revealed that total VE were associated solely with age or hypertension. However, analysis of the VE according to their origin showed an association of VE-A, but not VE-V, with anti-HSP60 (OR=2.326 [95% CI=1.157-4.673]). Furthermore, the concomitant presence of aPL with anti-HSP60 increased the risk of VE-A (OR=6.19 [95% CI=2.02-18.91]), but not VE-V (OR=1.09 [95% CI=0.36-3.28]). Finally, the presence of individual aPL (i.e., aCL, LA, or aβ2GPI) with anti-HSP60 also increased the risk of VE-A, with the strongest association observed for aCL (OR=8.67 [95%CI=1.97-38.08]). **Conclusions:** Our results demonstrate that anti-HSP60 are associated with VE-A, and that the concomitant presence of aPL (particularly aCL) with anti-HSP60 further enhances the risk of these events.

CS19 Clinical Trials

CS19.1

Some lessons from RA trials to make lupus trials even better

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The science and art of designing and implementing randomized clinical trials (RCTs) has developed immensely over the past decades. In rheumatology, the most succesful RCTs have been in rheumatoid arthritis (RA), where after some noted failures in the early 1990s, a large number of new treatments could demonstrate efficacy and safety sufficient to satisfy regulatory requirements and provide clinicians with useful initial knowledge of the new agent. Perhaps even more notably, many RCTs in RA – mostly investigator-initiated ones such as FinRACO, TICORA, BeST, SWEFOT and many others – have investigated not so much the specific agents as the optimal strategy to be employed when using these agents. In the case of SLE, fewer trials have been done and only very few have been succesful. Although this may to some extent be due to the medications having, in fact, limited efficacy in SLE, it is also clear that clinical trial design and implementation in SLE may, to some extent, be less than ideal. Here, I will discuss some specific lessons from RA clinical trials with potential relevance for the developing field of RCTs in SLE.

CS19.2

Trials and tribulations: what comes next?

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A new era in the treatment of systemic lupus erythematosus (SLE) may be dawning. Twelve years after the first approval of biologic therapy for patients with rheumatoid arthritis, the positive results of two large trials of a novel biologic therapy for SLE have raised hopes that a new approach to treatment may be at hand. This encouraging news follows several disappointments in trials of other biologic therapies and provides a timely moment to reflect on where we stand, what we have learned, and what may lie ahead. For belimumab, the next set of questions are likely to revolve around establishing the clinical setting that best suits this agent. For rituximab, it will be important to establish whether the disappointing results of this approach to B-cell therapy reflect shortcomings in trial design, inadequate statistical power, or an unequivocal failure of the drug. For abatacept, the looming question is whether either of two ongoing trials will constitute the first breakthrough in lupus nephritis.

Finally, for other novel therapies on the horizon, the recent successes will at the very least have set a new standard for comparison.

CS19.3

Outcome measures in SLE trials
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Most end-points used in clinical trials have not been validated in clinical trials but their use is based on other forms of validation. The BILAG index of disease activity and increasingly the improved revision, the BILAG 2004 index, are being used to measure disease activity and flare in outcome studies and trials alone or with other measures such as the SLEDAI. It is critical that investigators and monitors are trained to use the disease assessment methodology being used in a trial and that differences in definitions between indices are understood and applied. Trials may use an adjudication committee to assess disease activity or flare at entry to trial or for assessing whether end-points are met. For future trials it will be important to reflect on the lessons learnt from past trials including the Abatacept and Epratuzumab studies as well as to consider new ways of measuring flare. The Abatacept and Epratuzumab trials used cumulative flare and composite landmark analyses respectively. End-points should be chosen that will reflect the most clinically important differences between treatment groups. Consideration should be given to novel trial designs that encompass comparison of conventional treatment strategies with new drug treatment combinations associated with less use of traditional immunosuppressive drugs.

CS19.4

Evolution of the SRI: are composite endpoints the future?
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Numerous challenges to drug development have long confronted the lupus community. Despite these obstacles, the last decade has witnessed unprecedented activity in lupus clinical trials. Unfortunately, most of these trials were met with disappointment. While the reasons for failure are multifactoral, ineffective trial design and inadequate response endpoints have contributed to the negative outcomes. The Systemic Lupus Responder Index (SRI) is a composite responder index that grew out of a failed phase II study of belimumab. It fulfilled the criteria put forth by the FDA in their draft guidance document and was approved by regulatory authorities as the primary endpoint for two large phase III studies of belimumab, BLISS-52 and BLISS-76. The SRI's performance in these two successful studies supports the use of such a responder index in similarly-designed clinical trials.

CS19.5 & PO2.F.9

Flare assessment in systemic lupus erythematosus (SLE) patients treated with rituximab in the phase II/III EXPLORER trial
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Objectives: SLE patients (pts) with moderate-to-severe disease activity (≥1 BILAG A or ≥2 BILAG B domain scores) despite background immunosuppressives and corticosteroids, were randomized to placebo (PLA) or rituximab (RTX). Although differences in primary and secondary outcome measures were not observed, an exploratory analysis was performed to evaluate the im-

pact of RTX on disease flares. Our objectives were to assess in those pts who achieved response whether RTX affected: 1) time to moderate or severe flares, 2) annualized flare rates, and 3) prednisone usage during flares. ***Methods:*** Pts who achieved response (BILAG C, D, E scores for all domains before wk52) on monthly BILAG assessments were included in this analysis. Flares, defined as increased disease activity following achievement of response, were stratified as follows: Severe flare: ≥1 BILAG A or >3 BILAG B domain scores; A flare: ≥1 new BILAG A domain scores; Moderate flare: 2 BILAG B domain scores. Kaplan-Meier estimates were used to assess time-to-flare. Annualized flare rates were calculated using number of flares per patient with a Poisson regression model. Only flares that occurred after the protocol-mandated prednisone taper at 12 wks were included in the analysis of prednisone use during flares. ***Results:*** Responses were achieved in 58/88 (66.0%) PLA-treated and 127/169 (75.1%) RTX-treated pts during the study. No difference was found between rituximab and placebo in preventing or delaying moderate to severe flares. However, when BILAG A flares alone were examined, rituximab reduced the risk of an A flare after achieving a response by 52 weeks (hazard ratio=0.612; p=0.0524) and lowered the annualized A flare rate (0.86 ± 1.47 (SD) vs 1.41 ± 2.14; p=.038). Eighty-four of 169 (49.7%) rituximab-treated patients achieved low disease activity without subsequent A flares versus 31/88 (35.2%) patients in the placebo group (p=0.027). Prednisone rescue for A flares was similar in rituximab- (24%) and placebo-treated (14%) patients (p=.204). ***Conclusions:*** No conclusions about rituximab efficacy can be drawn from this post hoc analysis. This exploratory analysis suggests that assessment of BILAG A flares may distinguish potential treatment effects with more sensitivity than BILAG B flares, which capture modest changes in disease activity. If confirmed in other studies, this observation may help in the design of more robust clinical trial protocols.

CS20 World Wide Findings in Paediatric Lupus

CS20.1 & PO2.N.1

Defining and measuring global flares in juvenile systemic lupus erythematosus
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Objective: To develop a definition of global flare in juvenile Systemic Lupus Erythematosus (jSLE, defined as SLE onset ≤ 16 years) and determine candidate criteria for measuring jSLE flares. ***Methods:*** Pediatric rheumatologists answered two Delphi questionnaires to achieve consensus on a common definition of jSLE global flare and identify variables for use in candidate flare criteria. The diagnostic accuracy of these candidate flare criteria was tested with data from jSLE patients (n=98; 623 visits total). Physician-rated change in the jSLE course (worsening yes/no) between visits served as the criterion standard. ***Results:*** The 1st Delphi survey was sent to 299 pediatric rheumatology members of CARRA, PANLAR and PRES (53% response rate), and the 2nd one had a response rate of 84%. There was 96% consensus that "a flare of disease is a measurable worsening of jSLE disease activity in at least one organ system, involving new or worse signs of disease that may be accompanied by new or worse SLE symptoms. Depending on the severity of

the flare, more intensive therapy may be required". Variables suggested for use in jSLE flare criteria were: physician-rated disease activity (V1), disease activity index score (V2), Child Health Questionnaire physical score (V3), patient well-being, protein/creatinine ratio, anti-dsDNA antibodies, ESR, and complement levels. Candidate flare definitions based on percent change of some or all of the jSLE variables were at most 53% sensitive and 97% specific with areas under the receiver operating characteristic curves (AUC) all < 0.67. Using multiple logistic regression, we derived several candidate flare criteria with complex algorithms but AUC as high as 0.92 (sensitivity> 85%; specificity> 85%). CART analysis suggested that V1, V2 and V3 suffice to identify jSLE flares (AUC = 0.81; sensitivity = 64%; specificity = 86%). **Conclusions:** Consensus has been reached on a common definition of global disease flare in jSLE and promising candidate flare criteria have been developed. Further assessment of ease-of-use and accuracy in a prospective study is needed.

CS20.2 & PO2.N.2

Psychiatric illness of systemic lupus erythematosus in childhood

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Purposes: 1) To describe characteristic clinical, laboratory and imaging features; 2) To determine distinct entities in the spectrum of psychiatric disease of Juvenile SLE (JSLE) and 3) to report on treatment outcomes. **Methods:** Single centre cohort study of consecutive JSLE patients followed between 08/1985 and 12/2008. Patients were evaluated following standardized protocol. All patients were assessed by an experienced psychiatrist. Clinical features of psychiatric disease of Lupus were identified and classified according to ACR nomenclature except cognitive dysfunction. Cognitive dysfunction in this study defined as memory or attention deficits reported by patients/ parents, affecting academic performance. Psychiatric **Outcomes:** 1) response- no psychiatric symptoms, stopped anti-psychotic medications and Prednisone <50% maximal dose for at least 3 months; 2) remission- no psychiatric symptoms, stopped anti-psychotic medications and Prednisone ≤10mg/day for at least 3 months; 3) relapse- recurrence of symptoms (after response) requiring 50% increase in dose of Prednisone, change of 2nd line immunosuppressive not due to adverse effects. **Results:** 447 JSLE patients were followed during the study period: 12% (53) developed psychiatric disease of JSLE; 87% (46) females, median follow- up from psychiatric Lupus diagnosis 2.0 years (0.5-6.8). Half (27/53) had psychiatric disease at diagnosis of JSLE. Median interval from first psychiatric symptom to diagnosis was 60 days (1- 1460).Clinical features of psychiatric disease of JSLE: Psychosis-like symptoms seen in 75% (40) with hallucinations being predominant. Insight preserved in 70%. Novel symptom of visual distortions in 38% of those with psychosis. Clinically significant cognitive dysfunction present in 100%. No patient had isolated depression or anxiety. Specific investigations: 42 had Magnetic Resonance Imaging (MRI): 45% normal, 29% cerebral atrophy and 17% white matter changes. Lumbar puncture performed in 53% (28/53) at diagnosis: 29% had abnormally elevated total protein, 7% had elevated white cells. Treatment: Prednisone was started/ increased following protocol. 60% (24) of patients with psychosis required antipsychotic therapy. All but 3 were treated with 2nd line agents: 85% (45) azathioprine, 55% (29) cyclophosphamide and 28% (14) mycophenolate. **Outcomes:** 74%(39) responded, 25 attained remission (3 then relapsed), 6 relapsed, 8 improved but not attained remission. **Conclusion:** Psychosis and cognitive dysfunction were 2 distinct patterns found amongst children with Psychiatric Lupus. Psychosis of JSLE was different from adults' with preservation of insight and unique feature of visual distortion. 76% responded to standard therapy.

CS20.3 & PO2.N.3

Performance of a new health-related quality of life (HRQOL) measure in pediatric lupus across five countries

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Objective: Simple Measure of Impact of Lupus Erythematosus in Youngsters© (SMILEY©) is a novel, brief, 24-item health-related quality of life (HRQOL) assessment tool for pediatric systemic lupus erythematosus (SLE). Responses are in the form of 5-faces scale for easy comprehension. SMILEY© is valid in US-English and has been translated and adapted to nineteen additional languages. Currently, we are conducting cross-cultural validation of SMILEY©. Our objective herein was to test preliminarily the performance of SMILEY© in different geographic populations. **Methods:** Children ≤18 years with SLE and parents completed the appropriate SMILEY© translation, as well as gold standard quality of life (QOL) and physical function scales. Demographic, medication and SLE–related data were obtained. We compared the means of age, child reports of SMILEY© total score, PedsQL™ generic module total score, global quality of life (QOL) rating (administered with SMILEY© using the same style of responses), Child Health Assessment Questionnaire (CHAQ) disability index, and Systemic Lupus International Collaborating Clinics/ACR Damage Index (SDI). Depending on the data distribution of the above variables, we used one-way ANOVA or the Kruskal-Wallis (KW) test. **Results:** Eighty-five children (73 girls, 86%) participated from five countries: Argentina (n=11), Brazil (n=33), Italy (n=16), Netherlands (n=10), and Spain (n=15). The mean disease duration was 36 months (1 – 150 months). The number of children with current/prior steroid use was 74 (97%, n=76), and disease modifying anti-rheumatic drugs (DMARDS) was 74 (95%, n=78). On preliminary analysis, the means/medians of child reports of the SMILEY©, global QOL rating, PedsQL™ generic module, CHAQ disability index, and SDI were similar across the five countries (Table 1 lists p values) and compatible with an assumption of no statistically significant difference. The cumulative means, standard deviation, range, and number of subjects are provided in Table 1. The only significant difference was found in age. The mean age in Brazil was 12 years, but otherwise was 15 years. **Conclusion:** SMILEY© performed uniformly across countries on preliminary analysis. The lower mean age in Brazil is likely related to referral. Given the small sample, the difference in age is not likely to be meaningful. We are actively enrolling from the above centers and from additional centers to expand our sample.

Test	Mean/ median	Standard deviation	Range	Number of subjects	p value
Age (in years)	14	3	6 - 18	81	0.005 (ANOVA)
SDI	0.3 Median 0	0.8	0 - 4	73	0.7 (KW)
CHAQ	0.3 Median 0.2	0.4	0 - 1.5	24	0.2 (KW)
Global QOL rating (%)	80	20	20 - 100	81	0.9 (ANOVA)
SMILEY© total score (%)	69	15	26 - 97	81	0.6 (ANOVA)
PedsQL™ Generic total score (%)	75	18	24 - 100	60	0.3 (ANOVA)

CS20.4 & PO2.N.4

Primary immunodeficiencies in juvenile systemic lupus erythematosus patients

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Objectives: To evaluate the frequency of complement and antibody primary immunodeficiencies (PIDs) in Juvenile Systemic Lupus Erythematosus (JSLE) patients and to compare lupus patients with and without PID regarding demographic data, clinical features, disease activity and damage, treatment and occurrence of infections. *Methods:* Seventy-two JSLE (ACR criteria) patients (1 to 16 yrs at diagnosis) were analyzed for early components of the classical complement pathway (C1q, C1r/C1s, C4, C2, C3) and immunoglobulin levels (IgG, IgA, IgM, IgE, and IgG2 subclass). Statistical analysis was carried out according to Fisher´s exact test, Mann-Whitney test and Backward Stepwise multivariate analysis. *Results:* Nineteen patients (26%) had underlying PID. Complement PIDs were: 5 cases of C2 deficiency and 2 of C4 deficiency (all with persistently very low values in the presence of normal levels of other complement components, and SLEDAI <4), and 2 cases with complete C1q deficiency. All PID patients had normal C3 levels. Immunoglobulin deficiencies were: 4 cases with IgG2 deficiency (<20mg%), 3 with IgA deficiency (<7mg%), and 3 with IgM deficiency (<35mg%). One IgA deficient patient also presented C4 and C2 deficiencies. A clear gender bias was observed, since 54% of the boys (7/13) and 20% (12/59) of the girls presented an underlying PID (p=0.032; RR: 3.25; CI: 1.25–8.46). The 2 cases of infantile SLE (age at onset <2 years) were both males (one with C1q deficiency and other with IgM deficiency). A remarkably higher frequency of severe sepsis was observed in the PIDs group (31% vs 7.5%; p=0.017; RR: 4.2; CI: 1.32–13.2). Specific lupus clinical features (cutaneous, mucosal, neuro-psychiatric, cardiopulmonary, renal, hematological and articular manifestations and antiphospholipid syndrome) were uniformly alike in patients with and without PIDs. On the other hand, the median of the cumulative damage related to SLE (SLICC/ACR-DI) was significantly higher in immunodeficient subjects [1(0-5) vs 0 (0-3); p=0.0075]. Logistic regression showed that male gender (odds ratio=4.7; 95% CI=1.2–19.2; p=0.034) and SLICC/ACR-DI (odds ratio=2.5; 95% CI=1.13–4.8; p=0.007) were independent risk factors for PID (Nagelkerke R2=0.26). *Conclusions:* An exceedingly high frequency of antibody and complement deficiency was observed amongst JSLE patients, suggesting that these immunologic defects may contribute to the disease development. Our results command that these two groups of PIDs should be systematically investigated in early onset lupus.

CS20.5 & PO2.N.5

Bone mineral density in childhood and adult onset systemic lupus erythematosus (SLE): a cross-sectional comparative study

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Objectives: To compare the bone mineral density (BMD) in childhood and adult onset SLE patients *Methods:* Consecutive patients who fulfilled >=4 ACR criteria for SLE were screened for BMD at the hip, lumbar spine and whole body using the DXA scan (Hologic, Bedford, USA). Data on risk factors for osteoporosis were collected. Comparison was made between patients who had onset of their SLE in childhood (before the age of 18 years) and adulthood (at or after the age of 18 years). *Results:* 383 SLE patients consented for DXA scanning for their BMD. 39 (10.1%) patients had childhood onset SLE whereas 344 (89.9%) patients had adult onset SLE. The female to male ratio was 15:1. Compared to adult onset SLE patients, childhood onset patients were less likely to be chronic smokers (3% vs 12%) and post-menopausal (5% vs 39%); but had a lower body mass index (BMI) (20.9±3.3 vs 22.3±3.9 kg/m2), longer SLE duration at the time of DXA screening (129 vs 101 months),

and higher frequency of ever use of high-dose prednisolone (90% vs 65%), cyclosporin A (18% vs 13%) and cyclophosphamide (56% vs 18%). Despite a significantly younger age at the time of DXA scan in childhood onset SLE patients (24.4±5.8 vs 43.2±11.8 years; p<0.001), their BMD at the hip and lumbar spine was significantly lower than that of adult onset SLE patients (0.774±0.115 vs 0.819±0.116 g/cm2; p=0.03 and 0.861±0.131 vs 0.928±0.134 g/cm2; p=0.004). The proportion of patients having osteoporosis at the lumbar spine (30% vs 20%) and the hip (9% vs 6%) was also numerically higher in the childhood onset SLE patients. In a logistic regression model, childhood onset SLE (compared to adult onset SLE) was an independent risk factor for osteoporosis at the lumbar spine (RR 2.95 [1.07-8.14]; p=0.04) after adjustment for chronic smoking, alcohol drinking, menopause, BMI, SLE duration, use of corticosteroid for 3 years or more, and ever use of cyclophosphamide, cyclosporin A and exogenous estrogens. *Conclusions:* Childhood onset SLE patients had significantly lower BMD at the spine and the hip than adult onset SLE patients even after adjustment for SLE disease duration and other risk factors for osteoporosis including the long-term use of corticosteroids and immunosuppressive drugs. Failure to achieve a peak bone mass due to high-dose corticosteroid therapy during puberty is a plausible explanation for the observed low BMD in childhood onset SLE patients.

CS20.6 & PO2.N.6

Variability of mycophenolic acid (MPA) pharmacokinetics (PK) in childhood-onset systemic lupus erythematosus (cSLE) may be partly explained by UGT genotype

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Introduction and *Objectives:* Mycophenolate mofetil (MMF) is a prodrug that is pre-systemically hydrolyzed by esterases to the biologically active moiety, mycophenolic acid (MPA). MPA is mainly metabolized by UDP-glucurono-syltransferases (UGTs) in the liver, intestine, and kidney into the inactive 7-O-glucuronide (MPAG) metabolite. Since genetic variants in UGT1A8, 1A9, and 2B7 have been proposed to explain the large variability of MPA exposure (PK) in transplant patients, the utility of pharmacogenetic (PG) testing is currently being investigated for individualizing MMF therapy. The relevance of these UGT polymorphisms to MPA PK in cSLE has not been addressed, but may be helpful in better understanding the MPA exposure-effect relationship in cSLE. In our ongoing pharmacokinetic-pharmacodynamic study, the contribution of UGT genotype in relation to MPA PK in cSLE was investigated in an exploratory fashion. *Methods:* Full 9-hour pharmacokinetic profiles were obtained from cSLE patients (n=18; F:M=17:1; age 10-30 years, 43% Caucasian, 57% African-American, 81% non-Hispanic) who were on stable MMF treatment. The MPA PK parameters dose-normalized area under the curve from 0 to 9 hours (AUC0-9h), peak concentration (Cmax), time to peak concentration (Tmax), and oral clearance (CL/F) were assessed and analyzed by non-compartmental analysis. Genomic DNA was extracted using standard procedures and genotyped for UGT1A8*3 (830G>A), UGT1A9*3 (98T>C), 1A9-2152T>C, 1A9-440C>T, 1A9-331T>C, 1A9-275T>A and UGT2B7-900A>G by TaqMan assay and direct sequencing. *Results:* Large inter-patient variability in AUC0-9h (mean + S.D.: 38.7 + 19.8 mg*hr/L/gMPA, range: 14.9 - 95.4) and oral clearance (CL/F) of MPA (mean + S.D.: 27. 9 + 13.7 L/hr, range: 8.3 - 59.3) was observed. Patients with UGT1A9 -440T (-331C) or UGT2B7 -900G appeared to have a trend toward lower CL/F and higher MPA exposure (AUC) than the wild-type of all evaluated single nucleotide polymorphisms (SNPs). Patients with UGT1A9 -275A showed relatively high CL/F over a wide range. No patients with the UGT1A9*3 genotype were found. In an exploratory fashion, wild-type and -275A-carriers were analyzed and showed significantly higher CL/F compared to the rest of the carriers of SNPs 1A9 -440T (-331C) or 2B7-900G (p<0.05, Mann-Whitney's U test). *Conclusion:* Our preliminary data suggest that UGT1A9 and UGT2B7 poly-

morphisms may explain in part inter-individual differences in MPA exposure in patients with cSLE. Further studies in larger cohorts are needed to confirm the observed trends.

KS2 Pitfalls in the Development of New Lupus Drug

KS2.1

Pitfalls in the development of new lupus drugs
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The development of new medicines is facing unprecedented challenges as exemplified by increasing costs, lengthening timelines, and decreasing numbers of novel drug approvals. Efforts to develop new therapeutics for systemic lupus face additional challenges due to the heterogeneous nature of the disease and the lack of a well defined path. This presentation will sort through the steps involved in lupus drug development with a focus on key pitfalls and gaps. Illustration of key challenges will involve examples from published results of therapeutics being tested in lupus and from selected aspects of Amgen's lupus programs. Choosing drug candidates among the many compelling targets that are likely to influence lupus disease pathways represents a significant challenge, and the added value and predictive information from studies in mouse models of lupus is currently unclear. Biomarkers are essential in early-stage trials to quantify coverage of the target and the relevant biological pathway but they do not necessarily predict clinical effects. Insight into potential clinical benefit in early-stage trials has been difficult resulting in a reliance on relatively large phase 2 clinical trials to demonstrate the first evidence of clinical effect for novel agents. This gap with its associated major cost and delay to understanding impact on disease can be a major deterrent for companies considering lupus as the primary indication. Results from recent large clinical trials also emphasize the inherent challenges in designing later-phase clinical trials for agents that target a systemic multi-organ disease like lupus. Central to improving success is the ability to accelerate advancement of promising molecules and rapidly eliminate candidates with a low likelihood of success prior to conducting large clinical trials. Biomarkers that reliably predict clinical effect, alternative trial designs with narrowed organ-specific outcomes, or enhanced ability to measure general disease activity could greatly help span this gap. In summary, the opportunity to transform the therapy of lupus has never been greater, and surmounting key gaps will greatly enhance the possibility of success.

PL4 Lupus Epidemiology and Pathogenesis

PL4.1

How has murine lupus informed us about human lupus?
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The fundamental immunologic abnormality in lupus is the loss of tolerance to nuclear antigens. While the nature of the immune abnormalities that lead to this loss of tolerance in human SLE have proved elusive, study of mice with genetic deletions or transgenes that promote lupus has provided a conceptual basis for understanding the type of immune defects that lead to this breach of tolerance. In general these can be classified into three broad categories; 1) those that promote presentation of, or the response to, apoptotic debris in an immunogenic form; 2) those that affect B and/or T cell signaling resulting in abnormal stimulation of autoreactive lymphocytes; and 3) those that promote survival of autoreactive lymphocytes. In addition, genetic manipulations af-

fecting the inflammatory response initiated by deposited immune complexes modulate the extent of end organ damage. While study of induced-mutant mice has proved an excellent approach for identification of potential immuno-pathogenic mechanisms in SLE, they represent an extreme situation which is rarely duplicated in human SLE, where multiple genes act in concert to produce disease. Consequently, study of susceptibility loci in mice that spontaneously develop lupus-like autoimmunity may be more reflective of human SLE. Identification of these loci and the immune mechanisms through which they act to promote lupus has been greatly aided by the study of congenic mouse strains. In these strains a homozygous interval containing one or a small cluster of susceptibility genes derived from a lupus-prone mouse strain, has been introgressed on a lupus-resistant mouse strain, usually C57BL/6, enabling the study of each genetic locus in isolation. There are now a number of congenic mouse strains that have been produced from a variety of different lupus-prone mouse strains. Investigation of these strains has provided insights into the types of cellular abnormalities that promote lupus and how they interact with each other to produce the global lupus phenotype. As increasing numbers of genetic polymorphisms are identified that confer an increased risk for human lupus, their proposed function falls into similar categories to those identified in murine lupus models. Thus, the insights obtained from study of murine models are likely to be highly relevant to human disease.

PL4.2

Treatment of lupus nephritis
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Lupus nephritis is an important cause of renal failure. The severe complications that could result from disease or treatment are challenges to the clinician and the patient. At the same time, the treatment of lupus nephritis can be very rewarding, when one witnesses the reversal of severe disease and acute renal failure. The addition of cyclophosphamide to corticosteroid improved renal outcome, and this combination has become standard therapy since the 1980s. Acknowledging the many adverse effects of cyclophosphamide, the quest for new treatments continued. Over the past ten years there is an increasing trend to use the combination of corticosteroid and mycophenolate mofetil as induction immunosuppressive treatment for severe proliferative lupus nephritis, based on the data from clinical trials which showed that this treatment had at least comparable efficacy compared with conventional cyclophosphamide-based therapy. Recent data also suggest an impact of ethnicity on the comparative efficacy of immunosuppressive treatment and the propensity for adverse effects related to treatment. Against initial expectations, the results to date on biologic therapies that target specific molecules involved in the aberrant immunological or inflammatory responses in lupus have not been affirmative on their clinical efficacy in human lupus nephritis. This highlights the complexities of managing patients with lupus and our inadequate understanding of disease pathogenesis. Notwithstanding the setbacks, the vibrancy of both clinical and basic research in lupus nephritis justifies the optimism that treatment options will continue to increase, so that therapy can be tailored to suit the distinct characteristics of individual patients.

PL4.3

New therapeutic targets in SLE
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The last five years has seen the apparent dashing of several 'biologic' hopes in the treatment of SLE patients. A range of targeted therapies including abatacept, rituximab and LJP394 failed to meet their end points in double-blind controlled trials. However, good news has more recently emerged with the report that belimumab (an anti-BLyS monoclonal) and epratuximab (an anti-CD22) have met their primary end points in clinical trials and, for good measure; rituximab has been shown to be effective in a vasculitis trial. There is a

paramount need to optimize clinical trial design in SLE (1) and in particular the optimal method of capturing genuine flares (distinguishing them from 'grumbling disease') has proved difficult has yet to be established. Another issue is to avoid the 'over indulgence' of corticosteroids and concomitant therapies as this will inevitably blur the demonstration of any benefit by the therapy under investigation. With these important caveats, I think we can now realistically anticipate good news from a variety of on-going studies including trials of atacicept (which block the B cell activating factors BLyS and April); antibodies to interferon α and a spliceosomal peptide P140. The exciting thing about the biologic approaches is that they target cells and molecules for which there is evidence that they are actually involved in the pathogenesis of SLE. Traditionally, drugs ranging from steroids to mycophenolate (via azathioprine, methotrexate, cyclophosphamide and cyclosporine) were used largely on the principle that they worked for other diseases (notably cancer) or post-organ transplant so 'let's try it out on a few lupus patients'. We are truly living in an age in which we are moving rapidly from therapeutic serendipity to (immunological) sense!

(1) Isenberg D, Gordon C, Merrill J, Urowitz M. Lupus 2008; 17: 967-70.

Poster Presentations

PO1A Apoptosis

PO1.A.2

Cytopenias in systemic lupus erythematosus: the role of TRAIL
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Objective: Several mechanisms have been identified for the pathogenesis of systemic lupus erythematosus (SLE) - associated cytopenias. Apoptosis of peripheral blood cells has been implicated in SLE-associated cytopenias. Accordingly, the aim of the present study was to determine the level of serum TRAIL (Tumour Necrosis Factor-Related Apoptosis Inducing Ligand) and surface expression of TRAIL on lymphocytes of newly diagnosed SLE with cytopenias and their clinical correlation. **Methods:** The study population consisted of 4 groups. Group I included 20 SLE patients presenting with cytopenias and diagnosed as having SLE according to the ACR criteria. Group II comprised 10 newly diagnosed SLE patients with cytopenias. Group III included 10 patients having cytopenias secondary to conditions other than SLE or malignancy. Group IV consisted of 10 age-sex-matched healthy volunteers. All subjects underwent a detailed interrogation and physical examination. Disease activity was assessed by SLEDAI. Serology included ANA, antidsDNA and bone marrow examination in patients with cytopenias. Serum soluble TRAIL level was assayed by ELISA and surface TRAIL expression on lymphocytes was measured by flow cytometry. **Results:** Both membrane-bound and soluble TRAIL were significantly higher in SLE patients. Percent lymphocyte-expressing TRAIL tended to be higher in Group I. Neutrophil counts correlated negatively with levels of TRAIL both serum and lymphocyte bound. Serum TRAIL was significantly higher in Group III than in controls. Lymphocyte-bound TRAIL correlated negatively with the level of antidsDNA. Levels of TRAIL tended to be lower in patients with higher SLE-DAI. Bone marrow examination of SLE patients revealed that 4/20 patients had hypocellularity. Myelopoiesis and erythropoiesis were depressed in 30% of cases. **Conclusions:** TRAIL in both membrane-bound and soluble form is markedly increased in SLE, seems to be disease-specific and may contribute to pathogenesis. TRAIL has a role in inhibiting erythropoiesis and thus may play a role in anemia-complicated SLE. TRAIL also mediates neutrophil apoptosis in SLE and may be partially responsible for neutropenia. Apoptosis of blood cells goes some way to explain cytopenias, offers an accessible model of pathophysiology of disease in the different body systems and provides hope for a new era of apoptosis-based therapies.

PO1.A.3

Apoptotic neutrophils, a potential source of toll-like receptor (TLR) ligands in juvenile systemic lupus erythematosus
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Background: Juvenile-onset Systemic Lupus Erythematosus (JSLE) is a severe multi-system autoimmune disease, characterised by production of auto-antibodies against nuclear material. We have demonstrated increased and dysregulated neutrophil apoptosis in JSLE [1]. The toll-like receptor (TLR) family are essential components of the innate immune system. TLRs 3, 7-9 recognise auto-antigens typical of SLE. Up-regulation of TLRs in adult-onset SLE correlates with disease activity [2]. We have also demonstrated up-regulation of these TLRs in JSLE B and T cells. In addition, apoptotic neutrophils can result in auto-antigen exposure [3]. **Aim:** To determine whether apoptotic neutrophils in JSLE can induce TLR activation measured by IFN-α expression. **Method:** PBMCs isolated from healthy controls were incubated with commercial agonists for TLRs 3, 7, 8 and 9 for 24 and 48 hours, followed by RNA extraction and qPCR quantification of IFN-α mRNA expression. Control neutrophils were incubated with control serum and JSLE serum for 2 hours to induce apoptosis, quantified using Annexin V staining and flow cytometry. Control PBMCs were then incubated with these apoptotic neutrophils (24 and 48 hours) and IFN-α mRNA expression measured. IFN-α mRNA expression was normalised (fold difference) to 18s mRNA expression. **Results:** PBMC IFN-α mRNA expression was increased following stimulation of TLR 3 (x1.4fold), 7 (x1.4) & 8 (x2.4) relative to controls. Increased apoptosis was induced in neutrophils incubated with JSLE serum (15% apoptosis) compared to control (3%). IFN-α mRNA expression was increased markedly in PBMCs incubated with apoptotic neutrophils treated with JSLE serum (x19 fold) compared to PBMCs with nothing added. IFN-α mRNA expression in PMBCs incubated with control serum did not change significantly from that occurring in un-stimulated PMBCs (x1.2). **Discussion:** We have demonstrated that stimulation of TLRs 3,7 & 8 by their natural ligands on PBMCs leads to increased IFN-α expression. Incubation of control neutrophils with JSLE serum resulted in increased apoptosis compared to control serum. IFN-α expression in PBMCs was most markedly increased in neutrophils undergoing apoptosis induced by JSLE-serum. This suggests that there may be factor(s) within JSLE serum that induce neutrophil apoptosis that may result in TLRs activation that induces an inflammatory response.

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PO1B Biomarkers

PO1.B.1

Elevated levels of cytokines and chemokines in patients with SLE in a multi-center, multi-ethnic, US multi-institutional cohort
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Systemic Lupus Erythematosus (SLE) and Antiphospholipid Syndrome (APS) are two closely related autoimmune and multisystemic diseases. Approximately 30-40% of SLE patients present with antiphospholipid (aPL) antibodies and 50% of those have APS. Previous studies have indicated that cytokines and chemokines can be associated with SLE activity. However, whether those biomarkers are elevated in serum/plasma of SLE or APS patients and whether they are associated with disease activity is not clearly understood. **Objectives:** To examine if the levels of cytokines/chemokines in patients with SLE in samples from a multicenter multi-ethnic, and US multi-institutional cohort (LU-MINA) are elevated and correlate the levels of the biomarkers with disease activity (SLAM-R) and with damage accrual (SLICC-ACR Damage index). Methods: Fifty-six (56) sera, plasma from SLE patients (ACR criteria) was obtained from LUMINA. Patients that were on more than 10 mg prednisone/day, or on other immunosuppressive therapy were excluded from the study and were not on statins or on hydroxycholoroquine . In the SLE group (age range 16-67): 55% were African American, 23% were Caucasians, 12.5% were Hispanics from Puerto Rico and 8.5% were Hispanics from Texas, 87% were

females and 13% were males. Thirty-two healthy donors (age range: 18-65; 85% females, 15% males), without evidence of autoimmune or inflammatory diseases were used as controls (age range:18-65). Levels of IL1b, IL-6, IL-8, IFN-α, IP-10, MCP-1. VEGF, TNF-α, VEGF were measured in serum using a Millipore Millliplex™ Multiplex Assay, titers of IgG and IgM anticardiolipin antibodies (aCL), sE-sel, sVCAM-1 and sTF were detected by ELISA. and hsCRP by nephelometry. Cut-off values for the assays were determined using the 95th percentile of 32 controls. Nonparametric Kruskall-Wallis test was used to compare levels of biomarkers in SLE vs. controls and Spearman correlations were utilized to correlate levels of biomarkers with SLAM-R or SLICC scores. *Results:* aCL IgG, aCL IgM and hsCRP were elevated in 64%, 13% and 50%of the SLE subjects, respectively. IL-6, TNF-α, IFN-α, IP-10, sCD40L, sTF were significantly elevated in Lupus patients(<0.0001) The levels of IFN-α significantly correlated with SLAM-R scores (p=0.0546) and the levels of IL1-b and sVCAM-1 correlated with SLICC scores (p=0.0476 and 0.0009, respectively) in this group of SLE patients. *Conclusions:* Significant number of SLE samples had elevated levels of IgG aCL, hsCRP, IL-6, TNF-α, VEGF, IFN-α, IP-10, sTF, sCD40L and their titers were significantly different when compared to controls. In this group of selected SLE patients the levels of IFN-αcorrelated with disease activity (SLAM-R scores) and IL-1b and sV-CAM-1 levels were directly correlated with SLICC-ACR scores. This study underscores the importance of identifying biomarkers of disease that may help to predict disease activity and possibly to better address treatment of patients with SLE and APS

PO1.B.2

Serum levels of IL-33 and soluble ST2 and their association with disease activity in systemic lupus erythematosus
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Objectives: Interleukin (IL)-33 has recently been found to be the specific ligand of ST2, a IL-1 receptor family member that is selectively expressed on Th2 cells and mediates Th2 response. This study aims to measure serum levels of soluble ST2 (sST2) and IL-33 in patients with systemic lupus erythematosus (SLE) and to examine their association with disease activity. *Methods:* Seventy SLE patients were evaluated for disease activity determined by SLE disease activity index (SLEDAI), levels of anti-dsDNA antibody, C3 and C4. Fifty-seven patients were evaluated longitudinally on a second occasion. IL-33 and sST2 were measured by sandwich ELISA in the 127 SLE serum samples and compared to 28 age- and sex-matched healthy controls. *Results:* Serum sST2 level was significantly higher in active SLE patients (0.51±0.18ng/ml) compared to inactive patients (0.42±0.08ng/ml) (p=0.006) and normal controls (0.36±0.13ng/ml) (p<0.001). Patients who had active lupus nephritis (n=35) (0.52±0.21ng/ml) and those with non-renal active exacerbations (n=32) (0.49±0.12ng/ml) had significantly higher serum sST2 compared to inactive patients (p=0.02 and p=0.03 respectively). Among patients with biopsy proven nephritis, those with pure Class IV nephritis (n=7) (0.63±0.14ng/ml) had higher serum sST2 level compared to those who had Class III (n=2) (0.33±0.04ng/ml) and mixed Class IV/V nephritis (n=4) (0.46±0.04ng/ml) (p=0.006 by ANOVA). sST2 level correlated significantly with SLEDAI, anti-dsDNA antibody, prednisolone dosage and negatively with C3. Linear regression analysis showed that serum sST2 level was an independent predictive factor for modified SLEDAI excluding anti-dsDNA and complement score after controlling for age, sex, glomerular filtration rate and prednisolone dosage (regression coefficient: 8.5 95%CI 2.6-14.3) (p=0.005). There were significant changes in the serum sST2 level at the first and second evaluations among patients (n=13) with increased or decreased disease activity (0.53±0.20ng/ml and 0.41±0.09ng/ml for the occasions with higher and lower disease activity respectively) (p=0.02) with an effect size of sensitivity to change of d = 0.29 but not among those with stable disease (n=44) (0.46±0.12 vs 0.48±0.16 ng/ml) (p=0.34). Elevated serum IL-33 was comparable in frequency (4.3% vs 7.1%, p=0.62) and levels (p=0.53) between SLE patients and controls. *Conclusions:* Elevated

serum sST2 level in SLE patients was found to correlate with parameters of disease activity, sensitive to change in levels of disease activity and was not influenced by age, sex and renal function, suggesting a potential role as surrogate marker of disease activity in SLE. The level of IL-33, its specific ligand, is only infrequently detected in SLE serum by ELISA.

PO1.B.3

Neutrophil gelatinase-associated lipocalin (NGAL) and TNF-like weak inducer of apoptosis (TWEAK) as disease activity urinary biomarkers in lupus nephritis (LN)
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Objectives: 1) To evaluate urinary NGAL (uNGAL) and TWEAK (uT-WEAK) levels as biomarkers of active lupus nephritis (LN) in SLE patients. 2) To study the association of both biomarkers in patients with active LN, partial (PR) and complete (CR) response, non-renal flare and inactive SLE patients. 3) To calculate specificity/sensitivity ROC curves for these biomarkers to differentiate between active lupus nephritis, non-renal flare and ISLE patients. *Methods:* Five groups of patients and one healthy group control (n=35) were included in this cross-sectional study. Groups were the following: A) Patients with active LN (n=38) defined by an active renal sediment, urinary protein:creatinine ratio (uP/C) ≥ 0.5 and/or biopsy-proven renal disease; B) Patients with CR (n=29) defined by an uP/C<0.2 with an inactive sediment; C) Patients with PR (n=56) with an uP/C between 0.2-2.0; D) Patients with non-renal flare (n=23) defined by a SLEDAIs≥6; and E) Patients with inactive SLE (n=39) (SLEDAIs<6). In all patients both uNGAL and uTWEAK levels were measured by ELISA kits according to the manufacturer's instructions. *Results:* uNGAL (ng/mg creatinine) and uTWEAK (pg/mg creatinine) values were expressed as median, interquartile range (IQR). The values of both biomarkers were higher in ALN patients compared to the other groups.

Groups	uNGAL (ng/Cr mg)	uTWEAK (pg/Cr mg)
Active renal flare	0.409 (0.181-0.828)	0.78 (0.18-1.35)
Complete response	0.244 (0.120-0.394)	0.0 (0.0-0.47)
Partial response	0.26 (0.155-0.489)	0.36 (0.0-0.79)
Non-renal flare	0.190 (0.111-0.306)	0 (0-0.54)
Inactive SLE	0.14 (0.067-0.268)	0.40 (0.0-0.85)
Healthy control subjects	0.10 (0.055-0.224)	0 (0-0.52)

No significant differences in uNGAL values were found between active LN and patients with PR. On the other hand, we do not find statistically significant differences between uTWEAK levels of active LN and non-renal flare patients. For both biomarkers the differences between active LN and the rest of groups were statistically significant (p< 0.05). Therefore, the uNGAL levels were better to differentiated patients with active LN from non-renal flare patients, since AUC values of ROC profiles were equal to 0.73. UTWEAK showed better ROC profiles to differentiate active LN from CR patients with AUC values equal to 0.71. *Conclusions:* uNGAL seems to be a better biomarker for the diagnosis of active renal disease, whereas uTWEAK is better discerning patients with CR.

PO1.B.4

Urinary MCP-1 as a potential biomarker in juvenile systemic lupus erythematosus

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Objective: Urinary Monocyte Chemotactic Protein-1 (uMCP-1) is a cytokine expressed in response to pro-inflammatory stimuli [1] and correlates with a poor renal prognosis [2],[3]. We aimed to determine whether uMCP-1 could be a suitable biomarker for monitoring disease activity in Juvenile-onset Systemic Lupus Erythematosus (JSLE). **Methods:** Children (diagnosed <17 years) with JSLE and healthy controls were eligible for inclusion. Ethical approval and parental consent were obtained. Urine specimens (5-10mLs) were collected and infection excluded. Samples were spun and supernatants stored at -70°C. UMCP-1 concentration was quantified by enzyme-linked immunosorbent assay (ELISA) and standardised for urinary creatinine. Concentrations are expressed as mean values (± SEM). **Results:** Seventeen patients with JSLE were recruited: 8 males; mean age (range) at sample collection 15.2 years (7.5-17.9); healthy controls: (n=9) 2 males, 13.8 years (10.3-15.8). Twenty two urine samples were collected. JSLE patients had a significantly higher concentration of uMCP-1 than healthy controls: JSLE - 3106 pg/mg Cr (± 637 pg/mg Cr); controls 1034 pg/mg Cr (± 151 pg/mg Cr); p=0.009. Longitudinal data noted; over a four week period, a 15 year old female with grade II lupus nephritis, uMCP-1 changed significantly in relation to urine albumin creatinine ratio (uACR): (MCP-1: 2667, 7204, 3192 pg/mg Cr; uACR: 2.0, 19.7, 2.4 mg/mmol). In contrast, a 13 year old female with no demonstrable renal involvement, uMCP-1 correlated with a fall in serum dsDNA concentration over time: uMCP-1: 14,279, 5918, 1051pg/mg Cr; dsDNA: 110, 49, 45 IU/ml. **Conclusion:** Urinary MCP-1 concentrations were significantly higher in children with JSLE. UMCP-1 could have the potential to act as a biomarker of renal and/or overall disease activity in JSLE. Longitudinal assessment of this chemokine and determining its role in lupus nephritis is needed.

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PO1.B.5

Neutrophil gelatinase-associated lipocalin (NGAL) as a urinary biomarker of disease activity and severity in lupus nephritis

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Objectives: To evaluate urinary (uNGAL) and serum (sNGAL) NGAL levels as a biomarker of active lupus nephritis (LN) in SLE patients. To study the association of uNGAL and sNGAL levels in patients with active LN, partial (PR) and complete (CR) response as well as in patients with non-renal flare and inactive SLE. To establish the relationship between uNGAL levels, the clinical and biological parameters in patients with active LN and to calculate the specificity/sensitivity of this biomarker for distinguishing active renal from non-renal lupus. **Methods:** All patients fulfilled the ACR classification criteria for SLE. Active renal disease was defined by active renal sediment, a urinary protein:urinary creatinine ratio (uP/C)≥0.5 and/or a biopsy-proven renal disease. Complete remission (CR) was defined by an uP/C<0.2 with an inactive sediment and partial remission (PR) by an uP/C between 0.2-2.0. A non-renal flare was defined by a SLEDAIs≥6 and inactive disease by a SLEDAIs<6. This cross-sectional study included 5 groups of patients and one healthy group control (n= 35). Groups are as follow: a group of active LN (n=38), a group of patients with PR (n=56), with CR (n=29), with non-renal flare (n=23) and inactive SLE (n=39). For each patient laboratory parameters (anti-dsDNA, C3, C4, FBC, serum creatinine (sCr) and albumin, estimated glomerular filtration rate (eGFR by Cockcroft-Gault equation) and uSLEDAIs were measured. Both uNGAL and sNGAL levels were measured by ELISA kits according to the manufacturer's instructions (Bioporto, Denmark). ROC curves were used to calculate the specificity/sensitivity. **Results:** The uN-GAL values were expressed as median (ng/mL), interquartile range (IQR). In patients with renal flare, uNGAL was significantly higher (47.25 (25.08-88.05) than in those with CR (28.00 (17.10-47.50), PR (34.30 (15.63-61.10), non-renal flare (24.00 (16.30-41.60), inactive SLE (20.00 (12.50-37.80) and healthy control subjects (16.30 (9.14-28.70). Whereas, no significant differences in uNGAL values were found between active LN and patients with PR, the differences were statistically significant with the rest of groups (p<0.05). On the other hand, the sNGAL levels only could differentiate the active LN from the inactive SLE group (p = 0.0084). The uNGAL values in patients with active LN correlated significantly with uSLEDAIs (R = 0. 3527, p<0.0001), and uP/C ratio (R = 0.2282, p = 0.0018), eGFR (R = -0. 1784, p = 0.0151), and serum albumin (R = -0.2306, p = 0.0018). The uNGAL levels showed ROC profiles, with AUC values equal to 0.69, reflecting an acceptable sensitivity and specificity for discerning active LN from non-renal flare SLE patients. **Conclusions:** uNGAL is a promising potential biomarker of activity and severity in LN. Our results indicate that uNGAL can be a useful tool in the management of the patients with LN.

PO1.B.6

Hyperferritinemia is associated with serologic antiphospholipid syndrome in SLE patients

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Background and Aims: Hyperferritinemia may be a direct immunomodulator. The present study examines the association between hyperferritinemia and disease activity and organ involvement in a large cohort of lupus patients. **Methods:** The concentration of ferritin was assessed in 272 serum samples from lupus patients utilizing the LIASON Ferritin automated immunoassay method (DiaSorin S.p.A, Saluggia-Italy). Hyperferritinemia was defined as >341.2 ng/dl in men, >104.2 in women younger than 45 years of age and >232.3 in women 45 years of age or older. Disease activity was defined as present if SLEDAI>4 or ECLAM>2. Utilizing an EXCEL database, we compared elevated ferritin levels to manifestations grouped by organ involvement. **Results:** Of 272 lupus patients, 89% were female, the median age was 37 years old, and disease duration was 10.6 ± 7.7 years. The mean ECLAM was 3.2 ± 2.0 and the mean SLEDAI was 2.6 ± 2.8. Hyperferritinemia was found in 18.6% of SLE patients. Compared to normoferritinemic subjects, those with hyperferritinemia were thrombocytopenic (15.4% vs. 33.3%, p=0.003), had elevated lupus anticoagulant (11.3% vs. 29%, p=0.01) and marginally higher anticardiolipin antibodies IgG (12.1±8.9 vs.15.3±15.9, p=0.096). ECLAM was significantly higher in hyperferritinemic subjects (2.9±1.8 vs. 4.3±2.5 units, p= 0.04) and SLEDAI was marginally higher in hyperferritinemic subjects (2.4±2.6 vs. 3.4±3.2 units, p=0.1). No association was found between hyperferritinemia and cutaneous, joint, hematologic, renal, or neuropsychiatric manifestations of lupus. **Conclusion:** Hyperferritinemia was associated

with thrombocytopenia, elevated lupus anticoagulant and anti-cardiolipin antibody titers and may be an early marker for secondary antiphospholipid syndrome in SLE patients.

PO1.B.7

A pilot study: telomere length as a biomarker for osteoporosis in women with systemic lupus erythematosus (SLE)

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Objectives: Telomere shortening, a marker of cellular senescence, has been noted in the peripheral white blood cells (PBCs) of patients with low bone mineral density (BMD) in the general population. Patients with SLE are at an increased risk of low BMD. The aims of this study are to compare telomere length between patients with SLE and healthy controls and to test if telomere length is associated with BMD. ***Methods:*** A pilot study of 154 SLE patients and 152 controls were recruited from the parent study, SOLVABLE (Study of Lupus Vascular and Bone Long-term Endpoints). Data collected at the study visit included demographic information, osteoporosis risk factors, BMD, and buffy coats from PBCs were frozen at -80C. Genomic DNA was isolated from PBCs using the QIAamp DNA Blood Mini Kit (QIAGEN). Telomere length was quantitated using real time quantitative polymerase chain reaction (PCR) as previously described by Cawthon et al, with further modifications using an oligonucleotide standard. BMD was measured by dual-energy X-ray absorptiometry (DEXA) and reported as hip and spine Z scores. ***Results:*** The average telomere lengths (kb) in PBCs in SLE patients compared to controls was 5.83 kb vs 6.22 kb (p=0.42). In both patients and controls, as expected telomere length shortened with increasing age, but a trend was noted in patients with SLE showing shorter telomere lengths than controls at an earlier age. When telomere length was grouped into quartiles, SLE patients in the lowest quartile were significantly younger than controls in the lowest quartile of telomere length (42.4 vs 49.1 p<0.04) using a pair-wise comparison. Using logistic regression analysis, patients with SLE compared to controls were significantly more likely to have lower Hip Z score (OR 4.04 95% CI 1.74-9.37) and Spine Z score (OR 3.10 95% CI 1.52-6.35). The average telomere length tended to be lower in SLE compared to controls in patients with a Hip Z score <-1 (5.97 vs 7.12 kb, p=0.54) or Spine Z score <-1 (5.13 vs 7.17 kb, p=0.16). Using logistic regression analysis, the trend toward shorter telomeres in patients with low BMD in the hip and spine remained (OR 0.971, 95% CI 0.916-1.029; OR 0.978, 95% CI 0.922-1.037). When grouped into telomere length quartiles, SLE patients in the lowest 2 quartiles of telomere length trended toward having lower BMD in the spine (OR 0.89 95% CI 0.45-1.75). ***Conclusions:*** Patients with SLE had significantly lower BMD compared to controls. Patients with SLE and low BMD in the spine (defined as a Z score <-1) had a trend for shorter telomere lengths. Further research is warranted to test whether telomere length can be used as a biomarker to identify patients with SLE at risk for osteoporosis.

PO1.B.8

A pilot study: telomere length as a biomarker for cardiovascular disease (CVD) in women with systemic lupus erythematosus (SLE)

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Objectives: Telomere shortening, a marker of cellular senescence, has been noted in the peripheral white blood cells (PBCs) of patients with CVD in the general population. CVD is a major cause of morbidity and mortality and occurs at an earlier age in patients with SLE compared to the general population; and the risk of CVD in SLE is not explained by traditional risk factors. The aims of this study are to compare telomere length between patients with SLE and healthy controls and to test if telomere length is associated with premature subclinical CVD as measured by plaque index (PI). ***Methods:*** A pilot study of 154 SLE patients and 152 controls were recruited from the parent study, SOLVABLE (Study of Lupus Vascular and Bone Long-term Endpoints). Data collected at the study visit included demographic and CVD risk factors. PI was measured by B-mode carotid ultrasound. Buffy coats from PBCs were frozen at -80C. Genomic DNA was isolated from PBCs using the QIAamp DNA Blood Mini Kit (QIAGEN). Telomere length was quantitated using real time quantitative polymerase chain reaction (PCR) as previously described by Cawthon et al, with further modifications using an oligonucleotide standard. ***Results:*** The average telomere lengths (kb) in PBCs in SLE patients compared to controls was 5.83 kb vs 6.22 kb (p=0.42). In both patients and controls, as expected telomere length shortened with increasing age, but a trend was noted in SLE patients showing shorter telomere lengths than controls at an earlier age. When telomere length was grouped into quartiles, SLE patients in the lowest quartile were significantly younger than controls in the lowest quartile of telomere length (42.4 vs 49.1 p<0.04) using a pair-wise comparison. In patients with SLE and PI >0, there was a trend toward shorter telomere lengths in patients under 35 (5.04 kb vs 8.49 kb, p=0.33) and 35-44 years old when compared to controls (4.95 kb vs 6.16 kb, p=0.40). After controlling for age, there was a non-significant trend toward shorter telomere length in patients with PI>0 (OR 0.987, 95% CI 0.930-1.048). After controlling for age, patients in the lowest two quartiles of telomere length showed a non-significant trend of more plaque compared to patients in the upper two quartiles of telomere length (OR 1.062, 95% CI 0.53-2.13). ***Conclusions:*** Telomere length tended to be shorter in patients with SLE compared to controls. Patients with SLE and PI >0 trended toward shorter average telomere lengths at a younger age compared with controls. Further research is warranted to test whether telomere length can be used as a biomarker to identify patients with SLE at risk for CVD.

PO1.B.9

Serum TNF-like weak inducer of apoptosis (TWEAK) is a potent soluble biomarker of disease activity in systemic lupus erythematosus

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Objective: Disease activity in systemic lupus erythematosus (SLE) is frequently clinically and serologically determined by diverse traditional activity indices, such as SLE Disease Activity Index (SLEDAI), anti-dsDNA, complement proteins, and beta2-microglobulin (beta2-MG). Lupus nephritis (LN) contributes to increased mortality and morbidity in SLE. Recent studies revealed that TNF-like weak inducer of apoptosis (TWEAK), monocyte chemotactic protein-1 (MCP-1), and IFN-γ-inducible protein 10 (IP-10) reflect well disease activity, especially in LN. We investigated association of TWEAK, MCP-1, and IP-10 with traditional disease activity indices including SLEDAI, anti-dsDNA, complement proteins, and beta2-MG in SLE and then determined roles of these biomarkers in the renal involvement of SLE. ***Methods:*** Sixty-two female patients with SLE were consecutively enrolled for analysis of TWEAK, MCP-1, and IP-10 from serum and urine samples using ELISA. Laboratory parameters including anti-dsDNA, complements, urine protein:creatinine ratio, and beta2-MG were assessed. Clinical disease activity was determined by the SLEDAI. We also classified patients into 2 groups like these; active LN (n = 31) and non-LN (n = 31). Statistical analysis was performed using Spearman's correlation coefficient analysis and Mann-Whitney U test. ***Results:*** Serum TWEAK, IP-10, MCP-1 and urine beta2-MG correlated well with SLEDAI scores (p = 0.042, p = 0.042, p = 0.038, and p = 0.008, respectively). Also there were statistically significant correlation between serum TWEAK and anti-dsDNA antibodies (R = 0.303, p = 0.027) and inverse correlation between serum TWEAK and C3 (R = -0.253, p = 0.047) as well as C4 (R = -0.280, p = 0.028). Clinical parameters presenting with significance between active LN and non-LN include serum TWEAK and urine beta2-MG (p = 0.026 and p = 0.039, respectively). Additionally, serum TWEAK level is significantly associated with renal SLEDAI (R = 0.299, p =

0.018). **Conclusions:** Serum TWEAK is closely correlated with lupus disease activity and also may be considered a useful biomarker to indicate disease activity in patients with SLE.

PO1.B.10

Machine learning models using multiple low abundance protein biomarker levels is superior to clinical laboratories in diagnosing ISN/RPS class of lupus nephritis

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Objectives: Treatment and prognostication in lupus nephritis (LN) are often driven by renal biopsy findings, and traditional biomarkers are not predictive of renal pathology. We hypothesized that levels of multiple candidate low abundance urine proteins, when analyzed my multivariable machine learning techniques, would create more effective models of International Society of Nephrology/Renal Pathology Society class of nephritis (ISN/RPS Class) than biomarkers now available to clinicians. **Methods:** 95 subjects from the Charleston and Baltimore LN inception cohorts and the Genentech phase III trial of Rituxan in LN (LUNAR) study population were recruited. ISN/RPS Class was determined prior to induction therapy (as per the primary rheumatologist or the LUNAR study protocol). Urine samples were collected at entry for analysis. Urine levels of 52 candidate low abundance proteins (chemokines, growth factors, cytokines, and renal damage markers) were determined by the multiplex bead array or ELISA. Levels of individual markers were used to create receiver operating characteristics (ROC) curves, and those with ROC area under the curve (AUC) values > 0.65 were used to create multivariable models of LN Class at baseline using artificial neural network (ANN) and nearest related neighbor (NRN) machine learning modeling algorithms. Levels of proteins were either used alone to train models or were combined with baseline clinical variables as inputs. Clinical variables alone were also used to train a model for comparison. Input variables were Pr/Cr, DNA, C3, C4, serum Cr and the selected biomarker panel. The output variables were the individual biopsy classes. **Results:** The biomarker NRN models of class III, IV, V, and proliferative disease (ROC AUC 0.65, 0.83, 0.75, and 0.81 respectively) significantly outperformed the clinical models (ROC AUC 0.53, 0.76, 0.31, and 0.46 respectively). The most predictive markers for Class III and IV disease were IL6, IL1α, IL8, GM-CSF, MCP1, NGAL, IFNα2, IFN γ, and IL12. ANN modeling improved ROC AUC values for class II and V more than III and IV (ROC AUC 0.97, 0.95, 0.95, and 0.96 respectively). 93% of the diagnostic model's predictive power for Class IV disease derived from 11 variables: sIL2Ra, MCP1, IP10, IFNα2, IL1Ra, IL8, N-acetyl-beta-D-glucosaminidase (NAG), MIP1β, GMCSF, eotaxin, and IFNγ. **Conclusions:** This is the first study to systematically evaluate multiple biomarkers representing diverse pathogenic mechanisms by machine learning modeling techniques. It demonstrates that when markers of cell activation, migration, and damage are combined into a single model, diagnostic power is superior to models using traditional biomarkers.

PO1.B.11

Oxidative stress during exercise in lupus patients and controls

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Objectives: Recently, oxidative stress as measured by plasma F2 isoprostane has been linked to fatigue in SLE. The aim of this study was to investigate the relationship of F2 isoprostane to aerobic capacity and exercise tolerance in SLE patients and healthy subjects. **Methods:** Plasma F2 isoprostane provides a reliable index of oxidative stress. We measured change in F2 isoprostane from baseline during a bicycle exercise test in Lupus patients and healthy controls. Phlebotomy was performed via forearm catheter at baseline, at peak aerobic capacity, and at 30 minutes post exercise. Plasma was processed and frozen prior to measurement of F2 isoprostane by mass spec gas liquid chromatography. Physiologic measurements included peak aerobic capacity (V02peak), exercise duration, peak heart rate, ratings of perceived exertion (RPE). Fatigue was assessed with the Fatigue Severity Scale (FSS). We compared exercise parameters and change in plasma F2 from baseline in lupus patients and controls by t tests (significance threshold p=<.05). Spearman correlation coefficients were calculated to investigate the relationship between FSS, oxidative stress and exercise variables. **Results:** SLE patients (N=14) were similar to controls (N=8) in age, body mass index and baseline F2 isoprostane. Control subjects were able to reach a (p=0.0366) higher peak heart rates during exercise than the Lupus patients, even though there was no significant difference in RPE. There was a trend towards higher VO2peak and increased exercise duration in the controls. We observed a reduction in the exercise-induced oxidative stress response in lupus patients compared to healthy controls. In controls, peak exercise was associated with increased F 2 isoprostane reflecting transient low grade oxidative stress. F2 isoprostane was increased over baseline at peak exercise in the controls. Both fatigued (FSS>or =4) and not-fatigued (FSS<4) lupus patients had reduced F2 isoprostane at peak exercise. In both Lupus patients and healthy controls, F2 isoprostane returned to baseline during a 30 minute post exercise recovery period. **Conclusions:** We observed impaired generation of F2 isoprostane during peak exercise in lupus patients suggesting a defect in the protective adaptive response to exercise. In healthy persons, repeated bouts of oxidative stress during exercise provide a favorable stimulus resulting in adaptive up-regulation of anti-oxidant defenses. Failure of the adaptive response to oxidative stress generation during exercise could lead to a vicious cycle of oxidative stress, activation of redox sensitive signaling pathways and persistence of a pro-inflammatory milieu which could contribute to the pathogenesis of physical fatigue in SLE.

PO1.B.12

Impaired control of the tissue factor pathway of blood coagulation in Tasmanian patients with systemic lupus erythematosus

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Objectives: Thrombosis is a frequent manifestation in patients with systemic lupus erythematosus (SLE), although the precise mechanisms remain unclear. This study investigated whether the major physiological trigger of blood coagulation, the tissue factor (TF) pathway, was altered in SLE patients. Furthermore, we investigated potential associations between the TF pathway, the presence of antiphospholipid antibodies (APA), e.g., anticardiolipin (aCL) antibodies, lupus anticoagulants (LAC) and anti-beta2-glycoprotein-1 (anti-β2GP1) antibodies, and other abnormalities present in SLE. **Methods:** 101 subjects [40 SLE patients and 61 age- and sex-matched controls] were recruited from Tasmania, Australia. Markers of the TF pathway [TF, free and total tissue factor pathway inhibitor (TFPI) antigen, and TFPI activity], hypercoagulability [thrombin-antithrombin (TAT) complexes and prothrombin fragment 1+2 (F1+2)], inflammation [interleukin-6 (IL-6)], and endothelial cell damage [soluble E-Selectin (sE-Selectin)] were measured in the plasma of both patients and controls. Additionally, serum levels of APA (aCL IgG and IgM isotypes, LAC, anti-β2GP1 and anti-prothrombin antibodies) were also determined. **Results:** SLE patients had higher levels of LAC (p=0.0102), anti-β2GP1 (p=0.0139) and anti-prothrombin (p=0.0139) compared to normal controls. Furthermore, SLE Patients had almost twice the plasma levels of TFPI free antigen (patients vs controls; mean ± S.E.M) (11.64 ± 0.89 ng/mL vs 6.43 ± 0.42 ng/mL; p<0.0001), but approximately half the TFPI activity (0.66 ± 0.07 U/mL vs 1.22 ± 0.03 U/mL; p<0.0001), of normal controls. SLE patients had elevated TAT (18.18 ± 6.27 μg/L vs 4.79 ± 0.96 μg/L; p=0.01) and F1+2 (472.8 ± 51.6 nmol/L vs 355.9 ± 22.2 nmol/L; p=0.0212) compared to normal controls. Interestingly, there were no significant difference in the levels of aCL IgG, aCL IgM, TF, TFPI total antigen, IL-6 and sE-Selectin between normal

controls and SLE patients (all p>0.05). No TF pathway marker correlated with APA, inflammation or endothelial cell damage in SLE patients. **Conclusions:** Although a significant increase in the 'bioactive' free form of TFPI was demonstrated in SLE patients, this was not matched by a corresponding increase in TFPI activity. Indeed, the reduction in TFPI activity reflects significantly impaired functional control of the TF pathway in these patients. Moreover, changes to the TF pathway were not associated with abnormalities in SLE, including the presence of APA, type of APA, inflammation or endothelial cell damage. The results from this study suggest hypercoagulability in SLE may (in part) be due to reduced TFPI activity, a mechanism that appears to be independent of other abnormalities of SLE.

PO1.B.13

Monocyte surface expression of Fcγ receptor RI (CD64), a biomarker reflecting type-I interferon levels in SLE

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Introduction: More than half of SLE patients show evidence of excess Type I interferon (IFN-I) production, a phenotype associated with renal disease and certain autoantibodies. However, detection of IFN-I proteins in serum is unreliable and measurement of interferon stimulated gene (ISG) expression is expensive and time consuming. The aim of this study was to identify a surrogate marker for IFN-I activity in clinical samples for monitoring disease activity and response to therapy. **Methods:** Monocyte surface expression of Fcγ receptors, chemokine receptors and activation markers were analyzed by flow cytometry in whole blood from patients with SLE and healthy controls. FcγR expression also was measured in PBMCs from healthy controls cultured with Toll-like receptor (TLR) agonists, cytokines, or serum from SLE patients. Expression of ISGs was analyzed by real-time PCR. **Results:** Circulating CD14+ monocytes from SLE patients showed increased surface expression of FcγRI (CD64). The mean fluorescent intensity of CD64 staining correlated highly with the ISG expression (Mx1, Ifi44 and Ly6E). In vitro, IFN-I as well as TLR7 and TLR9 agonists induced CD64 expression on monocytes from healthy controls. Exposure of monocytes from healthy controls to SLE sera also up-regulated the expression of CD64 in an IFN-I-dependent manner. Decreased CD64 expression was observed concomitant with the reduction of ISG expression following high-dose corticosteroid therapy. **Conclusion:** Expression of CD64 on circulating monocytes is IFN-I inducible and highly correlated with ISG expression. Flow cytometry analysis of CD64 expression on circulating monocytes is a convenient and rapid approach for estimating IFN-I levels in SLE patients.

PO1.B.14

Hyperprolactinemia is correlated with anemia and proteinuria and may predict serositis in SLE patients

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Introduction: An important immune modulator, prolactin inhibits the negative selection and maturation of autoreactive B cells. In a previous study we found hyperprolactinemia in 21% of 100 lupus patients. We did not find correlation of hyperprolactinemia with disease activity, however hyperprolactinemia significantly associated with anemia and proteinuria. The present study investigated a larger cohort of patients seeking a linkage between hyperprolactinemia and either disease manifestations or disease activity. **Methods:** Prolactin level was measured in 256 out of 274 lupus patients using the LIAISON® Analyser (DiaSorin). Disease activity was defined as present if SLEDAI>4 or ECLAM>2. Subject's clinical laboratory and therapeutic data and also disease activity were compared by a dichotomized prolactin level defined as high (>498 mIU/L for women aged 45 years or younger; >392 mIU/L for post menopausal women and men). **Results:** The cohort was comprised of 274 lupus patients in age 13-77, median age 37, 89% females and disease duration of 10.6+ 7.7 years. Hyperprolactinemia was present in 46/256 (18%) subjects. Compared to normoprolactinemic subjects, those with hyperprolactinemia had significantly more serositis (40% vs. 22.4%, p=0.01). More specifically, pleuritis (33% vs. 11%, p=0.02), pericarditis (30% vs 12%, p=0.002) and peritonitis (15% vs 0.8%, p=<0.0001). Compared to others, hyperprotactinemic subjects exhibited marginally more proteinuria (65.5% vs. 46 %, p=0.09) and anemia (42% vs. 26%, p=0.065). Hyperprolactinemia was not significantly associated with other disease manifestations, serology or pharmacologic interventions. Disease activity measured either by ECLAM or by SLEDAI score was not associated with hyperprolactinemia. **Conclusions:** Hyperprolactinemia is more common in SLE patients, and is associated with serositis including pleuritis, pericarditis and peritonitis and with anemia and proteinuria, but not with other clinical, serological and therapeutic measurers or with disease activity.

PO1.B.15

Elevated serum YKL-40: predictors of preatherosclerosis and subclinical atherosclerotic manifestations in systemic lupus erythematosus?

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Objective: Patients with systemic lupus erythematosus (SLE) have excess cardiovascular morbidity and mortality due to accelerated atherosclerosis that cannot be attributed to traditional cardiovascular risk factors alone. YKL-40, a chitinase, is increased in serum of patients with acute myocardial infarction. In patients with stable coronary artery disease high serum levels of YKL-40 are associated with cardiovascular mortality. One prior study has shown that patients with SLE have increased serum levels of YKL-40. Flow-mediated vasodilation of the brachial artery (FMD) has become a broadly accepted indicator of endothelial dysfunction. Flow-independent vasodilation (FID) represents vascular smooth muscle function. Intima-media thickness of the common carotid artery (ccIMT) is a validated non-invasive anatomic measure of subclinical atherosclerosis. In a cross-sectional study including 94 patients with SLE we examined the relation between FMD, FID, ccIMT and high serum levels of YKL-40. **Methods:** FMD, FID and ccIMT were determined by means of ultrasonography. FMD and FID were determined in all SLE patients whereas ccIMT was only assessed in a random subset of 37 patients. YKL-40 was measured by radioimmunoassay in serum from all patients. Increase of serum YKL-40 was defined by means of an age-dependent regression model based on a population study. The following traditional and non-

traditional cardiovascular risk modifiers were assessed: male sex, age, blood pressure, smoking, body mass index, serum cholesterol, insulin resistance (HOMA model), C-reactive protein, SLEDAI, treatment ever with glucocorticoids, HCQs and other immunosuppressive drugs. **Results:** Twenty-eight patients (30%) had increased serum levels of YKL-40. The median FMD was 5.9% (range: 0 – 15%), the median FID was 17% (range: 4.9 – 37%) and the median ccIMT was 0.57 mm (range: 0.37 – 0.93 mm). Using non-parametric Mann-Whitney test we found high YKL-40 to be associated with low FID (P-value = 0.014) and high ccIMT (P-value = 0.010). There was no significant difference in FMD between high and low serum YKL-40 (P-value = 0.614). The difference in ccIMT remained significant in multivariate analysis including traditional and non-traditional cardiovascular risk modifiers (P-value < 0.001). The difference in FID between low and high serum YKL-40 was not significant in a multivariate analysis (P-value = 0.097). **Conclusion:** High serum YKL-40 was associated with ccIMT independently of the effects of traditional and non-traditional cardiovascular risk modifiers but not to FMD and FID. Prospective studies are needed to further substantiate if YKL-40 could be a marker of preatherosclerosis and subclinical atherosclerotic manifestations in patients with SLE.

PO1.B.16

Neither anti-dsDNA-antibodies nor C3, but type I interferon-regulated SIGLEC1 correlates longitudinally with disease activity in systemic lupus erythematosus

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Introduction: Contradictive reports exist about the longitudinal association of type I interferon regulated genes or proteins with disease activity in SLE. We recently described SIGLEC1, an adhesion molecule exclusively expressed on blood monocytes and tissue macrophages, as surrogate marker for IFN responses detectable by flow cytometry. In the present report, autoantibody titres, level of complement component 3 and the expression of SIGLEC1 were longitudinally monitored in parallel and correlated to disease activity of SLE patients. **Methods:** 25 outpatient and inpatient SLE-patients were visited in free time intervals (4 until 12 weeks) - in total 82 patient-visits. SLEDAI2k, BILAG2004, medication, standard laboratory, autoantibodies, complement components and SIGLEC1 were assessed. Differences of SLEDAI2k and BILAG2004 were correlated with changes of biomarkers using linear regression. **Results:** Neither anti-dsDNA-antibodies (SLEDAI2K: p=0.14; BILAG2004: p=0.53) nor C3 (SLEDAI2k: p=0.11; BILAG2004: p=0.37) were significantly associated with lupus activity over time. Only changes in expression of SIGLEC1 were significantly correlated with changes of BILAG2004 (p=0.005), but not with SLEDAI2k (p=0.44). **Conclusions:** SIGLEC1 outperformed routine biomarkers of SLE with respect to detect changes in disease activity and therefore, is a convincing parameter that can be easily and quickly measured in standard diagnostic labs to adjust appropriate immunosuppressive therapies in a more personalized approach. Furthermore, Siglec1 is a promising biomarker to accompany currently discussed IFN-directed therapies.

PO1.B.17

Levels of low abundance proteins three months after start of induction therapy reflect treatment response at one year

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Objectives: Lupus nephritis (LN) results in renal failure in up to 42% of patients after five years. However, traditional biomarkers and clinical indicators of treatment response often cannot detect treatment failure until irreversible damage to the kidneys has occurred. Therefore, a more reliable means of determining early response to induction therapy in LN is needed. We hypothesized that levels of multiple candidate low abundance urine proteins at the time of biopsy and three months after start of induction therapy would be different between treatment responders and nonresponders, offering the possibility of using the resulting panel to create predictive models of response to therapy. **Methods:** 62 subjects from the Charleston and Baltimore LN inception cohorts and the Genentech phase III trial of Rituxan in LN (LUNAR) study population were recruited. Urine samples were collected at entry and after three months of induction therapy (per the primary rheumatologist or per the LUNAR protocol for analysis). Urine levels of 17 candidate low abundance proteins (chemokines, growth factors, cytokines, and renal damage markers) were determined by the multiplex bead array or ELISA. American College of Rheumatology renal function response criteria for were used to determine responders (n=46) and non-responders/partial responders (n=16). Entry and three month urine candidate protein levels were compared between responders and partial/non-responders by Mann-Whitney U test or Student t-test, and p values < 0.05 were considered significant. Results were reported as mean ± standard error. **Results:** Only one marker, IFNα2, was higher in non-responders than responders (26 ± 8 vs. 19 ± 5 pg/ml) at baseline. Levels of the following proteins were greater in non-responders at three months: IFNα2 (34 ± 16 vs. 6 ± 2) IL1α (77 ± 35 vs. 10 ± 3), soluble IL2 receptor antagonist (Ra, 1143 ± 491 vs. 360 ± 72), IL8 (266 ± 123 vs. 39 ± 12), IL12 (408 ± 204 vs. 31 ± 14), cystatin C (331 ± 1 vs. 242 ± 8), N-acetyl-beta-D-glucosaminidase (NAG, 20 ± 8 vs. 3 1), and neutrophil gelatinase associated lipocalin (NGAL, 105 ± 3 vs. 30 ± 7). **Conclusions:** This study demonstrates that several biomarkers representing multiple pathogenic mechanisms measured 3 months after initiation of therapy reflect response at one year, offering clinicians an early window into treatment response. Machine learning algorithms will be used to create predictive models of response to therapy when one-year followup is complete for the 95 subjects in this multicenter cohort.

PO1.B.18

Vitamin D deficiency in Korean patients with systemic lupus erythematosus

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Objective: Vitamin D is a pleiotrophic hormone with immunoregulatory properties. Low levels of vitamin D were found in systemic lupus erythematosus (SLE) and other autoimmune diseases. We have investigated the prevalence of vitamin D insufficiency in SLE and the relationship between vitamin D and disease activity markers of SLE. **Method:** Blood samples were prospectively collected from SLE patients (n=104) and normal healthy controls (NC, n=49) from March 2008 to May 2008. The level of serum 25-hydroxyvitamin D (25(OH)D) was measured by radioimmunoassay and expressed as ng/mL. The levels of antichromatin antibodies were measured by enzyme linked immunosorbent assay (ELISA) and expressed as arbitrary unit (AU). The SLE patients were also evaluated for clinical, laboratory parameters, systemic lupus erythematosus disease activity index (SLEDAI) and systemic lupus international collaborative clinics/American College of Rheumatology damage index (SLICC/ACR DI). **Results:** The 25(OH) D levels of the SLE patients (42.49±15.08 ng/mL) were significantly lower than NC (52.72±15.19 ng/mL, p<0.001). Additionally, 17 SLE patients (16.3%) had vitamin D insufficiency, defined as 25(OH)D levels below 30 ng/mL and two in NC (4.1%). Three of SLE patients (2.9%) had vitamin D deficiency, defined as 25(OH)D below 20 ng/mL but none in NC. The risk of vitamin D insufficiency was 4.6 fold increased in SLE (p=0.032).

The serum 25(OH)D levels, adjusted with BMI were positively correlated with hemoglobin (β=0.256, p=0.018) and serum complement 3 (β=0.365, p=0.002). Any significant correlation wasn't found between the levels of serum 25(OH)D and disease activity markers like antichromatin antibody, anti-dsDNA antibody and SLEDAI. **Conclusion:** Serum vitamin D levels were lower and vitamin D insufficiency was more common in Korean SLE patients, however our study demonstrated that vitamin D levels might not be a good marker of disease activity.

PO1.B.19

Urine proteomics of active lupus nephritis

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Background: Non invasive test of urine has been developed to diagnose active lupus nephritis. Urine proteomic tool can discover novel biomarkers. We evaluated whether 2-dimension gel electrophroresis (2-DE) of urine could distinguish active lupus nephritis. **Methods:** Pre-biopsy samples from 50 mL of spot urine were collected and its supernatant was kept in -80 °C. Protein precipitation with ethanol and further separated by 2-DE was performed. Protein spot identification was analyzed by Imagemaster 2D platinum software. Selected proteins were validated by ELISA method. **Results:** Thirty urine samples were collected from biopsy-proven lupus nephritis (active =15, inactive = 15). Ten and five samples were from healthy volunteers and other glomerular diseases. Thirty-seven protein spots were differentially expressed between active and inactive LN. Most proteins are transferrin, kininogen, immunoglobulin, alpha-1 beta glycoprotein, prostaglandin D2 synthase, and zinc-alpha 2 glycoprotein (ZAG). Urine ZAG was significantly increased in active LN as compared to inactive LN and healthy volunteer (Table1).

Groups	ZAG(mean±SEM)
Healthy Volunteer	982.7±451.4
Inactive LN	2106±706.8*
Active LN	9271±1457*
Glomerular disease	14440±3773*

*p≤0.001 compare with Healthy volunteer

Conclusions: Novel urine biomarker (ZAG) has been identified by proteomic approach. Urine ZAG should be further evaluated for its potential non invasive test in lupus nephritis.

PO1.B.20

Lipoprotein (a), oxidised LDL and carotid atherosclerosis in patients with systemic lupus erythematosus

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Objective: The aetiology of atherosclerosis in systemic lupus erythematosus (SLE) appears to be multi-factorial. The inflammatory process may play a role in part by mediating alterations in lipids e.g. oxidation in addition, several studies have noted increased lipoprotein(a) [Lp(a)] in SLE patients. The aim of this study is to determine the association between lipid subtypes and subclinical atherosclerosis in a population with SLE. **Methods:** 168 women with SLE (≥4 1997 ACR criteria) and 56 healthy controls were studied. Sub-

clinical atherosclerosis was assessed using B mode Doppler ultrasound of the carotid artery to measure the carotid intima-media thickness (cIMT) and identify carotid plaque. Oxidised-LDL and Lp(a) were measured by ELISA and immunolatex enhanced immunoassay methods, respectively. **Results:** The mean (SD) age of the patients and controls were 52(11) and 47(14) yrs respectively. There was no significant difference in the mean (SD) cIMT between the two groups: 0.06 (0.01) vs 0.07 (0.1) cm; P=0.1. Patients with SLE tended to have a higher prevalence of carotid plaque 26% vs 14%; P=0.07. Oxidised-LDL was significantly higher among SLE patients [median (IQR) 76 (57, 99) vs 56 (42, 88) U/l, P=0.004]. Lp(a) also tended to be higher in SLE patients (p=0.08). In SLE and controls there was a significant correlation between oxidised-LDL and mean cIMT (R=0.15, P=0.04 and R=0.29, p=0.02 respectively). There was a positive association between oxidised-LDL and carotid plaque in SLE only (R=0.16, P=0.04). Lp(a) was significantly associated with carotid plaque (R=0.19, p=0.01) and weakly associated with mean IMT (R=0.13, p=0.09) in SLE but not controls. **Conclusions:** Oxidised-LDL and Lp(a) are associated with subclinical atherosclerosis in patients with SLE. Control of the inflammatory process may reduce oxidative stress and reduce the development of atherosclerosis in this population.

PO1.B.21

Relationship between markers of inflammation and common carotid intima-media thickness in patients with systemic lupus erythematosus and antiphospholipid syndrome

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Background: Low grade inflammation may present in patients (pts) with antiphospholipid syndrome (APS) in relation to specific risk factors, such as C-reactive protein (CRP) and cytokines. The only presence of antiphospholipid antibodies and traditional risk factors (TRF) of atherosclerosis cannot explain all spectrums of clinical features in APS pts. **Objectives:** to estimate levels of tumor necrosis factor α (TNFα), soluble TNFα-receptor 1 (sTNFα-R1) and high sensitive CRP (hs-CRP) in pts with systemic lupus erythematosus (SLE) and APS depending on common carotid intima-media thickness (IMT). **Methods:** A total of 156 pts (52 with primary APS (PAPS), 48 with SLE+APS and 56 with SLE without any APS manifestations) and 28 age– and sex–matched controls were assessed. They underwent electrocardiography, echocardiography, ultrasonography and laboratory testing including antiphospholipid antibodies and lipid profile of plasma. sTNFα-R1 and hs-CRP were measured in all pts and controls, TNFα – in 126 pts and 28 controls. TRF of atherosclerosis (according to the verified Framingham risk assessment formula) were assessed in all pts and controls. **Results:** Concentrations of TNFα and hs-CRP (3.59 [2.18; 6.48] pg/ml and 2.69 [0.95; 8.14] mg/l) were higher in groups of pts than in controls (0.34 [0.28; 0.89] pg/ml and 1.07 [0.43; 1.94] mg/l) respectively (p<0.05 in all cases), and did not differ between the pts` groups. Serum levels of sTNFα-R1 in SLE+APS pts (3.86 [2.24; 6.25] ng/ml) and in SLE pts (2.71 [2.10; 5.08] ng/ml) were significantly higher than in PAPS pts (2.25 [1.90; 3.26] ng/ml) and in controls (2.19 [1.93; 2.56] ng/ml), p<0.001. Atherosclerotic plaques (ATP) and thick IMT were registered with the same frequency in pts and controls: 14% and 25% vs 3% and 11%, respectively (p<0.05 in all cases), and were more frequent in pts older 50 years (p<0.05). Concentrations of sTNFα-R1 were significantly higher in pts with ATP (4.00 [2.90; 4.12] ng/ml) than in pts with thick (2.94 [2.30; 3.50] ng/ml) and normal IMT (2.18 [1.81; 2.55] ng/ml). There weren't any relationship between the TNFα, hs-CRP and IMT. Serum levels of sTNFα-R1 were correlated with IMT and Framingham risk score (Rs= 0.17 и 0.20, respectively, p<0.05), levels of TNFα – with concentrations of hs-CRP (Rs= 0.28, p<0.05). **Conclusion:** Increased levels of TNFα, sTNFα-R1 and hs-CRP were associated with thick IMT and the presence of ATP in pts with SLE with and without APS, but not with PAPS.

PO1.B.22

Serum fetuin-A (alpha HS-glycoprotein): correlation with other markers of disease activity in systemic lupus erythematosus

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Objective: To assess the serum level of fetuin-A, a negative acute phase reactant, in systemic lupus erythematosus (SLE) patients and to elucidate its correlation with clinical features, other laboratory parameters and overall disease activity. ***Methods:*** The study comprised 59 SLE patients (49 women and 10 men, mean age 44.7 years, mean disease duration of 6.2 years) diagnosed according to ACR criteria for SLE and 30 healthy age and sex matched controls. Disease activity was assessed by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Laboratory investigations included complete blood count, erythrocyte sedimentation rate (ESR), urine analysis, 24-hour urine protein measurement, serum creatinine, antinuclear antibody (ANA), anti-double stranded DNA (antidsDNA), complement component C3 and anti-C1q antibodies. Serum fetuin-A levels were measured by enzyme linked immunosorbent assay (ELISA). ***Results:*** Serum fetuin-A levels were found to be significantly lower in SLE patients compared to age and sex matched healthy controls, $p < 0.01$. Furthermore, the levels were significantly lower in active SLE patients compared to inactive SLE patients, $p < 0.05$. The results demonstrated a significant positive correlation between disease activity (SLEDAI) and serum fetuin-A levels, $p<0.001$; $r = 0.551$. There was a significant negative correlation between serum fetuin-A levels and ANA, anti-dsDNA, serum creatinine, 24-hour urine protein and C1qAb respectively ($p< 0.03$, $r = -0.488$; $p< 0.01$, $r = -0.521$; $p< 0.08$, $r = -0.401$; $p< 0.06$, $r = -0.411$; $p< 0.001$, $r = -0.585$ respectively). There was a significant positive correlation between fetuin-A levels and C3 levels, $p< 0.05$, $r = 0.546$. The findings showed that fetuin-A levels were significantly lower in patients with renal involvement than in to those without renal involvement, $p < 0.01$. Renal involvement was present in 21/59 patients. No correlation was found between age, disease duration and fetuin-A levels. ***Conclusion:*** The findings of the present study suggest that measurement of serum fetuin-A levels in SLE appears to be a useful addition to the clinical and other laboratory parameters and may be a useful biomarker in the monitoring of disease activity and progression particularly in the presence of renal involvement.

PO1.B.23

Evaluation of erythrocyte C4d to complement receptor 1 ratio in systemic lupus erythematosus by using CR1-2B11

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Increased erythrocyte-bound complement 4d (E-C4d) and decreased erythrocyte-bound complement receptor 1 (E-CR1) have been observed in systemic lupus erythematosus (SLE) patients and may be diagnostic biomarkers. The E-C4d/E-CR1 ratios might be augmented by the reciprocal changes in lupus patients. This study focused on more accurately measuring E-CR1 independent of polymorphisms, determining the ratio by using CR1-2B11 and examining its cut-off value to differentiate SLE from other disease. We enrolled 80 lupus patients, 72 patients with other diseases, and 72 healthy controls. A newly identified CR1-2B11 antibody specifically recognized an epitope of CR1 and was used to detect E-CR1. The E-C4d and E-CR1 were detected with indirect immunofluorescence staining and analyzed by flow cytometry. The E-C4d/E-CR1 ratios were calculated and compared with patient diagnosis. SLE patients had higher E-C4d levels than healthy controls and patients with other diseases (5.47 ± 0.5 vs. 0.64 ± 0.07 and 0.91 ± 0.09 mean fluorescence intensity (MFI) respectively, $P < 0.001$ for both). Conversely, E-CR1 levels in the SLE group were lower than in these two groups (1.94 ± 0.15 vs. 5.58 ± 0.24 and 3.74 ± 0.21 MFI, $P < 0.001$ for both). Furthermore, the E-C4d/E-CR1 ratios in SLE patients were significantly increased compared with the two groups (3.76 ± 0.38 vs. 0.12 ± 0.01 and 0.3 ± 0.03, $P < 0.001$ for both). The range of E-C4d/E-CR1 ratio in all patients is between 0.017 and 13.9.

The area under the curve in ROC curve was 0.992 (95% CI: 0.985 - 0.999) indicating a good performance of the value. Using a cut-off value > 0.53, we could distinguish SLE patients (n = 80) from healthy controls and patients with other disease combined (n = 144). The reciprocal changes in E-C4d and E-CR1 levels are augmented in SLE patients. Determination of E-C4d/E-CR1 ratios may provide a potential biomarker for SLE diagnosis.

PO1.B.24

Urine biomarkers of renal pathology in lupus nephritis

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Objectives: Kidney pathology is important for choosing therapy and monitoring disease progress in LN. Because it is not practical to biopsy an SLE patient at every renal flare, a non-invasive test that accurately reflects renal pathology is highly desirable. The objective of this study was to identify urine biomarkers that can be used as surrogates for specific pathologic kidney lesions, such as necrosis, crescents, inflammation, or fibrosis/scarring. ***Methods:*** 47 urine samples were obtained at the time of diagnostic biopsy for LN, fractionated to remove proteins larger than 30 kDa, and spotted onto weak cation exchanger protein chips (CM10 chips) for proteomic analysis by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS). Urine samples were grouped by pathologic findings according to the type of glomerular injury (defined as no endocapillary proliferation, endocapillary proliferation, cellular crescents, necrosis), or the degree of interstitial inflammation, or the degree of interstitial fibrosis and atrophy (defined as none, mild, moderate-severe). Differential protein expression was tested by ANOVA followed by t-test and a $p < 0.05$ was considered significant. ***Results:*** SELDI-TOF MS identified 169 protein ions between 2-20 kDa with a signal-to-noise ratio ≥ 15. Protein ions present in >50% of samples were chosen for statistical analysis. Thirteen urine biomarker candidates were differentially expressed between specific pathologic findings in LN biopsies: 4 for glomerular injury, 8 for interstitial inflammation and 7 for interstitial fibrosis and atrophy. To date 6 of these candidates have been identified, including isoforms of hepcidin, fragments of α1-anti-trypsin, and albumin. To verify SELDI-TOF MS findings, urine hepcidin was quantitatively measured by EIA and found to be increased specifically in moderate and severe interstitial inflammation. Urine hepcidin showed no correlation to liver fatty-acid binding protein or β2-microglobulin, both markers of proximal tubular injury. ***Conclusions:*** Urine protein profiling at the time of kidney biopsy for LN identified 13 potential biomarkers of specific renal pathologic lesions. One of these, hepcidin, may be a marker of renal interstitial inflammation. The lack of correlation with proximal tubular injury markers suggests that urine hepcidin is not increased because filtered hepcidin is not reabsorbed by the proximal tubule. This is consistent with our previous finding that hepcidin is expressed by interstitial leukocytes in LN.

PO1.B.25

E-selectin and VCAM-1 as biomarkers of disease in patients with SLE

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Introduction: Cellular adhesion molecules specifically E-selectin and Vascular Cell Adhesion Molecule-1 (VCAM-1) are produced in response to vascular activation and may reflect inflammatory or atherogenic stimuli. They are associated with increased cardiovascular risk in the general population. Some studies have found increased levels in lupus and correlation with disease activity, damage and specific disease manifestations such as nephritis and skin disease. We tested the hypothesis that circulating levels of VCAM-1 and E-selectin are increased in lupus, and that they correlate

with disease activity, overall damage and specific manifestations, in particular lupus nephritis and clinical cardiovascular disease. **Methods:** We conducted a cross sectional cohort-control study of patients with SLE and healthy controls. E-selectin and VCAM-1 were measured on fasting blood using standard ELISAs (R+D systems). We compared levels in patients and controls and also examined correlations with SLEDAI-2000, SLICC damage index and the presence of skin disease, nephritis and a history of cardiovascular events. **Results:** We studied 179 women with SLE and 69 healthy females. The mean (SD) age was 54 (11.17) and 49 (14.66) years respectively (P=0.019). There were significantly higher levels of E-selectin in SLE (P=0.009) however this increase was not found in any specific disease subgroup and did not correlate with disease activity, damage or cardiovascular events. Levels of VCAM-1 were not significantly different in the two groups (P=0.192) however in patients with active nephritis levels, VCAM-1 levels were significantly increased (P=0.009). Levels of VCAM-1 were increased in all smokers (P<0.0023). **Conclusions:** E-selectin is significantly raised in SLE and while not correlated with a specific disease subtype may reflect ongoing low-grade vascular inflammation in these patients. Although not significantly raised in lupus patients, VCAM-1 may be important in the pathogenesis of atherosclerosis as it is associated with smoking in both groups. The raised levels in active nephritis have been replicated in other studies and VCAM-1 may be a marker of disease activity and vascular risk in lupus nephritis.

PO1.B.26

Platelet C4d is associated with all-cause mortality in patients with systemic lupus erythematosus

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Objectives: Platelets bearing complement C4d (P-C4d) are reported to be specific for a diagnosis of systemic lupus erythematosus (SLE) and are associated with ischemic stroke. We investigated the association of P-C4d with all-cause mortality and prevalent cardiovascular disease (CVD) events in our longitudinal cohort of patients with SLE. **Methods:** We recruited 356 consecutive outpatients or inpatients with SLE since July 2001. Outcomes were all-cause death and cardiovascular events including myocardial infarction, coronary artery bypass graft, percutaneous coronary transluminal angioplasty, stroke, pulmonary embolism, deep vein thrombosis or other thrombosis. P-C4d status was determined by flow cytometry. Multivariable logistic regression was utilized to assess the independent association between P-C4d and each cardiovascular outcome variable. Multivariable Cox proportional hazard regression was performed to assess the independent association between P-C4d and all-cause mortality. **Results:** Mean age was 44.4 years (range: 18-81 years), 92% were female, and 81% were Caucasian. Mean SLE disease duration was 15 years at baseline. Mean duration of follow-up was 4.7±2 years. Seventy SLE patients (20%) had positive P-C4d at baseline. P-C4d-positive patients were more likely to have a history of renal disease, seizure disorder, hemolytic anemia, thrombocytopenia, anti-double stranded DNA (dsDNA) and/or antiphospholipid antibodies. Overall CVD event frequency was 21.6%. SLE patients with positive P-C4d had significantly more CVD events compared to those with negative P-C4d (35.7% vs. 18.2%, p=0.001). Positive P-C4d at baseline was associated with stroke, but not with other cardiovascular events (odds ratio 4.96, 95% confidence interval 1.75-14.06, p=0.003) after adjusting for age, race, smoking history, SLE disease duration, renal disease, dsDNA and antiphospholipid antibodies. The overall mortality was 3.9%. Causes of death were infection (n=4), cardiac arrest (n=2), congestive heart failure (n=1), cancer (n=2), hemorrhage (n=1), and unknown (n=4). Six of these 14 deceased patients had a history of cancer (ovarian carcinoma, lymphoma, lung cancer, anal squamous cell carcinoma). Positive P-C4d at baseline was associated with all-cause mortality (hazard ratio 7.92, 95% CI 2.13-29.48,

p=0.002) after adjusting for age, race, sex, SLE disease duration, renal disease, cardiovascular event, cancer, dsDNA and antiphospholipid antibodies. Baseline SLE activity and smoking history were not associated with stroke or all-cause mortality and did not attenuate the significant association between P-C4d and all-cause mortality. **Conclusions:** Platelet C4d is associated with all-cause mortality and stroke. Platelet C4d may be a prognostic biomarker as well as a pathogenic clue that links systemic inflammation, complement activation, and thrombosis.

PO1.B.27

Interleukin-18 (IL-18) in systemic lupus erythematosus: a novel marker of disease activity and a potential target for therapy?

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Objectives: Systemic lupus erythematosus (SLE) is an autoimmune disease with a complex pathogenesis. Cytokines and the ensuing inflammatory process play a vital role in SLE leading ultimately to irreversible organ damage. Interleukin-18 (IL-18) is a proinflammatory T helper-1 cytokine that induces interferon-gamma and may thus produce much damage in SLE. Accordingly, the aim of the present study was to investigate serum IL-18 in SLE patients before and after 4 months of hydroxychloroquine phosphate only therapy and to assess its effect on disease activity. **Methods:** A total of 66 SLE patients diagnosed according to the ACR criteria for SLE and 34 age-sex-matched healthy controls were recruited in this study. The serum levels of IL-18 were determined by ELISA. Serum levels of IL-6 and TNF alpha were also measured using the ELISA method. Anti-dsDNA antibody, CH50, C3, C4 and circulating immune complex levels were analyzed. Disease activity was measured using the SLE Disease Activity Index (SLEDAI). All parameters were measured before and 4 months after therapy. **Results:** At baseline serum levels of IL-18, IL-6 and TNF alpha were significantly higher in SLE patients when compared to the controls, p<0.005 and significantly higher in those SLE patients with higher SLEDAI, p< 0.05. After 4 months of hydroxychloroquine therapy, the mean serum levels of IL-18, IL-6 and TNF alpha decreased significantly in all patients, p< 0.05. IL-18 levels correlated positively and significantly with disease activity (SLEDAI score), p <0.05; r = 0.466 and with anti-dsDNA antibody titer, p< 0.05; r = o.484. IL-18 correlated significantly with TNF alpha, p < 0.05; r = 0.585. There was also a significant association between TNF alpha and SLEDAI score, p< 0.001; 0.623. **Conclusions:** The proinflammatory cytokine IL-18 is increased in SLE patients, indicating that it may play a crucial role in the inflammatory processes in SLE. Hydroxychloroquine therapy lowers the proinflammatory cytokines and is thus a valuable drug in SLE. Furthermore, as IL-18 correlates significantly with TNF alpha, TNF blockade could also be considered for use in SLE, particularly in active SLE. However, the development of a specific therapeutic agent for blocking IL-18 would be a welcome addition to the therapeutic armamentarium of this autoimmune disease.

PO1.B.28

Potential biomarkers identified from systemic lupus erythematosus patient peripheral blood B cell, T cell and myeloid cell transcriptomes

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Objectives: To characterize the transcriptomes of isolated leukocyte subsets obtained from SLE patients as compared to healthy controls and to determine whether proteins predicted to be differentially expressed by microarray an-

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alysis were associated with disease and might serve as biomarkers. ***Methods:*** Gene expression profiles of sorted peripheral blood leukocyte subsets including CD19+ B cells, CD3+CD4+ T cells and CD33+ myeloid cells from SLE patients (n=14) were compared to healthy controls (n=11). Analysis of Affymetrix HG-U133A chips was performed using a ≥ 1.5 fold differential expression and significance (p< 0.05) determined by applying the Welch t-test and the Benjamini Hochberg (BH) multiple testing correction to control for false discovery rates. Expression levels of cell surface and intracellular molecules were assessed by flow cytometry and plasma proteins were examined by ELISA. ***Results:*** Unique transcriptional profiles were observed in SLE CD19+ B cells (173 transcripts), CD3+CD4+ T cells (92 transcripts) and CD33+ myeloid cells (201 transcripts) as compared to controls. As predicted by the SLE transcriptomes, there was an increased frequency of Siglec-1 (CD169) positive SLE myeloid cells as compared to control myeloid cells. Likewise, the array transcripts for the endosomal molecules CD107a (LAMP1) and CD63 (LAMP2) were upregulated in SLE myeloid cells and correlated with active disease as did CD63 expression in SLE CD3+CD4+ T cells. High intracellular protein expression levels were observed for both CD63 and CD107a proteins in control and SLE leukocytes. Although activated cells can display these molecules on the cell surface, we rarely observed cell surface expression of CD63 or CD107a. SLE myeloid cells appeared to express increased levels of intracellular CD63 compared to controls. No consistent difference was noted for intracellular CD63 expression levels in T cells or for intracellular CD107a expression levels in T cells or myeloid cells. Elevated expression levels of CD38 transcripts were noted in SLE B cells which correlated with an increased frequency of CD38 positive B cells in SLE patients with active disease. Finally, elevated transcripts and protein expression for IFN-inducible molecules, such as Stat-1, were observed in SLE leukocytes. Increased expression of other IFN-inducible molecules are being validated at the protein level. Elevated levels of both plasma thioredoxin and galectin-3 were observed in some SLE samples as compared to controls consistent with the SLE transcriptomes. ***Conclusions:*** These studies suggest that leukocyte subsets in SLE express unique transcriptional profiles and this might translate to hyper-expressed proteins with potential pathogenic relevance in SLE.

PO1.B.29

**Lymphocyte-bound complement activation products
(LB-CAP) as biomarkers for SLE**

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Objectives: Systemic lupus erythematosus (SLE) is immunologically characterized by polyclonal lymphocyte activation, autoantibody production, and complement activation. Complement activation products bound to circulating lymphocytes, erythrocytes, and platelets have recently been shown to be specific biomarkers for diagnosing SLE. In this study, we explored the possibility that lymphocyte-bound complement activation products (LB-CAP) may serve as biomarkers for specific clinical/serological features and disease activity of SLE. ***Methods:*** A cross-sectional study was conducted which involved 224 patients with SLE, 179 patients with other diseases, and 114 healthy controls. LB-CAP on peripheral blood lymphocytes was measured by flow cytometry. Associations of LB-CAP with clinical and serological manifestations of SLE were investigated using logistic regression analysis. ***Results:*** Significantly elevated levels of C4d were detected specifically on T and B lymphocytes (designated T-C4d and B-C4d) of SLE patients. SLE patients with abnormally elevated (higher than the mean + 2 SD in healthy controls) T-C4d and B-C4d levels, as compared to those with normal T-C4d/B-C4d levels, were significantly younger (41.4 ± 11.6 y/o versus 45.2 ± 12.2 y/o; p = 0.034) and had longer disease duration (12.3 ± 9.8 years versus 8.3 ± 6.8; p = 0.002), suggesting a clinical disease that develops early and may be more active or serious in general. SLE patients with abnormal T-C4d/B-C4d levels were more likely to have a history of arthritis (odd ratio/OR = 3.0; 95% confidence interval/CI = 1.1-8.5; p = 0.025), serositis (OR = 2.7; 95% CI = 1.4-5.2; p = 0.001), renal disorders (OR = 2.0; 95% CI = 1.0-3.9; p =

0.035), hematologic disorders (OR = 2.7; 95% CI = 1.4-5.2; p = 0.002), and anti-dsDNA (OR = 5.1; 95% CI = 2.5-10.2; p < 0.001) and phospholipids autoantibodies (OR = 2.4; 95% CI = 1.4 – 5.2; p = 0.006). Notably, abnormal T-C4d/B-C4d was associated with lymphopenia (OR = 2.6; 95% CI = 1.3-5.3; p = 0.006), positive anti-dsDNA antibodies (OR = 6.2; 95% CI = 2.6-14.8; p < 0.001), and increased SLEDAI score (OR = 3.2; 95% CI = 1.7-6.1; p < 0.001) - parameters suggestive of recent active disease. ***Conclusion:*** These results suggest that LB-CAP, namely T-C4d and B-C4d, may serve as valuable biomarkers for organ involvement and disease activity in SLE patients. The potential use of T-C4d/B-C4d for clinical subsetting of SLE patients warrants further investigation.

PO1.B.30

Anti-C1q IgG levels neither forecast nor mark a lupus nephritis flare

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Objectives: The recognition of biomarkers of lupus nephritis (LN) that reliably identify, or more importantly forecast, a LN flare would greatly improve LN management. Anti-C1q antibodies have been shown to associate with LN, and reports have suggested that anti-C1q antibody levels can be used to forecast or mark a LN flare. However, this hypothesis has never been directly tested using regular (unbiased) serial measurements leading up to a LN flare. The present study tested this hypothesis. ***Methods:*** Anti-C1q IgG plasma levels were measured by ELISA in 38 normal individuals, and a cutoff of 3 standard deviations above the mean was used to identify anti-C1q positive samples. Single plasma samples were then tested from 101 SLE patients (66 with LN) enrolled in the Ohio SLE Study (OSS), a longitudinal study of flare pathogenesis in chronically active SLE patients who have been followed at regular bimonthly intervals for an average of over 4 years. The samples selected for this cross-sectional analysis were at the patients' first LN flare, or at baseline if they never experienced a LN flare. For the longitudinal analysis, anti-C1q positive patients who experience LN flares with available plasma samples at 8, 6, 4, and 2 months before, and at the time of LN flare (together termed a flare cycle) were tested for anti-C1q IgG. All samples from a flare cycle were assessed together. The same positive and negative controls were used in all patient assays, and all optical densities were normalized to the positive control. ***Results:*** For the cross-sectional analysis, 29% of the nonrenal SLE patients had positive anti-C1q IgG, compared to 64% of the LN patients (P < 0.001 by Fisher's exact test). For the longitudinal analysis, 21 LN flare cycles were identified from the anti-C1q-positive LN patients. No significant change was found in median anti-C1q IgG levels at flare (0.847 normalized OD, P = 0.235) or 2 months before flare (0.743, P = 0.488), compared to the levels at 8 months (0.909), 6 months (0.700), and 4 months (0.740) before flare (analyzed by Friedman's repeated measures ANOVA). ***Conclusions:*** The presence of plasma anti-C1q IgG is significantly associated with LN, confirming numerous previous reports. However, when comparing plasma levels at regular bimonthly intervals for 8 months leading up to and at the time of LN flare, anti-C1q IgG neither forecasted nor marked a LN flare.

PO1.B.31

Should therapy go beyond the control of immediate injury? Biomarkers of the vasculature and their association with longitudinal assessments in the induction phase of a randomized multicenter trial comparing mycophenalate mofetil and intravenous cyclophosphamide.

Robert, Clancy[1] the MMF/IVC Lupus Nephritis Induction Trial., Investigators i.[1] Kim, Mimi[2] Ginzler, Ellen M.[3]

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Objective: A major barrier to understanding and treating lupus nephritis (LN) is the paucity of sensitive and validated biomarkers. Adiponectin is expressed on the endothelium of all vessels in biopsies from patients with LN but decreased at inflamed areas. Adiponectin knockout mice suggest that adiponectin may be a key regulator of proteinuria. Increased expression of membrane EPCR in LN biopsies predicts a poor response to therapy. *Methods:* This study leveraged the LN induction trial comparing intravenous cyclophosphamide (IVC) and mycophenolate mofetil (MMF) to evaluate the relationship between clinical response of LN and adiponectin and sEPCR as a proxy for vascular "protective" molecules. Methods: Adiponectin, sEPCR, e-selectin, and nitric oxide (NO) were measured in 109 plasma from 48 patients in the LN induction trial. Response was evaluated using a prespecified primary endpoints related to urine protein/creatinine ratio and serum creatinine. Sample included visits 4 (4wks), 7 (15 wks) and 9 (24 wks-end of induction Rx). *Results:* A trend toward increased plasma adiponectin in responders vs nonresponders (19.2 ± 6.8 vs 16.4 ± 9.1 at visit 4; 13.0 ± 5.2 vs 11.7 ± 6.5 at visit 7; 13.7 ± 7.8 vs 10.9 ± 4.9 at visit 9) was observed. In patients with subnephrotic proteinuria (<3g/day urine protein; 63% of the total), plasma adiponectin was similarly increased in responders vs nonresponders at all visits. Moreover, when combining data across all visits nonreponders had significantly lower adiponectin (p=0.0032). There was a tendency of sEPCR to decrease in responders vs nonresponders (243 ± 164 vs 284 ± 167 at visit 4; 338 ± 271 vs 341 ± 197 at visit 7; 260 ± 104 vs 368 ± 216 at visit 9). In comparing MMF vs IVC, sEPCR, levels were significantly higher in the IVC group when data was combined over all visits (p=0.005). Consistent with evidence that therapy in the responder arm mobilizes vascular protective molecules, NO changed in the predicted direction (62 ± 47 vs 68 ± 66 at visit 4; 39 ± 46 vs 52 ± 65 at visit 7; 27 ± 33 vs 92 ± 55 at visit 9; p=0.02). Combining data across all visits, nonresponders had significantly higher NO levels than responders (p=0.046). sE selectin did not track with response. Conclusion: These results demonstrate that although MMF and CYC represent therapies which control immediate injury, MMF but not CYC may protect the vasculature, thereby attenuating the overall burden of disease.

PO1.B.33

Clinical significance of CD40 ligand in systemic lupus erythematosus (SLE)

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Objective: CD40/CD40L interactions are important in SLE pathogenesis by inducing T-cell mediated humoral immune responses. The aim of this study is to evaluate the role of sCD40L as a potential biomarker of SLE disease activity by correlating levels of this cytokine with clinical severity, cardiovascular risk factors and established markers of SLE activity. *Methods:* Twenty-five subjects with stable or disease exacerbation, ages 22-62 yrs, 2 males and 23 females, on various doses of steroids were evaluated in the Rheumatology clinic. The SLE Disease Activity Index (SLEDAI) was used to identify disease exacerbation that ranged from mild (2) to moderately severe (20), along with ANA, dsDNA, complement, urinalysis, urine protein-creatinine ratio and hematocrit levels. 13 of the 25 subjects were randomly selected and serum CD40L concentration via ELISA was measured and cardiovascular risk factors were assessed. Renal and hepatic profiles of these subjects were evaluated. All of the study subjects were on hydroxychloroquine. Results: sCD40L levels ranged from 0.26 to 3.9-ng/ml. Regression analyses using linear correlation

were prepared. sCD40L level was lower in 4 patients with SLE flare (high SLEDAI score) and was higher in 6 patients with stable SLE (low SLEDAI score) (p = 0.014, r2 = 0.55). Mean steroid dosages in flare vs. stable groups were 27 mg and 6 mg, respectively. In the 13 selected patients, 3 of 8 in the stable group versus all 5 patients in the flare group had cardiovascular risks.

Subject	Age	Sex	Disease category	BUN/ Creatinine	Prednisone Dose	SLEDAI	CV Risk Factors
1	34	F	Flare	52/4	20	20	HTN, Hypercholesterol
2	57	F	Flare	32/1.4	10	20	HTN, CVA
3	46	F	Flare	55/3.9	70	18	HTN, Recent HTN urgency
4	28	F	Flare	15/0.7	20	18	HTN, Hypercholesterol
5	55	F	Flare	17/0.8	7.5	19	HTN
6	42	F	Stable	20/1	13	4	Hypercholesterol, APLS
7	22	M	Stable	17/1.1	10	4	None
8	47	F	Stable	12/0.6	2.5	2	None
9	48	F	Stable	14/0.6	7.5	6	None
10	42	F	Stable	9/0.8	0	2	APLS
11	27	F	Stable	11/0.8	4	6	HTN, Morbid Obesity
12	33	F	Stable	13/0.8	6	10	None
13	24	F	Stable	14/0.8	0	2	None

Disease category (1) Stable (2) Flare
LRT-Liver related tests (ALT/AST/ALP/BIL) were normal in all subjects.
SLEDAI-SLE disease activity index
CV risk factors-cardiovascular risk factors
APLS-antiphospholipid antibody syndrome
HTN-hypertension

Conclusion: sCD40L plays a biologically active role in SLE with flares. Increased levels of sCD40L in serum and tissue are important in SLE pathogenesis. sCD40L concentrations correlated with subjects' cardiovascular risk profile, however corticosteroids in these patients can mask the secretion of sCD40L. Levels of sCD40L correlated with established markers for SLE flare (dsDNA, complement levels) in an inverse manner.

PO1.B.34

Oxidative stress markers and their correlation with severity of systemic lupus erythematosus

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Objective: Oxidative stress has been implicated as a contributing factor in various autoimmune diseases (ADs) including systemic lupus erythematosus (SLE). However, potential of oxidative stress in eliciting an autoimmune response, and its role in disease prognosis and pathogenesis in humans remains largely unexplored. This study investigates the status and contribution of oxidative stress in SLE. *Methods:* Sera from 72 SLE patients with various SLE scores (SLE disease activity index; SLEDAI) and 36 age- and gender-matched healthy controls were evaluated for oxidative stress markers, such as malondialdehyde (MDA)- and 4-hydroxynonenal (HNE)-protein adducts and their corresponding antibodies (anti-MDA- and anti-HNE-protein adduct antibodies), superoxide dismutase (SOD), and various autoantibodies, including ANA, anti-dsDNA, anti-Sm, anti-RNP and aCL. *Results:* Serum analysis showed significantly higher levels of MDA- and HNE-protein adducts and their corresponding antibodies, i.e., anti-MDA-and anti-HNE-protein adduct antibodies in SLE patients. Interestingly, our data showed not only increased number of subjects positive for anti-MDA- or anti-HNE-protein adduct antibodies, but also greater levels of these antibodies in SLE patients with higher SLEDAI (≥ 6), which were significantly higher than the lower SLEDAI group (<6). Data also showed a significant correlation between anti-MDA or anti-HNE antibodies and SLEDAI (r = 0.734 and 0.647 for anti-MDA and anti-HNE antibodies, respectively) suggesting a possible causal relationship

between these antibodies and SLE. Serum SOD was significantly lower in SLE patients with higher SLEDAI group (≥ 6) showing much lower SOD levels, suggesting a compromised antioxidant balance. Sera from SLE patients also had higher levels of various autoantibodies, including ANA, anti-dsDNA, anti-Sm, anti-RNP and aCL. *Conclusion:* Our findings support an association between oxidative stress and SLE. Stronger response in samples with higher SLEDAI suggests that oxidative stress markers may be useful in evaluating the prognosis of SLE as well as in elucidating the mechanisms of disease pathogenesis.

PO1.B.35

Monocyte chemoattractant-1 (MCP-1) as a urinary biomarker for the diagnosis of activity of lupus nephritis in Brazilian patients
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Background: Lupus nephritis is a major concern in systemic lupus erythematosus (SLE) and it affects more than 60% of patients during disease progression. Substantional evidence suggests that MCP-1 contribute to kidney injury in the SLE glomerulonephritis. *Objectives:* To evaluate urinary MCP-1 as a biomarker of renal activity in Brazilian lupus cohort using the ELISA test and compare it to other activity markers of the disease that are currently employed in clinical practice. *Methods:* Sixty female patients with a diagnosis of SLE and kidney involvement in treatment at the Rheumatology Sector of the Servidor Publico Estadual Hospital as well as patients without kidney involvement and a control group participated in the cross-sectional study. Patients were classified with regards to the disease activity based on clinical and laboratory parameters, such as alterations in urinary sediment, proteinuria, kidney function, renal clearance, C3, C4, native anti-DNA, the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and renal SLEDAI. The participants were distributed among four groups: Group 1 (30 lupus patients with kidney involvement in disease activity); Group 2 (30 lupus patients with the kidney condition in remission); Group 3 (15 lupus patients without kidney involvement); and Group 4 (17 apparently healthy individuals as controls). The levels of MCP-1 were measured by using a specific ELISA according to the manufacturer's protocols (BioSource International Inc.). *Results:* The mean MCP-1 (pg/mg creatinin) of Group 1 was significant higher than that of health controls and the others SLE patients (p<0,001). With the aim of applying this test in the detection of LN, a cut-off point was established using the results from the control group. The cut-off obtained was 596,2. Group 1 had a significantly greater frequency of positive results for urinary MCP-1 in comparison to the other groups (p<0,001), demonstrating that this is a promising chemokine for the screening of lupus patients in renal activity. With the aim of detecting disease activity in patients with LN, a new cut-off point was determined based on the results of the lupus patients with kidney involvement in remission. Setting specificity at 90%, the sensitivity of the test was 56.7%. *Conclusion:* The determination of this chemokine, using an accessible, easily performed immunoassay, contributes toward the diagnosis of lupus nephritis activity. Associated to other parameters employed in clinical practice, this biomarker represents an advance in the diagnosis and early establishment of adequate treatment for these patients.

PO1C Clinical Aspects - CNS, Renal, Skin, All Other

PO1.C.1

Antiphospholipid syndrome nephropathy in patients with systemic lupus erythematosus: prevalence, clinical manifestations and renal functional outcome
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Background: The presence of antiphospholipid antibodies (aPL) has been associated with small vessel renal injury and chronic renal ischemia. The renal histologic findings associated with antiphospholipid syndrome (APS) include thrombotic microangiopathy (TMA), fibrous intimal hyperplasia (FIH), fibrocellular and fibrous arterial and arteriolar occlusions (FAO), focal cortical atrophy (FCA) and tubular thyroidization (TT). The common clinical manifestations are hypertension, acute renal failure or chronic low-grade proteinuria. Although it has been suggested the term "associated nephropathy aPL", renal involvement has not yet included within the classification criteria of APS. *Objectives:* To determine the prevalence, clinical manifestations and renal functional outcome of patients with lupus nephritis and associated APS nephropathy. *Methods:* Inclusions criteria: a SLE diagnosis of (ACR criteria) nephritis requiring renal biopsy. Patients with systemic sclerosis, hemolytic-uremic syndrome, systemic vasculitis, thrombotic thrombocytopenic purpura, diabetic nephropathy and preeclampsia were excluded. The histological samples were all analyzed by the same pathologist. *Results:* Seventy-nine biopsies were included (70 female [88,6%]). The mean age at time of biopsy was $33,3 \pm 16,6$ years. The follow-up period (time between the renal biopsy and the last medical visit) was 73 ± 51 months. Nine (11,4%) renal biopsies met diagnostic criteria for APS nephropathy. Three (33.3%) showed acute APS nephropathy. Histological lesions founded: 3 (3.8%) cases of TMA, 4 (5%) of FIH, and 3 (3.8%) of FCA. The SLE patients with APS nephropathy showed higher duration of SLE at the time of renal biopsy (p=0.02) and higher rate of chronicity (p=0.01). They also had higher prevalence of interstitial fibrosis (p=0.04), tubular atrophy (p=0.02) and nephroangiosclerosis (p=0.03) when compared with the group without APS nephropathy. The presence of aPL was associated to APS nephropathy (p=0.009). Association of positive lupus anti-coagulant and anticardiolipin (but not isolated positivity) was related to the development of APS nephropathy (p<0.001). There was no association between APS nephropathy and the presence of hypertension, nephrotic syndrome, hematuria, proteinuria or elevated serum creatinine levels. No significant difference in complete renal response, partial renal response and no response between groups were found. Patients with TMA showed higher prevalence of APS (p<0.001), extrarenal arterial thrombosis (p=0.02) and venous thrombosis (p=0.02) along their evolution *Conclusion:* The prevalence of APS nephropathy was 11,4%. There was no association between APS nephropathy and clinical manifestations or laboratory features except for increased developmental risk of APS and arterial and venous thrombosis. The long-term renal outcome was similar in SLE patients with or without APS nephropathy.

PO1.C.2

Childhood systemic lupus erythematosus (SLE): analysis of clinical and immunological findings in 74 patients
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Objective: The aim of this report is to describe the first instance of the clinical features of childhood lupus erythematosus. To define the pattern of disease expression in patients with childhood onset (SLE). *Material And Methods:* We studied prospectively 74 patients with childhood-SLE who were seen con-

secutively either as inpatients or outpatients between 2000 and 2008. All the patients fulfilled the 1982 (ACR) revised criteria for SLE and had the disease at or before the age of 16 years. In 74 patients, defined as the initial manifestation clearly attributable to SLE, occurred before the age of 16, and they represent the childhood onset group described in this report. **Results:** A fifteen -year retrospective analysis of the clinical features and survival of 74 Iranian children with (SLE) was made. Sixty five (88%) patients from the childhood onset group were female and nine male (12%) (ratio female/male, 7/1). Range of age at onset was 3-16 years (Mean age10 + - 2). During the evolution of the disease, the childhood onset patients had the mode of presentation was as follows: 74% had skin involvement, 77% had musculoskeletal involvement, 43% had renal disease, 33% had hematological abnormalities, 24% had pulmonary involvement, 17% had central nervous system involvement, and 16% had cardiovascular disease. Anemia in 59% of patients. Autoimmune thrombocytopenia purpura in 45% cases, Leukopenia with lymphopenia was the presenting feature in 16 % cases. ESR >85 in 78% cases, and positive (C-reactive protein) in 59% patients. Hematuria was the most frequent finding in these patients (47%). Proteinuria was the second finding in our patients (43%). Raised BUN and creatinine was seen in (21%). The Coombs' test was positive in 21% children, false positive VDRL in 16% patients with childhood-SLE. ANA positivity was detected in 97% of cases at presentation; the mean titer was >1:160 in all patients except 2 cases. All 2 children who were ANA-negative had at least a malar rash, oral ulcer, and associated with several mild manifestation. Anti-d DNA was positive in 83 % patients. Antiphopholipid antibody was in 13% patients. 10% of patients with SLE will be anti-Sm positive, low C3 (85%), low C4 (41%), and low CH50 complement (85%). **Conclusions:** Childhood-SLE is not a common illness in the pediatric population. Although Childhood-SLE has been reported in children in first the 10 to20 years of life, it is rare in children under 5 years of age,childhood onset patients as presenting clinical manifestations, while malar rash , photosensitivity, musculoskeletal involvement, hematological abnormalities, and renal disease were more common during the evolution of the disease.

PO1.C.3

Factors predictive of thrombosis in a multiethnic cohort of SLE patients
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Purpose: Thrombosis is an important cause of morbidity and mortality in SLE and it occurs frequently at a younger age than in the general population. We have now explored the factors associated with time to the occurrence of thrombotic events in SLE patients to expand previous observations made on this cohort (larger number of patients and years of follow up). **Method:** SLE patients (ACR criteria), age ≥16 years, disease duration ≤5years (T0), African-American, Hispanic (Texan or Puerto Rican) or Caucasian ethnicity, from a longitudinal cohort were studied. An event was defined as the presence of thrombosis in either an arterial or a venous territory. Time to the first trombotic event was examined by multivariable (MV) Cox models (full and parsimonious) adjusting for pertinent baseline clinically and sociodemographic variables. **Results:** A total of 643 patients were studied, with a mean (SD) age of 36.4 (12.6) and time of SLE at enrollment of 1.4 (1.3) years, 90% were female and all ethnic groups were represented (Hispanic-Texas: 19%, Hispanic Puerto Rican: 16%, African American: 37%, Caucasian: 28%). At baseline, 80% of the patients had health insurance, 14% were smokers, and 33% were below the poverty line; 72% were hydroxychloroquine users, 3% had diabetes, 36% had hypertension, 3% antiphospholipid antibodies and 27% anti-DNA antibodies. Sixty five (13. 4%) patients developed a thrombotic event [stroke: 7.5%, claudication : 0.6%, myocardium infarction: 2.3%, angina: 2.8%, and deep venous thrombosis: 1.7%]. The parsimonius Cox model is shown in Table 1. **Conclusion:** As expected, age, poverty, smoking, damage accrual, antiphospholipid antibodies and higher doses of glucocorticoids

were independently associated with a shorter time to the first thombotic event. Health insurance seems to have a protective effect. Modifiable risk factors at the personal (smoking, high doses glucocorticoids) or societal (poverty, health insurance) should be acted upon to prevent these events and improve the outcome of our lupus patients.

Table 1. Cox Model for Baseline Variables Predictive of a Thrombotic Event

Variable	Odds Ratio	95% CI	p value
Age	1.05	1.03 - 1.07	<0.0001
Health Insurance	0.52	0.29 - 0.93	0.0271
Poverty*	1.60	0.95 - 2.70	0.0778
Smoking	1.89	1.03 - 3.49	0.0409
SDI †	1.65	1.41 - 1.93	<0.0001
Antiphospholipid antibodies‡	2.06	1.16 - 3.69	0.0144
Anti-DNA antibodies	0.55	0.30 - 1.02	0.0589
Glucocorticoid, maximum dose	1.01	1.01 - 1.02	0.0002

*As per the US Federal government guidelines adjusted for the number of persons in the household; † SDI, SLICC (Systemic Lupus International Collaborating Clinics) damage index; ‡IgG and/or IgM antiphospholipid and lupus anticoagulant.

PO1.C.4

Subclinical abnormalities in echocardiography and electrocardiography in patients with systemic lupus erythematosus
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Introduction: In patients with systemic lupus erythematosus (SLE), the cardiovascular system is frequently affected, and represents an important factor in determining their morbid-mortality and prognosis. **Objective:** To describe the echocardiographic and electrocardiographic findings in patients with SLE without cardiovascular symptoms. Method: In a cross-sectional study of 30 patients with SLE and 10 controls a M-mode echocardiography, Doppler and electrocardiography (EKG) was performed. The statistical analysis was conducted using non-paired t Student, Chi-square, Fisher exact test for small samples and test of correlation with Spearman's rho test. **Results:** Compared with the control group, patients with SLE had a higher prevalence of echocardiographic abnormalities and in the EKG. Pericardial effusion in 37%, concentric left ventricular hypertrophy 10% (3 vs1 p = 0.03), dilatation of right cavities 17%, dilated left cavities 3%, regurgitation : 20% mitral, tricuspid 53%, aortic 7% and pulmonary 10%. The ejection fraction was abnormal in 13% and diastolic dysfunction in 10% of the patients. 67% of cases had pulmonary hypertension (20 vs 1 p = 0.005). Sinus tachycardia was observed in 20% and 17% had left bundle branch hemiblock. **Conclusion:** We found a high prevalence of cardiac abnormalities, mainly of the pericardium, valvular and pulmonary vasculature in patients with SLE. Echocardiography is a sensitive method to detect these abnormalities and should be used routinely for evaluation of these patients.

Keywords: Echocardiography, systemic lupus erythematosus, cardiac abnormalities

PO1.C.5

Sensitivity and specificity of pleural fluid antinuclear antibodies in lupus pleuritis
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Introduction: serum antinuclear antibody (ANA) has long been serology test using for diagnosis SLE. Pleural fluid ANA was considered to be used in diagnosis lupus pleuritis. **Objectives:** to determine sensitivity and specificity of pleural fluid ANA titer >1:160 and ratio of pleural fluid to serum ANA ≥1 in order to distinguish lupus pleuritis from other etiologies. **Patients and**

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Methods: a cross-sectional study of 51 patients with pleural effusion (9 lupus pleuritis, 7 para-pneumonic effusion, 26 effusion from malignancy and 9 transudate effusion) attending at Maharaj Chiang Mai hospital was performed. ANA were tested by indirect immunofluorescence on Hep-2 cells. The cutoff value for diagnostic use was set at 1:160 and ratio of pleural fluid to serum ANA ≥1. *Results:* the sensitivity of pleural fluid ANA >1:160 was 88.9% and specificities were 88.33% when compared with all other effusion causes, 90.91% when compared with exudates effusion group (para-pneumonic effusion and effusion from malignancy) and 55.56% when compared with transudate effusion group. Using ratio of pleural fluid to serum ANA ≥1, the sensitivity decreased to 62.5% but the specificities did not change. *Conclusion:* this study provides further evidence that the pleural fluid ANA titer >1:160 is a sensitive and specific diagnostic biomarker in lupus pleuritis. However, pleural fluid ANA can see occasionally in other conditions.

PO1.C.6

Comparison of proteinuria determination by urine dipstick urine protein creatinine index (UPCI) and urine protein 24 hours in lupus patients

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Background: Urine dipstick has been recommended as an appropriate screening test for detecting protein in urine. Although a 24-h urine protein (24-hUP) collection is a standard way to measure the magnitude of proteinuria, the collection is difficult and most patients can not collect the specimen properly. A urine protein creatinine ratio (UPCI) has been developed and used as an alternative way to detect proteinuria. The UPCI has been shown to correlate well with the 24-hUP, and has been tested in lupus nephritis. *Objectives:* In this study, we aim to determine 1) over all agreement of the qualitative urine dipstick test results and the quantitative 24-hUP, 2) the sensitivity and the specificity of urine dipstick test value for the prediction of the 24-hUP in lupus patients; and 3) the correlation between the spot UPCI and the 24-hour UPCI with that of the 24-hUP. *Methods:* A cross-section study was conducted in 92 outpatients with SLE. All qualitative urine dipstick test values from 4 urine dipstick assays and the spot UPCI were obtained within 1 day of the 24-hUP collection. 149 samples were collected, but 91 samples were under and over collection and were excluded. Therefore, only 58 (39%) samples were left for analysis. *Results:* The agreement of the qualitative urine dipstick test values and the quantitative 24-hUP was poor (kappa = 0.2 – 0.4). The sensitivity of a > or = 2+ and > or = 3+ dipstick test result to detect > or = 0.50 g 24-hUP was 100%, while the specificity was 44% and 30% respectively. The correlation between the spot UPCI and the 24-hUPCI and the 24-hUP was significant (P < 0.0001, r = 0.83 and P < 0.0001, r = 1, respectively). *Conclusion:* Using urine dipstick test as a screening for proteinuria is sensitive to detect significant proteinuria, particularly when the dipstick test result is > or = 2+. However, clinicians should consider the quantitative 24-hUP collection for the diagnostic evaluation of lupus nephritis. According to high correlation with the 24-hUP; the spot UPCI, which is simple and inexpensive method, can be used interchangeably for follow-up proteinuria in patients with lupus nephritis.

PO1.C.7

Defective cerebral GABA-A receptor density in patients with systemic lupus erythematosus and central nervous system involvement. An observational study.

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Objectives: Gamma-aminobutyric acid-A (GABA-A) receptors play a crucial role in regulating neuronal excitability and cognitive functions. SPECT analysis of GABA-A receptors binding by 123I-labeled Iomazenil (123I-IMZ) has been applied in some neuropsychiatric (NP) disorders to investigate conditions where GABA-A receptor density (GRD) can be detected several pathophysiological conditions. In this study we investigate cerebral GRD in a small series of patients with systemic lupus erythematosus (SLE) and cognitive impairment characterized by recurrent, episodic memory loss. *Methods:* Nine female patients with SLE and cognitive alterations underwent to a clinical neuropsychiatric evaluation including digital video-EEG, brain MRI, 99m-Tc-ECD brain SPECT and 123-I-IMZ brain SPECT. *Results:* All the patients tested showed diffuse or focal reduced expression of rGABA-A by 123-I-IMZ brain SPECT, and most of them reveled neither EEG nor cerebral MRI abnormalities. *Conclusions:* The finding of a defective GRD obtained in these SLE clinical cases, focus on a cerebral functional abnormality whose relationship with the primary disease and with the cognitive disturbance is to be clarified. The impaired cerebral GABA-A receptor binding represents a previously unreported finding, which might be associated with CNS involvement in the course of SLE and induced by no obvious pathogenetic factors, as the disease-dependent vasculopathy, the presence of specific autoantibodies, a drug-induced receptor modulation, or, alternatively, an independent neuropathological status. Even though the series studied is small, the observation reported here represents a new finding open for further investigations in order to better assess the link between the imaging abnormality, NP manifestations, CNS involvement, and SLE.

PO1.C.8

Low-level proteinuria does not preclude significant renal pathology in lupus nephritis

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Objective: Diagnostic delay increases the risk of a poor renal outcome in lupus nephritis (LN). Patients with systemic lupus erythematosus (SLE) and suspected LN are typically referred to a diagnostic renal biopsy after detection of significant proteinuria. The aim of the present study was to describe renal histological findings in a group of LN patients with low-level proteinuria at time of first renal biopsy. *Methods:* Patients with urinary protein excretion <1000 mg/day at time of first renal biopsy were selected from a cohort of 100 Danish LN patients. Renal biopsy findings were classified according to the WHO criteria for LN. Activity and chronicity index scores were calculated using the scoring systems of the US National Institutes of Health. *Results:* In total, 13 patients (13%) displayed low-level proteinuria at time of first renal biopsy (median level of proteinuria: 600 mg/day; range: 0-900 mg/day). Three of these patients presented with hypertension. An elevated s-creatinine concentration was found in 4 patients, while 5 patients had haematuria and/or urinary cellular casts. One patient was diagnosed with WHO class I LN, 6 with class II LN, 2 with class IV LN, and 4 with class V LN. Active inflammatory and/or proliferative lesions were found in renal biopsies from 10 patients (median activity index score: 2.0; range: 0-9). Chronic renal damage

was observed in biopsy specimens from 9 patients (median chronicity index score: 2.0; range: 0-6). Among patients with proteinuria ≤500 mg/day (n=5), 4 patients had class II LN, and 1 patient had class V LN. Four out of five patients with proteinuria ≤500 mg/day presented with chronic renal lesions (median chronicity index score: 3.0; range: 0-6). **Conclusions:** These findings confirm that low-level proteinuria can be associated with significant renal pathology in LN, including membranous and proliferative nephritis. Our observations underscore the need for sensitive and specific methods for early detection of renal inflammation in SLE patients.

PO1.C.9

Depression is a risk factor for subclinical atherosclerosis in SLE

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Purpose: Women with SLE have increased rates of subclinical atherosclerosis compared to controls, as measured by coronary artery calcium (CAC) and carotid plaque. Women with SLE also exhibit higher prevalence of depression and depressive symptoms than controls. Although depression and other psychological factors have been linked to atherosclerosis in healthy women, their associations in women with SLE remain largely unexplored. The purpose of this study was to evaluate biological and psychological risk factors associated with subclinical atherosclerosis in women with SLE, as defined by presence of coronary artery calcium and/or carotid plaque. **Method:** In this cross-sectional study, 161 women with SLE without prior history of cardiac events completed comprehensive cardiovascular risk factor assessments, SLE disease activity assessments, and the Center for Epidemiologic Studies Depression scale (CES-D). Participants also completed an electron-beam computed tomography scan of the coronary arteries to determine the presence of CAC, and carotid artery ultrasound to detect presence of plaque. Subclinical atherosclerosis was defined as having either or both of these vascular indicators. In unadjusted logistic regression analyses, risk factors associated with subclinical atherosclerosis at p<0.15 were evaluated for inclusion in multivariable models. The final model was selected based on Akaike's Information Criteria. **Results:** The mean age of the participants was 50 years, and 88% were Caucasian. Mean SLE duration was 16 years. Most (68%) had taken corticosteroids, with median duration of 10 years of use. The mean CES-D score was 11.6 (SD= 9), with 27% of the participants scoring ≥ 16, a score consistent with clinically meaningful depressive symptoms. One hundred and one (63%) met criteria for subclinical atherosclerosis. Using multivariable logistic regression, SLE women with depression had nearly four-fold increased odds of subclinical atherosclerosis (OR= 3.85, 95% CI =1.37, 10.87) after adjustment for several traditional cardiovascular risk factors (age, hypertension, years of education), C-reactive protein (CRP), and waist-to-hip ratio. **Conclusion:** Depression is associated with atherosclerosis in women with SLE, independent of age, education, hypertension, CRP and adiposity. This is important in that mental health factors are modifiable. Interventions that reduce depressive symptoms may impact cardiovascular disease in SLE.

Table 1. Multivariable logistic regression analysis of risk factors for subclinical atherosclerosis in women with SLE (N=161).

Variable	Odds Ratio	95% CI	P Value
Age	1.11	(1.06-1.17)	<0.001
Years of Education	.82	(.68-.99)	0.037
Hypertension	2.50	(1.10-5.66)	0.028
Waist-Hip Ratio*			
2nd quartile: 0.77-<0.814	1.11	(.32-3.94)	0.867
3rd quartile: 0.814-<0.868	2.60	(.73-9.36)	0.142
4th quartile: ≥ 0.868	4.03	(1.12-14.49)	0.032
CRP	1.12	(1.01-1.23)	0.029
Depression	3.85	(1.37-10.87)	0.011

*Reference Group = 1st quartile of waist-hip ratio.

PO1.C.10

Pleuritis as the major risk factor for pulmonary tuberculosis in systemic lupus erythematosus (SLE)

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Introduction: A high prevalence of tuberculosis (TB) is observed in SLE, but there are scarce studies in literature addressing the predisposing factors for pulmonary TB in this disease. **Objective:** To evaluate potential risk factors for pulmonary TB in SLE patients. **Methods:** Clinical/ laboratorial features of 1,200 SLE patients (ACR Criteria) followed at the Lupus Clinic of Rheumatology Division were obtained from the electronic register database from 2001 to 2009. Pulmonary TB was diagnosed in 20 patients (1.7%) [TB+ group]. As control group [TB- group], were arbitrarily selected 40 patients without TB matched for age, gender, age at SLE diagnosis and disease duration. **Results:** All 20 patients of TB+ group presented confirmed pulmonary TB from 1 to 23 years after SLE diagnosis (7.6 +- 8.1), and none of them had TB before SLE diagnosis. Radiologic evaluation revealed that nonmiliary infiltrates was the predominant form in 16 patients (80%), followed by miliary infiltrates in 3 (15%), and pleural effusion in one (5%). The apparent higher frequency of previous exposure to TB contact in TB+ group than TB- group did not reach statistical significance (20% vs. 5%, p= 0.180). Frequencies of previous SLE involvements (cutaneous, articular, hematologic, renal, pericarditis and central nervous system) were alike in TB+ and TB- groups (p> 0.05). In contrast, prior pleuritis was more frequently observed in TB+ group (40% vs. 5%, p= 0.001), and the risk of pulmonary TB had an odds ratio (OR) of 12.74, 95% CI (Confidence Interval) 2.37-68.53. On the contrary, pneumonitis had similar frequency in both groups (p= 0.107). No differences in the frequencies of anti-dsDNA, anti-Sm, anti-Ro, anti-La and antiphospholipid antibodies were observed in patients with or without TB (p> 0.05). Immunosuppressive and corticosteroid (including daily dose of prednisone) therapies at the moment of TB diagnosis were also similar in both groups (p> 0.05). **Conclusion:** Our study has identified pleuritis as the major risk factor for pulmonary TB in SLE, reinforcing the need of a careful surveillance of this subgroup of patients.

PO1.C.11

Multiple renal artery aneurysms in a patient with systemic lupus erythematosus

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Objectives: Vasculitis is a known complication of patients with systemic lupus erythematosus (SLE). Inflammation of the vessels can result in the development of arterial aneurysms. Renal artery vasculitis is rare in patients with SLE, and documented only in a few case reports. We report an SLE patient who presented with severe hypertension. Multiple aneurysms of the right renal artery were identified and treated with endovascular embolization. **Methods:** A case was investigated retrospectively and literature was reviewed. **Results:** A 29-year-old woman presented with a 4-week history of asthenia, anorexia, low grade fever, edema, weight loss, arthralgia, and hypertension. On physical examination we observed elevated blood pressure (200/120 mm Hg). Oral aphthous ulcerations, elevated central venous pressure, tachycardia, and leg oedema were noted. Laboratory tests revealed: impaired renal function (creatinine 1,3 mg/dl), normocytic anemia (haemoglobin 10,2 g/dL), lymphopenia, positive Coombs´ test, a high erythrocyte sedimentation rate, and C-reactive protein level, and low albumin (2,7 g/dL). Complement C3 and C4 were low. Urine analysis revealed many erythrocytes, and 1,5 g protein excretion/24 hours. Antinuclear antibodies were positive. Testing for double-stranded DNA antibody was positive. Positive cryoglobulinemia was detected. Anti-phospholipid tests were negative. Other serologic tests for autoimmune disorders, and common viral and bacterial infections were negative or normal. A transthoracic echocardiogram was normal. Histologic examination of

renal biopsy specimens disclosed hypertensive changes without evidence of glomerulonephritis. This patient fulfilled the diagnostic criteria for SLE. She was treated with methylprednisolone (1 g/day intravenously for 3 consecutive days), and pulse cyclophosphamide, followed by oral prednisone 60 mg/day. The complaints of the patient had rapid resolution within a few days, but hypertension persisted despite antihypertensive therapy. A spiral computed tomography scan with intravenous contrast, followed by an angiogram were done and revealed multiple aneurysms of the right renal artery, which were treated with endovascular embolization. ***Conclusions:*** Clinicians should considerer the possibility of renal artery vasculitis with aneurysms in patients with SLE and hypertension. Visceral angiography may be a useful tool in these patients. Immunosuppressive therapy using steroids and cyclophosphamide, and arterial embolization, can be effective therapies.

PO1.C.12

Prospective analysis of neuropsychiatric events in an international disease inception cohort of SLE patients

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Objective: To determine the frequency, accrual, attribution and outcome of neuropsychiatric (NP) events and the impact on health-related quality of life over 3 years in a large inception cohort of SLE patients. ***Methods:*** The study was conducted by an international research network. Patients were enrolled within 15 months of SLE diagnosis. NP events were identified using the American College of Rheumatology (ACR) case definitions, and decision rules were derived to determine the proportion of NP events attributable to SLE and non-SLE causes. Physician assessment of outcome of NP events was recorded using a 7-point Likert scale (patient demise; much worse; worse; no change; improved; much improved; resolved), and patient perceived impact was determined by the mental component summary (MCS) score and physical component summary (PCS) score of the SF-36. Statistical analysis included Cox regression for examining the time to case resolution for NP events, multilevel ordinal regression for examining the association between explanatory variables and the probability of more favourable Likert outcome scores of NP events, as well as linear regression for SF-36 analyses. All analyses were adjusted for the correlation of multiple observations from the same patient. ***Results:*** There were 1206 patients (89.6% female) with a mean (SD) age of 34.5 (13.2) years. The mean disease duration at enrollment was 5.4 (4.2) months. Over a mean follow-up of 1.9 (1.2) years 486/1206 (40.3%) patients had one or more NP events. Eighteen of the 19 ACR NP case definitions were identified and the frequency of individual NP events varied from 47.1% (headache) to 0% (myasthenia gravis). NP events were attributed to SLE in 13.0 - 23.6% of patients (17.7 – 30.6% of NP events) using two a priori decision rules. The most frequent NP events attributed to SLE were seizures, mood disorders, cerebrovascular disease and acute confusional states. The outcome was significantly better for those NP events attributed to SLE (p<0.001), especially if they occurred within 1.5 years of the diagnosis of SLE. Patients with NP events, regardless of attribution, had significantly lower SF-36 summary scores for both mental and physical health over the study compared to those without NP events (estimate for MCS scores -9.7, p<0.001; estimate for PCS scores -3.3, p<0.001). There were 18/1206 (1.5%) deaths and in 4/18 (22.2%) the primary cause was attributed to NP events (intracranial hemorrhage (2), stroke (1), seizures (1)). ***Conclusion:*** NP events in SLE patients are variable in frequency, commonly present early in the disease course and adversely impact patients' quality of life over time. Events attributed to non-SLE causes are more common than those due to SLE, although the latter have a more favourable outcome.

PO1.C.13

Lupus nephritis: a 33-year experience

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Objective: Patients with Lupus nephritis as a serious manifestation of Systemic Lupus Erythematosus (SLE), may express increased frequency of other sever lupus manifestations. The aim of this study was to compare the clinical and paraclinical manifestations of patients with and without lupus nephritis. ***Method:*** We used the electronic database of Rheumatology Research Center (RRC), which registered clinical and paraclinical manifestations of 2200 SLE patients during 1976- 2009. Chi Square test was used to compare the two groups. Odds ratio and 95% Confidence Interval (CI) was used to present the strength of association (p-value<0.05). ***Results:*** Among 2200 lupus patients, 1468 patients (66.7%, 95% CI: 64.7-68.7) had lupus nephritis. Statistical analysis showed that constitutional manifestations, pulmonary involvement, cardiac involvement, neuropsychiatric manifestations, lymphopenia, thrombocytopenia, positive anti-dsDNA antibody, and low C3 were significantly higher, and discoid lesion was significantly lower in patients with renal involvement .

Manifestations	With Renal Involvement (n=1468)	Without Renal Involvement (n=732)	p-value	OR (95% CI)
Constitutional Symptoms	1013	391	<0.00001	1.9 (1.6- 2.3)
Malar Rash	913	419	0.025	1.2 (1.02- 1.4)
Discoid Lesions	180	150	<0.00001	0.54 (0.4-0.7)
Arthritis	800	350	0.003	1.3 (1.09-1.6)
Pulmonary Involvement	378	110	<0.00001	1.96 (1.5-2.5)
Cardiac Involvement	307	83	<0.00001	2.06 (1.59-2.65)
Neuropsychiatric Manifestations	568	197	<0.00001	1.7 (1.4- 2.1)
Lymphopenia	581	185	<0.00001	1.9 (1.6-2.4)
Thrombocytopenia	288	95	0.0001	1.6 (1.3- 2.1)
FANA	1190	541	0.0001	1.5 (1.2- 1.9)
Anti-DNA	1069	447	<0.00001	1.7 (1.416-2.061)
Antiphosphlipid Antibody	978	448	0.01	1.3 (1.05-1.5)
Low C3	834	243	<0.00001	2.65 (2.2-3.2)

Conclusions: Lupus nephritis is associated with more other major organs involvement and immunological abnormalities notably disease activity index such as high anti-dsDNA antibody and low C3.

PO1.C.14

Evans' syndrome and systemic lupus erythematosus. An analysis of clinical presentation and outcome of 20 cases

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Evans' syndrome (ES) is a rare disease characterized by the simultaneous or sequential development of autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia (ITP).It is a condition with unknown aetiology that results from an alteration of the immune system that produces multiple autoantibodies targeting red blood cells and platelets.This disease can be associated with an

underlying disorder such as lymphoproliferative disorders, commom variable immunodeficiency and systemic lupus erythematosus(SLE).We describe the characteristics and outcome of ES in 20 patients with SLE. The data from these patients ,all of them women, 13 white and 7 non-white, fulfilling strict inclusion criteria for ES are reported. The mean age at time of Evans' syndrome onset varied from 14 to 50 years. Four patients from 18 studied were under 16 years at onset of ES. All patients presented Evans' syndrome at onset of disease, both cytopenias occurred simultaneously in all patients. Although corticosteroids and/or intravenous immunoglobulin (IVIG) are commonly used in its treatment, no standard strategy has been established. All patients were given corticosteroids(oral in all and pulse in 5), but 10 of them (50%) required at least one other drug , including azathioprine(n=8),intravenous cyclophosphamide (n=4) and rituximab (n =1).Some patients received more than one drug. Splenectomy and intravenous immunoglobulin were not used. At time of analysis, 19 patients (95%) were in remission of treatment; 1 patient with severe systemic lupus (5%) had died. These data suggests that Evans' syndrome in SLE can have a good prognosis with a good response to treatment.

PO1.C.15

High sensitivity C-reactive protein is an independent risk factor for left ventricular hypertrophy in patients with lupus nephritis
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Objectives: To determine the prevalence of left ventricular hypertrophy (LVH) and its associated risk factors in patients with lupus nephritis (LN). *Methods:* 287 lupus nephritis patients (age 38.54±13.31, 262 female) were recruited at the time of outpatient visits from Jan 2005 to Dec 2008. Comprehensive interviews, physical examinations and laboratory assessment were carefully assessed in all subjects. Echocardiography was performed and left ventricular mass index (LVMI) was calculated by standard formula to quantify left ventricular (LV) status. Serum high sensitivity C-reactive protein (hs-CRP) level was detected by auto-immune scattering rate nephelometry. *Results:* The prevalence of LVH in this cohort was 78.75% (n=226), which was much higher than reported in healthy population of similar age. Hs-CRP level was significantly elevated in patients with LVH compared to those without [8.03(3.22-30.95) vs 3.93(1.48-9.48) mg/L, P<0.01]. To further understand the extent to which inflammation augment LVH, the patients were subdivided according to hs-CRP cut-off level (3mg/L) based on AHA/CDC reommendations. Among those who had higher hs-CRP levles ($\geq$3mg/L), LVMI was significantly increased (132.68±57.84 vs 113.67±29.17, P=0.018). In univariate analysis involving the entire variables, hs-CRP was positively correlated with LVMI (r=0.314, P=0.001). Multivariate regression analysis confirmed that hs-CRP was an independent risk factor (β=0.338, P=0.002) for LVMI in patients with LN. *Conclusions:* Our findings demonstrate that hs-CRP level is independently asssociated with LVMI, which suggest that measurement of hs-CRP may provide important clinical information to investigate and follow up the development of LVH in LN patients.

PO1.C.16

Diffuse alveolar hemorrhage in systemic lupus erythematosus : risk factors and clinical outcome: results from affiliated hospitals of Catholic University of Korea
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Objectives: This study was undertaken to investigate clinical characteristics of diffuse alveolar hemorrhage (DAH) in patients with systemic lupus erythematosus (SLE) and to determine risk factors and clinical outcomes of DAH in

SLE patients. *Methods:* Among the 1521 SLE patients admitted between January 1993 to June 2009 to affiliated hospitals of Catholic University of Korea, 21 SLE patients were admitted for DAH. The inclusion criteria for DAH was defined as new infiltrates on chest radiographs, an acute hemoglobin drop of at least 1.5g/dL in the absence of obvious source of bleeding, and one or more of the following signs: hemoptysis, hypoxemia, bronchoscopic or biopsy evidence of DAH. 83 SLE patients matched for age and sex who were admitted for other manifestations, were included as disease controls. Data based on medical records were analyzed retrospectively. *Results:* There were no significantly differing demographic characteristics between SLE patients with DAH and those with other manifestations. Multivariate analysis demonstrated coexisting neuropsychiatric lupus (P=0.002) and high SLE disease activity index scores (SLEDAI > 10) as independent risk factors in the development of DAH (P=0.029). Among the 21 SLE patients with DAH, 13 died during the admission period (in-hospital mortality rate:61.9%). Mortality was associated with infection and requirements of mechanical ventilation. *Conclusions:* Collectively, SLE patients who have neuropsychiatric manifestations or are in the active stage of the disease have an increased risk for developing DAH. Due to the high mortality of SLE patients with DAH, early recognition of risk factors and appropriate intervention is essential.

PO1.C.17

The relationships between serum vitamin D levels, vitamin D receptor polymorphism, anti-vitamin D autoantibodies, IL-17 and IL-23 levels, and clinical parameters in SLE patients
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Objectives: To investigate relationships between vitamin D status, vitamin D receptor (VDR) gene start codon Fok I polymorphism, the presence of anti-vitamin D autoantibodies, interleukin-(IL)-17, IL-23 levels, and clinical parameters in patients with systemic lupus erythematosus (SLE). *Methods:* The study included 49 patients with SLE. Serum concentrations of 25(OH)D3 were measured with electrochemiluminescence immunoassay (ECLIA) in an automated analyzer (Elecsys 2010- under international control of Vitamin D External Quality Assessment Scheme -DEQAS). Fok I genotyping was performed based on real time polymerase chain reaction (RT-PCR), that identified FF, ff and Ff. In order to detect antibodies directed against 1,25(OH)2D3, and determine serum levels of IL-17 and IL-23 in SLE patients, enzyme-linked immunosorobent assays (ELISA) were employed. *Results:* The serum concentration of 25(OH)D3 in patients with SLE during summer time was 18.47±9.14 ng/mL, and was significantly decreased as compared with those of the control group (31.27±12.65 ng/mL) (p=0.0005). During winter time a trend toward lower concentration of 25(OH)D3 in SLE patients was revealed, however it did not reach statistical significance in comparison to those of control (respectively, 11.71±7.21 ng/mL vs 16.01±8.46 ng/mL; p=0.054). 25(OH)D3 levels were significantly lower in SLE patients with renal disease or leucopenia as compared to SLE patients who did not have these manifestations (respectively, p=0.006 and p=0.047). Vitamin D deficiency in systemic lupus erythematosus was also associated with low interleukin-23 levels, but not lower interleukin-17 levels, anti-1,25(OH)2D3 autoantibodies or vitamin D receptor gene start codon Fok I polymorphism. Autoantibodies directed against 1,25(OH)2D3 were detected in 4 patients with SLE. No significant difference in 25(OH)D3 serum concentrations was found between SLE patients with and without these autoantibodies. Additionally, anti-1,25(OH)2D3 autoantibodies were not associated with clinical or laboratory findings including IL-17, and IL-23 levels. Serum concentrations of IL-23 were significantly lower in patients with vitamin D deficiency (p=0.037). The frequency of the VDR Fok I polymorphism in SLE patients was FF-9.52%; Ff-59.52%; ff-30.95%. No relationships were found between the Fok I polymorphism and the clinical and laboratory profiles of the SLE patients. *Conclusions:* Vitamin D deficiency in SLE patients, may have other causes besides that resulting from sun

avoidance. From the presented findings, it is associated with renal disease, leucopenia, and low interleukin-23 levels, but not lower interleukin-17 levels, anti-1,25(OH)2D3 autoantibodies or the vitamin D receptor gene start codon Fok I polymorphism. It is generally advisable to recommend supplemental vitamin D in SLE patients year round.

PO1.C.18

Unusual presentations of antiphospholipid syndrome: report of three cases
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Objectives: Clinical presentation of antiphospholipid syndrome (APS) may vary widely. Therefore, its early detection requires a strong index of suspicion, especially when thrombosis occurs at unusual sites or non-specific symptoms predominate in early stages. We report three cases of APS with unusual clinical presentations. **Methods:** Three cases were investigated retrospectively and literature was reviewed. **Results:** Case 1: A 32-year old woman presented during her 6 month of pregnancy with severe depression, anxiety, and hypertension. At 32 weeks of gestation foetal ultrasound disclosed signs of intrauterine growth retardation. Therefore, the patient underwent caesarean section, which resulted in a live birth. Autopsy of placenta showed widespread infarctions. MR imaging of the brain revealed multiple small foci of increased T2 signal intensity in the periventricular and deep white matter, consistent with microvascular ischemic lesions. High titres of lupus anticoagulant and anticardiolipin (IgM and IgG) and antibeta-2 glycoprotein I (IgM and IgG) antibodies, were observed. Laboratory tests showed low titre of anti-nuclear antibody (ANA) positivity. Other serologic tests for autoimmune disorders were negative. The patient received therapeutic anticoagulation during puerperium, and a progressive recovery of the psychiatric disorder was achieved. Case 2: 26-years-old male presented with recurrent episodes of transient visual loss and blurred vision in both eyes. He has a medical history of an episode of loss of vision in the right eye one year previously. Funduoscopy disclosed a normal right eye and an ischemic area caused by an arterial obstruction in the superotemporal quadrant of the left eye, that was confirmed by fluorescein angiography. High titres of lupus anticoagulant and anticardiolipin (IgM and IgG) and antibeta-2 glycoprotein I (IgM and IgG) antibodies, were observed. A cardiac evaluation was performed with echocardiography and carotid artery ultrasonography, and findings of both were normal. The patient received anticoagulant therapy. Case 3: 46-year-old male presented with a 1-week with history of worsening of chronic renal insufficiency and pain in the left leg. Venous ultrasonography disclosed deep venous thrombosis. MR imaging of the aorta and abdominal arteries revealed occlusion of the left renal artery. High titres of lupus anticoagulant and anticardiolipin (IgM and IgG) and antibeta-2 glycoprotein I (IgM and IgG) antibodies, were observed. The patient received anticoagulant treatment. **Conclusions:** Physicians should considerer the diagnosis APS in patients with clinical presentations such as psychiatric disorders and leukoencephalopathy during pregnancy, retinal vascular occlusions, and renal failure associated to deep venous thrombosis.

PO1.C.19

Primary cardiac disease (PCD) in systemic lupus erythematosus (SLE) of Latin American patients. Data from the multinational inception GLADEL cohort.
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Background and Purpose: Cardiac involvement is one of the major concerns in the management of SLE patients. The aim of this study was to investigate the prevalence of PCD, associated factors and mortality in SLE using data from GLADEL, a multi-ethnic, multinational Latin American cohort. **Methods:** SLE patients with a recent SLE diagnosis ($\leq$2 years) were recruited and followed longitudinally. PCD was defined as the presence of pericarditis, myocarditis, endocarditis, arrhythmias and/or valve abnormalities related to SLE pathogenic mechanism. Socioeconomic-demographic, clinical, serologic and therapeutic variables were compared between patient with/without PCD. Those significant variables at p$\leq$0.20 in these UV analyses were included into logistic regression models with stepwise selection with PCD endpoint. **Results:** Of the 1437 GLADEL cohort patients included in this study, 202 (14%) developed PCD (pericarditis 164, myocarditis 7, endocarditis 1, arrhythmia 23 and valvulopathy 35). Delay to SLE diagnosis was shorter in PCD patients (median 4.0 vs. 6.1 months, p=0.0002). In the UV analysis PCD was statistically more frequent in patients of African-Latin American origin (ALA) vs. Caucasian (OR:1.8, IC:1.18-2.80) and in lower-middle/lower vs. upper/upper-middle economic status (OR:1.8, CI:1.01-3.21). PCD patients have less chance to have had skin disease previous to SLE diagnosis (OR:0.7, CI:0.46-0.99), but more chance to have had lung disease (OR:4.7, CI:2.45-9.20), previous PCD manifestations (OR:7.1, CI:4.98-10.4), anti-DNAds (OR:1.68, CI:1.05-2.7), anti-SSB/LA (OR:2.36, CI:1.27- 4.39) or low C3 (OR:2.38, CI:1.43-3.95). PCD patients had more chance to present infections (OR:1.9, CI:1.39-2.53), hypertriglyceridemia (OR:2.2, CI:1.31-3.57), hypercholesterolemia (OR:1.8: CI:1.15-2.68) during follow-up and more chance to receive prednisone in a medium (>20-<60mg/day) or high dose ($\geq$60mg) (OR:4.41, CI:1.58-12.34 and OR:4.47, CI:1.60-12.49, respectively), intravenous cyclophosphamide (OR:2.77, CI:2.05-3.75) and hemodyalisis (OR:5.14, CI:3.05-8.68). Patients with PCD had higher activity (SLEDAI) (median 12.0 vs. 10.5, p=0.0057) and damage score (SLICC/ACR) (1 vs. 0, p=0.0002) at SLE diagnosis and higher damage score at follow-up term (2 vs. 1, p<0.0001). Patients with PCD were significantly associated with mortality (16.8 vs. 4.1%, p<0.0001).In the MV analysis the presence of PCD during follow-up was associated to ALA ethnicity vs. Caucasian (OR:2.22, CI:1.01-4.87), PCD previous to SLE diagnosis (OR:4.99, CI:2.63-9.48), hypertriglyceridemia (OR:1.82, CI: 1.02-3.23), intravenous cyclophosphamide (OR:3.27, CI:1.81-5.91) and hemodyalisis (OR:5.68, CI:2.25-14.32). On the contrary, renal disease previous to diagnosis of SLE decrease the probability of having PCD during follow-up (OR:0.33, CI:0.17-0.61). **Conclusion:** ALA ethnicity, as well as some clinical, serological and therapeutics variables were associated with PCD in Latin-American SLE patients. A higher damage index and mortality associated with PCD should remind us of the importance of early diagnosis and appropriate treatment.

PO1.C.20

Pleuropulmonary compromise in systemic lupus erythematosus (SLE) of Latin American prospective inception cohort (GLADEL)
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Pulmonary involvement in systemic lupus erythematosus (SLE) is common (50%), with clinical manifestations ranging from a benign course to potentially catastrophic manifestations. **Objective:** The aim of this study was to investigate the prevalence of pleuropulmonary compromise, to analyze the socioeconomic-demographic, clinical and serological features, and to assess

their influence on the prognosis, in terms of mortality in patients with SLE. *Patients and Methods:* SLE patients from 34 centers of 9 Latin-American countries (Argentina, Brazil, Chile, Colombia, Cuba, Guatemala, Mexico, Peru and Venezuela) with a recent SLE diagnosis (≤2 years) had been recruited and followed longitudinally. Patients were subdivided into those without pleuropulmonary (WPPC), with pleuropulmonary compromise (PPC) and with pulmonary compromise (PC). Odds ratio with 95% CI was used to measure the strength of association between variables. Kaplan Meier survival curve was examined. Results were confirmed by univariate (UV) and multivariate (MV) logistic regression analysis *Results:* Of the 1,480 included in GLADEL cohort, 90% were female. Median age (years) at onset was 26 and at diagnosis 27. The median time of follow up was 55 months. Two hundred ninety six patients had PPC (20%), 244 pleurisy (17%) and 90 PC (6%). Of these 90 patients with PC, 28 had pulmonary hypertension (31%), 25 pneumonitis (28%), 16 pulmonary fibrosis (18%), 14 pulmonary hemorrhage (16%), 12 thrombosis (13%), 9 shrinking lung (10%) and 3 had pulmonary infarction (3%). The UV analysis showed that PC was significantly associated with SLE hematological (8.0% vs. 2.2%, p<0.0001), cardiovascular (9.9% vs. 3.2%, p<0.0001), neurological (8.4% vs. 5.1%, p<0.02) and renal disease (7.4% vs. 4.6%, p<0.03). The MV analysis confirmed the association of PC with hematological (OR=3.18, CI: 1.65-6.13) and cardiovascular (OR=2.81, CI: 1.75-4.51) manifestations. The MV analysis showed that the presence of PC increased the risk of death by more than 5 times (OR: 5.25, CI: 2.92-9.42). When different types of PC were evaluated, pulmonary hypertension (OR: 4.93, CI: 1.96-12.42) and pulmonary hemorrhage (OR: 12.60, CI: 3.79-41.85) increased significantly the mortality. The overall cumulative estimated probability of survival at 6 years was significantly lower in patients with PC (73 vs. 93%, p<0.001). *Conclusion:* The prevalence of PPC was less frequent than in other series. The PC showed a significantly association with hematological and cardiovascular involvement. SLE patients with PC had poorer survival, being pulmonary hypertension and pulmonary hemorrhage the main causes of death.

PO1.C.21

Renal transplantation in patients with systemic lupus erythematosus: 22 years experience from a single centre
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Objectives: To determine the clinical characteristics and outcome of renal transplantation in patients with SLE from our center. *Methods:* To analyze SLE patients with renal failure due to lupus nephritis, who were treated with renal transplantation in our Hospital from January 1986 to December 2008. *Results:* 40 renal transplantations were performed in 29 SLE patients (24 females (83%), mean age at transplantation 33±8 years (range 17 – 53). In 20 cases, only one transplantation was performed. The analysis included only the first transplantations performed in our centre. In 27 patients (93%), we obtained pre-transplantation histological diagnosis (according to the WHO classification): 22 (76%) type IV, 2 (7%) type III, 2 (7%) type II and 1 (3%) type VI. Six patients (20%) were positive for antiphospholipid antibodies (APL). Positive serologies for hepatitis virus were detected in 10 patients (9 HCV, 1 HBV). Twenty-six patients were on haemodialysis in the pre-transplantation period and 6 were on to peritoneal dialysis. Twenty (69%) transplantations were from deceased donors and 9 from living donors. The mean time elapsed between the diagnosis of lupus nephritis and the start of dialysis was 43±40 months, the time on dialysis was 62±52 months and the time on follow-up was 73±67 months. In 18 (62%) patients renal biopsy was performed for impairment in the renal function. Recurrence of lupus nephritis in renal allograft and flare-ups of lupus activity were not observed in this study. The graft survival rates were 76% at 5 years, 69% at 10 years and 62% at the end of the study. The patient survival rate was 93% at the end of the study. Graft rejection occurred in a total of 11 patients, 6 out of the 9 (66%) positive for HCV and 5

out of the 20 (25%) negative for HCV (exact Fisher test p = 0,047; OR = 6; CI = 1,08 – 33,4). In one case, graft rejection occurred twice, both produced by thrombotic microangiopathy, although studies were persistently negative for APL. Prior to her third transplantation, APL were detected as positive, which indicated anticoagulation immediately after transplantation, with a good evolution of this new graft. In the re-transplanted group (9 patients) a total of 20 transplantations were performed. There was a deterioration of renal function with re-entry into dialysis in 13 of them, 7 of whom had positive serology for HCV. There were 2 deaths in this group. *Conclusion:* Renal transplantation is a good alternative for renal replacement therapy in patients with SLE, but the existence of a thrombotic disease associated with the APL or the coexistence of HCV infection are related with the development of graft rejection.

PO1.C.22

Antiphospholipid syndrome in male lupus patients
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Background: Antiphospholipid syndrome (APS) is characterized by vascular thrombosis, and/or pregnancy morbidity associated with anticardiolipin antibodies (aCL) and lupus anticoagulant (LAC). Incidence of APS and antiphospholipid antibodies (aPL) in men is not known. We analysed 146 male patients with SLE to estimate frequency of aPL and aPL-associated complications. *Materials and Methods:* We studied 146 male patients (pts) fulfilling at least four of the American College Rheumatology criteria for the classification of SLE. Mean age was 30,5 years (range 15-64 years), mean disease duration was 84 month and ranged from 2 to 504 month. APL was measured by standardized ELISA. Presence of LAC was detected by according to the quidelines of the International Society on Thrombosis and Haemostasis. Diagnosis of APS was based on the Sapporo criteria. Additionally we analyzed frequency of aPL-associated symptoms not included in the revised criteria (heart valve disease, livedo reticularis, thrombocytopenia, neurological manifestations). *Results:* Vascular thrombosis developed in 39 from 146 male pts (26,7%), recurrent thrombosis occurred in 27 out of 39 pts (69,2%). Number of thrombosis varied from 1 to 7 in one pts. 24 pts (61,5%) had only venous thrombosis, 8 pts (20,6%) – merely arterial trombosis. Presence of arterial and venous localization was register in 7 (17,9%) pts. Positive titers of IgG-aCL (>25 GPL) was observe in 57/125 pts (39,3%), positive titers of IgM-aCL (> 25 MPL) – in 36/145 (24.8%) pts. LAC was positive in 22 from 60 pts (36,7%). 39 male pts (26,75) satisfied classification criteria of definite APS. Pts with APS had higher frequency livedo reticularis (33,3%), pulmonary hypertension (20,5%), heart valve disease (46,2%) in comparison to pts without APS (10,3%, 5,6% and 16,8% respectively, p<0,05 in all cases). Statistically differences were not observed in frequency of thrombocytopenia and neurological involvement between pts with and without APS. Clinical manifestations of APS added to SLE symptoms in 28 (72%) pts (average 2-199 month after SLE onset). In 9 pts (23%) aPL-associated signs preceded SLE-related manifestations. Only 2 men (5%) developed APS and SLE simultaneously. *Conclusions:* We observed high incidence of definite APS in male patients with SLE. Main localization of thrombosis was vein vessels. Some aPL-associated manifestations not included in The Sapporo criteria (livedo reticularis, heart valve disease, pulmonary associated) were more frequently in patients with APS. Clinical manifestations of APS may precede SLE symptoms.

PO1.C.23

A concurrent case of systemic lupus erythematosus and ankylosing spondylitis
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Objectives: some co-existence case of systemic lupus erythematosus (SLE) and ankylosing spondylitis (AS) was described in literature but this co-existence is very rare. *The case:* our case is 26 years old female that referred for

1 year inflammatory low back pain. In physical examination she had bilateral positive FABER test and both sacroiliac tenderness and considerably limitation of motion in sagittal and coronal movement of spine. She had positive HLA B27 and bilateral symmetric grade 2 sacroiliitis in radiography. According to modified New York, 1984 criteria diagnosis of AS established. 6 month later she visited because of arthritis in PIP, MCP, wrists, elbows, knees and bilateral sacroiliacs. She had history of photosensitivity, oral ulcers, hair loss and urticarial rash. In further study she had positive FANA with homogenous pattern and positive Anti DNA (ds) but with normal complement. She met ACR criteria for SLE. Continuously she had spinal pain. **Conclusions:** this is a rare combination of SLE and AS disease. Sacroiliitis is seen 6% of SLE patients but symptomatic sacroiliitis and AS is rare. An unusual combination of genetically determined markers may cause increase rise for the development of both disorders.

PO1.C.24

Cardiovascular risk factors predict rapid progression of atherosclerosis in lupus erythematosus

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Background: Patients (pts) with systemic lupus erythematosus (SLE) have a high cardiovascular (CV) risk, which may be due to a predisposition to atherosclerosis. **Objectives:** To estimate the rate of atherosclerosis in SLE cohort and to identify factors predictive of rapid progression. **Methods:** 227 SLE (156 women, 71 men, mean age was 35,6 ±0,7, disease duration – 132,9±7,7 months) pts fulfilling ACR criteria for SLE were included in the analysis. A high resolution ultrasound scan of the common carotid arteries was performed in pts with SLE. Digitized scans were used to measure the intima-media thickness (IMT). Traditional risk factors, SLE-related risk factors and biomarkers (hs-CRP, CD40L, sTNF-α, neopterin) of CV disease risk factors were recorded. We used linear regression analysis to identify baseline factors that were predictive of rapid atherosclerosis progression. **Results:** Ninety seven (43 %) pts had atherosclerosis (20 % - the IMT>0,9 mm, 24 % - carotid artery plaque). The mean (m) common carotid IMT was 0,77 (0,01) mm. Univariate linear regression model of increased IMT included traditional (age, gender, Framingham risk score, hypertension, dyslipidemia, high body mass index, smoking, family history of premature coronary disease, uric acid), SLE-related risk (disease duration, revised damage index (SLICC), SLEDAI-2K, duration of nephritis, duration prednisone, prednisone cumulative dosage) factors and levels of CD40L, sTNF-α. In multivariate prognostic model of increased IMT included age, Framingham risk score, hypertension, levels of systolic blood pressure (SBP), duration of nephritis, immunosuppressant (see table 1). **Conclusion:** Subclinical atherosclerosis was associated with traditional risk and lupus-related factors (duration of nephritis and absence of immunosuppressive therapy) in pts with SLE.

	Unstandardized Coefficients, B	Standardized Coefficients, Beta	p
(Constant)	0,1998		0,185
Age, year	0,011	0,472	0,001
Framingham risk score, %	0,002	0,331	0,003
BMI, kg/m2	0,006	0,598	0,0001
Hypertension, %	-0,012	-0,558	0,002
SBP, mm Hg	-0,122	-0,255	0,024
Duration of nephritis, months	0,076	0,117	0,223
Immunosuppressant	-0,003	-0,085	0,405

PO1.C.25

Autoantibodies and possible carbamazepine-induced lupus erythematosus: a clinical challenge

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Objectives: Drug-induced lupus erythematosus (DILE) has been associated with various medications including anti-convulsants like carbamazepine (CBZ). Imunologically in DILE unlike idiopathic systemic lupus erythematosus (SLE) less than 5% are associated with anti-dsDNA and ENA and less than 1% associated with hypocomplementemia. Anti-histone antibodies are positive in more than 95% of the individuals. Our case is unusual for the absence of anti-histone and dsDNA antibodies, and the presence of hypocomplementemia and leukopenia. **Methods:** A 34-year-old Caucasian lady developed epilepsy post surgery (January 2003) for a congenital arterio-venous malformation and was started on CBZ (October 2003). Five years later, she presented with an acute onset, photosensitive, scaly plaques on sun-exposed areas of her body which initially cleared with a topical steroid but recurred months later, when a skin biopsy revealed feature consistent with lupus erythematosus. Laboratory data revealed a positive IgG ANA (1:320) and anti Ro-SSA, reduced complement C4 and C3, leukopenia (WCC: 2.6, neutrophils 1.03 and lymphocytes 0.88) and negative dsDNA, RNP, Sm and IgG and IgM cardiolipin. Mepacrine 100mg was initiated for skin manifestations. She, subsequently developed myalgia, a generalized arthralgia and sicca symptoms in spite of continuing mepacrine. Serial serology was negative for anti-histone and anti-dsDNA antibodies repeatedly. The patient was reluctant to discontinue the CBZ as she had experienced numerous relapses and adverse effects whilst on Lamotrigine and Phenytoin prior to commencing CBZ and on switching from CBZ twice. She preferred to persist with the therapy despite the skin and musculoskeletal manifestations. However, should major organ involvement arise, management could prove challenging if she wishes not to withdraw CBZ. **Results:** Several cases of CBZ induced SLE have been reported in the literature. All but one was associated with anti-histone positivity. Despite the lack of standard diagnostic criteria for DILE, temporal association with a putative drug and resolution following withdrawal of therapy has been the best diagnostic tool. **Conclusions:** In this lady's case, withdrawal of CBZ, in view of the fact that she is anti-histone and dsDNA negative, and a resulting resolution of symptoms would be diagnostic. Unfortunately her reluctance to stop CBZ leaves us with a diagnostic dilemma. The auto-antibody profile in DILE is not predictive of severity or extent of manifestation of symptoms. In our patient it is difficult to ascertain the course of the condition on continued challenge from a likely drug source.

PO1.C.26

The medical outcomes study (MOS) sleep scale in lupus patients

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Objectives: To evaluate and to identify correlates of the psychometric properties of the Medical Outcomes Study (MOS) Sleep Scale in Systemic Lupus Erythematosus (SLE) patients **Methods:** Sleep in 118 SLE patients was assessed using the self-administered MOS Sleep Scale. Bivariate correlations were determined using a Pearson's correlation matrix between each of the six MOS sleep subscales and each potential predictor variable (age, educational level, SLEDAI, current prednisone dose, body mass index, visual analog pain scale, Beck Depression Inventory, State-Trait Anxiety Inventory). Serial hierarchical multiple regression analyses were computed to test the importance of demographic, clinical and psychosocial factors to the six sleep subscales. **Results:** The average age of this lupus population was 45.4 years; 92% were female and 57.6% were African-American. They had a mean SLEDAI score of 3.93 (range 0-22). SLE patients' mean±SD MOS Sleep scores were generally poorer than the US general population in all six

subscales: sleep disturbance (45.29±28.78), snoring (30.59±33.88), awakening with shortness of breath or headache (21.36±29.09), sleep adequacy (39.92±24.02), daytime somnolence (35.82±21.45) and sleep quantity (6.30±1.67). Pain significantly correlated with all sleep subscale scores (r= 0.40, 0.25, 0.52, -0.27, 0.34 and -0.20, respectively, all p<0.05). Depression also strongly correlated with all scores (r= 0.39, 0.32, 0.37, -0.40, 0.40 and –0.27, respectively, all p<0.01). Anxiety significantly correlated with only the first five subscale scores (r= 0.32, 0.38, 0.38, -0.24, 0.43, respectively, all p<0.05). Number of years in school correlated negatively with sleep disturbance and daytime somnolence, and positively with sleep quantity (all p<0.05). Body mass index correlated positively with snoring and awakening with shortness of breath, and negatively with sleep quantity (all p<0.05). Results of a multivariate regression model using forward selection of variables with p<0.20 showed that depression is a significant predictor of sleep adequacy (β=-0.79, p=.003). Depression also trended towards association with sleep disturbance (β=0.72, p=.054). Anxiety trait significantly predicted daytime somnolence (β=0.72, p=.002) and snoring (β=1.41, p<.005). Among disease-related measures, prednisone dosage predicted daytime somnolence (β=0.56, p<.005). Pain scale significantly predicted awakening w/ shortness of breath/pain (β=0.45, p<.005). SLEDAI and pain trended towards association with sleep disturbance (p=0.062, p=0.061, respectively). **Conclusions:** Subscale scores of the MOS Sleep Scale suggest that SLE patients have greater sleep problems relative to the general population. Psychosocial factors, particularly depression and anxiety, play significant roles in predicting sleep abnormality. Disease activity and pain appear to make modest contributions to sleep disorder after adjustment for covariates.

PO1.C.27

Myocardial ischemia in the absence of obstructive coronary artery disease in patients with systemic lupus erythematosus
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Objective: Prior studies have demonstrated the presence of perfusion defects using adenosine stess cardiac magnetic resonance imaging (CMR) in the setting of microvascular disease with open epicardial coronary arteries. Our purpose was to use CMR to evaluate for microvascular coronary artery disease (CAD) in SLE women with anginal chest pain (CP). **Methods:** Twenty adult female SLE patients with anginal CP in the last 6 months and low to moderate SLE disease activity index (SLEDAI) were enrolled. Patients with known atherosclerosis or CAD were excluded. CMR was performed at 1.5 Tesla with 0.05 mmol/kg gadolinium first-pass perfusion three-slice stress followed by rest imaging, function and delayed enhancement imaging. CMR images were analyzed by visual semi-quantitative 5 point scoring in 16 segments for presence and extent of wall motion abnormality, hypoperfusion at rest and stress, or myocardial scar. Perfusion defects were categorized by the percentage of myocardium abnormal. SLE patients also underwent 64-slice coronary computed tomography angiography (CCTA). CCTA images were analyzed by consensus of two imaging cardiologists for the following: 1) coronary calcium score (CAC) and 2) plaque type and location, and 3) degree of coronary luminal narrowing using a 5 point scale. **Results:** Eighteen subjects (mean age 41.3±11 years, mean SLE duration 13.2±12 years, 100% ANA positive, 56% dsDNA Antibody positive) had complete data available for analysis. Nine of 18 (50%) SLE patients displayed subendocardial perfusion defects on stress CMR. All had normal ventricular function and no scar. On CCTA, only 2 of these 9 subjects showed evidence of coronary artery plaque or calcification; one had CAC score of 5.9 (minimal calcification, 60th percentile for age and gender) and the other had an ulcerated plaque in the left anterior descending artery with a CAC score of 0. The Framingham risk score was ≤1% in all subjects except for in 1 subject where it was 2%. **Conclusion:** In our pilot sample of SLE patients with anginal CP, we observed the striking finding that 50% of our study group had abnormal

stress myocardial perfusion by CMR in the absence of obstructive CAD on CCTA. The detection of subendocardial perfusion abnormalities suggests microvascular coronary involvement as a potential cause of the cardiac ischemic findings. Further validation testing of adenosine CMR in a larger SLE population is warranted.

PO1.C.28

CT findings of lung changes in systemic lupus erythematosus: correlation with disease duration
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Objective: To evaluate lung changes in systemic lupus erythematosus (SLE) and to analyze the relationship between the extent of lung abnormalities and duration of SLE. **Materials and Methods:** We studied 41 patients (1 man, 40 women; mean age, 42 years) with systemic lupus erythematosus (SLE) who showed airway or interstitial lung disease on thin-section chest CT scans. Mean duration of disease was 9 years. Patients in whom pulmonary abnormalities were clinically thought to be due to infection or drug toxicity were excluded. Lung parenchymal abnormalities that included airspace consolidation, ground-glass opacity (GGO), reticulation, honeycombing, bronchovascular bundle thickening, nodules, bronchiectasis, traction bronchiectasis, and air trapping were assessed retrospectively by two chest radiologists. The relationship between the extent of each CT finding and duration of SLE were analyzed. One or 2 predominant HRCT findings were identified for each case, and patients were classified according to the predominant pattern. **Results:** The most frequent pulmonary abnormality was GGO (54%), followed by traction bronchiectasis (46%), reticulation (27%), honeycombing (27%), bronchiectasis (24%), consolidation (15%), air trapping (15%), bronchovascular bundle thickening (7%), and nodules (7%). None of these abnormalities correlated with the duration of SLE. We identified 15 patients with bronchiolitis pattern, 11 patients with nonspecific interstitial pneumonia (NSIP) pattern, 10 patients with usual interstitial pneumonia (UIP) pattern, 3 patients with organizing pneumonia pattern, and 2 patients with lymphocytic interstitial pneumonia pattern. **Conclusion:** Airway disease was more common than individual interstitial pneumonia patterns in patients with SLE. No correlation was found between duration of disease and the extent of CT abnormalities.

PO1.C.29

Analysis of clinical features of meningitis in Korean patients with systemic lupus erythematosus
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Objectives: Meningitis is a rare complication in systemic lupus erythematosus (SLE), which can lead to a fatal outcome. Meningitis is divided into septic and aseptic meningitis. The purpose of this study is to determine the demographic, clinical, laboratory features and outcomes of the meningitis in Korean patients with SLE. **Methods:** We conducted a retrospective medical record review of 1420 patients who were diagnosed as SLE in the rheumatology department of Seoul St. Mary's and St. Mary's Hospital in Korea between January 1997 and June 2009. We identified 20 patients (mean age 29.7±2.5 years) who developed septic or aseptic meningitis. The clinical characteristics, laboratory data, brain imaging findings and prognosis of these patients were analyzed. **Results:** In 11 cases, causative microorganisms were revealed ("septic meningitis"). Cryptococcus neoformans was identified in 5 patients, Listeria monocytogenes in 2 patients, Neisseria meningitidis, Streptococcus agalactiae, Haemophilus influenzae, and Mycobacterium tuberculosis in 1 patient, respectively. The other 9 patients were diagnosed as aseptic meningitis. Patients with septic meningitis were older than those with aseptic meningitis at the time of diagnosis of meningitis

(P=0.025). The most common manifestation was headache followed by fever and nausea in both types of meningitis. Mental changes were more frequently observed in patients with septic meningitis (P=0.005) although the presence of abnormal findings in brain imaging and prognosis did not differ significantly. Leukocyte counts in CSF were higher in patients with septic meningitis (P=0.044). The level of CSF protein tends to be higher in aseptic meningitis group (P=0.053) and the level of CSF glucose was lower in septic meningitis group (P=0.036). Plasma leukocyte count as well as neutrophil count was higher in patients with septic meningitis than in those with aseptic meningitis (P=0.037 and P=0.020, respectively). Conclusions: Meningitis was observed in 1.4% of the Korean patients with SLE. In 55% of the meningitis cases, microorganisms were isolated and Cryptococcus neoformans was identified most frequently. Alteration of mental status was more common in cases of septic meningitis. In addition, plasma leukocytosis and neutrophilia as well as CSF pleocytosis and hypoglycemia were more prominent in patients with septic meningitis.

PO1.C.30

Unusual presentation of antiphospholipid syndrome with cardiac failure and cutaneous thrombotic microangiopathy

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Objectives: Since the initial description of the antiphospholipid syndrome (APS) the spectrum of clinical manifestations has broadened, and it has become one of the most systemic conditions. Occasionally, the APS may present with predominant occlusive thrombosis of small vessels. We report a case of a patient who developed acute heart failure and cutaneous nodules, with histologic evidence of subcutaneous thrombotic microangiopathy. **Methods:** A case was investigated retrospectively and literature was reviewed. **Results:** A 38-year-old woman with a previous history of breast cancer and thrombocytopenia, presented with 2-week history of dyspnoea and a skin rash. On physical examination she had a blood pressure of 150/100 mm Hg. Localized cutaneous areas with a mottled blue discoloration consistent with livedo reticularis, and nodular skin lesions were observed on the lower extremities. Laboratory tests revealed: lymphopenia, a high erythrocyte sedimentation rate, and C-reactive protein level. Complement C3 and C4 were low. High titres of anticardiolipin (IgM and IgG) and antibeta-2 glycoprotein I (IgM and IgG) antibodies, were observed. Antinuclear antibodies, and anti-double-stranded DNA antibodies tests were positive. Other serologic tests for autoimmune disorders, and common viral and bacterial infections were negative or normal. High titres of anticardiolipin (IgM and IgG) and antibeta-2 glycoprotein I (IgM and IgG) antibodies, were observed. Antinuclear antibodies, and anti-double-stranded DNA antibodies tests were positive. Other serologic tests for autoimmune disorders were negative. The ECG demonstrated sinus tachycardia, and diffuse ST-T segment abnormalities. CT scan of the chest revealed marked enlargement of the cardiac silhouette, pulmonary congestion, and patchy infiltrates in the lower lobes. Transthoracic echocardiography demonstrated severe left ventricular dysfunction. Endocarditis was excluded by transesophageal echocardiography. A gammagraphic study of the heart revealed left ventricular akinesia.Histologic examination of a cutaneous biopsy specimens disclosed a diffuse thrombotic small vessel involvement of the dermis and the subcutaneous tissue. The patient was diagnosed of APS and systemic lupus erythematosus, and started treatment with anticoagulants, steroids, cyclophosphamide, diuretics, and ACE inhibitors. At 2-years follow-up, the patient was asymptomatic. In this patient, the close temporal relationship between the microvascular thrombotic lesions of the skin and the cardiomyopathy suggest that both disorders were produced by the APS. **Conclusions:** Clinicians should considerer the diagnosis of APS in patients with acute cardiac failure and cutaneous lesions.

PO1.C.31

Comparison of the clinical manifestations and disease severity of systemic lupus erythematosus (SLE) among Vancouver residents of Asian and Caucasian origin

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Objectives: To compare Caucasian and Asian SLE patients followed by selected rheumatologists in the Greater Vancouver area for: 1) clinical manifestations and pattern of organ involvement; 2) Disease severity using the SLICC damage index. **Methods:** A retrospective medical chart review was conducted for the period 1999-2005 of rheumatology practices selected for large numbers of lupus patients of Asian origin. To be included patients had to meet 1982 ACR criteria for SLE and be Caucasian or Asian. Ethnicity was determined by chart review or by asking the treating rheumatologist if missing from chart. Asian ethnicity included Chinese, Japanese, Korean, Philippino, and Vietnamese. East Indians were not considered as Asians. Data were extracted using a standard form that included: demographics, SLE duration, 1982 ACR criteria, SLE treatment, SLICC, and involvement of the following systems, using predefined criteria derived from the SLAM: skin, myositis, arthritis, haematological, renal, cardiovascular, pulmonary, gastrointestinal, and neuropsychiatric manifestations. **Results:** Our sample included 165 Caucasians and 102 Asians (Mean age: 41.1 vs 44.9 years, resp. and 88.6% vs. 95% were female, resp.). Asian patients had more frequent renal involvement than Caucasians: proteinuria (41% vs. 17%, resp.), biopsy proven glomerulonephritis (39% vs. 13%), renal insufficiency (15% vs. 4%) and end-stage renal failure (8% vs. 2%); as well as haematological manifestations: lymphopenia (80% vs. 60%) and thrombocytopenia (37% vs 22%) (all p < 0.05). Caucasian patients had more frequent skin disease compared to Asians: discoid rash (15% vs 7%) and photosensivity (48% vs 30%), as well as neuropsychiatric manifestations, mainly cognitive impairment, anxiety, mood disorders and migraine headaches, but severe CNS involvement, such as vasculitis, CVA, seizures or psychosis, were not more frequent. The risk of SLICC score of 2 or more versus SLICC of 0 or 1, after controlling for age, gender, disease duration and smoking in a binary logistic and multinomial regression, was greater in the Asian group (OR 1.868 95% CI 1.001-3.485 p = 0.0498). **Conclusion:** In our sample of Vancouver residents, people of Asian origin had a different pattern of SLE manifestations, with more renal and haematological, but less CNS disease, compared to Caucasians. There was a significant difference in disease severity between the two groups using the SLICC damage index suggestive of a more severe disease in Asians.

	Caucasian N (%)	Asian N (%)	P value
Mucocutaneous	152 (92%)	97 (95%)	0.346
Musculoskeletal	144 (87%)	92 (91%)	0.469
Hematological	122 (75%)	94 (92%)	0.0001
Cardiovascular	56 (31%)	28 (27%)	0.26
Respiratory	53 (32%)	22 (22%)	0.062
Gastrointestinal	5 (3%)	8 (8%)	0.076
Neuropsychiatric	107 (65%)	32 (31%)	0.0001
APLS	35 (21%)	13 (13%)	0.08
Renal	43 (26%)	56 (55%)	0.0001

PO1.C.32

**Hidroxichloroquine and metabolic syndrome
in patients with systemic lupus**
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Background: The prevalence of the metabolic syndrome (MS) is increased in inflammatory and inmune processes. In patients with systemic lupus erythematosus (SLE) this condition predisposes to cardiovascular disease concerning its morbidity and mortality. It has been reported a beneficial effect with antimalarial drugs such as hydroxychloroquine (HCQ) on lipid and glucose metabolism, but the potential protective effect on MS is controversial. **Objective:** To describe the prevalence of MS, insuline resistance and secretion using homeostasis model assessment (HOMA IR) and disease activity (SLEDAI) in patients with SLE with and without HCQ therapy. **Methods:** A total of 51 patients with SLE (per the American Collegue of Rheumatology classification criteria) were prospectively evaluated (October 2007-May 2009). MS was assessed using the World Health Organization (WHO) classification. Group 1: Patients without HCQ in the last 6 months. Group 2: Patients with HCQ $\geq$ 3 months. Dependent variable: treatment with HCQ. Independent variable: HOMA-IR ($\geq$ 2,11) Covariables: age, sex, median disease duration, median glucocorticoids dose (GC) in last 6 months, SLEDAI, Body Mass Index (BMI), waist ($\geq$88 cm), hypertension ($\geq$140/90), TG ($\geq$150mg/dl), HDL($\leq$40mg/dl),fasting glucose (>110 mg/dl). Statistical analysis: Significance level was set at probability value p $\geq$0.05. Chi-square and Fisher´s exact test were used for comparison of categorical variables or percentages. **Results:** There were 51 patients, 49 of whom were women (96%). Group 1: 23/51 (45%); mean age: 38 years (DS: 13.72); mean duration of the disease: 8.9 years (DS: 7.33); mean GC dose: 6.7 mg (DS: 5.96). Group 2: 28/51 (55%); mean age: 34 years (DS: 13.47); mean duration of the disease: 7.3 years (DS: 7.15); mean GC dose: 5.1mg (DS: 6.14).

	HOMA IR; $\geq$ 2,11	Waist; $\geq$88 cm	Hypertension; $\geq$ 140/90	TG; $\geq$150 mg/dl	HDL; $\leq$40mg/dl	MS (%)	SLEDAI $\geq$4
Group 1	10/23 (43.5%)	13/23 (56.5%)	6/23 (26%)	6/23 (26%)	11/23 (47.8%)	9/23 (39.1)	10/23 (43,5%)
Group 2	11/28 (39.3%)	6/28 (21.4%)	1/28 (3.6%)	5/28 (17.4%)	5/28 (17,9%)	2/28 (7.14)	6/28 (21.4%)
p	1	0.02	0.05	0.136	0.04	0.01	0.16

Conclusions: In this study we found that patients with SLE under treatment with HCQ presented lower disease activity and that the prevalence of MS was significantly lower. In this group there was no evidence of significant decrease in sensitivity to insulin (HOMA IR). Treatment with antimalarial drugs plays a rol in the inflammatory procces of SM and shows a protective effect in patients with SLE.

PO1.C.33

**Regenerative nodular hyperplasia of the liver in systemic lupus
erythematosus: case report and review of the literature**
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Objectives: Regenerative nodular hyperplasia (RNH) of the liver represents a rare, but clinically important and likely under-recognized manifestation of systemic lupus erythematosus (SLE). To date, reported therapy in the available literature has focused on the treatment of associated portal hypertension and its sequelae, but not the underlying immunopathology. We describe a case in which systemic therapy for SLE was associated with evidence of improvement of RNH of the liver. **Methods:** Case report and review of the literature. **Results:** A 27-year old female from Guyana was diagnosed with SLE on the basis of cutaneous, serosal, renal, and hematologic involvement and elevated antibodies to Smith and double-stranded DNA. Additional initial workup revealed elevations in serum alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT), with mild elevations in aminotransferase levels and low albumin, but normal bilirubin and coagulation profile. Abdominal ultrasonography and computed tomography revealed multifocal hepatic nodules, portal venous dilatation and splenomegaly, but no ascites. Guided transcutaneous liver biopsy revealed only periportal inflammation, but no RNH, likely due to sampling error. Studies were negative for bacteria, fungi, parasites, mycobacteria, fibrosis and malignancy. Testing for hepatitis B and C, cytomegalovirus, antibodies to smooth muscle, mitochondrial and liver/kidney microsomal 1 antigens were negative, as was testing for anticardiopin antibodies and lupus anticoagulant. Most of the features, but not the hepatic biochemical abnormalities, improved with initial treatment including tapering prednisone and hydroxychloroquine. Subsequently, following a period of noncompliance, the patient presented with florid SLE including panniculitis and mononeuritis multiplex, necessitating more aggressive treatment including corticosteroids, intravenous pulsed cyclophosphamide and intravenous gammaglobulin. With this combination, the panniculitis and mononeuritis multiplex improved, as did the biochemical evidence of RNH. **Conclusions:** RNH of the liver is a rare (0.3% of cases by one estimate), but an important and likely under-recognized manifestation of SLE, with sequelae that can include portal hypertension with associated ascites and esophageal varices. The mechanisms are thought to include vasculitis, or, in some cases, primary thrombosis in the setting of antiphospholipid antibodies. Less than 25 cases of RNH of the liver in association with SLE have been reported, and therapies have focused on controlling portal hypertension (e.g. with non-specific beta blockade or porto-systemic shunting) and its sequelae (e.g. local treatment of esophageal varices). In the current case, systemic immunosuppressive treatment of severe SLE was associated with hepatic biochemical improvement, suggesting alteration of the suspected underlying vasculitic mechanism of RNH.

PO1.C.34

**Abnormal regional cerebral blood flow in systemic lupus
erythematosus patients with memory impairments**
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Objective: Previous single-photon emission computed tomography (SPECT) studies have demonstrated multifocal hypoperfusion areas in systemic lupus erythematosus (SLE) patients. To our knowledge, there are no studies that explore the association between memory impairment and regional blood flow (rCBF) changes in SLE patients using statistical parametric mapping (SPM) analysis. This study investigated the association of rCBF changes with memory impairment in SLE patients using SPM. **Methods:** 19 SLE patients (mean age 36.1±8.6, range 17~47) with subjective memory complaints underwent 99mTc ECD SPECT. Their SPECT images were analyzed by SPM2 software. The Korean-Wechsler Adult Intelligence Scale (K-WAIS) and Rey-Kim Memory Test (RKMT) were used to evaluate cognitive functions of the patient group objectively. On the basis of the Intelligent Quotient (IQ) - Memory Quotient (MQ) difference score, the patients were classified into 2 groups: a group with pronounced memory impairment (n=8) and a group without memory impairment (n=11). **Results:** There was no significant difference between 2 groups in clinical and demographic characteristics. However, we found decreased rCBF in the posterior cingulate cortex (PCC) in patients with pronounced memory impairment. **Conclusion:** This is the first study using SPM analysis of SPECT images in SLE patient complained of memory impairments. The PCC hypoperfusion may play a significant role in the memory function of SLE patients. *Key words:* SLE, memory impairment, SPECT, SPM

PO1.C.36

Clinical features, disease activity and damage accrual in systemic lupus erythematosus patients. Data from a Cuban cohort.

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Background and Purpose: systemic lupus erythematosus (SLE) is a chronic disease with flare-up and remissions episodes. An active disease may lead to a damage accrual. The aim of this study was to determine the prevalence of activity and damage accrual using data from SLE Cuban cohort. ***Methods:*** SLE patients from 2 centers were recruited and followed-up longitudinally. Demographic characteristics, cumulative clinical manifestations, laboratory data, disease activity using both SLEDAI and MEX-SLEDAI indexes (at diagnosis, 3, 6 and 9 years of follow-up) and Systemic Lupus International Collaborating Clinics damage index (SLICC/ACR) were compared between patients in an univariable (UV) and multivariable (MV) logistic regression models. ***Results:*** of the 102 patients included in this study, 93.1% were female. The mean age at disease onset was 28 years (SD 10) and at diagnosis was 32 years (SD 10). The mean disease duration was 9 years. Eighty six percent of the patients fulfilled ACR criteria at the time of diagnosis and 94.1% during follow-up. Most frequently accumulated clinical manifestations were arthritis (86.7%), photosensitivity (67.6%), malar rash (58.8%), fever (53.9%), fatigue (35.2%) and renal disease (24.5%). Disease activity evaluation using SLEDAI and MEX-SLEDAI showed active disease at the time of diagnosis in 100% of the patients (by both indexes), at 3 years: 60.7% and 59.8%, at 6 years: 35.3% and 37.2% and at 9 years: 19.6% and 16.7% respectively. Median SLEDAI and MEX-SLEDAI scores were: at diagnosis 10 and 5 and at 3 years 4 and 2 respectively. Evaluation at 6 and 9 years of follow-up the result was 0 by both indexes. The UV analysis performed to evaluate associated risk factors to lupus activity showed a significant association only with non-Caucasian ethnicity (OR: 2.54, CI: 1.06-6.18). Five hundred and six flare-up episodes were registered in a 732 cumulated years. An incidence of 0.69 flare-up/patient/year was observed. UV and MV logistic regression analysis showed significant association between damage accrual with non-Caucasian ethnicity (OR: 3.19, CI 1.23-8.38), a disease of more than 5 years (OR: 3.25, CI: 1.09-10.12) and with ≥3 flare-up during 1 year (OR: 3.70, CI: 1.26-10.96). ***Conclusion:*** in this cohort, active disease was present in 100% of the SLE patients at diagnosis but in less than 20% at the end of follow-up. Non-Caucasian ethnicity was the only risk factor associated with active disease. Damage accrual was significantly associated with non-Caucasian ethnicity, a disease of more than 5 years and with 3 or more flare-ups in 1 year.

PO1.C.37

Risk factors for arterial thrombosis in patients with lupus nephritis

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Objectives: To study the risk factors for arterial thrombosis in patients with lupus nephritis. ***Methods:*** Between 1996 and 2007, patients with SLE who fulfilled the ACR criteria for renal involvement were identified from our lupus cohort database. The cumulative incidence of arterial thromboembolic events since the diagnosis of lupus renal disease was analyzed by Kaplan-Meier's plot. The effect of blood pressure control (mean systolic and diastolic blood pressure over time since the diagnosis of lupus nephritis), renal insufficiency (mean creatinine clearance [CrCl] over time as estimated by the MDRD formula) and other traditional and non-traditional risk factors on the risk of arterial thrombosis were studied by Kaplan-Meier and Cox regression analysis. ***Results:*** 232 patients with lupus nephritis were studied (88% women; mean age at diagnosis of lupus nephritis 35.3±15 years). Renal biopsies were performed in 211 (91%) patients - 100 (43.1%) patients had diffuse prolifera-

tive nephritis and 54 (23.3%) had membranous lupus nephritis. After a total follow-up of 1646 patient-years (mean 7.1 years) since the diagnosis of lupus renal disease, 20 arterial thromboembolic events occurred in 28 patients (incidence 12/1000 patient-year). The cumulative hazard for arterial events at 5 and 10 years after diagnosis of lupus nephritis was 3% and 18% respectively. In a Cox regression model, age, sex, SLE duration, chronic smoking (>=3 years), diabetes mellitus, the presence of antiphospholipid antibodies, menopause, dyslipidemia (LDL ≥4.1 mmol/L or HDL ≤1.0 mmol/L), long-term use of prednisolone (>=3 years), mean CrCl of <60ml/min and a mean systolic blood pressure of ≥130mmHg ever since the onset of lupus nephritis was not significantly associated with arterial thrombosis. However, a mean diastolic blood pressure of ≥85mmHg since the diagnosis of lupus nephritis was a strong independent risk factor for arterial thrombosis (HR 33.5 [95%CI 4.45-252]; p=0.001). Patients with histological membranous lupus nephropathy were not at higher risk of arterial thrombosis. ***Conclusions:*** In this large cohort of patients with lupus nephritis, histological classes, renal insufficiency and the mean systolic blood pressure were not significantly associated with arterial thrombosis. However, the mean diastolic blood pressure was a strong independent factor for the development of arterial thrombosis. Vigorous control of the diastolic blood pressure to a target of 85mmHg is recommended to reduce the risk of arterial thromboembolism.

PO1.C.38

Is osteoporosis a predisposing factor for subclinical coronary atherosclerosis in systemic lupus erythematosus (SLE)?

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Background and Objectives: Patients with SLE are prone to accelerated atherosclerosis, which is contributed by traditional and SLE-specific risk factors. Some of these factors such as chronic smoking, menopause, persistently elevated cytokines as a result of disease activity and long-term medications such as corticosteroids and cyclosporin are also unfavorable for bone mineral density (BMD). This cross-sectional study aims to evaluate the relationship between low BMD and subclinical coronary atherosclerosis in patients with SLE. ***Method:*** Consecutive patients who fulfilled ≥4 ACR criteria for SLE and had at least one vascular risk factor but without any ischemic symptoms were screened for osteoporosis and coronary atherosclerosis by DXA scan (Hologic, Bedford, USA) and multi-detector CT scan (Agatston calcium scores), respectively. Risk factors for atherosclerosis and osteoporosis were identified. Association between BMD values and the Agatston calcium scores was studied by correlation and regression analyses. ***Results:*** 144 SLE patients were studied (94% women). The mean age was 46.4±10.5 years and the mean SLE duration was 9.8±7.5 years. The frequency of vascular risk factors was as follows: smoking ≥3 years (10.4%), menopause (57%), diabetes mellitus (3%), hypertension (41%), body mass index ≥27kg/m2 (15%), LDL ≥2.6 or HDL ≥1.0 mmol/L (52%), antiphospholipid antibodies (36%), glucocorticoid treatment≥3 years (78%). Thirty-one (22%) patients had osteoporosis at either the hip or lumbar spine (Z score <-2.5). Patients who had osteoporosis at the hip or spine had higher Agatston calcium scores than those without, but the difference was not statistically significant (37.7±80 vs 20.3±60; p=0.27). The proportion of patients who had abnormal coronary calcification (Agatston score ≥1) was also higher in osteoporotic than non-osteoporotic patients (42% vs 27%) but again statistical significance could not be achieved (p=0.13). The odds ratio of coronary atherosclerosis in patients with osteoporosis was 1.90 [0.82-4.40]. Age was an independent risk factor for both the occurrence of coronary atherosclerosis (relative risk 1.11 [1.04-1.19]; p=0.003) and osteoporosis at the hip / spine (relative risk 1.09 [1.01-1.18]; p=0.03). In a logistic regression model, osteoporosis had a positive relationship but was not significantly associated with coronary atherosclerosis after adjustment for age, sex and other risk factors such as SLE duration, smoking, hypertension, diabetes mellitus, hyperlipidemia, antiphospholipid antibodies, long-term glucocorticoid treatment, menopause and obesity. ***Conclusions:*** SLE patients with low BMD tend to have a higher risk of coronary atherosclerosis. A bigger sample size may be able to show a significant relationship.

PO1.C.39

Ultrasound in the assessment of Achilles tendon alteration in SLE

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Articular involvement, most frequently arthralgias or non erosive arthritis, is one of the most frequent clinical manifestation of Systemic Lupus Erythematosus (SLE). Few studies have evaluated joints and tendons involvement in SLE patients. In two studies, the ecographic analysis of tendons of a limited number of SLE subjects has showed the presence of finger flexor tenosynovitis and inflammation and thickness reduction of hand tendons. The purpose of this study was to evaluate inflammatory Achilles tendon involvement in SLE patients through three-dimensional ecography. ***Material And Methods:*** We enrolled 10 SLE patients (F:M=9:1) referring to our Rheumatology Unit, with a medium age of 37.2 (±8.6) and disease duration of 10,8 years (±11.3). A three-dimensional ultrasound study (using a Logiq 9, General Electric Medial Systems, Milwaukee, WI) was performed by a rheumatologist who was not experienced in ultrasound, specifically trained for using the volumetric probe on Achilles tendon. The images volumes analysis was performed, at least 30 days after, by a specialist in muscle-skeletal ultrasound, unaware of the clinical picture of patients. Only two patients referred the presence of calcaneal pain. ***Results:*** The ultrasound showed tendinous alterations in all patients; 6 of 10 patients had calcific enthesopathy (bilateral in two cases). A mild swelling of the retrocalcaneal bursa (bilateral in three cases) was displayed in one-half of the patients, whereas only one patient (complaining of calcaneal pain) had tendinous power Doppler signal. Calcaneal bone profile was irregular in two cases (with bone erosions in one patient). Only in one case there was the increase of the thickness of the tendon. No Achilles tendon tears were observed. ***Conclusions:*** The results of this study confirm the frequent tendinous and peri-tendinous involvement in SLE patients and show the presence of both inflammation and degeneration signs (usually not accompanied by clinical symptoms). Volumes, and not single images, acquisition could allow a ultrasound follow-up of patients.

PO1.C.40

Antiphospholipid antibodies predict future arterial events: results from the Montreal Antiphospholipid Study (MAPS) at eleven years of follow up.

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Objectives: To determine the role of antiphospholipid antibodies (aPL) in predicting new arterial (VE-A) and venous (VE-V) vascular events in an ongoing prospective cohort of individuals. ***Methods:*** Demographic and clinical data were obtained at baseline and semiannually. Events were confirmed by medical record review. Blood samples collected at baseline and annually for four years were assayed for IgG/IgM anticardiolipin (aCL), lupus anticoagulant (LA), and IgG/IgM anti-β2-glycoprotein I antibodies (aβ2GPI). Kaplan-Meier and proportional hazard survival analyses were used to compare time to new VE-A or VE-V in aPL-positive (aCL>40 and/or LA and/or aβ2GPI positive) versus aPL-negative individuals. Multivariate regression analyses were performed using new VE-A or VE-V as outcome variable and aPL positivity as predictor variable. Covariates for outcome VE-A were age, gender, family history of cardiovascular disease (FMH), smoking, systemic lupus erythematosus (SLE), hypertension (HTN), diabetes mellitus (DM), previous VE-A, activated protein C resistance (APCR), and hyperhomocysteinemia. Covariates for outcome VE-V were age, gender, SLE, previous VE-V, anticoagulation therapy (ACT), APCR, antithrombin III, and factor V Leiden and MTHFR mutations. ***Results:*** 414 persons with mean (SD) age 46.4 (14.1) years were enrolled: 83.1% were female; 53.3% had FMH, 20.5% had SLE, 27.8% were smokers, 15.9% had HTN, 5.6% had DM, and 16.4% had had previous

VE-A or VE-V. Fifty-nine (14%) individuals were aPL-positive at baseline; 38 became aPL-positive during follow-up for a total of 97 (23.4%). During 11 years of follow-up (median 9.5 [IQR=4.0, 10.6] years), 52 (12.6%) individuals sustained 80 new events (33 had VE-A; 14 had VE-V; 5 had both). The proportion of VE-free survivors at 11 years was 88% (95% CI=84%, 92%) for aPL-negative and 67% (95% CI=54%, 81%) for aPL-positive individuals. The proportion of VE-A-free survivors was 91% (CI = 88%, 95%) for aPL-negative and 76% (CI = 65%, 89%) for aPL-positive individuals. For VE-V-free survivors, the proportions were 97% (CI = 95%, 99%) for aPL-negative and 83% (CI = 73%, 95%) for aPL-positive individuals. VE-A were predicted by aPL positivity [HR= 2.52 (CI=1.17, 5.47)], age [HR=1.05 (CI=1.02, 1.07)], DM [HR=3.93 (CI=1.66, 9.32)], smoking [HR=2.31 (CI=1.15, 4.64)], and previous VE-A [HR=4.77 (CI=2.32, 9.82)]. VE-V were predicted by previous VE-V [HR=4.00 (CI=1.35, 11.87)], ACT [HR=4.60 (CI=1.50, 14.08)], and APCR deficiency [HR=5.21 (CI=1.95, 13.90)]. The effect of aPL positivity on VE-V could not be accurately estimated from our data [HR=1.86 (CI=0.67, 5.20)]. ***Conclusion:*** aPL positivity independently predicts VE-A, whereas the effect of aPL on VE-V is less clear.

PO1.C.41

Prolongation of the corrected QT interval in anti-Ro/SSA positive adults with SLE

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Electrocardiographic (ECG) abnormalities such as prolongation of the corrected QT interval (QTc) are known to occur in newborns who passively acquired anti-Ro/SSA antibodies through maternal transfer. In adults, studies of the association between QTc prolongation and anti-Ro/SSA are conflicting. Prolonged QTc can lead to life-threatening arrhythmias. Hence, the identification of risk factors for this syndrome is important. ***Objective:*** To examine whether anti-Ro/SSA antibodies are associated with an increased risk of QTc prolongation in a large SLE cohort. ***Methods:*** Patients fulfilling ACR criteria for SLE were enrolled at their first clinic visit. All patients were followed prospectively on an annual basis, at which time medication use, measures of disease activity (SLEDAI) and damage (SLICC/ACR damage index [SDI]), and laboratory data were collected. All registry participants were invited to undergo a 12-lead resting ECG at the time of their annual research visit between June 2002 and May 2007. Results of the last ECG obtained and corresponding clinical and laboratory data were analyzed. QTc greater than or equal to 440msec was considered prolonged. Bayesian Information Criterion (BIC) was used to select factors more likely to be associated with the outcome of prolonged QTc and multivariate logistic regression models were performed to assess the association between anti-Ro/SSA antibodies and prolonged QTc. Other potentially associated factors examined included age, disease duration, SLEDAI, SDI, potassium and magnesium levels, antimalarials, and beta-blockers use. ***Results:*** For the 278 subjects with an ECG performed during the study, most (91%) were female, mean age was 44.8 years (SD=14.8), and mean disease duration was 12.9 years (SD=11.0). The prevalence of anti-Ro/SSA positivity was 41.9% and QTc prolongation was present in 6.5% of the entire cohort. Only 38.8% of patients with a normal QTc were anti-Ro/SSA positive compared to 72.2% of patients with a prolonged QTc. Of these, the 271 patients (97.5%) with complete data were used for the multivariate analysis. The only factors associated with prolongation of QTc were anti-Ro/SSA (OR 3.8; 95%CI 1.3–11.0) and SDI (1.3; 1.1 – 1.6). This corresponds to an almost 4-fold risk for prolonged QTc in the presence of anti-Ro/SSA. ***Conclusion:*** Anti-Ro/SSA antibodies were associated with QTc prolongation in a large SLE cohort. Patients positive for anti-Ro/SSA antibodies may benefit from ECG testing. Those identified with QTc prolongation should receive counseling, including education about drugs that may put them at risk for life-threatening arrhythmias.

PO1.C.42

**Findings on conventional MRI of the brain
in active neuropsychiatric SLE**

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Objective: The clinical manifestations of systemic lupus erythematosus with primary involvement of the nervous system (primary neuropsychiatric SLE or NPSLE) are highly diverse and their etiology is incompletely understood. The aim of this study was to provide an inventory of abnormalities on conventional brain MRI in patients with primary NPSLE and to interpret the findings in relation to possible underlying pathogenetic mechanisms. **Methods:** The first brain MRI exams of the first episode of active primary NPSLE of 74 patients were retrospectively reviewed. All patients fulfilled the revised 1982 ACR criteria for SLE and were classified according to the 1999 ACR case definitions for NPSLE syndromes. Patients with a history of brain disease or secondary NPSLE and patients in whom other mechanisms unrelated to SLE caused the neuropsychiatric symptoms were excluded. **Results:** The principal findings were 1) punctiform, focal or patchy hyperintensities in WM (49% of all patients) or both WM and GM (5%) suggestive of vascular inflammation (vasculitis) or multifactorial autoimmune mediated mechanisms of vascular occlusion or narrowing (vasculopathy with ischemia), 2) more widespread, confluent hyperintensities in the WM, suggestive of chronic hypoperfusion due to the same mechanisms, 3) diffuse cortical GM lesions, most likely due to an immune response to neuronal components (12%) and 4) absence of MRI abnormalities, despite active signs and symptoms (42%). None of the MRI patterns, including normal MR images, appeared characteristic for a separate ACR NPSLE syndrome. A remarkable finding was the frequent occurrence (12%) of small cerebellar defects, which were probably old subclinical infarcts. **Conclusion:** Several distinct brain MRI patterns were observed in patients with active NPSLE, suggestive of different pathomechanisms. To advance our understanding of the various processes leading to NPSLE, the radiological manifestations may be a good starting point and useful for categorization of patients in further research.

PO1.C.43

**Incidence and long- term prevalence of neurological manifestations
in an inception cohort of 1480 SLE patients (NPSLE).**

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Neurological manifestations of LES patients (NML) at diagnosis are partially known; however, the incidence of them during follow up is unknown. The object of the present was to show the NML at diagnosis and during the follow up. Up to the moment of the study, GLADEL cohort (Grupo Latin American Del Estudio de Lupus) had evaluated 1480 LES patients, with 5.5 years of median time follow up. We formed 3 groups for showing data: 1) patients with NML at diagnosis, 2) with NML during the follow up (no one of them had NML at diagnosis), and 3) patients without NML up to the moment. For group 1, 2 and 3 respectively: women 90.1%, 87%, 91% (p=0.18); Age in years at beginning of symptoms 29±12, 28±12 and 29±12 (p=0.81); Age in years at diagnosis 31±13, 30±12 and 30±12 (p=0.29); Urban residence 89%, 91%, 91% (p=0.70), and ethnicity: white 43%, 34%, and 43%, mestizo 42%, 53%, and 41%, African Latino American 11%, 10% and 14%, others 5%, 4% and 2% (p=0.0001).

Variable	Group 1 n (%)	Relapses in group 1 n (%)	Group 2 n (%)
Central and pheripheral nervous system			
Seizures	73 (4.9)	49 (3.3)	60 (4.1)
Motor and sensitive disturbances	27 (1.8)	21 (1.4)	72 (4.9)
Sensitive disturbances	21 (1.4)	15(1)	42(2.8)
Stroke	21(1.4)	16(1.1)	32(2.2)
Cranial Nerves disturbances	19(1.3)	25(1.7)	55(3.7)
Motor disturbances	16(1.1)	14(0.9)	24(1.6)
Polineurophaty	12(0.8)	5(0.3)	13(0.9)
Extra pyramidal manifestations	9(0.6)	7(0.5)	8(0.5)
Vertigo	8(0.5)	8(0.5)	21(1.4)
Mono neuritis multiplex	8(0.5)	1(0.1)	14(0.9)
Syncope	7(0.5)	1(0.1)	5(0.3)
Aseptic Meningitis	4(0.3)	1(0.1)	5(0.3)
Coma	3(0.2)	2(0.1)	1(0.1)
Pseudo tumor cerebri	3(0.2)	1(0,1)	1(0.1)
Transverse myelitis	1(0.1)	6(0.4)	7(0.5)
Autonomic neuropathy	-	1(0.1)	-
Pychiatric and neuropsychological manifestations			
Mood changes	34(2.3)	20(1.4)	60(4.1)
Confusional state	27(1.8)	10(0.7)	42(2.8)
Psychosis	27(1.89	18(1.2)	37(2.5)
Organic brain syndrome	16(1.1)	8(0.5)	23(1.6)
Memory loss	10(0.7)	6(0.4)	23(1.6)
Dementia	--	1(0.1)	1(0.1)
Total of patients with at least 1 manifestations (N=1480)	207(14)	124(8.4)	318(21.5)

PO1.C.44

**Psychiatric syndromes in patients with systemic lupus
erythematosus and rheumatoid arthritis**

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Objective: To examine the frequency and reliability of depression and anxiety in patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). The cause of psychiatric syndromes in SLE patients with is mutifactorial and includes primary immunopathogenic mechanisms, nonspecific sequelae of chronic disease, and concurrent illnesses. Depression and anxiety are more common in patients with SLE than in RA patients. **Methods:** Forty-three patients with SLE were matched by age and sex to 43 patients with RA attending ambulatory clinics in a single academic medical centre. All fulfilled the American College of Rheumatology (ACR) classification criteria for either SLE or RA. Anxiety and depression levels were assessed with the Beck Depression Inventory and Beck Anxiety Inventory. Health related quality of life (HRQOL) was evaluated by the SF-36. **Results:** The patients were well matched with regard to age, sex and disease duration. There were no significant differences in self-reported HRQOL, anxiety and depression symptoms between the 2 groups. **Conclusion:** Psychiatric syndromes such as depression and anxiety regardless of etiology, are common in both SLE and RA patients. Depression and anxiety in SLE patients may represent global changes in the central nervous system that require ongoing evaluation and treatment. Severe chronic pain accompanied by progressive joint destruction, disability, and disfigurement in RA patients increases the risk of depression and anxiety.

PO1.C.45

Framingham scores and individual cardiovascular disease (CVD) risk factors in patients with systemic lupus erythematosus (SLE) without past history of cardiovascular events

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Objective: To determine the baseline Framingham score and prevalence of individual CVD risk factors in female subjects with SLE without past history of CVD in the Health Improvement and Prevention Program (HIPP). *Methods:* Our cohort consisted of 287 females, meeting at least 4 of the ACR classification criteria for SLE. CVD risk profile including: family history, smoking, diabetes, menopause, systolic, diastolic blood pressure (SBP, DSP), body mass index (BMI), physical activity, serum levels of triglycerides (TG), total cholesterol (TC) LDL, HDL-cholesterol, homocysteine and high sensitivity C-reactive protein (hsCRP) were documented for all patients. Hypertension was defined as SBP>130 and DBP>85. Patients with BMI>25 and >30 were considered overweight and grossly obese respectively. Serum TG>1.7mg/l; TC>5.5 mg/l; LDL>2.5; HDL<1.3mg/l, homocysteine >13 and CRP>1 were considered abnormal and risk factors for CVD. Ten years Framingham scores were calculated. *Results:* The mean (SD) age for the cohort was 43.6(13.1) years, SLE duration was 11.5(10.3) years, 38.6% (n=128) had family history of CVD, 23.2% (n=77) were smokers, 42.5% (n=141) had a sedentary lifestyle, 31% (n=103) were post-menopausal and 0.9% (n=3) had history of diabetes. The mean (SD) BMI was 25.8 (6.1), 34.5% were overweight and 18.1% were morbidly obese. The mean SBP and DSP were at 121.9 mmHg (14.7) and 75.9 mmHg (11.1), respectively. 22.9% of the patients had abnormal SBP and 13% had abnormal DBP. The mean (SD) serum profile were as follows; triglycerides: 1.2(0.6) mg/dl, total cholesterol: 4.7(1.1) mmol/l, LDL-cholesterol: 2.6 (0.8) mmol/L, HDL-cholesterol: 1.7 (0.6) mmol/L, TC/HDL ratio: 3.0(1.0), homocysteine: 10.1 (4.3) μmol/L and CRP: 3.2 (6.2) (mg/L). Abnormal serum levels were seen in 14.2 % for TG, 21.2% for TC, 50.8% for LDL, 18.4% for HDL, 16.7% for homocysteine and 62.7% for CRP. The distribution of total CVD risk factors were as follows: 31% had only 1, 44 % had 2 – 4 and 25 % had ≥5 risk factors. 259 patients had complete data for calculation of Framingham score for developing CVD in the next 10 years. 43.2% (n=112) were at low risk with a chance of 1-5 % and 22.8% (n=59) 6-10%. At moderate risk were 22.4% (n=58) with a chance of 11-20% and 11.6% (n=30) were at high risk with chance of > 20%. *Conclusion:* CVD risk factors are commonly seen in female patients with SLE. Given the high prevalence of CVD risk factors, immediate screening and interventions are needed for CVD risk reduction in this patient population.

PO1.C.46

Clinical correlates of renal vascular lesions (RVL) in lupus kidney disease

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Objective: To determine the clinical significance and prognostic value of RVL detected in renal biopsies of patients with systemic lupus erythematosus (SLE). *Methods:* Two-hundred and seven renal biopsies scored according to the International Society of Nephrology/Renal Pathology Society revised 2004 criteria for Lupus Nephritis were used. RVL were defined as: 1) thrombotic microangiopathy (TMA), 2) arterial fibrinoid necrosis, 3) lupus vasculopathy and/or uncomplicated vascular immune deposits. Demographic, renal and vascular outcomes, overall mortality, disease activity measured by SLE-disease activity index-2000 (SLEDAI-2K) and organ damage assessed

by SLICC damage index (SDI) were evaluated. *Outcomes:* In this study 207 biopsies from 162 patients were examined. TMA was seen in 16 biopsies (16.6%) and 18 had lupus vasculopathy (7 vasculopathy, 11 vascular immune deposits). Three patients had both pathologic findings and 131 patients had neither pathologic finding (controls). Demographic features and SDI were similar between patients with TMA or lupus vasculopathy and controls at baseline. Compared to controls, patients with TMA on biopsy had a higher prevalence of hypertension (35.9% vs. 68.8%; p=0.01) but there was no difference in amount of arteriolar sclerosis found on biopsy between the two groups. During the course of follow-up, the prevalence of thrombotic events was significantly higher in patients with TMA (37.5%, p=0.04) and in those with lupus vasculopathy (38.9%, p=0.02) compared to controls (15.3%). Patients with TMA had similar SLEDAI-2K to controls at the time of renal biopsy. Conversely patients with lupus vasculopathy had higher adjusted mean SLEDAI-2K (16.6 ± 9.8 vs 11.4 ± 7.4, p=0.008) and met more ACR criteria at the time of biopsy compared to controls (6.0 ± 1.6 vs 4.9 ± 1.9, p=0.025). At last follow-up 18.3% of the control group, 18.8% of those with TMA (p=0.967) and 27.8% of those who had vasculopathy on renal biopsy had died (p=0.348). Neither RVL was associated with smoking, dyslipidemia or diabetes. *Conclusion:* Renal RVL including TMA and lupus vasculopathy may be predictive of thrombotic events in the absence of traditional risk factors; however, at the time of renal biopsy, patients with TMA had a higher incidence of hypertension and those with lupus vasculopathy had higher mean disease activity scores. Despite the increased incidence of thrombosis, we could not conclude that there was an excess mortality associated with TMA or lupus vasculopathy on renal biopsy.

PO1.C.47

Tai Chi on psychological well-being: systematic review and meta-analysis

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Background: A growing list of psychological states including stress, anxiety, mood disturbance, and depression has been linked to many chronic disorders such as Lupus. Thus, there is an urgent need for inexpensive and effective strategies to promote psychological well-being and improve general heath status for people with Lupus. Although physical activity and exercise appear to improve psychological health, the quantitative effects of Tai Chi on psychological well-being have rarely been examined. We systematically reviewed the effects of Tai Chi on stress, anxiety, depression and mood disturbance in eastern and western populations. *Methods:* Eight English and 3 Chinese databases were searched through March 2009. Randomized controlled trials, non-randomized controlled studies and observational studies reporting at least 1 psychological health outcome were examined. Data were extracted and verified by 2 reviewers independently. The randomized trials in each subcategory of health outcomes were meta-analyzed using a random-effects model. The quality of each study was assessed which takes into account whether a study described randomization, blinding, and withdrawals/dropouts. *Results:* We reviewed 2579 English and Chinese articles and retrieved 61 full-text articles for detailed evaluation. Twenty-one studies were eliminated for not reporting original or relevant psychological outcome data. Ultimately, forty studies were identified for data abstraction and critical appraisal totaling 3817 subjects. Approximately 29 psychological measurements were assessed. Twenty-three of 33 randomized and nonrandomized trials reported that 1 hour to 1 year of regular Tai Chi significantly increased psychological well-being including reduction of stress (effect size [ES], 0.66; 95% confidence interval [CI], 0.23 to 1.09), anxiety (ES, 0.66; 95% CI, 0.29 to 1.03), and depression (ES, 0.56; 95% CI, 0.31 to 0.80), and enhanced mood and emotion (ES, 0.45; 95% CI, 0.20 to 0.69) in community-dwelling healthy participants and in patients with chronic conditions. Seven observational studies with relatively large sample sizes reinforced the beneficial association between Tai Chi practice and psychological health. *Conclusions:* Overall, evidence suggested short and long-term Tai Chi is associated with improvements in psychological well-being among healthy adults and patients with chronic conditions in eastern and western populations. The definitive conclusions were limited due to variation in designs, comparisons and heterogeneous outcomes, however, these results are promis-

ing and warrant a high-quality, well-controlled, randomized trial of Tai Chi for Lupus condition. Knowledge about the physiological and psychological effects of Tai Chi exercise may lead to new complementary and alternative medical approaches to treat chronic medical conditions and better inform clinical decisions.

PO1.C.48

Anti-phospholipid antibodies are related with cardiac involvement in SLE patients

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Objectives: To compare the prevalence and nature of cardiac involvement between SLE patients with anti-phospholipid antibodies (aPL) positive and aPL negative antibodies. **Population and Methods:** Consecutive non-selected patients fulfilling the 1997 ACR Classification Criteria for SLE within the Coimbra Lupus Cohort were included. Patients were submitted to transthoracic echocardiography by a single operator (RT), blinded for SLE aPL status. Evaluation was performed with classic M-Mode, 2 D, color, continuous, pulsatil and tissue Doppler assessing dimensions, left and right ventricular systolic and diastolic function, and valvular heart disease. Sixty-one patients were evaluated and classified into two groups based on the presence of at least one positive aPL determination. Results were compared by T-Test for continuous variables and Chi-2 test for categorical variables. p<0.05 was considered statistically significant. **Results:** Sixty one patients were included; 36 with aPL (group A) and 25 without aPL (group B). Demogaphic, cardiovascular risk factors and SLEDAI were similar between patients. Left ventricular (LV), right ventricular (RV) dimensions and LV ejection fraction did not show difference between groups. With respect to mitral disease, leaflet thickening, calcification and mitral regurgitation were similar between groups, although the severity of the regurgitation was higher among aPL patients (mitral jet area: 4.2±3.9 vs 1.8±1.5 cm2, p=0,026). Findings related to aortic valve disease show no difference. None of the patients presented valvular vegetations. Circumferential pericardial effusion was more frequently observed in SLE patients with positive aPL antibodies (36.0 vs 8.3% p=0.008), but in all cases the effusion was classified as mild. Longitudinal contraction of RV, assessed by TAPSE (Tricuspid annular plane systolic excursion), was slightly lower in aPL (2.2±0.4 vs 2.4 ±0.5 cm, p=0,047). Estimated systolic pulmonary artery pressure was similar between them as well as contraction and relaxing myocardic velocity assessed by tissue doppler. **Conclusion:** SLE Patients with aPL antibodies present a higher risk of pericardial effusion, more severe mitral disease and myocardial function impairment compared to Lupus patients without aPL antibodies. These results support the pathogenic role of aPL in cardiac abnormalities and the importance of echocardiographic assessment in these SLE patients.

PO1.C.49

Should systemic lupus erythematosus patients be submitted to routine echocardiography evaluation?

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Objectives: To evaluate the prevalence of ecocardiographic abnormalities in systemic lupus erythematosus (SLE) patients compared to healthy controls. **Population and Methods:** A cross-sectional and controlled study with healthy subjects was conducted. Consecutive non-selected patients, from a single outpatient clinic, fulfilling the 1997 ACR classification were selected. Two groups were created; lupus patients (group A) n=62; controls (group B) n=31. Patients and controls were submitted to transthoracic echocardiography by a single operator (RT). Classic M-Mode, 2 D, color, continuous, pulsatile and tissue doppler were performed assessing dimensions, left and right ventricular systolic function, diastolic function, valvular and pericar-

dial disease. Data was compared with T-Test for continuous variables and Chi-2 test for categorical variables. p<0.05 was considered statistically significant. **Results:** 93 subjects were included. 82.3% of SLE patients were female (with a mean age =39.8±14.7 years, mean disease duration =11.2±7.0 years and mean SLEDAI =3.8±3.2). Groups were similar with respect to demographic data. Left ventricular (LV), right ventricular (RV) dimensions and LV ejection fraction (58.3±6.7 vs 60.7±5.6%, p=0.09) were within the normal range in both groups and no difference was observed between them. With respect to mitral disease, leaflet thickening and mitral regurgitation (67.7 vs 45.2%, p=0.036) were more prevalent in SLE patients, as was the severity of the regurgitation (mitral jet area: 2.7±2.8 vs 1.3±0.5 cm2, p=0,04). A similar finding was reported to aortic valve disease, although there was a similar rate of tricuspid regurgitation between groups. Estimated systolic pulmonary artery pressure was similar between them (25.9±6.0 vs 24.4±6.0 mmHg, p =0.29). Circumferential pericardial effusion was more frequently in SLE patients (19.4 vs 3.2% p=0.034), and in all the cases the effusion was classified as mild. Markers of LV diastolic function were different between groups. The E/A ratio was lower for group A (1.3±0.4 vs 1.5±0.3 p=0.05) and the E/E' lower for the control group. Myocardial LV septal and RV free wall basal segments contraction and relaxation velocities assessed by tissue Doppler were lower for the lupus patients (S septal wave – 0.10±0.2 vs 0.11±0.03 m/s p=0.03). **Conclusions:** Valvular heart disease and pericardial effusion were more frequent in our SLE group, as were changes in LV and RV myocardial function indexes, although not clinical relevant. It makes that the role of echocardiography evaluation performed by routine in non-selected SLE patients remains questionable.

PO1.C.50

Don't forget what natural SLE history is teaching us when establishing clinical-genetic correlations

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Objectives: To evaluate how important natural SLE history of patients cohort is to reach a correct interpretation of the clinical-genetic studies. **Methods:** Retrospective evaluation of a subgroup of patients from the Hospital Geral de Santo António (HGSA), Porto, Portugal, SLE cohort, followed by internists and nephrologists, using the M. Petri John Hopkins hospital protocol. **Results:** The cohort of HGSA SLE patients is one of the biggest in Portugal including, until June 2006, 451 patients. We evaluated the natural history in a subgroup of patients, about a third part (144/451, 31,9%), with similar epidemiological characteristics regarding sex, age and mortality (9,1% versus 10,6% in the global cohort) and cumulative clinical manifestations - mucocutaneous 84%, haematological 79,2%, articular 77,1%, renal 44,4%, neurological 29,9% and serositis 19,4% - except for neurological (13% in the global cohort where the involvement was characterized mainly by ACR criteria). It stands out that the cutaneous and articular manifestations appear predominantly in the first year of clinical disease, but 9,1% and 10,8% of these manifestations, respectively, appear after 5 years. In half of the patients with renal involvement it is manifested also in the first year, although in a fourth part it only appears after 5 years of evolution. For serositis and haematological it occurs in 21,4% and 22,9%, respectively, and in 37,2% in neurological involvement. **Conclusions:** Some important organ involvement like kidney, which split SLE patients in two very sharp groups, may occur in a large percentage only after 5 years of clinical evolution. That implies a potential and significant bias in clinical genetic correlations, so that must be taken into account in those studies and lupus patients included should preferentially have more than 5 years of clinical disease.

PO1.C.51

Lupus nephritis does not increase risk of echocardiography abnormalities

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Objectives: To investigate whether lupus nephritis, as a marker of severe disease, predicts echocardiography abnormalities in SLE patients. *Population and Methods:* Consecutive non-selected patients fulfilling the 1997 ACR Classification Criteria for SLE and followed in the Coimbra Lupus Cohort were included. Patients were submitted to transthoracic echocardiography by a single operator, blinded for SLE clinical status. Evaluation was performed with classic M-Mode, 2 D, color, continuous, pulsatile and tissue Doppler targeting dimensions, left and right ventricular systolic and diastolic function, and valvular heart disease. Patients were evaluated and classified into two groups based on the presence or absence of biopsy-proven lupus nephritis. Comparisons were done using T-Test for continuous variables and Chi-2 test for categorical variables. p<0.05 was considered statistically significant. *Results:* We evaluated 61 patients (female 82.3%, mean age =39.8±14.7 years, mean disease duration =11.2±7.0 years, mean SLEDAI =3.8±3.2). Demographic and cardiovascular risk factors were similar in patients with (n=36) and without (n=25) lupus nephritis. No differences were observed between groups regarding Left ventricular (LV) ejection fraction, LV shortening fraction, and Right ventricular (RV) function assessed by Tricuspid annular plane systolic excursion (TAPSE). Contraction and relaxing myocardial velocity, indexes of diastolic function, were normal and similar between groups. Patients with lupus nephritis did not present a higher risk of pericardial effusion.

Echocardiography parameters	SLE patients without nephritis	SLE patients with nephritis	p-value
Left ventricular (LV) ejection fraction (%)	57.6±5.3	58.6±7.7	0.57†
LV shortening fraction (%)	41.8 ±32.7	36.5±5.5	0.34†
TAPSE (cm)	2.5±0.5	2.3±0.4	0.126†
Mitral regurgitation (%)	62.5	74.3	0.334‡
Aortic regurgitation (%)	4.2	20	0.081‡
Tricuspid regurgitation (%)	91.7	88.6	0.699‡
Pericardial effusion (%)	20.8	20	0.938‡
PSAP (mmHg)	25.7±5.5	26 ±6.5	0.855†
Basal septal S wave (m/s)	0.1±0.01	0.1±0.03	0.074†

‡Chi-2Test; † t-Test

Conclusion: Lupus nephritis does not seem to predict heart abnormalities in SLE.

PO1.C.52

SLE clinical manifesations – Klinelfelter versus normal men

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Background: Men with systemic lupus erythematosus (SLE) have more severe disease than women with SLE. There are reports of increased renal and neurological SLE manifestations as well as increased rates of thrombocytopenia and autoimmune hemolytic anemia. In addition, SLE affected men are more likely to have serositis. Certain autoantibodies may be more common among SLE-affected men compared to SLE-affected women, including anti-dsDNA, anti-RNP and anti-Sm. We previously have reported that Klinefelter's syndrome (47,XXY) is found in excess among men with SLE. We undertook this study to determine whether SLE-affected men with 47,XXY had manifestations of disease similar to those of SLE-affected men with 46,XY. *Methods:* All patients were from the Lupus Family Registry and Repository and were confirmed to have at least 4 of 11 ACR SLE classification criteria by chart review, interview and questionnaire. X chromosome number was determined by karyotype or FISH. Statistical comparisons were made by chi square analyses with Yates correction. *Results:* There were a total of 176 men with SLE, of whom 5 (2.8% or 1 in 35) also had Klinefelter's syndrome. Each SLE-affected man with Klinefelter's was matched for ethnicity and duration of illness with as many SLE-affected, 46,XY men as possible. No 47,XXY, SLE-affected man had discoid rash, serositis, neurological disease, anti-RNP, anti-Sm, autoimmune hemolytic anemia, or thrombocytopenia, while these manifestations were found among SLE-affected, 46,XY men (see Table).

	46,XX SLE men	47,XXY SLE men
	n=176	n=5
discoid	25	0
serositis	71	0
neurological	16	0
AIHA	16	0
thrombocytopenia	39	0
Anti-RNP	21	0
Anti-Sm	4	0

However, no findings reached statistical significance because of the small number of Klinefelter men. *Conclusions:* These findings suggest that Klinefelter men with SLE have manifestations that are less severe than 46,XY men.

PO1.C.53

The prevalence of CNS lupus in Canada: tesults from the 1000 Faces of Lupus cohort

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Objectives: Neuropsychiatric systemic lupus erythematosus (NPSLE) can vary widely depending on the definition used and has been found to range from 12 to 80%. We determined the prevalence of NPSLE and associated factors in 1000 Faces of Lupus, a large multi-centre Canadian cohort. *Methods:* Adults enrolled who satisfied the ACR classification for SLE were included and had completed SLEDAI, SLAM and/or SLICC damage indices. NPSLE was defined as: (i) NPSLE by ACR diagnostic index which has strict criteria consisting of psychosis and seizure in which a metabolic etiology had been excluded, ACR, SLEDAI, SLAM and SLICC with indices with (ii) and without (iii) minor nonspecific NPSLE manifestations, and (iv) ACR and SLEDAI indices. Factors associated with NPSLE were explored using univariate and multivariate regression. *Results:* Total cohort size was 1417; 86% were female, with mean ± standard deviation (SD) age at study entry and disease duration of 41.0 ± 16.3 and 11.7 ± 10.2 years respectively. Subgroup size and characteristics were dependent on the specific definition of NPSLE. Group (i) contained N=1253 with NPSLE prevalence of 6.4% (n=80). Group (ii) contained 681 and NPSLE prevalence of 38.6% (n=263). Group (iii) had 586 and NPSLE prevalence of 28.7% (n=168). Group (iv) had 1125 and NPSLE prevalence was 10.2% (n=115). In univariate analysis, Aboriginals had increased prevalence of NPSLE at 12.5%, Caucasian 61.1%, African 6.9% and Asian 19.4% (p=0.04) in group (i). Anti-Ro and antiphospholipid (aPL) antibody + were also significant in this group. aPL+ remained significant in groups (ii) and (iii). In group (iv) absence of anti-Sm and presence of anti-Ro were significant. In multivariate analysis, anti-Ro and aPL (i) and anti-Ro+ and lack of anti-Sm (iv) were significant. Age at diagnosis, disease duration, anti-DNA, RNP and anti-cardiolipin antibody alone were not found to be significant. *Conclusions:* Prevalence and factors associated with NPSLE varied depending on the definition used. Prevalence of NPSLE was nearly two-fold greater in Aboriginals. NPSLE may be less in this database than other publications as NPSLE may be decreasing, or due to selection bias of those who enter an observational cohort. NPSLE was associated with aPL and often anti-Ro and varied by ethnicity.

PO1.C.54

Prevalence of mood and anxiety disorders in young women with systemic lupus erythematosus (SLE)

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Objective: To determine prevalence of mood and anxiety disorders in young women with SLE. **Methods:** We screened consecutive female SLE patients followed in a longitudinal cohort between 2007/2008. We excluded patients with age $\geq$ 50 years and comorbidities [i.e., hypertension (BP >140/90 on 2 occasions or 1 occasion with concomitant antihypertensive medications), renal insufficiency (creatinine > 200 mmol/dl on 1 occasion), transient ischemic attack or stroke, scleroderma features, diabetes, drug abuse, or malignancy]. Healthy age-matched women were selected as controls. SLE patients were assessed for disease activity [SLE Disease Activity Index (SLEDAI)], damage [Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI)], and presence of fibromyalgia; patients completed the Becks Depression and Becks Anxiety Inventory (BDI and BAI). Both scales consist of 21 items, each describing a common symptom of depression/anxiety. The respondent is asked to rate how much he or she has been bothered by each symptom over the past month on a 4-point scale ranging from 0 to 3. The items are summed to obtain a total score that can range from 0 to 63. Correlations between mood disorders and age, disease duration, disease activity, damage, current prednisone use, and the presence of fibromyalgia were examined. **Results:** One hundred and twenty seven patients < 50 years were screened, 52 fulfilled the inclusion criteria, and data are currently available on 41 patients (mean age 34.08, SD 8.85). Sixteen controls (mean age 33, SD 8.3) participated. Mean SLE duration was 8.16 years (SD 6.8), with a mean SLEDAI of 4.8 (SD 4.5). Spontaneous complaints of mood disorder were reported by 7 patients, 4 reported anxiety and 3 depression and no controls reported a mood disorder. No/minimal depression was observed in 24/41 (58.5%) patients and in 15/16 (94%) controls, mild depression in 10 (24.3%) patients, moderate depression in 6 (14.6%) patients and 1 (6%) control and severe depression in 1 (2.4%) patient. No/minimal anxiety was observed in 21/41 (52.5%) patients and in 13/16 (84.2%) controls, mild anxiety in 13 (32.5%) patients and 3 (15.7%) controls, and moderate anxiety in 5 (12.2%) patients and severe anxiety in 2 (4.9%) patients. We observed a correlation between anxiety and disease activity (r=0.6; p=0.01), but no other significant associations were observed. **Conclusion:** Mood disorders were frequently observed in young SLE patients and most patients did not have spontaneous complaints. Anxiety was worse in patients with higher disease activity. This study suggests that SLE patients should be routinely screened for mood disorders as they may not be apparent during a routine clinical visit.

PO1.C.55

Analysis of 641 patients with systemic lupus erythematosus (SLE): a single center experience from Turkey

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Introduction: The aim of this study was to determine the clinical and laboratory manifestations and the SLICC damage scores of 641 SLE patients followed-up at the Rheumatology Unit of Istanbul Faculty of Medicine. **Patients and methods:** The records of 641 patients attending the SLE clinic between 1980 and 2009 were retrospectively analysed. **Results:** All patients fulfilled at least 4 of the revised ACR criteria for SLE. Eighty-seven % of the patients was women. The mean age of the patients was 39.7±13.4 years (range 12-78) and their mean duration of disease was 112,5 ± 83.4 months (range 1-420). Musculoskeletal manifestations were observed in 70% of the patients whereas mucocutaneous in 65%, renal in 44% and neurological in 6%. The

most prevalent histopathology of lupus nephritis was class IV (21.5%) followed by class V (14%). Antiphospholipid syndrome was present in 20% of the patients. Anti-DNA antibodies were present in 73%, anti-Sm in 20% and anti-cardiolipin antibodies in 30% of the patients. The most common site for damage was musculoskeletal system (43%). Renal damage was present in 41% of the patients and neuropychiatric in 40%. There were 7 patients who developed cancer during their follow-up. The mean duration of disease in patients with damage (n= 309) was significantly higher compared to patients without a damage (n=300) (126.16±86.3 vs 96±75 months, p=0.000). There was a positive correlation between disease duration and SLICC damage scores (r=0.274, p=0.000). The mean duration of follow-up was 95.6±88 months. No information could be gathered for the current status of 152 patients. Of the rest, 9 were dead. The mean SLICC scores of patients who died was not significantly higher than that of living (2.44 vs 0.96, p=0.083). The cause of death was lupus renal disease in 2, lupus cardiac involvement and infection in 2, infection in 1, a probable cerebrovascular event in 1. In the remaining 3, no reason was identified. **Conclusions:** The findings of this study are comparable to those of other studies. Musculoskeletal, renal and neuropychiatric damage were found to be more frequent in this cohort. Although it is hard to draw a conclusion about survival in this cohort, it seems that damage is not the only factor for the patients' cause of death.

PO1.C.56

Different patterns of cognitive function in SLE may be associated with anti NMDA receptor and not with anti-ribosomal P/neuronal autoantibodies.

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Objectives: Cognitive impairment (CI) in systemic lupus erythematosus (SLE) reveals a sub cortical pattern and has been associated with depression and active disease, but its frequency is controversial, varying from 20-80%, and its cause remains unknown. Although auto antibodies against neural antigens in the brain might play a role, so far there is no clear data supporting this possibility. The aim of this study was i) to assess the presence of neuropsychiatric disorders and CI in patients with SLE ii) to evaluate their possible association with antibodies cross reacting with NR2 subunit of the N-methyl-d-apartate (NMDA) glutamate receptor and anti-ribosomal P (Anti-P) plus anti-neuronal antibodies in serum in non-selected Chilean women with SLE. **Methods:** Fifty-two patients with SLE, median age of 33 years (interval 17-64), with a median disease duration of 3 years (0-21) and with formal education of 14 (9-19) years were recruited in an 18 month period. Median disease activity SLEDAI 2K was 8 (0-25) and damage SDI 0 (0-6). All patients were assessed using the Cambridge neuropsychological test automated battery (CANTAB) which included different cognitive domains: episodic memory, executive functions, attentional function, and the structured interview (MINI-PLUS) for psychiatric disorders (DSM-IV). Autoantibodies were assessed by ELISA (NMDA and anti-P) and by immunofluorescence in neuroblastoma N2a cells (anti-neuronal). Anti-NMDA reactivity was found in 7 (19%) of 37 patients and Anti-P/neuronal in 16 (30.8%) out of 52 patients. **Results:** Twenty-seven (51%) patients presented at least one of the 26 common psychiatric disorders: major depression in 12 (23%) and lupus psychosis in 1 (2%). Fourteen (26%) patients presented low performances in CANTAB memory tests (performances < -1 SD), 22 (42%) deficit in sustained attention tests, and 26 (50%) deficit in executive function tests. Six out of 7 patients with positive Anti NMDA receptor antibodies had significant attention impairment versus 9 out of 21 without Anti NMDA antibodies (Fisher´s exact test P value of 0.011). No mnemonic domain deficits were associated with Anti-P/neuronal antibodies. Major depression was not associated with these auto antibodies. **Conclusions:** Specific areas of cognitive function in SLE might be associated

with the presence of anti-NMDA while not associated with Anti-P/neuronal auto antibodies.

PO1.C.57

A/H1N1 influenza vaccination in patients with systemic lupus erythematosus: safety and immunity

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Objectives: To determine the safety and immunity of A/H1N1 influenza vaccination in systemic lupus erythematosus (SLE) patients. *Research design and methods:* The study population comprised twenty-one SLE patients and fifteen healthy control subjects, who underwent split-virion, inactivated monovalent A/H1N1 vaccination during December 2009- January 2010. Sera were obtained before and three weeks after the vaccinations. At each visit, SLE disease activity index (SLEDAI) scores and the autoantibodies were measured for SLE patients. The haemagglutination inhibition (HAI) test and sera immunoglobulin (Ig)G were calculated according to the World Health Organization (WHO) procedure to evaluate the antibodies response to A/H1N1 influenza vaccine of the SLE patients. We also collected contemporary medications and past seasonal influenza vaccinations records to analyze the interaction between vaccinations and autoimmunity of SLE patients. *Results:* The mean age of enrolled population was 34.3 years and 39.4 years in SLE patients and control subjects, respectively. The SLEDAI scores for SLE patients were 4.1 at vaccination and 4.5 three weeks later. The seroprotection rate was 73.7 % in SLE patients, while 78.6% in healthy control subject. The seroconversion rate was 80.0%, compared with 91.7% in the normal controls. Both groups had met the criteria according to the European Committee for Proprietary Medicinal Products (CPMP) guidance. The clinical disease activity and SLEDAI scores before and after vaccination did not differ significantly in SLE patients, though the anti-cardiolipin immunoglobulin (Ig)G increased after vaccination without apparently clinical manifestations. *Conclusions:* The A/H1N1 influenza vaccine is safe and effective in SLE patients without obvious clinical detrimental appearance. Immunosuppressive agents such as azathioprine and prednisolone might lead to lower humoral immunity.

PO1.C.58

Abdominal pain in a patient with systemic lupus erythematosus: initial presentation of acquired C1-inhibitor deficiency. Case report and literature review.

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Acute abdominal pain in a patient with systemic lupus erythematosus (SLE) represents a challenging task, given the wide range of differential diagnoses, including infrequent conditions. We report the case of a 14-years-old female patient with SLE, in course of treatment for nephritis with Methylprednisolone and Cyclophosphamide, that presented severe abdominal pain, diarrhea, nausea, hypotension and abdominal distension without signs of peritonitis. Concomitantly, she suffered recurrent attacks of nonpruritic painless swellings of the head, neck and extremities, with 48-72 hours of duration and residual hyperchromic macula. Computerized tomography and abdominal ultrasonography revealed dramatic edema of the large and small bowel walls with no evidence of bowel dilatation or pneumatosis intestinalis. Skin biopsy showed superficial and perivascular dermatitis. Musculoskeletal ultrasonography revealed thickening of subcutaneous tissues and heterogeneity of muscular fibers. The diagnostic of type I acquired angioedema was confirmed by decreased levels of C1-INH, C4 and C2. Traditional therapies include fresh frozen plasma, epslon-aminocaproic acid and danazol. Danazol was started at 200, increased to 400 mg. There was good efficacy, relapse free. After four

months, C1-INH level was normal. The relevancy of this case report lies in the differential diagnosis of abdominal pain in SLE, situation in which angioedema is not among the prime suspects. The relevant literature is reviewed.

PO1.C.59

Thrombosis in systemic lupus erythematosus: contribution of factor V Leiden, prothrombin G 20210 mutation, antiphospholipid antibodies and clinical risk factors

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Objective: To detect factor V Leiden, prothrombin G20210 mutation, antiphospholipid antibodies and clinical risk factors of thrombosis in systemic lupus erythematosus (SLE) in order to suggest the role of thrombo-prophylaxis. *Methodology:* The study was conducted on 45 egyptian female SLE patients who used to attend the Outpatients Clinics of Rheumatology & Rehabilitation and internal medicine departments . Patients were subjected to history taking and thorough clinical examination. Disease activity was assessed using SLEDAI. SLE cumulative organ damage was scored using SLICC damage index. Clinical risk factors of thrombosis were detected such as dyslipidemia, hypertension, diabetes mellitus, smoking, family history of thrombosis, varicose veins, the use of contraceptive pills, malignancy, trauma and surgery. Patients were subjected to complete blood picture, serum creatinine, lipid profile, fasting and post-prandial blood sugar, ANA and anti ds-DNA, LA and aCL antibodies and Real time PCR for detection of factor V Leiden and prothrombin G20210 mutation. *Results:* Nine SLE patients had history of venous thrombosis and 6 with arterial. SLICC damage index was significantly higher in patients with thrombosis vs. those without ($p<0.05$). The frequencies of varicose veins, contraceptive pills intake, dyslipidemia and hypertension were non-significantly higher among patients with venous thrombosis. Renal disease, dyslipidemia and hypertension were found to be significant risk factors for arterial thrombosis. APL antibodies were detected in 33.3%, LA in 22.2% and aCL antibodies in 15.6%. Two patients were positive for LA and aCL antibodies. The frequency of FVL and prothrombin gene mutation was 15.6% and 2.2% respectively, all carrying the heterozygous form of mutation. LA and FVL were found to be highly significant risk factors for venous thrombosis. ACL antibodies were high significant risk factors for arterial thrombosis. Prothrombin gene mutation was not associated with thrombosis, aPL antibodies or FVL. The frequency of venous thrombosis among SLE patients who were carriers of FVL in the presence of LA was 75%, higher than that in carriers of normal FV genotype with positive LA (50%) or carriers of FVL in the absence of LA (66.7%). The annual incidence of venous thrombosis among SLE patients with one abnormality was 1.8% while among those with 2 abnormalities (LA + FVL, LA + aCL antibodies) was 3.6%. *Conclusions:* SLE patients require closer follow up for potential thrombotic complications to identify patients with more than one risk factor where thrombo-prophylaxis should come into consideration.

PO1.C.60

Dyslipoproteinemia in patients with systemic lupus erythematosus treated with rituximab and its correlation with lupus activity

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Objective: Lipid abnormalities contribute to the increased risk of premature atherosclerosis in patients with systemic lupus erythematosus (SLE). This study was undertaken to investigate the prevalence of dyslipoproteinemia and

to evaluate the influence of disease activity on the lipid profile of patients with severe SLE requiring treatment with rituximab (anti-CD20 monoclonal antibody). **Methods:** Design: Spanish multicenter retrospective study of patients with severe SLE who had failed standard immunosuppressive therapy requiring B-cell depletion therapy (BCDT) with rituximab. The levels of total, HDL and LDL cholesterol and triglycerides and the atherogenic index (total/HDL cholesterol) were measured before the infusion of rituximab. Dyslipoproteinemia wad diagnosed if the patient was on lipid-lowering therapy, total cholesterol levels were higher than 240 mg/dl or triglyceride levels were higher than 180 mg/dl. Lupus activity was retrospectively assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI-2K) and serum C3, C4 and anti-double-stranded DNA antibody levels. **Results:** One hundred eighteen patients with refractory SLE treated with BCDT with rituximab were examined. The mean age was 39 years (SD 12.1). One hundred six (90%) were females. The most represented races were Caucasian (n=108) and Hispanic (n=7). The mean duration of the disease was 7.3 years (SD 6.5). The levels of total, HDL and LDL cholesterol at the time of BCDT were 196.8, 61.1 and 103.8mg/dl, respectively. The atherogenic index was 3.6. The triglyceride level was 152.0 mg/dl. Fifty-four (45.8%) of the 118 patients had dyslipoproteinemia. Twenty-two (18.7%) of the patients were on treatment with lipid-lowering agents. Hypercholesterolemia and hypertriglyceridemia were diagnosed in 14 (11.9%) and 29 (24.6%) of the 118 patients, respectively. At the time of BCDT, the mean value of SLEDAI-2K was 14.2 (SD 9.3). At that time, there was a significant correlation between the activity of the disease measured as SLEDAI-2K and the triglyceride levels (p=0.001). **Conclusion:** Nearly half of the patients with severe SLE who had failed standard immunosuppressive therapy may have dyslipoproteinemia and it may be underestimated. The activity of the disease correlates with that abnormal lipid profile. A therapeutic strategy based on an aggressive treatment to control inflammation including BCDT and the early use of lipid-lowering drugs is recommended for lupus patients who had failed standard immunosuppressive therapy.

PO1.C.61

Hyposmia and psychopathology in systemic lupus erythematosus

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Objectives: 1) to assess odor identification capacities in SLE patients; and 2) to explore possible associations between hyposmia and psychopathology in patients with SLE. Methods: 54 patients with SLE in the nonactive phase of their illness (96.3% women; mean age=41.24, s.d.=11.71; mean education=11.20, s.d.=5.29; mean disease duration=11.02 years, s.d.=7.82; 31.5% with CNS involvement) were selected from the CHP's Clinical Immunology Unit. The participants underwent a neurological examination, answered the Hospital Anxiety and Depression Scale (HADS), and performed the Smell Identification Test (B-SIT). A cut-off score of <8 (i.e., 1st percentile of healthy demographically matched subjects) was used to classify hyposmia on the B-SIT. Non-parametric tests (i.e., Chi-square and Mann-Whitney) were used to analyze the results. **Results:** SLE patients with impaired performance on the B-SIT (13.5%) had higher levels of depression (mean=11.43, s.d.=2.51 vs. mean=5.34, s.d.=4.1; p=0.001) and anxiety (mean=13, s.d.=3.61 vs. mean=8.64, s.d.=3.77; p=0.008). The current intake of benzodiazepines (44.4%) was associated (p=0.002) with hyposmia (100% of patients with hyposmia were taking benzodiazepines). No significant associations (p>0.05) were found with age at onset, disease duration, Central Nervous System involvement (31.5%), or current intake of antidepressants (38.9%) and prednisone (66.7%). **Conclusions:** The present study documented odor identification deficits in SLE patients. These results extend Shoenfeld and colleagues' (2009) findings of low odor threshold and poor olfactory discrimination in SLE patients. However, there is an apparent inconsistency between these two studies regarding a putative association between impaired olfaction and neuropsychiatric manifestations of SLE. The present study provided evidence for an association between hyposmia and psychopathology in SLE. This finding is consistent with the literature. Pause and colleagues (2001) reported that patients with major depression had low olfactory threshold. The resection of the olfactory bulb in rodents has been found to result in depression-like behaviors (Kelly et al., 1997; Song & Leonard, 2005).

PO1D Complement

PO1.D.1

Molecular basis of complement C1r deficiency in a male African American patient with systemic lupus erythematosus (SLE)

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Objectives: Homozygous deficiencies of early components for complement activation are among the strongest genetic risk factors for human SLE. Between 70 and 90% of human subjects deficient in complement C1q, C1r, C1s or C4 developed SLE or lupus-like diseases. Twelve cases of C1r deficiency were reported but no causative mutations have been identified. This is the first report on elucidating the molecular basis of a C1r deficiency. **Patient and Methods:** The proband is an African American male who developed SLE at 3 months of age. He had biopsy-proven discoid lupus, seizures and transverse myelitis, diffuse proliferative glomerulonephritis with glomerular basement membrane staining for C1q and C3 but not C4, and consistently negative ANA, anti-dsDNA and anti-Sm autoantibodies. From this patient, the coding regions of the *C1R* gene with 11 exons located at chromosome 12p13 were PCR-amplified and sequenced to completion. A sequence-specific-primer (SSP)-PCR coupled with StuI-RFLP was developed to screen for the novel *C1R* mutation in the patient's family and in 181 unrelated black SLE samples. Complement assays including radial immunodiffusion and immunofixation were employed to determine protein levels and polymorphisms. **Results:** Serum complement analyses showed zero CH50 activity, reduced levels of C1s (22μg/mL; reference range: 31-45μg/mL) and undetectable C1r in the patient. Unexpectedly, C3 and C4 protein levels were 2156μg/mL (reference range: 1045-1670μg/mL) and 2238μg/mL (reference range: 445-740μg/mL), respectively. Immunofixation experiments confirmed high levels of C4A and C4B and elevated levels of C3F and C3S proteins in both the patient and his mother. DNA sequencing revealed a C to T mutation at position 6392 in exon 9 of the *C1R* gene, resulting in a mutation from Arg-380 to a premature stop codon. The patient's mother is heterozygous for this mutation. However, the R380X mutation was not detectable in 181 African American SLE patients. **Conclusions:** Similar to homozygous C1q or C4 deficiencies, C1r-deficient patients develop SLE. Unlike most SLE patients, C4 and C3 protein levels in C1r-deficiency patients are consistently high. This phenomenon underscores the independent roles of classical pathway complement proteins in the protection against SLE pathogenesis. Mutations leading to early complement component deficiencies and SLE, with the exception of the 2-bp insertion into codon-1213 of *C4A*, are observable mainly in probands' families or isolated populations. Such observations support the concept that genetic variants with large effect for a common disease (i.e. disease causative variants) only exist at very low frequencies in the population.

PO1E   Cutting Edge of Lupus Research

PO1.E.9

Estradiol differentially regulates Foxp3 in human SLE T cells

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Objectives: The forkhead transcription factor FOXP3 is essential for regulatory T cell (Treg) function. Estradiol is a female sex hormone that influences the gender biased autoimmune disease systemic lupus erythematosus (SLE) and regulates Foxp3 gene expression in mouse T cells. The purpose of this study was to investigate if estradiol controls Foxp3 expression in human T cells. ***Methods:*** Thirteen females with SLE were enrolled in this study. Their disease activity ranged from mild to active, median SLEDAI value of 6 (range 2-18). The age of the patients ranged from 24 to 51 years with a median of 42 years. Seven of the patients were Caucasian, five were African American, and one was Asian. Ten healthy females were enrolled in the study as controls, with age ranging from 21 to 51 years, median of 37 years. To control for the effects of SLE medications, five patients with rheumatoid arthritis (RA) were also enrolled in the study. The age of the RA patients ranged from 28 to 48 years with a median of 42 years. T cells were purified from blood samples by negative selection. T cell samples were activated after 18 h of culture for 4 h with PMA (10 ng/ml) and ionomycin (0.5 μg/ml). To test the effect of estradiol on activated T cells, estradiol-17β (10-7 M) was added (or not) to half of the replicate cultures for the entire culture period. Total RNA was isolated from the T cell samples, converted into cDNA and Foxp3 expression was measured by real-time PCR. ***Results:*** Estradiol increased Foxp3 expression (p = 0.017) in activated normal T cells. In ten of thirteen activated SLE T cell samples estradiol decreased Foxp3 expression, with substantial decreases more likely in patients under 40 years of age (p = 0.005). Neither activated nor resting SLE T cell samples showed increased Foxp3 expression in response to estradiol. Three RA T cell samples showed increased Foxp3 in response to estradiol indicating that estradiol suppression of Foxp3 in SLE T cells was not due to medications. ***Conclusions:*** These findings indicate that estradiol increases Foxp3 expression in activated normal T cells but suppresses Foxp3 in SLE T cells. The suppressive estradiol effect was more likely in SLE patients under 40 years of age. The results suggest that differential regulation of Foxp3 could contribute to the gender bias in SLE through an estrogen sensitive age-dependent mechanism.

PO1.E.10

Perspectives of toll-like receptor (TLR) blockade for treating lupus: inhibitory oligonucleotides differentially affect TLR7-induced activation of B cells depending whether a co-signal through the B cell receptor for antigen is simultaneously provided

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Objectives: Single-stranded RNA-sensing TLR7 receptor plays a well established role in the pathogenesis of SLE. For example, BXSB male mice develop spontaneous lupus due to the duplication of the TLR7 gene. MRL-Fas lpr/lpr mice lacking TLR7 survive longer and have less kidney pathology. Much less is known whether simultaneous co-engagement of BCR with TLR7 may affect responses to suboptimal TLR7 agonists and antagonists. ***Methods:*** We addressed the role of BCR and TLR7 cross-talk in pre-diseased BXSB male and female B cells. We purified total splenic B cells by magnetic sorting and then stimulated them with various TLR7 ligands (e.g. CL-075, R-848, R-837, CL-097, and Loxoribine). B cell cycle entry and apoptosis protection was measured by acridine orange flow cytometry. Polyclonal IgM and IL-6 secretion were determined by ELISA. In some experiments, anti-IgM F(ab')2 anti-bodies were combined with suboptimal concentrations of TLR7 ligands and with various 32-mer inhibitory oligonucleotides. ***Results:*** B cells from BXSB male mice in comparison to female controls responded more vigorously to low affinity TLR7 ligands. Furthermore, when the BCR was co-engaged with TLR7, B cells secreted at least 10 times more IL-6. Phosphorothioate-based 32-mer oligonucleotides (ODN) were capable of blocking TLR7-induced, but not BCR-induced, B cell activation. This inhibition appeared to be ODN-backbone dependent, but sequence independent. Adding TGC triplets to the 5' end of an oligonucleotide failed to generate more powerful TLR7 inhibitors. When BCR and TLR7 were co-engaged a differential effect of TLR7 antagonists on IL-6 secretion versus G1-M entry/Apoptosis protection was observed. While at high nanomolar concentrations TLR7 antagonists were still capable of suppressing IL-6 secretion, though with decreased potency compared to non-BCR engaged B cells, they completely failed to block BCR and TLR7-ligand induced cell cycle entry/apoptosis protection. ***Conclusions:*** our results suggest that while TLR7 blockade may be of benefit for treating animal lupus, simultaneous co-engagement of BCR and TLR7 may result in undesirable augmentation of B cell survival/proliferation favoring autoreactive B cell selection, theoretically worsening autoimmunity.

PO1.E.11

Clinical utility of changes in cyto/chemokines as markers of disease activity in systemic lupus erythematosus

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Objective: Lupus patients have elevated plasma levels of a number of cytokine/chemokines suggesting that fluctuations in the concentration of these soluble mediators may mirror changes in SLE disease activity. In a preliminary screen of 20 cyto/chemokines plasma concentrations in SLE patients, IP-10, MCP-1 and sVCAM were found to be elevated in active SLE patients. We therefore examined the relationship between changes in disease activity and alterations in cyto/chemokine plasma levels. ***Methods:*** Patients (n = 74) satisfying 4 or more ACR criteria were recruited from the University of Toronto Lupus Clinic. 21 healthy controls were also recruited. The plasma concentration of the 3 analytes was determined by Luminex assay. All patients underwent at least 2 assessments during the study period. Disease activity was measured by the SLEDAI-2K and used to segregate patients into a high (≥ 7) and low/moderate (< 7) disease activity groups. A modified SLEDAI-2K was calculated by subtracting the contribution of anti-dsDNA antibodies and complement from the global score. The Mann Whitney non-parametric test was used for comparisons between groups. The statistical significance of correlations was determined by linear regression analysis. ***Results:*** IP-10, MCP-1 and sVCAM were significantly elevated in SLE patients versus controls (p < 0.0001 for all). The concentration of all 3 analytes was significantly increased in patients with high versus low/moderate disease activity (IP-10; p = 0.02: MCP-1; p = 0.007: sVCAM; p = 0.0002). There was a moderate positive correlation for IP-10 (r = 0.42, p = 0.0002), MCP-1 (r = 0.50, p < 0.0001) and sVCAM (r = 0.48, p < 0.0001) and the SLEDAI-2K at inception. This association was comparable to that seen for traditional biomarkers (dsDNA or complement (C3)) and the modified SLEDAI-2K (anti-dsDNA (r = 0.52, p < 0.0001) and C3 (r = 0.40, p = 0.0004)). There was a moderate positive correlation between the change in concentration of each analyte and the change in SLEDAI-2K, between the first and 12-month visits (IP-10 (r = 0.56, p < 0.0001), MCP-1 (r = 0.58, p < 0.0001) and sVCAM (r = 0.59, p < 0.0001)). This was stronger than the association between the change in the modified SLEDAI-2K and the change in anti-dsDNA (r = 0.43, p = 0.0001) or C3 (r = 0.33, p = 0.0041). Longitudinal analysis (≥ 3 or more assessments) of 34 patients showed that changes in IP-10 mirrored changes in disease activity with high fidelity in 50% of cases. ***Conclusion:*** This study suggests that the IP-10, MCP-1 and sVCAM may be useful biomarkers to monitor disease activity functioning as well, if not better, than traditional biomarkers.

PO1.E.12

Urinary cyto/chemokines correlates with renal histopathology in systemic lupus erythematosus

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Objective: Renal biopsies are the gold standard for the assessment of renal disease in SLE patients. Urinary biomarkers have been proposed as a less invasive alternative in the management of lupus nephritis (LN). In a preliminary screen of 20 cyto/chemokines urine concentrations of IP-10, MCP-1, adiponectin, IL-15 and sVCAM were found to be elevated in patients with active LN. In this study, we examined the relationship between renal histopathology and the urine concentration of these 5 analytes at the time of biopsy. **Methods:** Twenty-two SLE patients undergoing renal biopsy were recruited and plasma and urine were obtained coincident with biopsy. 19 controls were recruited. The plasma and urine concentration of 5 analytes was determined by a Luminex assay with urine concentrations corrected for creatinine excretion. Patients were segregated into active (ISN/ASN; III to V; $n = 19$) and inactive (chronic, $n = 3$) based on renal histopathology. Elevated urinary analyte concentration was defined as values ≥ 2 standard deviations above the mean in controls. Mann Whitney non-parametric test was used for comparisons between groups. The statistical significance of correlations was determined by linear regression analysis. **Results:** The urine concentration of IP-10 ($p < 0.0001$), MCP-1 ($p < 0.0001$), adiponectin ($p = 0.0004$), IL-15 ($p = 0.0006$) and sVCAM ($p = 0.0073$) were significantly elevated in SLE patients versus controls. Elevated urine concentrations of MCP-1, IP-10 adiponectin and IL-15 were seen in 82.3%, 68.1%, 54.5% and 40.9% respectively. As only 22.7 % of patients had elevated urine sVCAM this analyte was excluded from further analysis. To assess if increased urinary analyte concentrations discriminate between active and inactive LN, the absolute number of elevated values for all four analytes were summed to create a global score (maximum = 4). Patients with active renal lesions had a statistically significant increase ($p = 0.03$) in the global score versus patients with established fibrosis. All 3 patients with fibrosis had scores ≤ 1 whereas the majority (73.8 %) of patients with active renal lesions had scores ≥ 2. In patients with proliferative lesions (ISN III or IV) 85.7% patients ($n = 14$) had scores of 2 or greater whilst 75 % ($n = 4$) patients with membranous LN (ISN V) had scores of 1 with a single patient having a score of 4. **Conclusions:** Urinary elevation in selected cyto/chemokines effectively discriminate between active and chronic renal lesions. The elevation ≥ 2 analytes was sufficient to identify proliferative renal lesions. These results suggest that urinary cyto/chemokines reflect underlying renal pathology and may serve as alternatives to renal biopsy.

PO1.E.13

Time to development of lupus nephritis

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Background/**Purpose:** Lupus nephritis is often present at time of diagnosis of SLE, but in some patients it may occur later in the course of disease. We investigated the first occurrence of nephritis in an inception cohort of SLE patients followed in a single centre and examined predictive factors for late occurrence of nephritis. **Methods:** Inception patients seen in clinic within one year of diagnosis of SLE were selected and followed in an observational cohort study. Lupus nephritis was defined as sterile hematuria and/or pyuria, granular casts, proteinuria (>500mg/24hr), or elevated serum creatinine (defined as > 120μmols/l) on two or more consecutive visits, or dialysis, transplant or WHO renal biopsy $\geq$ class 2. Incidence of lupus nephritis was determined for each year following entry into the clinic. In patients with no lupus nephritis, a Cox survival regression analysis was run, using the values at 1st clinic visit to pre-

dict development of future Lupus nephritis. Included in the model were sex, disease duration, SLEDAI-2K, SLICC/ACR damage index (SDI), steroids, antimalarial, immunosuppressants, race, complement and dsDNA. Selection of variables retained in the model was done through the stepwise approach. Kaplan-Meier curves were done for significant predictors **Results:** In a cohort of 633 inception patients with SLE, 382(57 %) did not have lupus nephritis at inception. There were 87% female, mean age at SLE diagnosis 36yrs. 77% Caucasian, 8% Black, 65% Chinese, 10% other. Their disease duration at first clinic visit was 0.24 yrs; SLEDAI-2K was 8.63 and SDI 0.07. 46% were taking glucocorticoids, 35% antimalarials and 8% immunosuppressants. The mean serum creatinine was 73%, 46% had low complement and 48% elevated anti-DNA antibodies. Of the 382 patients, 107 (28%) eventually developed lupus nephritis, 77% of them within the first 5 years. Cox regression analysis revealed that only anti-DNA antibody was a statistically significant predictor with HR = 1.59 (95% CI 1.01, 2.48, p=0.04). Kaplan-Meier curve comparing the development of nephritis between patients with normal vs. elevated anti-DNA antibody at 1st clinic visit showed a statistically significant difference (Wilcoxon p=0.04) **Conclusion:** 28% of patients with SLE without nephritis at inception develop lupus nephritis later in their course. The majority occur within the first 5 years. Some patients developed nephritis later. The only predictor for future development of lupus nephritis is the presence of anti-DNA antibody at inception.

PO1.E.16

Systemic lupus: long term remissions with intravenous pulsed methylprednisolone and cyclophosphamide and no daily oral steroids (the "lupus cocktail")

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Objective: To follow up on a report presented 15 years ago on the treatment of serious systemic lupus with periodic pulses of methylprednisolone and cyclophosphamide plus usual doses of hydroxychloroquine and no daily oral steroids. (1) **Methods:** Periodic assessments and therapeutic adjustments in the initial cohort of patients plus new individuals, entered in the protocol consisting of hydroxychloroquine 200 mg twice a day orally, methylprednisolone 1000 mg intravenously given in three (3) hours, every six hours for 3 doses, followed by cyclophosphamide 750 mg per square meter of body surface within a drip of normal saline at 100cc per hour. One hour before and one hour after cyclophosphamide intravenous mesna up to 2.5 grams per square meter of body surface to protect the urinary bladder epithelium from the noxious effect of cyclophosphamide metabolites was also given. This regimen was administered once a month for six months and then every three months for two years, and then once or twice a year as "maintenance" for up to five years. A very important point in this protocol is the absence of daily use of corticosteroids in between the pulses and thereafter, as is the case in past and current approaches to the treatment of SLE. **Results:** The clinical and laboratory improvements reported in Ref.1 were noted again in the new patients by now reaching 50 individuals. The severe toxicity associated with the use of daily oral steroids was conspicuously absent with no cases of a vascular necrosis recorded, reduced rate of osteoporosis, lack of opportunistic infections, absence of Cushinhoid features, and lack of weight gain provided that no daily use of oral prednisone took place. In the original cohort, remissions lasting 6 to 10 years document the treatment consisting of maintenance of hydroxychloroquine and non-steroidals prn. **Conclusions:** The use of pulsed methylprednisolone and cyclophosphamide as described associated with daily oral hydroxychloroquine is effective in systemic lupus and may lead to clinical and laboratory remissions. The elimination of oral daily steroids from the regimen clearly reduces the toxicity of these agents without diminishing the beneficial clinical response. Additionally, this is a low cost treatment when compared with recently developed ones.

Reference: (1) The Lupus Cocktail, Lupus: Vol. 4, Supplement 2, March 1995, page 114

PO1.E.17

SLE patients show evidence of premature biological senescence

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Background: Patients with SLE have premature mortality compared to the general population. The main causes of death include coronary heart disease and infection. These observations suggest a phenotype of premature senescence in SLE. Telomere length (TL) can be used to assess overall biological ageing. We hypothesise is that patients with SLE will demonstrate reduced TL compared to a control population. **Methods:** Telomere length was measured in a cross sectional study using real time quantitative PCR in females with SLE (4 or more ACR 1997 criteria) and age-matched healthy female controls recruited from the local community. SLE factors and traditional cardiovascular risk factors were noted and assessed against TL in a larger cross-sectional SLE population. **Results:** We studied 63 SLE patients vs. 63 controls with a median (IQR) age of 50.8 (37, 59) and 49.9 (32, 60) yrs respectively. The median relative TL was significantly reduced in SLE patients (0.97 vs 1.5, p=0.0017). In our cross-sectional study we assessed 163 SLE patients with a median (IQR) age of 52 (44, 60) years; disease duration of 13.0 (7, 23) years; SLEDAI 1 (0, 4) and SLICC DI 1 (0, 2). 55 (34%) were positive for anticardiolipin antibody or lupus anticoagulant, 36 (22%) had renal involvement and 79 (48%) were current steroid users. Therapy and traditional cardiovascular risk factors were not correlated with telomere length. **Conclusions:** Whole blood telomere length, a marker of biological senescence, is significantly reduced in SLE patients compared to controls. Telomere length was not correlated with cardiovascular risk factors or SLE disease activity. Telomere length may be a risk marker for future cardiovascular morbidity or mortality however the mechanism within SLE that results in premature biological senescence is not fully recognised. The predictive value of telomere length as a biomarker of future risk of damage/mortality requires prospective evaluation.

PO1.E.18

Deoxyribonuclease 1-mediated clearance of circulating chromatin prevents from cell activation and inflammatory cytokine production

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Objectives: Chromatin fragments represent major autoantigens in systemic lupus erythematosus (SLE). They are composed of DNA and histones and are present in the circulation due to impaired clearance mechanisms in SLE. Increased chromatin concentrations may favor the break of the peripheral tolerance partly upon direct activation of immune cells. Deoxyribonuclease 1 (DNase1) is known to degrade isolated DNA or nuclear DNA. However, its impact on circulating chromatin-organized DNA is less clear. Since DNase1 activity is impaired in SLE, we have investigated whether DNase1 may control chromatin clearance and chromatin-induced cell activation. **Methods:** Chromatin was purified from calf thymus in order to get mono- and oligonucleosomes. Chromatin degradation was analyzed in vitro in human plasma and serum or plasma from wild type (WT) and DNase1-knockout (KO) mice by agarose gel electrophoresis. In vivo chromatin clearance was estimated in WT and DNase1-KO mice upon intravenous injections of chromatin and analyzed by agarose gel electrophoresis. Chromatin-induced activation of total spleen cells prepared from WT and DNase1-KO mice was estimated by flow cytometry (CD69 expression) and ELISA (cytokine secretion). **Results:** Chromatin fragments are degraded in mouse sera/plasmas and human plasmas into mononucleosomes or smaller fragments, espe-

cially upon activation of the plasminogen system by heparin, which leads to histone degradation. The degradation occurs in a DNase1-dependent manner since no degradation is observed in DNase1-deficient plasma or when serum DNase1-like3 is inhibited. Moreover, the in vivo clearance of circulating chromatin is impaired in DNase1-KO mice as compared to WT mice. Nucleosomes significantly induce the activation of spleen cells from both WT and KO mice, leading to CD69 up-regulation and the secretion of the pro-inflammatory cytokines IL-6 and IL-12. Importantly, the activation by nucleosomes was significantly stronger in DNase1-KO spleen cells. On the contrary, LPS activated cells from both WT and KO cultures to the same extent, showing that DNase1-deficiency has a specific impact on nucleosome-induced cell activation. Plasma from healthy individuals and SLE patients are currently being compared. **Conclusions:** DNase1 is involved in the degradation of circulating chromatin and protects from nucleosome-induced activation of cells, likely antigen-presenting cells. DNase1 plays therefore an important role in the clearance of circulating chromatin. A low DNase1 activity in combination with a low fibrinolytic activity, as reported in SLE patients, may partly favor the break of the peripheral tolerance.

PO1.E.19

Nucleosome-induced neutrophil activation occurs independently of toll-like receptor 9 and endosomal acidification in systemic lupus erythematosus

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Objectives: Nucleosomes are major autoantigens in systemic lupus erythematosus (SLE) and are believed to play a key role in disease development. Circulating nucleosomes are detected in SLE patients and have been shown to activate neutrophils. Toll-like receptor 9 (TLR9) is expressed in neutrophils and is activated by different types of DNA. Since nucleosomes are partly composed of DNA, we have investigated whether TLR9 and endosome acidification are required for nucleosome-induced neutrophil activation. **Methods:** Human and mouse neutrophils were freshly isolated from blood by density centrifugation or from bone marrow by positive selection using magnetic beads, respectively. Neutrophils were cultured with purified nucleosomes or TLR ligands in the presence/absence of polymyxin B, chloroquine, ammonium chloride or a TLR9 inhibitor. Nucleosome endocytosis was analyzed by confocal microscopy. Cell activation was estimated by measuring cytokine secretion by ELISA and up-regulation of cell surface molecules by flow cytometry. The consequences on TLR9 expression were analyzed. Neutrophils from wild-type and TLR9-knockout mice were compared. **Results:** Nucleosomes, LPS, R848 and CpG, but not GpC, oligonucleotides activate human neutrophils, leading to IL-8, IL-6 and TNF secretion as well as CD11b up-regulation. Plasmas from lupus patients activate also normal neutrophils and activation was blocked upon depletion of nucleosomes, confirming the role of circulating nucleosomes in neutrophil activation. Free nucleosomes accumulated in the cytoplasm upon endocytosis. Nucleosome-induced activation was not inhibited by polymyxin B, chloroquine, ammonium chloride or the TLR9 inhibitor, indicating that activation occurred independently of endotoxins, endosomal acidification and TLR9. Moreover, both neutrophils from wild-type and TLR9-knockout mice were activated by nucleosomes, as estimated by MIP-2 secretion and CD11b up-regulation, confirming that TLR9 was not required. Although nucleosome-induced neutrophil activation is TLR9-independent, nucleosomes induced the up-regulation of intracellular TLR9 in some donors. **Conclusions:** The role of TLR9 in SLE development is still a matter of debate. Here we show that neutrophil activation by nucleosomes occurs independently of TLR9 and does not require endosomal acidification. The signalling pathway used is thus different from the classical pathway for unmethylated CpG-DNA. TLR9 might play different roles in nucleosome-induced innate immunity and anti-nucleosome autoimmunity.

PO1.E.20

Low copy-number of complement *C4A*, the presence of HLA-DR3, and the presence of HLA-DR2 are independent and additive risk factors for human systemic lupus erythematosus (SLE)

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Objective: The major histocompatibility complex (MHC) in chromosome 6p21.3 has the strongest genetic association with human SLE. Because of the extremely complex polymorphisms, frequent and continuous gene copy-number variations (CNV), and strong linkage disequilibria among candidate genes, identifying the disease causal variant(s) in the MHC posts a great challenge for SLE genetics. The objective of this study is to dissect the roles of candidate genes HLA-*DRB1* and complement *C4* in human SLE. ***Methods:*** The study population includes 744 SLE patients and 760 unrelated healthy controls of European ancestry, recruited mainly from the mid-western US (476 patients and 461 controls) and Sweden (268 patients and 299 controls). Rigorous and definitive genotyping techniques by Southern blot analyses of *Taq*I and *PshA*I-*Pvu*II digested genomic DNA, and quantitative real-time PCR were performed to determine the copy-numbers of total *C4*, *C4A* and *C4B*. Two-digit genotypings for HLA-*DRB1* were performed by twenty independent SSP-PCRs for each DNA sample. Chi-square analyses and 2-tailed t-tests were applied to compare categorical data and means of continuous data, respectively. ***Results:*** The copy-numbers for total *C4*, *C4A* and *C4B* vary mainly from 2 to 6, 0 to 5, and 0 to 4, respectively. Significant differences were observed between SLE and controls for the CNV of *C4A* (χ^2=65.2; p=2.4x10^{-13}), and frequencies of HLA-*DRB1*03* (DR3, χ^2=45.2; p=1.5x10^{-10}) and HLA-*DRB1*15* (DR2, χ^2=6.89; p=0.032). Low *C4A* copy-numbers (i.e., 0 or 1 copy), HLA-*DR3* and HLA-*DR2* together are present in 71.2% of SLE patients, compared to 53.2% in controls [odds ratio (95% CI): 2.18 (1.76-2.70); p=7.1x10^{-13}]. The presence of 0, 1 and ≥2 copies of *C4A* are strongly (but not absolutely) correlated with homozygous, heterozygous and absent HLA-*DR3*, respectively, both in healthy subjects (r^2=0.435, χ^2=374.0; p=1.2x10^{-79}) and SLE patients (r^2=0.381, χ^2=463.9; p=4.2x10^{-99}). Using subjects with non-low *C4A*, non-*DR3* and non-*DR2* as the reference group, the odds ratios for SLE with low *C4A* only was 2.22 (95% CI: 1.11-4.43; p=0.031), *DR3*+ only was 1.81 (1.21-2.71; p=0.004), and *DR2*+ only was 1.65 (1.26-2.16; p=0.0002). Notably, the odds for SLE increased to 2.80 (2.09-3.76; p=3.6x10^{-12}) among subjects with both low *C4A* and *DR3*, and 2.71 (1.10-6.64; p=0.037) among subjects with both low *C4A* and *DR2*. ***Conclusion:*** Low gene copy-number of complement *C4A*, the presence of HLA-*DR3*, and the presence of HLA-*DR2* each can be an independent risk factor for human SLE. However, higher additive odds for SLE are present among subjects with low *C4A* plus HLA-*DR3* or HLA-*DR2*.

PO1.E.21

Pulmonary fibrosis: long-term survival with pulsed cyclophosphamide and methylprednisolone without daily steroids. Twenty years follow up

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Objective: To report on the clinical evolution and long term outcome of patients with Pulmonary Fibrosis treated with this protocol previously described at the IV International Conference on Lupus Erythematosus in 1995 (1). ***Methods:*** Follow up of the initial cohort and observations on newly enrolled patients receiving the original protocol of pulsed cyclophosphamaide and methylprednisolone monthly for six months and then quarterly for two years and then once or twice a year as " maintenance" without administration of daily oral steroids. ***Results:*** The first patient did very well for 2 years but when she moved to another state and lost to follow up. The second patient, 34 years of age, female, entered in the protocol because her idiopathic Pulmonary Fibrosis was not improving on 30 milligrams of prednisone daily. She had also developed avascular necrosis of her left hip requiring a prosthetic joint. Her abnormal pulmonary function tests normalized in one year, her O2 saturation also normalized, and she is asymptomatic and working full time 21 years later, with her right hip unaffected by the steroids given in pulses. The third patient consulted after she was given 1 one month to live by a world expert in scleroderma at a "world class tertiary academic facility because of her Terminal Pulmonary Fibrosis. She remained alive for four years with significant softening of her thickened skin, improvement of pulmonary function tests and improvement of her physical functional capacity. She died of causes unrelated to her Pulmonary Fibrosis. The fourth patient, female 36 year of age, presented with the acute edematous phase of sclerodera involving her limbs, face and trunk plus evidence of interstitial pulmonary manifestations. Upon entering the protocol, the edematous face resolved quickly, the pulmonary manifestations became quiescent and both her pulmonary function tests and O2 saturation normalized. She is now working full time 17 years after starting the treatment. Newly arrived patients are leading normal lives and experiencing remissions of up to seven years. Long Term toxicity of the regimen is remarkably low: Reduced rates of osteoporosis, no avascular necrosis, no opportunistic infections, no Iatrogenic cushing's ***Conclusions:*** This protocol is associated with Long Term survival and very low toxicity in patients with Pulmonary Fibrosis.
Reference:

(1) Cima,M: Pulsed Corticosteroids and Cyclophosphamide in combination for Pulmonary Fibrosis, Lupus: Vol. 4 supplement 2 March 1995

PO1.E.22

Loss of cytokine-induced PD-L1 expression in pediatric SLE

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Objectives: Immune mechanisms implementing tolerance to self antigens can be modeled in the autologous mixed lymphocyte reaction (aMLR). Negative regulatory factors like programmed death ligand-1 (PD-L1) may normally play an important role in controlling lymphocyte responses to self antigens, and thus prevention of autoimmune diseases like SLE, in which T and B lymphocytes lose normal tolerance to self. We hypothesized that in SLE PD-L1 expression on antigen presenting cells (APC) is lost secondary to aberrant T lymphocyte activity, which in turn may lead to further loss of lymphocyte regulation through chronic APC stimulation. ***Methods:*** Serial samples were obtained from 27 pediatric controls and 37 pediatric patients with SLE (21 samples were drawn during active disease, SELENA-SLEDAI score >4, and 33 during remission). PD-L1 expression on CD11c$^+$ CD14^{high} monocytes (Mo) and CD14^{low} myeloid dendritic cells (mDC) was assayed in cultured peripheral blood cells by flow cytometry. PD-L1 mRNA expression was assayed by reverse transcriptase Real Time Q-PCR. Cytokines in culture supernatants were assayed by multiplex ELISA. ***Results:*** PD-L1 expression was induced during the aMLR on a mean of 80% of healthy Mo and 26% of mDC, whereas cells from pediatric patients with active SLE expressed lower levels of PD-L1 (mean 52% on Mo and 19% on mDC, p=0.00003, p=0.08). Expression of the positive costimulatory factors CD80, CD86 and MHC Class II was low/normal in SLE, suggesting that loss of PD-L1 may contribute to the reportedly high stimulatory activity of SLE mDCs. PD-L1 expression on active SLE Mo and mDC could be restored by normal T cells or by supernatant from healthy PBMCs. Both IL-10 and TNF-α secretion correlated with PD-L1 expression, and either cytokine could restore PD-L1 expression on SLE Mo. Conversely, SLE cells produced TGF-β and PD-L1 expression on healthy Mo was suppressed by TGF-β. ***Conclusions:*** During an inflammatory response patients with SLE may be lacking an important process to limit autoreactive lympho-

cyte activity: the local, cytokine-mediated induction of PD-L1 expression on antigen presenting cells. Moreover, therapeutic agents that inhibit TNF-α may induce autoantibodies in part by preventing PD-L1 induction, thereby impeding an important mechanism of T and B lymphocyte regulation.

PO1.E.23

New approach in therapy of female SLE patients by fulvestrant (faslodex), an estrogen selective receptor downregulator.
A one-year study with faslodex in women with SLE and followed by one to two years off faslodex; clinical and serologic parameters in the double-blind placebo controlled trial.

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Several autoimmune diseases, including SLE, predominantly affect females during their childbearing years. Estrogen plays a role in upregulation of intracellular signals by binding to estrogen receptors (ER). Faslodex competes for receptor binding in vitro and inhibits estrogen action in immune cells. *Objectives:* To determine the clinical status and the immunological parameters of female lupus patients at the end of the one year study in which they received monthly Faslodex and report the long term effects of Faslodex in those same female SLE patients after 1 to 2 years off Faslodex. *Methods:* Sixteen cycling female SLE patients participated in the one-year, double blind, placebo controlled study; randomized to receive Faslodex, 250 mg or placebo on a monthly basis to coincide with their menstrual cycle. Study required monthly labs: ANA, anti-dsDNA, CH50, C3, C4 and routine hematology, chemistry and UA. The Systemic Lupus Disease Activity Index (SLEDAI) was measured at each visit and a bone density done pre-study and at end-of-study. The labs and SLEDAI were repeated at visit 15, three months off study drug. Patients with active nephritis or cerebritis were excluded. The randomization revealed 8 on Faslodex, and 8 on placebo and the 1 to 2 year visits revealed 2 subjects from each group lost to follow-up. *Results:* The study revealed a significant drop of SLEDAI in the Faslodex group from 8.75 pre to 3.75 post one year of Faslodex (p=0.02). Anti-dsDNA dropped from 9.25 to 5.75 IU/mL (p=0.01). There were no significant changes in ANA, CH50, C3, C4, bone density, liver enzymes, or serum creatinine following Faslodex therapy. Standard therapy (prednisone, azathioprine, hydroxychloroquin) for lupus were reduced or stopped in 3 patients from the Faslodex group, but not in the placebo group. In the same Faslodex group, a persistent drop of the SLEDAI and anti-dsDNA was seen in the 1 to 2 year follow-up. Serum estrogen levels did not significantly change in the Faslodex group when compared to the placebo group. *Conclusion:* Blocking estrogen receptors in vivo by an estrogen selective receptor downregulator could be considered as a new therapeutic approach for moderately active female SLE. Studies have been designed to study the effects of estrogen on regulatory cells and if Faslodex selectively blocks ERα or ERβ or both receptors. Future studies are needed to treat SLE patients with a larger dose of fulvestrant for a longer period in order to achieve—if possible—long term remission.

PO1.E.24

Sex bias in mouse lupus: beyond the sex-hormones.
A gene expressional analysis of myeloid cells.

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Systemic Lupus Erythematosus is a prototypic systemic autoimmune disease with a strong female bias (9:1) and no known etiology or treatment. Our previous studies have found a sex hormone independent potential of female BM to transfer accelerated disease development to either sex in our murine model of spontaneous lupus. Further analyses found no differences in the lymphoid cell populations including all B and T cell subset, but a

significantly higher number of myeloid Gr1^{high}CD11b^+ in males. The intrinsic virulent property of female BM and the immunosuppressive role of Gr1^{high}CD11b^+ in cancer suggest that the Gr1^{high}CD11b^+ cells play a role in inferring protection to males from the disease. The complete genetic profiling of these cells along their developmental pathway will provide significant insight into the sex bias and causes of lupus. *Objectives:* The recent study is aimed at identifying if sex specific gene expression differences occur during the development of Gr1^{high}CD11b^+ cells and if so, where such differences are first measurable; BM, spleen or transitional phase. *Methods:* We used prepubescent NZWxNZB F1 mice to obtain BM single cells. CMP (Lin-IL-7Rα-Sca1-CD34+c-kit+FCγR^{low}), GMP (Lin-IL-7Rα-Sca1-CD34+c-kit+FCγR^{high}), splenic Gr1^{high}CD11b^+ and BM Gr1^{high}CD11b^+ cells were sorted by high speed cell sorting (FACSAria I, BD biosciences). The sorted cells were analyzed for purity and subjected to RNA isolation followed by microarray analysis. The differential gene expression analysis was carried out in Bead Studio using female samples as the reference and appropriate normalization. The differential score for the genes were accessed for statistical significance and a cut off of <-20 or >20 applied. *Results:* There is no gene expression differences in the two earliest myeloid progenitors (CMP and GMP). The BM Gr1^{high}CD11b^+ cells show 214 genes differentially expressed between males and females, while mature splenic Gr1^{high}-CD11b^+ cells show 535 genes differentially expressed. Further analyses of these genes suggested that female Gr1^{high}CD11b^+ cells show a higher transcriptional activity indicating faster maturation. *Conclusion:* In this study we identified differential gene expression in BM derived male and female Gr1^{high}CD11b^+ cells in our lupus mouse model, suggesting a sex bias in the BM microenvironment that modulates these cells differentially in males and females. Further studies are evaluating if these factors are responsible for driving further maturation of Gr1^{high}CD11b^+ cells in females predominantly and possibly a loss of suppressive activity.

PO1.E.25

Sex hormones and gender influence the function of regulatory T cells in SLE patients

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Immunophenotyping of PBMC from SLE patients (n~40) indicated significantly reduced numbers of CD4+CD25hi Foxp3+ T cells and CD8+Foxp3+T cells (p<0.02) as compared to healthy matched controls (n ~20) and autoimmune disease control RA patients (n=30). Numbers of CD4+ and CD8+ regulatory T cells are decreased in healthy females compared to healthy males (p<0.01). Both CD4+CD25hi and CD8+CD25hi subsets in males had 3-4-fold higher Foxp3 mRNA compared to females. Stimulation of PBMCs with b-estradiol (30pg/ml) decreases Foxp3 expression in healthy females but not in age matched healthy males. At higher doses (60,150 pg/ml) estrogen has little effect in either sex. Estrogen decreases Foxp3 mRNA and protein expression in both female and male SLE patients' CD4+CD25– T cells (p<0.05). An Inhibitor of b-estradiol (ERa) increased apoptosis in male SLE patients only. These data suggest that estrogen affects the T regulatory compartment.

PO1.E.26

Dexamethasone and cyclophosphamide resistant plasma cells in inflamed kidneys and bone marrow of NZB/W mice

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Antibodies contribute to the pathogenesis of many chronic inflammatory diseases, including autoimmune disorders and allergies. They are secreted by proliferating plasmablasts, short-lived plasma cells and non-proliferating, long-lived plasma cells. Long-lived plasma cells refractory to immunosuppression are critical for the maintenance of both protective and pathogenic antibody titers. Here, we studied the spleen, bone marrow and inflamed kidneys of lupus prone NZB/W mice for the localization of autoreactive and non-autoreactive long-lived plasma cells and their response to high-dose dexamethasone and/or cyclophosphamide. Treatment depleted plasmablasts and short-lived plasma cells in the spleen. In contrast, all bone marrow plasma cells, including anti-DNA secreting cells were refractory to both drugs. Remarkably, in the inflamed kidneys a substantial fraction of plasma cells including anti-DNA secreting cells survived the treatment. These results indicate that the inflammatory environment contributes to the survival of both, autoreactive and non-autoreactive plasma cells. This may have relevance in chronic-inflammatory processes. Therefore, new strategies targeting pathogenic plasma cells have to consider long-lived bone marrow plasma cells, but also plasma cells in inflamed organs. This could be the key to finding a curative approach to the treatment of chronic inflammatory antibody-mediated diseases.

PO1.E.27

Deregulation of mir-21 expression affects key pathways that contribute to the aberrant phenotype of SLE T lymphocytes

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Objective: MicroRNAs (miRNAs) have the potential to maintain the balance between immune activation and tolerance by regulating gene expression. We investigated the role of this new class of gene modulators in the deregulation of the immune response in human SLE. *Methods:* TaqMan miRNA arrays were used to examine the expression of 365 miRNAs. The expression of miRNAs and their target genes were validated by real time PCR and Western blot analysis. The effect of mir-21 on anti-CD3/CD28–induced T cell proliferation and cytokine production was examined by transfection assays. T-/B- lymphocyte co-cultures were set up and plasma cell differentiation was assessed by flow cytometry and anti-nuclear antibody (ANA) measurement. *Results:* Analysis of the miRNA profile of hSLE identified a total of 27 differentially expressed miRNAs in the PBMCs, B and T lymphocytes of normal and SLE patients. Among them, miR-21, miR-25, miR-106b and miR-148b highly correlated with disease activity with mir-21 displaying the highest correlation in all cellular subpopulations examined (r2>0,92). Compared to healthy T cell, SLE T cells significantly up-regulated miR-21 upon anti-CD3/CD28–activation and displayed increased proliferation. Silencing of mir-21 reversed several features of the aberrant phenotype of lupus T cells, including increased proliferation, production of IL-10 and sustained co-stimulation, as determined by CD40L expression. Of interest, antago-mir-21–transfected T cells had reduced capacity to drive lupus B cell differentiation into CD19+CD38+IgD- plasma cells. Analysis of mir-21 gene- targets revealed that PDCD4, a protein that has been recently shown to be important for the immune response, was significantly decreased in lupus patients. *Conclusion:* Mir-21 is a key regulator of processes such as proliferation, co-stimulation and cytokine production that contribute to the aberrant phenotype of SLE T lymphocytes. Thus, we show that mir-21 is not just an activation marker but is might implicated in the development of the abnormal immune response in SLE.

PO1.E.28

UV irradiation promotes the effect of TLR7 agonist induced lupus like disease in autoimmune prone NOD mice

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Purpose: The role of environmental precipitants in autoimmunity such as systemic lupus erythematosus (SLE) remains unclear. We wished to determine whether UV alone or UV in the presence of TLR7 activation would induce lupus-like disease in an autoimmune mouse model and to explore underlying mechanism(s). *Methods:* 6 week old female NOD (non obese diabetic) mice received repeated weekly 5000 j/m2 UVB radiation or 25 ug of topical imiquimod or both. Control mice were left un-treated. For comparison, non-obese diabetic resistant (NOR) mice, MYD88-/- NOD mice and TLR9 -/- NOD mice were treated with combination therapy (imiquimod+UV). Serum was collected for detection of anti-nuclear antibodies (ANA), desmoglein 3 (Dsg 3) antibodies and IFNα by ELISA and detection of pro-inflammatory cytokines by cytokine bead array. Peripheral blood was collected for cell surface or intra-cellular staining using flow cytometry. Apoptotic cells in the skin were detected using TUNEL assay and IgG deposition in the kidneys were detected by direct immunofluorescence. Immunohistochemical studies were conducted to determine the expression of IFNα inducible gene myxovirus A (MxA) and high mobility group box 1 (HMGB1) in skin. PAS staining of kidney identified the presence of glomerulosclerois. Freeze-thawed splenocytes were intra-dermally injected to simulate UV-induced cell death. *Results:* Imiquimod treatment enhanced ANA and Dsg3 Ab production in NOD mice. Imiquimod+UV induced MXA and HMGB1 expression in the skin and glomerulosclerosis. Systemic immune activation was detected following combination therapy but not single therapy as evidenced by IL-6, TNFα, IFNγ, MCP-1. Serum IFNα was significantly elevated in NOD mice following combination therapy. Combination therapy up-regulated TLR-7 and IFNα expression in the peripheral blood PDCs of NOD but not NOR or MyD88-/- NOD mice. TLR9-/- NOD mice demonstrated enhanced auto-antibody production, and serum TNFα, IL-6 and IFNα levels compared to NOD mice. UV induced cell death was unlikely to be the only promoter of IFNα release as freeze-thawed dead cell injection at time of imiquimod application did not promote comparable IFNα levels to UV therapy. *Conclusions:* These studies demonstrate that environmental factors combined with inflammation induce autoimmune like disease and suggests UV enhances and TLR9 activation diminishes the effect of TLR7 engagement in autoimmune prone animals.

PO1F   Epidemiology

PO1.F.2

Adherence to cervical cancer screening in SLE patients

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Introduction: Women with SLE have an increased incidence of cervical dysplasia (CD) compared to the general population; the long-standing immunosuppressive treatment, especially Cyclophosphamide and Azathioprine, might have a causal relationship. Screening programs (SP) with the Papanicolaou (Pap) smear test have significantly reduced the number of cervical Cancer (CC) invasive cases by early diagnosis and treatment of precancerous lesions. However, it is well known that the adherence rate to the cancer screening programs is generally low both in general population and in chronic diseases such as diabetes. In 2005, Bernatsky S et al observed a low adhesion rate to recommended CC screening in a cohort of Canadian SLE patients. In Italy, organized SP are promoted and addressed to women aged from 25 to 64 years old, personally invited by the Health System (with a call-recall system) to undergo a free Pap test every three years. *Objectives:*

(i) To evaluate the adherence to cervical cancer screening programs in an Italian cohort of SLE patients; (ii) To establish disease-related factors possibly influencing the patients behaviour. ***Material And Methods:*** *Patients*: Inclusion criteria: SLE diagnosis based on ACR classification criteria, female sex , age > 25 and < 64 years. *Controls:* Aged- matched healthy women recruited from our Unit health personnel and from patients friends or relatives. *Data collection:* Face by face or telephone interview. ***Results:*** One hundred forty one SLE female patients have been enrolled (mean age 48.18 years, min 27- max 64). Of these, 34 (24.1%) underwent the test yearly by the personal gynaecologist, 58 (41%) regularly answered to the SP. Of the remaining 49, 23 (16.3%) never answered to the SP and 26 (18.4%) did the test only occasionally in presence of gynaecological symptoms. Overall, 49 (35%) of the SLE patients and 16 (38.3%) of the controls did not perform a Pap test during the previous three years. ***Conclusion:*** Among our SLE cohort, in agreement with previous observations, the adherence rate to the cervical cancer screening program resulted low and an important percentage of patients (35%) did not follow adequate cancer preventive measures. However, we also noticed that a significant proportion of patients performed the Pap test most frequently than what proposed by the Regional SP and some disease- related variables might explain these differences in preventive behaviours.

PO1.F.3

Survival and mortality in patients with lupus nephritis
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Objectives: The clinical outcome of patients with severe lupus nephritis has improved over the past few decades due to improvements in immunosuppression and supportive care. This study aimed to examine the survival and predictors of death in Chinese patients with lupus nephritis in the current era. ***Methods:*** We reviewed the records of all patients who have attended our SLE Clinic from Jan 1971 to Dec 2008. Survival curves were plotted and the causes of death identified. ***Results:*** 287 patients were included with a mean follow up of 18.0±8.4 years. 230 (80.1%) patients had a history of renal involvement, with Class III or IV lupus nephritis ± membranous features. The 5-, 10-, and 20-year survival rates were 98.9%, 98.1 % and 90.9% respectively. Death occurred in 27 (9.4%) patients, with 85% occurring after 10 years of follow up. Infection (44.8%), malignancy (20.7%) and cardiovascular disease (17.2%) were the leading causes of death. The 5-, 10- and 20-years renal survival rates were 98.6%, 96.6% and 85.3% respectively in patients with renal involvement. The overall standardized mortality ratio (SMR) was 6.3 compared to the local age-and-gender-matched general population, while the SMR in patients with renal involvement, endstage renal failure, malignancy, and cardiovascular disease were 6.4, 26.1, 13.0, and 11.4 respectively. ***Conclusion:*** Although the survival of patients with SLE has improved, lupus nephritis is associated with a 6.4-fold increase in the risk of death, and endstage renal failure confers a 26-fold excess risk of death, the latter being more than twice the risk associated with malignancy or cardiovascular disease.

PO1.F.4

Coronary calcification in SLE: comparison with Multi-Ethnic Study of Atherosclerosis (MESA)
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Objectives: Women with lupus (SLE) have been demonstrated to have a marked increase in risk for myocardial infarction compared with the general population. CT coronary artery calcium (CAC) is a measure of subclinical atherosclerosis associated with risk for cardiovascular (CV) events in women with SLE. The purpose of this study was to determine whether the prevalence of CAC is higher in female SLE patients compared with the participants in the Multi-Ethnic Study of Atherosclerosis (MESA) who were free of CV events and SLE at the baseline exam. ***Methods:*** CAC was measured in 88 female SLE patients enrolled in the Lupus Atherosclerosis Prevention Study (LAPS) and 583 female MESA controls from the Baltimore Field Center aged ≥ 45 years without evidence of clinical cardiovascular disease. Poisson regression with robust variance estimation was used to estimate the ratio of CAC prevalence between SLE and MESA controls, controlling for demographic and CV risk factors. ***Results:*** Mean ages were 53.8 (SD=7.1) years (SLE) and 63.3 (SD=10.1) (MESA). 28 percent of SLE and 55 percent of controls were African-American. Sixty percent of SLE and forty seven percent of MESA controls had coronary calcification. In all age groups, SLE patients had a higher prevalence of CAC than MESA controls

Table 1. Proportion (%) with coronary artery calcium (>0), by study group and age

Age Group	SLE Patients (n=88)	MESA Controls(n=583)
45 – 54	33/57 (58%)	31/144 (22%)
55 – 64	13/22 (59%)	52/144 (36%)
65 – 74	5/7 (71%)	120/211 (57%)
75+	2/2 (100%)	70/84 (83%)

After controlling for age, ethnicity, diabetes, hypertension, hyperlipidemia, smoking, education, and BMI, SLE patients had a significantly higher prevalence of CAC than controls (Prevalence Ratio 1.8 (1.5, 2.3))

Table 2. Association between predictors and presence of coronary calcium based on a multivariable prevalence-ratio regression model.

Variable	Prevalence Ratio (95% CI)	P-value
SLE patients vs. MESA controls	1.8 (1.4, 2.3)	<0.0001
Age (per 10 years of life)	1.5 (1.4, 1.6)	<0.0001
Caucasian vs. NonCaucasian	1.3 (1.1, 1.5)	0.0013
Diabetes	1.2 (1.0, 1.5)	0.042
Total cholesterol >200mg/dl	1.0 (0.9, 1.2)	0.65
Hypertension	1.2 (1.0, 1.4)	0.020
Ever smoked	1.3 (1.2, 1.5)	0.0001
BMI 25-29 vs. <25	1.0 (0.9, 1.3)	0.67
BMI 30+ vs. <25	1.1 (0.9, 1.4)	0.24

Conclusion: SLE is associated with a greater prevalence of CAC in women than MESA controls even after adjusting for traditional cardiovascular risk factors. We previously demonstrated that inflammatory markers and degree of disease activity are not associated with CAC in SLE. Future studies are needed to determine the etiology of increased subclinical atherosclerosis in this population.

PO1.F.5

Systemic lupus erythematosus in Iran: a study of 2200 patients over 33 years
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Objective: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with worldwide distribution and wide variety in the natural history among different ethnic and geographic groups. The aim of this study was to

explore the manifestations of SLE in Iranian patients. *Method:* The study was based on the Iran Rheumatology Research Center (RRC) database, which registered clinical and Para clinical manifestations of SLE patients, during 1976-2009. *Results:* A total of 2200 SLE patients (1975 female and 225 male) were studied. The female to male ratio was 8.8:1 and the mean age at the presentation was 24.4±11.6 Years. Prevalence of clinical manifestations included: Musculoskeletal (85.5%), Cutaneous (83.4%), Renal (66.7%), Neuropsychiatric (24.0%), Pulmonary (22.2%), Cardiac (17.7%), and Hematologic (67%).There were seen positive FANA in 86.5% and anti-DNA in 82.1% of patients. Overlap syndrome and positive family history with other autoimmune diseases were detected in 8.2% and 3.4% of patients respectively.

	Number	Percentage
Musculoskeletal Manifestations	1880	85.5
Arthralgia	682	31
Transient Arthritis	1150	52.3
Articular Erosion	21	1
Avascular Necrosis of Bone	105	4.8
Myositis (biopsy)	64	2.9
Cutaneous Manifestations	1835	83.4
Malar rash	1332	60.5
Discoid lesion	330	15
Oral Ulcer	861	39.1
Photosensitivity	1252	56.9
Hair loss	1145	52
Renal Manifestations	1468	66.7
Proteinuria	1214	55.2
Cellular Cast	534	24.3
Hematuria	928	42.2
Renal biopsy	776	35.3
Type I	3	0.1
Type II	83	3.8
Type III	190	8.6
Type IV	413	18.8
Type V	75	3.4
Neuropsychiatric involvements	527	24.0
Convulsion	291	13.2
Psychosis	111	5
Peripheral neuropathy	131	6
Pulmonary Manifestations	488	22.2
Pleuritis/ pleuresia	362	16.5
Lupus pneumonitis	45	2
Pulmonary hypertension	4	0.2
Cardiac Manifestations	390	17.7
Pericarditis	209	9.3
Myocarditis	63	2.9
Valvular lesions	87	4.0
Hematologic Manifestations	1474	67.0
Leucopenia	777	35.3
Hemolytic anemia	91	4.1
Thrombocytopenia	383	17.4
Immunologic Manifestations	-	-
FANA	2017	86.5
Anti-dsDNA	1878	82.1
Low C3	1077	49
Low C4	1107	50.3
Anti- phospholipid antibodies	372	16.9

Conclusion: The prevalence of cutaneous and renal involvement in our patients were similar to those in nearby countries (with similar climate), while the prevalence of joint and hematologic involvement were similar to the European countries (with similar ethnicity). We may conclude genetic or climate factors may lead to different presentations of lupus.

PO1.F.6

Non-Hodgkin's lymphoma (NHL) in systemic lupus

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Purpose: To describe histology, prognostic factors, treatment and outcome of NHL in Systemic Lupus (SLE). *Methods:* Lymphomas were identified via cancer registry linkages at nine centres, and through hospitalization and billing information at two centres. Histology, prognostic factors at lymphoma diagnosis, treatment, and outcome were assessed from medical records. Prognostic factors were based on the International NHL Prognostic model, including age, elevated serum LDH, advanced stage, extra-nodal disease, and poor performance status *Results:* Of 47 patients who developed NHL after SLE diagnosis, 43 were female. At the time of lymphoma diagnosis, mean age was 53.6 years (SD 14.5), and mean SLE duration 13.8 years (SD 9.2, median 12.7). Of the 34 cases where detailed histology information was available, 20(59%) were diffuse large B-cell lymphomas (DLBC). In addition there were three follicular lymphomas, eight marginal zone lymphomas, and one each of Burkitt's, Mantel, and Lyphoplasmacytic lymphoma. None of the patients with marginal zone lymphomas had Sjogren's syndrome. Data on lymphoma stage (Ann Arbor) was available for 28 cases; among these, advanced stage was seen in 19 (68%). Most (79%) had ≥2 risk factors for poor prognosis. Treatment data was available for 28/47 cases. Fourteen were treated with chemotherapy alone, 4 with radiation therapy alone, 2 with surgery alone and 6 with more than one therapy. Chemotherapy consisted primarily of standard CHOP (Cyclophosphamide, Adriamycin, Oncovin, Prednisone). Rituximab was included in chemotherapy of 7 subjects, with an initially favorable response in terms of the lymphoma. Three were treated with stem cell transplants (autologous or allogenic).Two patients were not treated, one due to advanced lymphoma stage and poor prognosis. At the time of data collection, 20 of the 47 cases were deceased, including all but 3 of the subjects with poor prognostic factors at presentation. *Conclusion:* These data confirm that the most common lymphoma histology in SLE is DLBC. The DLBC lymphomas make up about a third of NHL in the general population, but were twice as prevalent as this in our SLE sample. The predominance of DLBC histology in SLE may support the role of chronic antigen stimulation in the pathogenesis of lymphoma development in SLE. Our preliminary data suggest that SLE subjects with poor prognosis after NHL diagnosis may be identified on the basis of the International NHL Prognostic Factors model. It appears that most SLE patients diagnosed with NHL are offered standard therapy. Continued follow-up of the subjects, and expansion of the study sample, is ongoing.

PO1.F.8

Long-term follow-up of Danish patients with SLE: increased incidence of virus associated tumours?

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Objectives: Patients with systemic lupus erythematosus (SLE) seem to experience an increased prevalence of oncogene virus infections such as Human Papilloma Virus (HPV) due to inherent or treatment-induced immunosuppression. However, due to the latency of cancer development long-term

follow-up studies are needed to study a possible increased incidence of virus associated cancers and premalignant tumours in SLE patients. The aim of the study was to investigate whether SLE is associated with long-term cancer risk with special focus on HPV virus associated cancers, dysplasia and carcinoma in situ of the uterine cervix, and other virus associated cancers. *Methods:* A hospital-based cohort of 576 SLE patients was linked to the national Danish Cancer Registry which collects information on all patients in Denmark with cancer since 1943. The cohort was followed for cancer from the date of the SLE diagnosis until the end of 2006 for a total of 7803 person years. Expected number of cancers was calculated from the total number of person-years under observation and national cancer incidence rates. *Results:* The SLE patients were on average followed 13.9 years (range 0-38.7 years). The patients experienced an increased risk of malignant tumours based on 61 cases observed during follow-up compared with an expected of 38.4 (SIR=1.6(95%CI:1.2-2.0)). The increased risk was observed in both sexes and after both short and long-term follow-up. The increased risk was observed for HPV associated malignant cancers as anal cancer SIR=26.9(95%CI:8.7-83.4) and vulva cancer SIR=9.1(95%CI:2.3-36.5) but not for cervical cancer SIR=0.6(95%CI:0.1-4.5), however an increased risk of cervical dysplasia and carcinoma in situ of the uterine cervix SIR= 1.8(95%CI:1.2-2.7) was observed based on 24 cases. Furthermore, an increased risk of liver cancer SIR=9.9 (CI 95%:2.5-39.8), bladder cancer SIR=3.6(95%CI:1.4-9.7), non-Hodgkin´s lymphoma SIR=5.0(95%CI:1.9-13.3) and non-melanoma skin cancer SIR=2.5(95%CI:1.5-4.1) was observed. *Conclusions:* In this cohort, SLE patients experienced an overall increased risk of malignant tumours and dysplasia and carcinoma in situ of the cervix. The increased cancer risk was mainly attributable to cancers of the vulva, liver and bladder, anal cancer and non-Hodgkin lymphoma. These neoplasias have individually been associated with polyoma virus and known oncogenic viruses such as HPV, hepatitis B and C and Epstein Barr virus. Release of the oncogenic potential of these viruses may relate to treatment-induced immunosuppression or features of SLE in itself, which will be the subject of further analyses of this cohort.

PO1.F.9

Impact of lupus on patients' employment, family relationships, and overall well-being

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Introduction: Limited research has evaluated the impact of lupus and its treatments on the lives of patients. This survey of adults with lupus in the United States was conducted to explore the effect of lupus on employment, overall well-being and activities of daily living, as well as patients' experiences with treatment. *Methods:* Participants completed a web-based questionnaire over the Internet during April and May 2009. *Results:* 531 respondents with lupus completed the questionnaire; 93% were female, and 86% were 20–50 years of age. 71% identified themselves as Caucasian, 10% as African descent and 3% as Asian. 41% reported >4 flares/year, and 28% estimated 3–4 flares/year. Most respondents were taking several medications, including NSAIDs, antimalarials, corticosteroids and cytotoxics/ immunosuppressants. Only 44% were 'satisfied' or 'very satisfied' with current treatment. 89% reported that lupus has affected their careers, with 48% indicating a highly significant effect. 30% were unemployed due to lupus, and 44% reported missing 1–30 days of work per year due to illness. The most common symptoms of lupus experienced by participants were fatigue (96%); arthritis, muscle pain/weakness or tendonitis (93%); and skin rashes, oral/nasal ulcerations, or hair loss (72%). 99% of respondents reported that lupus affected their physical well-being, and 96%, their daily activities, with 52% and 45%, respectively, citing a highly significant effect in these areas. Additional areas affected by lupus included mental health (91% of respondents), social life and relationship with friends (90%), and relationships with a partner or family (70% and 73%, respectively). Understanding from family

and friends was considered to be the most helpful form of support (71%), followed by the ability to speak with healthcare professionals (57%). *Conclusions:* These results indicate that people affected by lupus experience frequent symptoms which they identify as flares, with a significant impact on employment, physical well-being and everyday living. Fatigue, joint and muscle pain, and skin disorders are the most common physical symptoms reported, and may have a high impact on quality of life regardless of current standards of care. Most patients with lupus are not satisfied with the effects of the treatments they are taking, and place a high priority on communications with family, friends and healthcare professionals.

PO1.F.10

Differences of systemic lupus erythematosus clinical features between two large European population subgroups

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Objectives: Clinical heterogeneity of Systemic Lupus Erythematosus (SLE) between ethnic groups is partially explained by a genetic component. A recent report showed evidence supporting and extending this hypothesis[1]. In this work, SLE phenotypic differences were observed between two large subgroups of the European population, Northern and Southern, that have been shown as the most clear subpopulations in whole genome studies. Our aim was to replicate these findings. *Methods:* We compared frequencies of the ACR SLE classification criteria and mean age of disease onset between 475 patients from the North of Europe (Germany, The Netherlands, Hungary, The Czech Republic and Slovakia) and 1080 patients from the South of Europe (Spain, Italy, Greece and Portugal) by logistic regression, incorporating patient gender and time of follow-up as covariates. We selected for analysis only the clinical features showing low variability (< 30 %) between the recruiting centres in the Northern and in the Southern subgroups: serositis, immunologic disorder, malar rash, photosensitivity, arthritis, hematologic disorder, antinuclear antibodies and mean age of disease onset. *Results:* Two of the previously reported associations and the direction of their changes were replicated. Photosensitivity was 9.4 % more prevalent among patients from the Northern group (P = 0.00056), while arthritis was 10.7 % more prevalent in patients from the Southern group (P = 1.7 x 10^{-6}). The previously reported difference in prevalence of immunologic disorder was not replicated. *Conclusions:* Our study reinforces the evidence of a degree of SLE phenotypic variation between the Northern and Southern European subpopulations. These differences could be partially explained by the different genetic structure of the two subpopulations of Europeans. Identification of these relationships contributes to the understanding of SLE clinical heterogeneity and indicates the need to account for ancestry in SLE epidemiological studies within Europeans.
References:

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PO1.F.11

Lupus in Asian Canadians: the 1000 Canadian Faces of Lupus study

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Purpose: There are few reports of systemic lupus erythematosus (SLE) in North American Asians. We describe differences in disease expression, disease activity and damage between Asian (ASN) and Caucasian (CAU) SLE patients in a multicentre Canadian cohort. ***Methods:*** SLE patients were enrolled in a multi-centre cohort and followed annually. Sociodemographic factors, diagnostic criteria, disease activity, treatment, damage, and self-reported disease activity and health were collected using standardized tools. Patients reporting ASN or CAU ethnic origin were abstracted, and results were compared. Cross-sectional data were analyzed, testing for differences in sociodemographic and clinical factors and patient reported measures between the two ethnic groups in univariate analyses; significant variables from univariate analyses were included in multivariate regression models. ***Results:*** 1388 patients were studied, including 1113 CAU and 275 ASN. 330 patients reporting other ethnic backgrounds were excluded. Disease onset was younger (ASN= 22±12 yrs, CAU 33±15 yrs; $p<0.001$) and disease duration (ASN= 9±8 yrs, CAU 14±11 yrs; $p<0.001$) and age (ASN= 32±15 yrs, CAU 47±16 yrs; $p<0.001$) were lower in ASN compared to CAU. Income was similar; high school completion was higher in ASN (ASN 92%, CAU 84%; $p=0.001$). SLEDAI scores and number of ACR criteria met were similar. ASN had more frequent renal criteria (ASN 60%, CAU 34%; $p<0.001$), less frequent arthritis criteria (ASN 64%, CAU 80%; $p<0.001$). ASN were more frequently treated with prednisone (ASN 80%, CAU 64%; $p<0.001$), cyclophosphamide (ASN 18%, CAU 12%; $p=0.007$) and mycophenolate (ASN 22%, CAU 10%; $p<0.001$). Mean damage scores were higher in CAU but this was no longer significant after controlling for age and disease duration. SLAM scores were higher in CAU (ASN= 5.0±4.2, CAU 6.5±4.7; $p<0.001$). Self-reported flares, fatigue, disease activity and symptom scores (SLAQ) were lower in ASN. SF-36 physical (PCS) and mental (MCS) component scores were higher in ASN. In multivariate analyses, Asian ethnicity remained a significant predictor of better PCS ($\beta=3.3$, 95% CI 0.5,6.1;$p=0.02$) and MCS ($\beta=-2.9$, 95% CI 0.3,5.5;$p=0.03$) and lower SLAQ ($\beta=-5.0$, 95% CI -6.1,0.1; $p=0.01$) when age, disease duration, damage, immunosuppressives, ACR criteria met, education, and SLEDAI were included. ***Conclusions:*** Although lupus was at least as severe in ASN Canadians, (younger onset age, more renal involvement, and more exposure to immunosuppressives), ASN patients reported fewer disease flares, lower levels of disease activity and fatigue, and better physical and mental health compared to CAU. Cultural attitudes may influence patient perceptions of disease activity and overall health.

PO1.F.12

The Georgia Lupus Registry: trends in the prevalence of early SLE manifestations across age at diagnosis in a population-based registry

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Objectives: Although it is accepted that age at onset of SLE influences phenotypes, most studies compared SLE features between two predefined age groups in selected, predominantly Caucasian individuals. We described trends in the prevalence of SLE manifestations occurring within 5 years of diagnosis in a population-based registry that seeks complete ascertainment of all potential pediatric and adult patients with SLE, most of whom (77%) were high-risk blacks. ***Methods:*** The Georgia Lupus Registry is a population-based registry designed to estimate the incidence (2002-2004) and prevalence (2002) of SLE in Atlanta, Georgia. Case-finding utilizes multiple sources. Potential cases from 100% pediatric hospitals and pediatric rheumatologists, 96% adult hospitals and 100% high yield adult rheumatologists were ascertained. Nearly 250 demographic and clinical elements from medical records of potential cases were abstracted. 1,423 cases met our case definition: ≥ 4 ACR criteria or 3 ACR criteria with a final diagnosis of SLE by a rheumatologist. Among them, 488 patients with a disease duration <=5 years were analyzed. We plotted 36 clinical and immunological features in 5 groups, according to age at diagnosis. Linear trends in the prevalence of SLE manifestations across increasing age at diagnosis were tested using the Cochran-Armitage test. Significant trends were also tested by race and gender. ***Results:*** Table 1 shows the SLE manifestations with significant trends across age at diagnosis. Blacks (77%) and females (88%) showed similar decreasing trends. Trends in whites and males were not reliable due to small sample size. 29 other SLE manifestations did not reveal significant trends.

Table 1: Early SLE Manifestations by Age Groups (% and 95%CI)

SLE Manifestations	Age Group					Trend test p-value
	1-19 (n=63)	20-29 (n=96)	30-39 (n=114)	40-49 (n=104)	≥ 50 (n=111)	
Malar Rash	41.3 [37-46]	34.4 [31-38]	21.1 [19-24]	23.1 [21-26]	14.4 [12-17]	<0.0001
Cutaneous Vasculitis	23.3 [19.9-27.3]	7.4 [5.8-9.5]	8.0 [6.5-10]	5.8 [4.5-7.6]	5.6 [4.3-7.3]	0.0014
Lupus Nephritis	64.5 [60-69.7]	41.9 [38.6-45.5]	31.4 [28.4-34.6]	31.3 [28.3-34.6]	28.0 [25.3-31.1]	<0.0001
Leukopenia	70.5 [66.7-74.5]	50.5 [47.2-54.2]	52.7 [49.6-56]	53.9 [50.7-57.4]	43.2 [40.2-46.5]	0.0072
Anti-DNA	83.6 [80.5-86.9]	63.0 [59.7-66.5]	56.6 [53.4-59.9]	48.5 [45.1-52]	35.9 [32.9-39.3]	<0.0001
Anti-Sm	53.7 [48.7-59.2]	45.8 [42-50]	30.9 [27.6-34.5]	28.6 [25.3-32.3]	17.4 [14.9-20.4]	<0.0001
Hypocomplementemia	77.8 [74.3-81.4]	54.2 [50.8-57.7]	37.7 [34.8-40.9]	30.8 [27.9-34]	27.9 [25.2-31]	<0.0001

Conclusions: This population-based study in a predominately black lupus cohort showed a decreasing prevalence of autoantibodies, lupus nephritis, and mucocutaneous manifestations across increasing age at diagnosis. Notably, aging was not associated with increasing prevalence of any lupus manifestations. This may suggest that immune senescence may decrease responses to environmental triggers or other factors and modify the clinical presentation of SLE.

PO1.F.13

High prevalence of osteopenia in SLE patients in India

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Objective: With increasing longevity of SLE patients, Osteopenia and Osteoporosis are becoming very important morbidity in patients with SLE. Vitamin D deficiency is believed to be common in India. It is expected that Osteopenia and Osteoporosis will also be very common in Indian patients with SLE. Thus it was aimed to look for the prevalence of osteoporosis in SLE patients in India. *Method:* In this ongoing study twelve patients of SLE visiting the Rheumatology OPD were evaluated for osteoporosis. Age, duration of disease, weight and height, medication, intake of milk, menopausal status, and cumulative steroid intake was noted for all patients. Disease activity was scored by SELENA SLEDAI. ESR, CRP, Serum calcium, serum alkaline phosphatase tests were done and samples for vitamin D levels have been stored. BMD of Lumbar spine (L2-L4), non dominant hip and the non dominant distal forearm were measured by DXA. *Results:* Out of the 12 patients 11 were females and 1 male. The mean age was 28.2 years. Of the 11 females only 1 was postmenopausal. All the patients received corticosteroids with the mean peak dose of 48 mg/day. 3 patients had osteoporosis at one or more site, while 8 patients had osteopenia (t score between -1.0 to -2.5) at one or more site. More patients had low BMD at forearm than at the other 2 sites. *Conclusion:* Patients of SLE are thus at higher risk for osteoporosis than in western countries.

PO1.F.14

Prevalence of lupus nephritis in an urban Hispanic lupus cohort

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Objectives: Lupus Nephritis (LN), a feared manifestation of SLE, is more common and severe in ethnic minorities, including Hispanics. Vila et al. noted that of Hispanics with SLE, Puerto Ricans from the island of Puerto Rico have lower rates of LN compared to other Hispanics, namely Mexicans and Central Americans in Texas (13.6% v 41%). Our goal was to investigate this in an urban Hispanic cohort. *Methods:* The Einstein Lupus Cohort (ELC) is a prospective, multiethnic cohort recruited from SLE clinics at the Montefiore and Jacobi Medical Centers affiliated with the Albert Einstein College of Medicine in Bronx, NY. Enrollment of prevalent and newly diagnosed cases of SLE began in 2002 and is ongoing. Data are collected at enrollment and at every visit. Patients self-identify racial and ethnic identities from the following groups: African American, Afro-Caribbean, Hispanic, White: non-Hispanic, Asian, and Other. Of those who report Hispanic ethnicity, country of origin is recorded. For the purposes of this study, LN was defined as biopsy proven disease. *Results:* Our cohort includes 119 (38%) Hispanic lupus patients, with a median age of 42. The largest sub-groups are Puerto Rican (34%; n=40) and Dominican (16%; n=19). The remaining 50% are from various Latin American countries, or did not specify country of origin. Of these 40 Puerto Ricans, 16 have LN for a prevalence rate of 40%. 50% have proliferative Class III or IV disease (n=8), 25% have membranous disease (n=4), and the remaining 20% have mixed class on biopsy (n=3). The LN class was not known for one patient. Although there was no significant difference in median GFR in Puerto Ricans with and without LN, 25% of the LN patients did have moderate to severe renal disease with GFR≤ 40. When adjusted for LN, age, gender, urine protein to creatinine ratio and anti-dsDNA antibody status, Puerto Ricans have increased odds of moderate to severe kidney disease when compared to other Hispanics (OR: 1.23). *Conclusions:* The prevalence of LN in our urban Puerto Rican population is 40%, a rate much higher than that reported in Puerto Ricans from the island of Puerto Rico. Studies are needed to further elucidate differences in LN prevalence in Hispanic sub-groups. Differences in the prevalence of LN in Puerto Rican populations from the U.S. and on the island of Puerto

Rico should be explored; specifically whether these differences are due to socio-economic factors, acculturation, environmental exposures or genetic differences.

PO1.F.15

The incidence of biopsy-proven lupus nephritis in Northern Portugal: pointing to a European homogeneous distribution of the disease

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Purpose: The incidence of Systemic Lupus Erythematosus (SLE) is thought to be lower in Southern Europe, which could be related to the hygiene hypothesis and/or lack of data/methodological problems. Renal involvement is a major complication of SLE and is a strong determinant of morbidity and mortality. There have been a few previous studies of the epidemiology of lupus nephritis in Europe. Our aim was to establish the incidence of biopsy-proven lupus nephritis in northern Portugal during 1996 - 2005 and compare it with other European lupus cohorts, in order to discuss that the incidence in Southern Europe is not inferior like initially thought. *Method:* SLE patients with biopsy-proven lupus nephritis were identified from renal biopsy databases of 3 tertiary Hospitals, including more than 95% of renal biopsies in SLE patients in that period. The denominator data for the northern of Portugal were ascertained from the 2004 census. *Results:* We identified 237 cases of biopsy-proven lupus nephritis: the incidence was 6.4 per 1.000,000 population per year (95% confidence interval [95% CI] 4.8-9.4), based on a population of 3.727.310 habitants. *Conclusion:* This first estimate incidence of biopsy-proven lupus nephritis in Portugal, shows no important differences between this Portuguese Lupus Cohort and the one reported in Spain 5.6 per 1000000 per year (Rivera et al), but an higher incidence than those observed in England 4.0 (Patel M et al.2006), Italy 2.6 (Schena FP 1997) and Check Republic 3.2 (Rychlik et al 2004). The traditional vision of lower incidence of SLE and severe SLE in southern Europe is probably due to absence of data or it could represent a past situation. Nowadays in Iberia, the incidence is even higher than those observed in other European cohorts, even sought the authors believe that the incidence distribution of the disease in Europe tends to be homogeneous.

PO1.F.16

Cigarette smoking and disease specific patient reported health outcomes in patients with systemic lupus erythematosus.

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Objective: To determine the effect of cigarette smoking on disease specific patient reported health outcomes (LupusQOL-US) in systemic lupus erythematosus (SLE). *Methods:* The data were extracted from an ongoing prospective study on health related quality of life in patients with SLE. Consecutive consenting adult SLE patients seen in the rheumatology clinic at an academic hospital were enrolled from September 2006 to April 2008, and detailed clinical and demographic variables were collected from 216 enrolled SLE patients. Disease specific patient reported health outcomes were estimated by LupusQOL – Systemic Lupus Erythematosus Specific Quality of Life measure. LupusQOL has 8 domains: Physical Health, Pain, Planning, Intimate Relationships, Burden to Others, Emotional Health, Body Image and Fatigue. Smoking was defined as present if the subject reported

smoking at the time of the study. A Mann Whitney test was used for non parametric data between the cases 'smoker' and controls 'non-smoker'. P < 0.05 was considered significant on two tailed test. ***Results:*** The mean (± S.D) age of the participants was 42 ± 13 years and 93 % were females. The ethnic composition was: African American 60%, Caucasian 20%, Hispanic 14% and Asian 6%. Fifteen percent of subjects reported that they were "currently smoking" at the time of the study. Smokers had worse disease specific health outcomes compared to non smokers, specifically with regard to the physical health, pain, planning, emotional health and body image domains of the LupusQOL (mean ± S.D, median) : physical health (34.2 ± 20.3, 40.6 vs. 46.3 ± 19.8, 50; p 0.005), pain (33.7 ± 21.8, 33.3 vs. 45 ± 22.9, 50; p 0.022), planning (36.4 ± 25, 45.8 vs. 51.2 ± 23.3, 58.3; p 0.004), emotional health (39.5 ± 24.5, 50 vs. 53.4 ± 18.6, 57.1; p 0.008) and body image (39.8 ± 24.4, 50 vs. 56.7 ± 20.4, 58.3; p 0.005), respectively. ***Conclusion:*** Cigarette smoking is associated with worse disease specific patient reported health outcomes in SLE. Special attention should therefore be given to patients with SLE with regard to smoking history and smokers should be counseled and treated aggressively to reduce their cigarette exposure.

PO1.F.17

Fibromyalgia and health related quality of life in patients with systemic lupus erythematosus.

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Objective: To determine whether the presence of fibromyalgia (FM) has an effect on health related quality of life (HRQOL) in patients with systemic lupus erythematosus (SLE). ***Methods:*** The data were extracted from an ongoing prospective study on HRQOL in SLE. Consecutive consenting adult SLE patients seen in the rheumatology clinic were enrolled from September 2006 to April 2008. Demographic and clinical data were collected from 216 patients. HRQOL was assessed by MOS-SF-36, EuroQol-5D (EQ-5D) and LupusQOL – US. SF-36 has 8 domains: Physical Functioning, Role Physical, Bodily Pain, General Health, Vitality, Social Functioning, Mental Health and Role Emotional. It provides two summary scores: Physical Component Score (PCS) and Mental Component Score (MCS). The EQ-5D has the following domains: Mobility, Self care, Usual Activities, Pain/Discomfort and Anxiety/Depression, along with an EQ-5D summary Index. LupusQOL has 8 domains: Physical Health, Pain, Planning, Intimate Relationships, Burden to Others, Emotional Health, Body Image and Fatigue. Chi-Square test and Mann Whitney test were used for comparisons between SLE patients with and without FM. P < 0.05 was considered significant. ***Results:*** The mean (± S.D) age was 42 ± 13 years and 93 % were females. African American constituted 60%, Caucasian 20%, Hispanic 14% and Asian 6% of the cohort. Subjects with FM had worse HRQOL than those without (mean ± S.D, median): SF36: Physical Functioning (40.8 ± 28.4, 35 vs. 58 ± 28.1, 55; p 0.001), Role Physical (23.8 ± 34, 0.0 vs. 41.4 ± 41.3, 25; p 0.010), Bodily Pain (41.3 ± 21.3, 41 vs. 55 ± 27.8, 51; p 0.005), Vitality (38.8 ± 23.3, 40 vs. 49.8 ± 21, 50; p 0.014), Social Functioning (49.1 ± 25.8, 50 vs. 62.1 ± 27.3, 62.5; p 0.011) and PCS (29 ± 9.3, 26.2 vs. 37 ± 11, 37.5; p 0.001). EQ5D: Usual activities for extreme problems (11.3 % vs. 2.4 %, p 0.006), Usual activities for some problems (68.2 % vs. 58 %, p 0.006), Pain/discomfort for extreme problems (23.2 % vs. 9.7 %, p 0.019), Pain/discomfort for some problems (65.1 % vs. 64.6 %, p 0.019) and EQ-5D summary index (0.66 ± 0.21, 0.70 vs. 0.74 ± 0.18, 0.77; p 0.032). Lupus QOL: Physical health (38.7 ± 16.4, 42.8 vs. 46.3 ± 21, 50; p 0.020), Pain (35.7 ± 19, 33.3 vs. 45.8 ± 23.6, 50; p 0.008), Burden to others (38 ± 22.6, 37.5 vs. 47 ± 23.1, 50; p 0.045) and Fatigue (26.4 ± 22, 25 vs. 38.1 ± 24, 41.6; p 0.008). ***Conclusions:*** In SLE patients, the HRQOL is worse in those with FM than without. All HRQOL domains appear to be significantly affected by FM in SLE.

PO1.F.18

Does systemic lupus erythematosus affect desire for intimacy?

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Objectives: Systemic Lupus Erythematosus (SLE) may pose physical function limitations and affect vitality, pain and emotional health. The disease or medications may decrease sex drive or cause vaginal dryness. Thus factors sexual health may be at risk. Study of sexual health is important for improving overall health and well-being of SLE patients. We aimed to study 1) the prevalence of low sexual desire (LSD), and 2) to determine the correlates of LSD in SLE. ***Methods:*** Previously collected data pertaining to demographic, clinical and quality of life data on 89 SLE subjects were analyzed. A self administered questionnaire requested their response to the question "During the past 4 weeks, how often did lupus interfere with you desire to have intimate contact?" during the development of a lupus specific health status questionnaire. Five Likert scale options ranging from "None of the time" to "all of the time" were provided. Patients were categorized as having LSD if they responded to "most of the time" or "all of the time". Prevalence of LSD was calculated by obtaining the percentage of subjects with LSD against all the responders. The variables tested for correlates of LSD included demographics, disease measures (disease activity-SLEDAI, disease damage-SLICC/SDI), and health related quality of life HRQOL (EQ5D and SF-36 domains). T-tests for continuous variables and chi square test for categorical variables were used. A p-value of 0.05 was considered statistically significant on two tailed tests. ***Results:*** The prevalence of LSD was 15.7%. The mean (SD) age of the LSD and non LSD subjects were 44.00 (13.18) and 46.37 (12.16), p=0.51. Disease activity and damage were greater among LSD than non LSD group, but not statistically significant: mean SLEDAI (SD) 5.79 (6.16) vs 5.11 (4.69), p=0.63 and SLICC (SD) 1.50 (1.60) vs 1.19 (1.65), p=0.51. The LSD subjects had significantly decreased mobility (Mean (SD) 1.77 (0.43) vs 1.45 (0.50), p=0.03), decreased ability to perform usual activities (Mean (SD) 2.00 (0.57) vs 1.53 (0.55), p=0.01), increased pain/discomfort (Mean (SD) 2.15 (0.37) vs 1.77 (0.53), p=0.01), and increased anxiety (Mean (SD) 1.85 (0.55) vs 1.45 (0.55), p=0.03) on EQ5D domains than subjects without LSD. Similarly on SF36, LSD subjects had a significantly worse HRQOL as compared to those without LSD: social functioning (Mean (SD) 37.50 (21.04) vs 65.87(25.6), p=0.00), mental health (Mean (SD) 59.38 (19.03) vs 70.7 (19.49), p=0.05) and physical component summary score (Mean (SD) 27.54 (8.87) vs 38.47 (10.91), p=0.00). ***Conclusions:*** SLE subjects with poor HRQOL is associated with lower sexual drive. This may affect their overall quality of life. To improve overall QOL, we should screen patients with significant decline in their HRQOL for sexual health issues.

PO1.F.19

Body image in patients with systemic lupus erythematosus

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Purpose: Body Image (BI) is likely important to the quality of life in Systemic Lupus Erythematosus (SLE), but it has not been well studied in this disease. We compared BI-related quality of life ("BI-related QOL") in SLE subjects with healthy controls, and studied its associations with patient demographics, disease features and health status. ***Methods:*** Using the Body Image Quality of Life Inventory (BIQLI), we assessed 87 SLE and 78 healthy non-SLE subjects for demographics, BMI, health status, disease activity, disease damage, disease and serology. Between group differences were analyzed using t-test, chi-square test, and correlation and regression analyses. ***Results:*** Mean ages (± SD) were 42.4± 13.1 and 38.7±13.2 years for SLE and control subjects, respectively; BIQLI scores were 19.9± 33.2 and 41.6±24.8 (p=0.001). The internal consistency reliability of the 20 items of the BIQLI was excellent (Cronbach alpha=0.96). Among SLE patients, BIQLI scores correlated inversely

with overall disease damage (r= [-0.32], p<0.001), irreversible cutaneous damage (r= [-0.28], p=0.01), alopecia on disease activity (r=[-0.24], p=0.02), self reported history of a diagnosis of depression (r= [-0.37], p<0.001), and directly with age (r= [0.21], p<0.05) and health status (r=[0.37], p<0.001). BIQLI did not correlate with BMI, overall disease activity, corticosteroid use or fibromyalgia. On hierarchical regression analysis, modifiable covariates for BIQLI included alopecia disease activity and depression among SLE subjects. ***Conclusions:*** SLE patients have worse BI-related QOL than individuals without SLE, and this is associated with decreased health status. Younger patients and those with cutaneous disease activity or damage, or depression are more likely to have poor BI related QoL. Effective BI-related interventions for SLE might include prevention, treatment and rehabilitation strategies directed at cutaneous disease activity, damage and depression.

PO1.F.20

Employment, work disability and health related quality of life in systemic lupus erythematosus

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Objective: Health related quality of life (HRQOL) in the context of employment and work disability in systemic lupus erythematosus (SLE) has not been well studied. We assessed the association of demographics, disease and HRQOL in employment and work disability among SLE subjects. ***Methods:*** 210 subjects with SLE completed HRQOL assessments using SF36 and EQ5D instruments. Self reported current employment (EQ5D item) and work disability status, and demographics were obtained. Disease activity and damage were assessed using SLEDAI and SLICC-ACR. Serologic and clinical disease features were determined from medical chart review. Retired individuals (n=20) were excluded from analysis. SF6D and EQ5D summary scores were obtained for HRQOL. Comparisons were made using Chi square analyses, analysis of variance, and student t test for demographics, disease status and HRQOL, stratified by our primary outcomes of self reported current employment status and disability benefits status. Pearson's correlation coefficients between the primary outcomes measures with demographics, disease features and HRQOL were determined. ***Results:*** 92% of the subjects were women. The mean age was 40.1 ± 12.2 yrs. Ethnic composition of the cohort was: 59% African American, 19% Caucasian, 16% Hispanic and 7% Asian. 56% were employed at the time of the study. Other main work activities were: 13% "Housekeeping", 6% students, 7% seeking work, 7% disabled and 11% "other" activities. When specifically asked, 28% of the subjects reported receiving work disability benefits. Of the employed subjects 11% were on disability benefits as compared to 50% and 47% of those reporting " house keeping" and "other" as their main activity respectively (p=0.001). Currently employed status correlated with education achieved (r=0.27, p=0.001), marital status (r=0.22, p=0.003), disease activity (r= -0.15, p=0.05), disease damage (r= -0.22, p=0.004), depression (r= -0.16, p=0.03), deep venous thrombosis (r= -0.19, p=0.01), leukopenia (r= 0.15, p=0.04), hypertension (r= -0.20, p=0.01) and HRQOL (SF6D [r= -0.30, p=0.001], EQ5D [r=-0.29, p=0.001]). Disability benefit status correlated with gender (r=0.19, p=0.01), deep venous thrombosis (r=0.25, p=0.00), depression (r=0.29, p=0.001), disease damage (r= 0.25, p=0.001) and HRQOL (SF6D [r= -0.29, p=0.001], EQ5D [r=-0.29, p=0.001]). Conclusions: Half of the SLE subjects were currently employed and 28% were receiving work disability benefits. Disease activity and HRQOL are associated with employment status, whereas disease damage and HRQOL are associated with disability benefits status. Depression, disease activity and HQROL are modifiable variables; and disease damage is preventable. Aggressive screening and management for end organ activity and damage, along with targeted interventions for depression and HRQOL may also have a favorable impact on work and disability in patients with SLE.

PO1.F.21

Systemic lupus erythematosus (SLE) in older adults: the influence of age on clinical and serologic expression of SLE

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Objective: SLE is a multisystem inflammatory disease occurring commonly in females of child-bearing age, however SLE may also present for the first time in older adults in which case the clinical picture tends to differ from that of younger patients. Accordingly, the aim of the present study was to assess the differences in clinical and laboratory features between older and younger SLE patients. ***Methods:*** Twenty-five late onset SLE patients with disease onset after 60 years of age were compared to 25 SLE patients with onset at 15-40 years of age at the time of diagnosis. Patients were recruited from the outpatient clinic of our institution. All patients fulfilled the ACR criteria for the diagnosis of SLE. Disease activity and severity was assessed by the SLE disease activity index (SLEDAI) and the number of SLE criteria fulfilled when SLE was first diagnosed. Laboratory investigations included complete blood count, liver and renal function tests, urine analysis, proteins in urine, chest x-ray, ECG, echocardiography, ANA, antidsDNA, ANA, complements 3 and 4, anticardiolipin antibody and lupus anticoagulant. ***Results:*** The female predominance was reduced in the older group and the duration from disease onset to diagnosis was longer in the older group. The most frequent clinical manifestations in the older SLE patients were serositis (52%), arthritis (48%), malar rash (44%) and glomerulonephritis (44%). The incidence of renal insufficiency was significantly higher in the older compared to the younger SLE patients, p< 0.05. The most common laboratory features in the older SLE patients were ANA (100%) and blood disorders (leucopenia 84% and lymphopenia 82%). Immunologic abnormality rates were lower in older compared to younger SLE patients. ***Conclusions:*** Clinical and laboratory features are different between older and younger SLE patients. Late onset SLE patients should thus be given greater attention to avoid delays in diagnosis or misdiagnosis and care should be directed towards specific potentially life-threatening problems such as the greater incidence of renal insufficiency in older SLE patients.

PO1.F.22

Environmental and hormonal risk factors for the development of lupus nephritis

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Objectives: Nephritis is a common and serious organ involvement in patients with lupus, especially African-Americans (AA). Other than race, little is known about risk factors for development of lupus nephritis in humans. This study examined hormonal and environmental determinants of biopsy-proven lupus nephritis (LN) compared to lupus patients without nephritis. ***Methods:*** The Carolina Lupus Study (CLU) is a population-based case-control study of hormonal, environmental, and genetic risk factors for SLE in the southeastern US. An additional 100 LN patients from the CLU study area and time period comprised the nephritis CLU (CLN) inception cohort. Data collection for both cohorts was a structured 60-minute interview including female reproductive history, lifetime job history, smoking history, use of moonshine and family history of kidney disease, dialysis, hypertension and diabetes. We used logistic regression to examine the association between exposures and risk of developing LN. All analyses are limited to exposures and reproductive history before diagnosis of lupus or LN. Associations were estimated as the odds ratio (OR) and 95% confidence interval (CI), and were adjusted for age, state, race and education. ***Results:*** In 56 patients (36%), the diagnosis of LN was concurrent with the diagnosis of SLE, in 53 patients (36%) within 2 years

of diagnosis, and in 47 patients (28%) more than 2 years after SLE diagnosis. Age at menarche was significantly older among CLN compared to CLU patients without nephritis. Occupational exposure to silica dust or mercury was not significantly associated with risk of LN. Likely or possible solvent exposure was associated with a significantly lower risk of nephritis; (OR=0.22, 95% CI 0.06, 0.76 in whites; OR=0.49, 95%CI 0.26, 0 in AA). There was no association with smoking history after adjusting for covariates. Reported use of moonshine was significantly higher among LN patients (OR=3.3; 95% CI 1.3, 8.6), higher among membranous compared with proliferative nephritis (17.87 95% CI 1.55-205.89). Family history of kidney disease was stronger in whites (OR 6.91 95% CI 1.68-28.36) vs AA (OR 2.3 95% CI0.95-5.61) as was family history of hypertension (OR 2.76 95% CI 1.04-7.27) vs. AA (OR 1.12 95% CI 0.59-2.15). Family history of dialysis was confined to AA (OR 3.39 95% CI 0.82-14.07). *Conclusions:* Our findings provide evidence of an increased risk of LN associated with later age at menarche, past use of moonshine and family history of kidney diseases. Null associations were seen for most other exposures and risk factors examined excepting use of Goody's powders among AA.

PO1.F.23

Silica persistent innate immunity and lupus
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Silica has been associated with a 4 fold increased risk for Lupus. Sarcoidosis and Lupus often coexist. Recently sarcoidosis has been associated with silica, as an FDA approved inert ingredient in brand name medications, and in the first responder- fire fighters and policemen of NYC-following 9/11, where the dust contained 18% silica by weight. The new finding that it can come from the inactive ingredients of brand name medications which are licensed and approved by the FDA, is a wakeup call to all1. All of the connective tissue diseases2 3 4, as well as vasculitis with renal involvement8, have already been linked to silica exposure. What is less well known is that almost all of the anti-inflammatory medications used for the treatment of arthritis contain as inactive ingredients silicone gel, silicon dioxide (silica), aluminum OH (lake), and titanium dioxide. Silica, and aluminum hydroxide (alum) are known to activate the innate immune system, and their intracellular cytosolic sensors, Inflammasomes. While most of the auto-immune diseases are caused by persistence of adaptive T and B cell immune responses, against self or altered self antigens, researchers are beginning to question whether they really emanate from persistent innate immune activation. We propose that persistent innate immune activation from persistent silica exposure results in the generation of the pro-inflammatory cytokines IL-1 , IL-12, IL-17, IL-6, IL-18, IFN γ and TNFα, prostaglandins and reactive oxygen species. These cause alteration of self antigens & dsDNA demethylation rendering them immunogenic. The induced adaptive immunity to these altered self antigens is then responsible for SLE. This hypothesis is favored over EBV virus as the major risk factor, and IL-18 as the marker for silica exposure and inflammasome activation in SLE. The ubiquitous presence and the mechanism of silica immune activation will be discussed.

PO1.F.24

Late-onset systemic lupus erythematosus: prevalence, survival, causes of death in a monocentric cohort of 330 Italian patients
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Background: Late-onset Systemic Lupus Erythematosus (LOSLE), defined as disease onset over 50 years, is not frequent, and it is considered rare over 65 years. It is associated with insidious onset, more benign course, but higher mortality. *Objectives:* To evaluate the prevalence of LOSLE in a monocentric

cohort of Caucasian Italian patients, survival, causes of death. *Methods:* Information were retrieved from the database of patients with SLE attending our institution from 1970 to 2008, diagnosed according to the 1982 ACR criteria; clinical and serological manifestations, at the time of diagnosis and in the successive follow-up, were checked for. Death events, causes of death and survival rates were calculated. *Results:* Data from 330 patients (W 298 and M 32) were analyzed. The mean age at diagnosis was 40.9 years; 96 patients out of 330 (29%) had LOSLE at the time of diagnosis, and 27 of them were over 65 (8%). During the follow-up 34 patients died: 18 had their disease onset over 50 years (14 W, 4 M) and 7 of them were diagnosed over 65 years (5 W, 2 M). Kaplan – Meier survival curves, stratified by age at diagnosis (more or less 50 years), showed a significant divergence (p<0.0001), demonstrating a worse survival for LOSLE patients. The mean age of death, in patients diagnosed after 65 years (5 W, 2 M), was 86,6 years for women and 77,9 for men, and the causes of death were heart failure, Alzheimer, cancer, myocardial infarction, infections. Among patients, diagnosed between 50 and 65 years (9 W, 2 M), the mean age of death was 73.5 and 70.5 years for women and men respectively, and the causes of death were infections, cancer, hepatic failure, neuropsychiatric complications, cardiac failure, haemolytic acute anaemia. Patients, diagnosed before 50 years (15 W and 1 M), died for infections, neuropsychiatric complications, cancer, uraemia, heart failure; the mean age of death was 52.7 for women and 49 for the man. *Conclusions:* SLE onset over 65 years does not seem associated with a significant decreased life expectancy, appeared less severe and the causes of death were mainly associated with aging process. In patients who developed the disease between 50 and 65 years, SLE related complications, treatment side effects and aging-related comorbilities seem to play a major role in determining the conditions leading to death; in younger patients (< 50 years) mortality did prove principally due to disease activity and therapeutic complications.

PO1.F.25

Mortality trends related to systemic lupus erythematosus, state of Sao Paulo, Brazil, 1985-2007: a study using multiple-cause-of-death
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Objective: to study the mortality related to Systemic Lupus Erythematosus (SLE) over 23 years. *Methods:* The mortality data came from the annual multiple-cause-of-death files of the Sao Paulo State Data Analysis System Foundation. All deaths certificate on which SLE was listed on any line were selected. Sex, age and causes of death were the variables studied. Statistical analyses were performed by chi-square test; p< 0.05 was considered significant. *Results:* A total of 4,815 deaths related to SLE were identified; in 3,133 SLE was considered as underlying and in 1,682 as associated causes of death. The mean age of death was 37.5 (±15.6) y.o and 90% were women. For SLE as underlying cause, the main associated causes of death mentioned were: renal failure (29.7%), cardiovascular diseases (25.3%), pneumonias (25.7%) and septicemia (22.9%). Comparing the five earliest calendar years studied (1985-1989) to the latest one (2003-2007) we observed a significant decreasing of renal failure (p<0,001) and increasing of pneumonias (p<0,001), septicemias (p<0,001) and cardiovascular diseases (p<0,007), specially hypertensive diseases (p<0,001) and acute myocardial infarction (AMI) (p=0,004). For SLE as associated cause of death, the main underlying causes were: cardiovascular diseases (27.5%), respiratory diseases (18.37%), certain infectious and parasitic diseases (12.0%), digestive diseases (9.2%) and genitourinary diseases (9.2%). Comparing the five earliest to the latest calendar years of the study we observed a significant decreasing of respiratory diseases (p<0,001) and an increasing of neoplasms (p=0.049). Sixty-one percent of all deaths due to AMI as underlying cause of death in SLE occurred in individuals younger than 50 years, whereas in the general population it occurred only in 14% (p<0.001). The observed/expected ratios of deaths related to SLE were 1.91 for pneumonia and 4.48 for septicemia, independently of age, however higher ratio for AMI was observed only in individuals younger than 50 years at death (2.2). *Conclusions:* In Sao Paulo State the deaths related to SLE occurred at young age. Infections and cardiovascular diseases are the most important associated causes of death

in subjects with SLE and they are increasing in the recent years. Our results shows AMI as underlying cause of death are more frequent than expected in SLE population only in individuals younger than 50 years. A high percentage (35%) of death related to SLE is underestimated in primary statistics, which selects only SLE as an underlying cause of death.

PO1G Genetics snd Epigenetics

PO1.G.4

Both estrogen and female sex contribute to disease development in an epigenetic model of lupus
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Objective: Systemic lupus erythematosus (SLE) is an autoimmune disease primarily afflicting women. The reason for the gender bias is unclear. Impaired T cell DNA methylation contributes to human lupus-like autoimmunity by causing overexpression of genes normally suppressed by DNA methylation. DNA demethylation affects women more than men because women have two X chromosomes, one of which is silenced by DNA methylation. The relative contributions of estrogen and T cell DNA demethylation to the increased susceptibility of women to lupus are unknown. We therefore used a transgenic mouse model with an inducible T cell DNA methylation defect to compare the effects of estrogen and DNA demethylation on the development of autoimmunity in male and female mice. *Methods:* We used BL6xSJL transgenic mice that inducibly express a dominant-negative MEK (dnMEK) selectively in T cells when fed doxycycline (DOX). The dnMEK decreases DNA methyltransferase expression, causing DNA demethylation and lupus-like autoimmunity. To examine the role of estrogen in this model, male and female transgenic mice were neutered and implanted with time-release pellets delivering placebo or a supra-estrus dose of estrogen, and given DOX or not in their drinking water. Anti-DNA antibodies were measured by ELISA, and methylation-sensitive gene expression by RT-PCR. *Results:* DOX induced IgG anti-dsDNA antibodies in intact and neutered, placebo-treated control female transgenic mice. No anti-DNA antibody was observed in DOX-treated intact or neutered, placebo-treated male transgenic mice. DOX induced even greater amounts of anti-dsDNA antibodies in neutered females given high dose estrogen, but in contrast to earlier reports in NZB/W mice none were detected in neutered males given high dose estrogen. No anti-DNA antibody developed in the absence of DOX treatment or without the transgenes. DOX also increased expression of CD40L protein in CD4+ T cells of female but not male mice consistent with demethylation of the second X chromosome in the females. Glomerular IgG deposits were found in the kidneys in 4 of 4 female but 0 of 4 male transgenic mice. No IgG deposits were found in either female or male transgenic mice in the absence of DOX treatment. Platelet thrombi were also observed in the small vessels of the lungs of 4 of 6 female mice but in none (0 of 6) of the male mice. *Conclusions:* These results indicate that both estrogen and female gender contribute to the female predisposition in lupus susceptibility, likely through hormonal and epigenetic X chromosome effects, respectively.

PO1.G.5

Lack of association between beta2-glycoprotein I gene polymorphisms with antiphospholipid antibodies in Spanish patients with antiphospholipid syndrome
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Objectives: To determine the presence of polymorphisms at codons 247 (Val247Leu) and 316 (Trp316Ser) of beta2-glycoprotein I (β2GPI) gene in Spanish patients with antiphospholipid syndrome (APS) and healthy controls and their possible correlation with the development of antiphospholipid antibodies (aPL) and associated clinical manifestations. *Methods:* Polymerase chain reaction amplified codons 247 and 316 from β2GPI gene and the presence of polymorphisms was detected by restriction fragment length polymorphism analysis (PCR-RFLP) using Rsa I (Val247Leu) and BstB I (Trp316Ser) restriction endonucleases. Antiphospholipid antibodies were detected by ELISA. Allele frequencies and genotypes in patients and control subjects were compared and correlations between the presence of antiphospholipid antibodies with clinical thrombotic and non-thrombotic manifestations in APS patients were performed. *Results:* APS patients and healthy controls presented neither differences in prevalence of codon 247 (Val247Leu) (32.4% vs. 38% respectively, P= 0.44) nor in codon 316 (Try316Ser) (18.2% vs. 10% respectively, P= 0.11). Heterozygous genotypes of Val247Leu polymorphism (V/L) affected 23.4% patients and we had 7 cases (9.1%) of homozygous L/L genotype, not different from controls (P= 0.26). Heterozygous T/S genotype was found in 14 patients (18.2%) and 10 controls (10%) and no cases of homozygous (S/S) were found. No significant correlation was found between the presence of a particular polymorphism and the presence of anticardiolipin, lupus anticoagulant or anti-beta2-GPI antibodies. Clinical variables as type of APS, existence and type of thrombosis and neurological (migraine, epilepsy), cutaneous (livedo, ischemic ulcers) manifestations as well as heart valve disease did not correlate with any of the studied polymorphisms of β2-GPI in our Spanish population. *Conclusion:* Polymorphisms at codons 247 (Val247Leu) and 316 (Trp316Ser) of β2GPI gene in Spanish population are similarly expressed in APS patients and the general population. We found no association with, or protection from, the production of aPL for either both polymorphisms and seem not to be involved in the serological and clinical features of the disease.

PO1.G.6

Associations of genetic polymorphisms in CD40 with susceptibility to SLE in Korean populations
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Objectives: Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease and genetic factors play an important role in its pathogenesis. IRF5 and STAT4 as well as MHC region are well-known SLE-associated genes, but the genetic contributions of others are unclear. CD40 mediates co-stimulatory signals that activates and differentiates B-cell, and recent meta-analysis of two published genome-wide association study (GWAS) of rheumatoid arthritis (RA) revealed CD40 as a novel RA-susceptible gene. Therefore, we investigated genetic associations of CD40 with SLE, as another autoimmune disease, in Korean populations. *Methods:* A total of 785 SLE patients and 1,020 healthy controls belonging to the Korean population were enrolled into this study. We selected nine SNPs of CD40 gene

(rs1535044, rs1800686, rs1883832, rs4810485, rs1535045, rs73115010, rs6074028, rs11569333 and rs3765456). Nine SNPs were genotyped using the single-base extension method. The statistical analysis is carried out with logistic regression using age and sex as covariates. The associations of rs3765456 with SLE-related phenotypes were analyzed using multiple regression. ***Results:*** The rs3765456 showed significant association with the risk of SLE (OR=1.34, P-value=0.007, Pcorr=0.04) in dominant model. The rs11569333, rs1883832, rs4810485, haplotype 1 (GGTTCTAGA), and haplotype 3 (GACGTCGGG) were also associated with the risk of SLE, but statistical significance is disappeared after correction for multiple comparison (Table). We further analyzed the associations with phenotypes of SLE. The rs73115010 and rs6074028 showed increased risk of arthritis in recessive model (OR=2.81 and 2.76, P-value=0.002 and 0.004, Pcorr=0.02 and 0.03, respectively) and the rs1535044 was significantly associated with the production of anti-Ro antibody in co-dominant and dominant model (OR=2.57, P-value=0.006, Pcorr=0.04). The rs3765456 also showed associations with butterfly rash, serositis and lupus nephritis (OR=0.69, 1.51 and 0.58, P-value=0.04, 0.03 and 0.05), but was not statistically significant after P-value correction. ***Conclusions:*** In our study, genetic polymorphisms of CD40 were associated with susceptibility to SLE and its phenotype, suggesting CD40 as a novel candidate gene in SLE. To confirm our findings, replications in large populations and different ethnicity is required.

SNP	Allele	Minor Allele Frequency	Co-dominant	-	Dominant	-	Recessive	-
-	-	(SLE/Control)	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
rs1535044	G>A	0.036/ 0.046	0.80 (0.54-1.19)	0.27	0.81 (0.54-1.21)	0.31	.	.
rs1800686	G>A	0.299/ 0.299	1.00 (0.85-1.18)	0.99	0.97 (0.79-1.20)	0.80	1.08 (0.75-1.56)	0.68
rs1883832	C>T	0.355/ 0.325	1.14 (0.97-1.33)	0.11	1.27 (1.02-1.57)	0.03	0.99 (0.72-1.38)	0.97
rs4810485	G>T	0.358/ 0.327	1.14 (0.98-1.33)	0.10	1.26 (1.02-1.57)	0.03	1.02 (0.74-1.42)	0.90
rs1535045	C>T	0.289/ 0.297	0.97 (0.82-1.14)	0.67	0.93 (0.75-1.14)	0.47	1.06 (0.73-1.53)	0.77
rs73115010	T>C	0.300/ 0.308	0.95 (0.81-1.11)	0.51	0.89 (0.72-1.10)	0.29	1.07 (0.75-1.53)	0.72
rs6074028	A>G	0.277/ 0.289	0.91 (0.77-1.07)	0.27	0.85 (0.68-1.05)	0.12	1.04 (0.72-1.51)	0.84
rs11569333	G>A	0.033/ 0.053	0.62 (0.42-0.93)	0.02	0.63 (0.42-0.94)	0.02	.	.
rs3765456	G>A	0.355/ 0.322	1.17 (1.01-1.37)	0.04	1.34 (1.09-1.66)	0.007*	1.02 (0.74-1.41)	0.91
ht1	GGTT CTAGA	0.325/ 0.296	1.14 (0.97-1.33)	0.11	1.27 (1.03-1.57)	0.03	0.98 (0.69-1.39)	0.91
ht2	GGCG CTAGG	0.193/ 0.209	0.90 (0.76-1.07)	0.23	0.81 (0.65-1.02)	0.07	1.06 (0.73-1.55)	0.76
ht3	GACGT CGGG	0.180/ 0.195	0.90 (0.76-1.07)	0.24	0.79 (0.63-1.00)	0.05	1.14 (0.77-1.68)	0.52

PO1.G.7

Significant association of the GABRP polymorphisms with systemic lupus erythematosus in a Korean population

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Background: The balance between Th (T helper lymphocyte type) 1 and Th2 subsets is very important in the immune response to pathogens and allergies. SLE is a Th2 predominant autoimmune rheumatic disease. The γ-aminobutyric acid (GABA) inhibits T cell response to antigen through the u-subunit of γ-aminobutyric acid receptor (GABRP). The GABRP is located on chromosome 5q34 and expressed on murine T cells. ***Aims:*** To determine a possible association between GABRP gene polymorphisms and the susceptibility to SLE, the genotype and allele frequencies of the GABRP gene polymorphisms were analyzed between SLE patients and healthy controls. ***Methods:*** We screened the polymorphisms of the GABRP based on their location, minor allele frequencies and linkage disequilibrium (LD) analysis from NCBI SNP database. Genotyping of the GABRP gene was performed by high resolution melting (HRM) method with 164 SLE patients and 527 healthy controls. ***Results:*** A statistically strong association was observed between the GABRP polymorphisms and SLE. The genotype frequencies of the g.-4623T>A, g.24875C>T and g.25241G>A in SLE patients were significantly different from those of healthy controls (P <.0001, 0.007 and 0.014, respectively). The frequencies of haplotypes TCACCG and ACACTA were significantly different between SLE patients and healthy controls (P = 3.9E-04 and 3.4E-26, respectively). ***Conclusion:*** The g.-4623T>A, g.24875C>T and g.25241G>A polymorphisms of the GABRP gene are significantly associated with the susceptibility to SLE.

PO1.G.8

Genetic interactions reveal a novel B-cell signaling pathway in systemic lupus erythematosus

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Background: Epistasis or genetic interaction might explain larger genetic effects on the susceptibility to diseases than single-gene associations and help define functional pathways with potential therapeutic targets. ***Aim:*** To identify genes that modify the susceptibility to SLE through their interaction with the B-cell scaffold protein with ankyrin repeats gene (BANK1). ***Methods:*** We searched for genetic interactions in the Affymetrix 100k genome-wide scan performed in 256 cases and 515 controls from Sweden. A subsequent replication study included two independent multicenter cohorts of European-Americans (n=676 cases and 850 controls) and Europeans (n=1265 SLE cases and 1506 controls). We developed a genotypic interaction test based on contingency tables for all possible genotype combinations between pairs of SNPs with r2 <.80 and calculated a Pearson S score of interaction association and its chi-squared P value. Each interacting combination was tested against the hypothesis of independence to derive an epistasis score (Se) and a P value (Pe)

was obtained through permutation. ***Results:*** BANK1 showed genetic interactions with 29 genes, including the B-cell tyrosine kinase (BLK) and the inositol 1,4,5-triphosphate receptor 2 (ITPR2). One fifth of SLE patients (21%) vs. 8 % of controls were homozygous for the risk alleles of polymorphisms in these three genes with a significant epistatic effect (Pe < 0.0002). The interactions BANK1xITPR2 and BANK1xBLK were replicated in two independent European-American (P = 2.1 x 10-6) and European sets (P = 4.11 x 10-9). The data was verified using multifactor dimensionality reduction (MDR). Moreover, BLK co-immunoprecipitated and co-localized with BANK1 in co-transfected HEK-293T. Exogenous expression of BANK1 in human Daudi B cells curbed BLK from reaching the plasma membrane with the subsequent accumulation in cytoplasmic compartments. Expression of BANK1 and BLK but not ITPR2 was modulated by IFNα. ***Conclusions:*** BANK1, BLK and ITPR2 are genetically and functionally interacting partners and through their protein-protein interactions might result in a novel B-cell signaling pathway regulated by type I interferons. This pathway may affect B-cell responses to self-antigens in human lupus.

PO1.G.9

Independent association of variants in the class III gene, MSH5, and the class II gene, HLA-DPB1, at the MHC in a Spanish SLE cohort

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Objectives: Variants within the MHC region demonstrate the greatest genetic risk for lupus in European and Chinese populations. Recent high-density SNP genotyping studies have demonstrated multiple independent signals across the MHC in northern European cohorts. We therefore undertook a high-density SNP study of the MHC in a southern European Spanish lupus cohort in order to further refine association signals in this haplotypically diverse European population. **Methods:** We genotyped 11640 SNPs in 466 cases and 469 controls using a custom Illumina chip. 4743 SNPs were informative for major and European ancestry, 6045 SNPs were located within the MHC region (29 – 33.5 Mb) and 852 SNPs were located in putative autoimmune loci outside the MHC region. We incorporated these data with classical typing at the HLA-DRB1 locus - performed in 99% of the post-QC cohort. **Results:** Following QC measures 390 cases and 401 controls were put forward for analysis. Logistic regression analysis demonstrates that the peak signal at the MHC arises from SNPs in the class III gene, MSH5. The most highly associated SNP is rs3130490 (OR=3.08, p=1.04x10-7) and is located in an LD block that spans the RCCX module. This top signal replicates that observed in lupus cohorts of northern European origin (IMAGEN I study). In contrast to our previous northern European data, the peak southern European signal shows only moderate/weak LD with HLA-DRB1*0301 (r2=0.297) suggesting that variants in the class III region of the MHC, rather than class II, may be the primary drivers of association in SLE in this population. Conditioning on the top SNP (rs3130490) reveals a number of potentially independent signals, the best of which is the class II SNP, rs3129768, located between HLA-DRB1 and HLA-DQA1 (OR=2.04, p=1.04x10-6). This SNP shows moderate LD with HLA-DRB1*1501 (r2=0.54). Again this contrasts with our northern European data where the main secondary association signal was observed with variants in strong LD with HLA-DRB1*1501 (r2~0.98). Furthermore, conditioning on both rs3130490 and rs3129768 demonstrates association with SNPs close to and including HLA-DPB1; the best being rs3117213, (OR=1.73, p=1.58x10-5). This SNP has previously shown association with ACPA-positive RA, and is independent of the known RA HLA-DRB1 risk alleles. HLA-DRB1 analysis demonstrates association with HLA-DRB1*0301 (OR=1.86, p=1.03x10-5), HLA-DRB1*1501 (OR=1.68, p=2.38x10-3) and HLA-DRB1*0801 (OR=3.03, p=7.8x10-3). **Conclusion:** Southern European MHC associations in SLE exhibit significant contrasts with those observed in northern Europeans particularly with regards to the roles of MSH5 and HLA-DPB1.

PO1.G.10

Complement C4 gene copy number is not tagged by flanking MHC SNPs or haplotypes

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Objectives: The complement C4 locus is in the class III region of the MHC and exhibits copy number variation. Complement C4 null alleles, which describe the non-expression of C4A or C4B protein, have shown association with a number of diseases including the autoimmune disease, systemic lupus erythematosus (SLE). However, most studies to date have used protein immunophenotyping and not direct interrogation of the genome to determine C4 null allele status. Moreover, a lack of accurate C4 gene copy number (GCN) estimation and tight linkage disequilibrium across the disease-associated MHC haplotypes has confounded attempts to establish whether or not these associations are causal. **Methods:** We have therefore developed a high throughput assay in the form of a paralogue ratio test (PRT) in association with two restriction enzyme digest variant ratio tests (REDVRs) to determine total C4 GCN, C4A GCN and C4B GCN. We determined total C4 GCN, C4A GCN and C4B GCN in 89 HapMap CEU samples using our novel assay, Southern blot analysis and a quantitative PCR (qPCR) technique, in order to assess C4 GCN variability in this extensively genotyped cohort. Furthermore, we wanted to investigate the correlation between surrounding SNPs and complement C4 copy number in the CEU population under study. In order to corroborate our results, we genotyped 163 individuals of northern European ancestry from the 1958-British Birth Cohort (BBC), for C4 GCN using the PRT/REDVR method. High-density SNP data at the MHC was available for all 163 subjects from a previous study. In order to determine the relationship between the complement C4 locus and surrounding SNPs we calculated the correlation coefficient, r2, between SNP genotypes and integer copy number estimates for C4A and C4B using standard linear regression in both cohorts. **Results:** In the densely genotyped CEU cohort we show that our PRT/REDVR method is accurate and reproducible, particularly at low C4 GCN (where disease association is observed), when compared to gold standard Southern blot copy number estimation and qPCR. We find a broad range of C4 GCNs in the CEU and the 1958-BBC populations under study. In addition, SNP-CNV analyses show only moderate levels of correlation and therefore do not support the use of SNP genotypes as proxies or tagSNPs for C4 GCN (best SNP is rs1269852 for C4A GCN, r2=0.62 in CEU and r2=0.74 in 1958-BBC). **Conclusion:** There are no surrogate SNPs for the common C4A or C4B gene deletions in the CEU and 1958-BBC cohorts under study.

PO1.G.11

Association of *TNFAIP3 and TNIP1 SNPs with systemic lupus erythematosus in a Japanese population*

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Objectives: *TNFAIP3* (encoding TNF-α-induced protein 3, A20) and *TNIP1* (TNFAIP3 interacting protein 1) are thought to be involved in inhibition of NF-κB activation. Recently, association of both genes with systemic lupus erythematosus (SLE) was demonstrated by genome-wide association studies both in the European-American and Chinese populations. In this study, we investigated whether the *TNFAIP3* and *TNIP1* SNPs were associated with SLE also in a Japanese population. **Methods:** Case-control association studies were conducted on the *TNFAIP3* SNPs, rs13192841, rs2230926 and rs6922466, in 318 Japanese SLE patients and 444 healthy controls, and the *TNIP1* SNP, rs7708392, in 365 patients and 513 controls. Association of *TNFAIP3* rs2230926 with *TNFAIP3* mRNA levels was examined using the mRNA expression data of the HapMap samples obtained from the GENEVAR database. **Results:** Among the *TNFAIP3* SNPs, association of rs2230926 G allele with SLE was replicated in Japanese (SLE: 10.7%, control: 7.5%, P=0.033, Table 1). When the patients were stratified by the presence of nephritis, the association was observed only in the SLE patients with nephritis (P=0.010, Table 1). On the other hand, association of rs13192841 and rs6922466 were not detected. The mRNA database analysis revealed that presence of *TNFAIP3* rs2230926 G allele was associated with lower mRNA expression of *TNFAIP3* (P=0.013), raising a possibility that this SNP not only results in an amino acid substitution (F127C), but also reduced expression of *TNFAIP3*. *TNIP1* rs7708392 C allele was also replicated Japanese (SLE: 76.3%, control: 69.9%, P=0.0030, Table 1), confirming the observations in European-Americans. This SNP is in strong linkage disequilibrium with rs10036748, reported to be associated with SLE in Chinese. Interestingly, the risk allele frequency was considerably greater in Japanese (0.70) than in European-American populations (0.24). Similarly to *TNFAIP3*, the association was more strongly observed in the SLE patients with nephritis (P=0.0018, Table 1). **Conclusions:** These findings in a Japanese population supported that *TNFAIP3* and *TNIP1* are shared SLE susceptibility genes in European-American and Asian populations.

Table 1. Association of *TNFAIP3* and *TNIP1* with SLE in Japanese.

	Genotype			Allele	Allelic association P	OR(95%CI)
TNFAIP3						
rs2230926	G/G	T/G	T/T	G		
SLE	6 (1.9)	56 (17.6)	256 (80.5)	68 (10.7)	0.033	1.47 (1.03-2.09)
nephritis +	4 (2.4)	33 (19.6)	131 (78.0)	41 (12.2)	0.010	1.70 (1.13-2.56)
nephritis -	2 (1.4)	21 (14.5)	122 (84.1)	25 (8.6)	0.55	1.16 (0.72-1.87)
Controls	1 (0.2)	65 (14.6)	378 (85.1)	67 (7.5)		reference
TNIP1						
rs7708392	C/C	C/G	G/G	C		
SLE	216 (59.2)	125 (34.2)	24 (6.6)	557 (76.3)	0.0030	1.39 (1.12-1.72)
nephritis +	121 (60.8)	69 (34.7)	9 (4.5)	311 (78.1)	0.0018	1.54 (1.17-2.02)
nephritis -	91 (56.9)	55 (34.4)	14 (8.8)	237 (74.1)	0.15	1.23 (0.93-1.63)
Controls	251 (48.9)	215 (41.9)	47 (9.2)	717 (69.9)		reference

PO1.G.12

Association of CD226 gene with systemic lupus erythematosus through impaired mRNA processing in T cells

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Objective: CD226 has recently been associated with a number of autoimmune diseases with the SNP rs763361 been proposed as the putative causative variant. We here report a fine mapping of the gene locus and the genetic association of several SNPs with systemic lupus erythematosus in Europeans, together with functional analyses to give a better understanding on the mechanisms behind the gene association and potential contribution of these variants. **Methods:** Genetic association of 12 SNPs in the CD226 gene was conducted using 1194 SLE patients and 1454 healthy controls from a European multicenter collection. Genotyping was performed using a GoldenGate Custom Genotyping Assay and a BeadXpress Reader from Illumina or Taqman assay and the statistical association analyzed with PLINK v1.07. Gene expression was analyzed by quantitative real-time PCR and SYBR Green for signal detection, using total RNA purified from PBMCs from healthy donors. Surface detection of the protein was performed by a three-color flow cytometry using leukocytes isolated from healthy donors, where total lymphocytes, CD3+CD4+, CD3+CD8+, CD3-CD56+, CD3-CD19+ and CD3+CD56+ cells were analyzed. Expression analysis of reporter plasmids including different alleles of the risk haplotypes of the CD226 3'-UTR region was assessed by transfection of HEK293 cells and dual luciferase assay. **Results:** A risk haplotype (P = 3.96x10-6) was detected in the 3'-UTR region of the gene, and revealed rs727088 as the strongest associated variant (PCMH-adjusted = 0.0098). Expression analysis showed that the risk haplotype correlated with decreased levels of CD226 transcripts and protein levels in the surface of T helper cells (CD4+), Cytotoxic T cells (CD8+) and NK T cells (CD3+CD56+), but not NK cells (CD3-CD56+) or B cells (CD19+). Luciferase assays suggest that rs727088 is the main polymorphism responsible for altered gene expression. **Conclusion:** Our data does not support Gly307Ser as main functional variant within CD226 gene and indicates rs727088 located in the 3'UTR region as the potential causative SNP, by a mechanism that alters protein expression in T cells, potentially involving mRNA processing and/or stability.

PO1.G.13

Allelic and genotype frequencies of -871 C/T SNP of BAFF gene in systemic lupus erythematosous

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Objectives: BAFF is a potent activator of B cells it has been involved in proliferation and differentiation of B cells. Recent studies in murine models and humans correlate high levels of BAFF and lupus erythematosus systemic (SLE). For these reasons BAFF is a candidate gene for susceptibility to SLE in humans and we analyzed the genotype and allele frequencies of the SNPs at position -871 C/T of this gene in Colombian patients with SLE. **Patients**

and Methods: Patients diagnosed with SLE according to ACR criteria (129) from HUSV de Paúl and healthy controls (112) were included. DNA was obtained by DNAzol. PCR of BAFF gene fragment was performed and the product was digested with restriction enzyme AciI. The digestion products were evaluated in agarose electrophoresis and allelic and genotypic frequencies were estimated using a simple counting procedure. The Hardy-Weinberg (HW) equilibrium was evaluated with the Fisher test. Genetic analyses were performed in the GENEPOP package and statistical analysis in SPSS version 15. *Results:* 82.9% of patients were female. Allele frequencies and genotype of patients and controls were in HW equilibrium. The allele frequencies of patients and controls were: 0.50 for the C allele and 0.50 for allele T and 0.51 for the C allele and 0.49 for allele T, respectively. The genotype frequencies in patients and controls were: 0.29 for CC, 0.41 to 0.29 for CT and TT, and 0.23 for CC, 0.56 to 0.20 for CT and TT. There were no statistically significant differences in both allele and genotype frequencies. The correlation between genotype frequencies with anti-DNA, anti-RNP, anti-Ro, anti-La and anti-Sm antibodies did not show any statistically significant difference, however there was a trend with anti-Sm antibody p = 0.053. The genotype frequency in patients with renal involvement show 31.0% patients were CC, 44.8% were CT and 24.1 % were TT, data showed no significant difference. *Conclusions:* There was no association between BAFF polymorphisms -871 C /T and the presence of ENAs in patients with SLE. CC and CT tended to be found more frequently in patients with SLE and anti-Sm. The frequencies of SNP-871C/T in patients with renal involvement not showed significant differences. BAFF polymorphism -871 C / T was not associated with SLE in our group.

PO1.G.14

Genetic/epigenetic modeling of male lupus

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Objectives: Lupus afflicts women more often than men. Hormones and having two X chromosomes contribute to the female predilection. However, why lupus develops in men with normal hormones and one X chromosome is unknown. Standard paradigms postulate that lupus requires predisposing genes and exposure to environmental agents that trigger the disease. We hypothesized that lupus disease activity is directly proportional to total genetic risk, and inversely proportional to T cell DNA methylation levels. We also hypothesized that men with normal hormone levels and one X chromosome require a greater genetic risk, lower T cell DNA methylation levels, or both, to achieve a lupus flare equal in severity to women. *Methods:* Men and women with lupus were recruited from the University of Michigan clinics and the Oklahoma Lupus Cohort at OMRF. Peripheral blood mononuclear cells were isolated using density gradient centrifugation, and CD4+ T cells were separated using magnetic beads. Genotyping of 17 confirmed lupus-associated genetic loci was performed using TaqMan genotyping assays. A total lupus genetic risk score was calculated using an additive model. Bisulfite DNA sequencing was used to quantify DNA methylation in the promoter sequences of two known methylation sensitive genes, KIR and perforin. Global CD4+ T cell DNA methylation was measured using ELISA to measure total deoxymethylcytosine (dmC) content. *Results:* Using the equation SLEDAI=[total genetic risk]/[T cell DNA methylation] we compared 26 lupus men and 24 women with a range of SLEDAIs. We found that men require a greater genetic score / DNA methylation ratio than women to achieve a similar SLEDAI score when using either KIR methylation or perforin methylation (p=0.003 for each) as a measure of T cell DNA methylation. These results were confirmed by measuring total deoxymethylcytosine (dmC) content in CD4+ T cells from 24 women and 16 men with lupus. In contrast, analysis of genetic risk or DNA methylation alone revealed no difference between men and women. *Conclusions:* Our data indicate that men require both a higher genetic risk and a lower CD4+ T cell DNA methyla-

tion levels to achieve a lupus flare equivalent in severity to women. These findings support the contention that interaction between genetic predisposition and environment as reflected by DNA demethylation play a role in the pathogenesis of lupus.

PO1.G.15

Weak association of systemic lupus erythematosus clinical features with susceptibility alleles

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Objectives: We intended to analyze if nine newly identified SLE susceptibility loci were influencing the presence of some of the best defined SLE clinical features. *Methods:* We compared the genotypes of a representative SNP in each of nine SLE-associated loci, in *ITGAM, STAT4, C8orf13-BLK, IRAK1-MECP2, BANK1, TYK2, KIAA1542, PXK* and *1q25.1*, between patients with and without each of 11 clinical features: ten of the ACR classification criteria (except ANAs) and age of disease onset. Data of up to 1561 patients were used. *Results:* The most consistent and significant result was the association of the rare allele of *rs13277113* in the *C8orf13-BLK* locus with increased risk of nephritis (O.R. = 1.35, P = 0.00026). The reported association of STAT4 with early age of disease onset was replicated. Not so the other described associations of this locus, but joint analysis of a previous report with our data lent further support to association of *STAT4* with oral ulcers and immunologic disorder. Combined analysis of *ITGAM* data from a recent report and our study showed more doubtful association of this locus with immunologic disorder, nephritis and discoid rash, although our study, separately, did not replicate these associations. Some other results were suggestive of the presence of additional phenotype-genotype associations, but they were not significant after correction for the number of tests. *Conclusions:* Our results together with previous studies indicate that clinical variability of SLE is influenced by the SLE susceptibility alleles. However, the effects are weak and difficult to reproduce, indicating the need of phenotype-specific genetic studies.

PO1.G.16

Pathway approach to genetic analysis of early SLE autoimmunity with viral, anti-autoimmunity antibodies among African American sample

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Objectives: Systemic lupus erythematosus (SLE) is a complex disease with many debilitating or even fatal clinical manifestations. Genetic determinants of SLE are supported by high twin concordance rates, linkage study findings and the identification of variants associated with disease. However, lack of complete concordance among monozygotic twins suggests an environmental "trigger" for disease (such as viral infection) is likely important. Further, recent immunochemistry studies have implicated immune targets of several antibodies as the first structures of Sm B', Ro, and Sm D1 as some of the earliest signs of autoimmunity. These are unique to SLE and, curiously enough, closely related to the antibodies that arise in some lupus patients against Epstein Barr Virus Nuclear antigen-1. Understanding how genes influence the production of these early autoimmune structures, as well as an individual's response to infection and how that response progresses to benign and ultimately pathogenic autoimmunity is critical in understanding the etiology of systemic autoimmunity. In this study, we collected the following epitope and viral assay data for 558 SLE patients self-reported as African-American: Anti-SmB', Anti SM D1 and anti 60kD R epitopes (PPPGMRPP, PPPGRPP, Ro169), Epstein-Barr Virus (EBV) – viral capsid antigen (EBV-VCA), cytomegalovirus (CMV), peptides from EBV nuclear antigen 58 (EBNA-58), an EBV "Mosaic" antigen (EBNA-Mosaic), herpes simplex virus type 1 (HSV-1, herpes simplex virus type 2 (HSV-2). **Methods:** We demonstrate using Structural Equation Modeling (SEM) the predictive directions of the complex gene by environment interactions influencing the early immune response as measured by the above epitopes. Specifically, we examine single-nucleotide polymorphism (SNPs) found to be significant in univariate analysis at a significant level ≤ 0.01, including SNPs within genes from common pathways into a single model as well as the viral and epitope data that are most biologically plausible. **Results:** Preliminary results indicate that six SNPs within the tyrosine-protein kinase gene (TEC) and mitogen-activated protein kinase gene (MAP3k7) form two associated (path loading = 0.46, p< 0.001) latent genetic constructs, which in turn are predictive of first, a viral latent factor comprising EBNA-58 and EBV-VCA (path loading = .20, p< 0.01) and second, a autoimmune construct comprising PPPGM-RPP and PPPGRRP (path loading = .06, p < 0.05). Loading coefficients for each indicator are provided to demonstrate the predictive contributions of these indicators to the model. **Conclusions:** This paper contributes to the existing epigenetic literature by portraying the genetic-environmental-biological interplays using an increasingly popular pathway method.

PO1.G.17

Polymorphism 4G/5G in the promotor region of PAI-1 gene. Association with autoimmune parameters in SLE.

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Polymorphism 4G/5G in the promoter region of the plasminogen activator inhibitor type 1 (PAI-1) gene has been implicated in thrombosis and coronary disease. Autoantibodies against oxidized LDL (oxLDL), oxLDL/

β2GPI complex and phospholipids have been identified as possible factors in the development and progression of thrombosis in SLE. *Aim:* To evaluate the association between the polymorphism 4G/5G of the PAI-1 gene and antibodies against oxLDL, oxLDL//β2GPI complex, antiphospholipids (anticardiolipin, antiphosphatidilserine and β2GPI) and anti-prothrombin in SLE. **Methods:** We analyzed 62 SLE patients (ACR 1982) for the genotypes of the polymorphism 4G/5G of the PAI-1 gene by PCR-RFLP´s using BslI as the restriction enzyme. The profile of the different antibodies was determined mainly by ELISA method. The associations of the different genotypes with the autoantibodies were analyzed by Kruskal-Wallis and U-Mann Whitney tests (p value <0.05). **Results:** The distribution of the allelic and genotypic frequencies among SLE patients and control group were similar. Patients with 5G/5G genotype showed increased levels of aCL-IgM antibodies (11.3IU/ml) different to those with the 4G/4G genotype (3.8IU/ml) (p<0.05). This subgroup, 5G/5G also showed high levels of antibodies against oxLDL/β2GPI (IgM and IgG) (29.1 and 24.5 IU/ml respectively) than the patients with the genotype 4G/4G (21.5 and 16.5 IU/ml respectively) (p=0.07); when we compared these antibodies with the 4G/5G genotype (17.9 and 17.6 IU/ml) it was statistically significative. **Conclusions:** The genotype 5G/5G was associated with an increase in the aCL-IgM antibodies levels, with respect to those patients with 4G/4G genotype and high levels of antibodies against oxLDL/β2GPI complex (IgM and IgG) when it was compared with SLE patients with the genotype 4G/5G.

PO1.G.18

Replication of SLE loci in European-American males

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Objectives: Systemic Lupus Erythematosus (SLE) is an autoimmune inflammatory disease with a strong genetic component, which predominantly affects women with a 9:1 female:male ratio. A genome wide association study (GWAS) and its replication cohort, the Large Lupus Association Study #1 (LLAS1), were performed in women of European-American (EA) ancestry and identified SLE susceptibility variants in *ITGAM, PXK, KIAA1542* and confirmed several previously associated loci (Nat Genet 40:204-210, 2008). Here, we report confirmation of genetic associations with SLE in the EA males from LLAS1. **Methods:** We studied 8633 SNPs selected from the female LLAS1-GWA case-control study in a replication cohort of 213 EA males affected with SLE and 709 EA male controls. We performed a standard chi-square analysis in the males and conducted a Fisher's combined p-value for the combined result of males and females. We required primary associations to be significant for the risk allele in the males cohort at P < 0.05 and P < 10^{-6} in the combined analysis of the four female cohorts plus male cohort. **Results:** The most significant associations were within the MHC region in chromosome 6p21, where all prior associations in females were confirmed in males; this was also the case for the association with *IRF5*. We also identified highly significant associations with markers in a 700 Kb segment on chromosome 8p23.1 containing *XKR6, BLK* and *C8orf12*. The associations with *PXK* and *KIAA1542* did not reach statistical significance in the male-only cohort. Table 1 shows the most relevant associations identified.

	Male Cohort			Male + Female Cohorts
Gene	P value	OR	95% CI	P value
1. Significant association in all cohorts (male + 4 female cohorts)				
HLA region	4.46×10^{-14}	2.42	1.92 - 3.06	9.18×10^{-31}
HLA region	3.04×10^{-13}	2.72	2.06 - 3.58	5.68×10^{-62}
IRF5/TNPO3	9.85×10^{-6}	1.87	1.41 - 2.48	1.96×10^{-22}
XKR6	2.44×10^{-4}	1.50	1.21 - 1.87	1.54×10^{-12}
rs10798269	5.28×10^{-4}	0.65	0.51 - 0.83	1.44×10^{-9}
BLK	1.48×10^{-3}	1.43	1.15 - 1.78	2.96×10^{-11}
ITGAM	0.025	1.38	1.04 - 1.84	2.29×10^{-11}
2. Significant associations in males only				
USH2A	5.70×10^{-6}	1.71	1.35 - 2.16	4.92×10^{-5}
rs11913002	7.30×10^{-5}	1.55	1.25 - 1.93	
XKR6	5.39×10^{-4}	0.68	0.55 - 0.85	6.35×10^{-5}
XKR6	3.14×10^{-3}	1.39	1.12 - 1.72	4.25×10^{-5}

Conclusions: The EA male replication cohort supports many of the associations previously identified in the LLAS1-GWA study in females, and suggests that some sex differences may exist. These include a tendency for larger odds-ratios in males than those observed in females at several loci, and a candidate association to *USH2A* in males-only. Furthermore, we found significant association to multiple SNPs in a segment of 8p23.1 that encodes for at least three transcripts that are expressed in immune cells. This region has been proposed as a candidate for SLE-association mainly in Asian populations.

PO1.G.20

Fcγ receptor 2b genetic polymorphisms are associated with systemic lupus erythematosus in Koreans

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Objective: Fcγ receptor 2b is unique in its ability to transmit inhibitory signals, and recent animal studies demonstrate a role for Fcγ receptor 2b deficiency in the development of autoimmunity. To evaluate whether genetic polymorphisms of Fcγ receptor 2b gene are associated with the pathogenesis of SLE, we investigated single nucleotide polymorphisms (SNPs) of Fcγ receptor 2b gene in a Korean population. ***Method:*** Blood samples were collected from Korean SLE patients (n=297) and normal healthy controls (NC, n=300) from the rheumatology clinic at the Ajou University Hospital. Genomic DNA was extracted and variation screening of Fcγ receptor 2b gene was carried out in 40 SLE patients and 40 NC We identified possible polymorphisms in the Fcγ receptor 2b gene between intron4 and intron6 region, which was 2.3 kb-sized. Additionally, we amplified that and the detected SNPs were genotyped using direct sequencing for SLE patients (n=257) and NC (n=260). ***Results:*** The mean age of SLE patients was 34.5 ± 12.4 years and 86.5% were women. The mean age of NC was 26.4 ± 5.3 years and 66.7% were women. We have identified 695 T>C (rs1050501) polymorphism in the exon5 region, and 10950 T>G (rs6666965) polymorphism in the intron5 region in the Fcγ receptor 2b gene. The 695T>C polymorphism was significantly associated with SLE (codominant and recessive anlaysis, p=0.008 and p=0.014, respectively). No association was found in the 10950 T>G polymorphism, however, the 10950 T>G polymorphism was statistically significantly associated with lymphopenia (p=0.036). ***Conclusions:*** These data suggest that genetic polymorphisms within Fcγ receptor 2b gene may be associated with disease susceptibility and phenotype of SLE in Koreans.

PO1.G.21

Association of androgen receptor gene polymorphism with damage in systemic lupus erythematosus

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Objective: Although most SLE patients are women, male patients tend to have more severe disease and poorer outcome. A polymorphic CAG repeat in the androgen receptor (AR) gene located on the X chromosome is known to modulate androgen-mediated transactivation activity, with shorter length of CAG repeats (n ≤ 17) conferring enhanced AR function in vitro. We investigated whether the CAG repeat length might be associated with damage in SLE. ***Methods:*** The polymorphic CAG trinucleotide microsatellite in exon 1 of AR was genotyped by PCR. Patients' clinical information was collected from chart review. Damage was assessed using the Systemic Lupus International Collaborating Clinics (SLICC)/ ACR Damage Index (SDI), a standardized, validated damage instrument. Association of the length of CAG repeats with SDI was analyzed using the Spearman correlation test, Chi square test, and logistic regression analysis. ***Results:*** 78 male and 310 female SLE patients from multiple ethnic groups were genotyped for AR CAG repeat (n [interquartile range] = 12-32 [17.5-21]). A negative correlation was observed between AR CAG length and SDI in male SLE patients (r=-0.33, p = 0.0032). The lowest quartile of CAG repeat length (n ≤ 17) was significantly associated with a SDI >1 in both male and female SLE patients (OR = 5.28 [1.57-17.76], p = 0.0044 and OR = 2.03 [1.18-3.50], p = 0.0096, respectively). A significantly higher proportion of female patients with the lowest quartile of CAG repeat length developed damage (SDI>1) after 18-20 years of disease duration compared to other females (p = 0.006). Using logistic regression analysis in the combined study of both genders, shorter CAG repeat length (n ≤ 17) was identified as an independent risk factor for increased damage (SDI > 1) in SLE (OR = 2.18 [1.26-3.75], p = 0.005). Other factors associated with damage included longer disease duration (OR = 1.06 [1.03-1.09], p = 7.5×10-5), higher cumulative prednisone dosage (OR = 2.22 [1.65-2.99], p = 1.5×10-7), and younger age at disease onset (OR = 1.02[1.00-1.03], p = 0.047). ***Conclusions:*** These data suggest that shorter CAG repeat length in AR, which confers enhanced AR activity, is a risk factor for severity of SLE. The number of CAG repeats may serve as a prognostic biomarker in SLE.

PO1.G.22

Trans-ethnic association studies fine map the SLE-susceptibility locus OX40L

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Objectives: We have established the TNF superfamily member *OX40L*, as a SLE susceptibility gene in Europeans. This gene operates at the T-cell-APC interface as a late-stage lymphocyte activator. Several genetic polymorphisms in the 5' region of the gene predispose to SLE and form an overtransmitted 98kb haplotype ($P_p < 10^{-5}$, after permutation, OR=1.6, 95% CI=1.27-1.89). Strong LD between SNPs in this region in Europeans has prevented further resolution of the association signal; however the associated risk haplotype correlates with a 6-fold increase in *OX40L* transcript and OX40L protein levels. Our objective is to resolve the association of this locus with SLE. **Methods:** We adopted a trans-ethnic mapping approach complemented by 454 sequencing; 50 SNPs were employed in a SE Asian population (1119 cases, 1347 controls), 67 SNPs identified from Yoruba Hapmap data in an African-American cohort (657 cases, 929 controls) assembled by the PROFILE group and 67 SNPs in our UK European Lupus cohort (920 cases, 1457 controls). We examined 20 key SNPs in an additional cohort of Afro-Caribbean's, recruited in the UK, to further resolve the association signal in West Africans. In a complimentary strategy to define functionally relevant polymorphisms, the *OX40L* locus (120kb encompassing the gene and 5' region) was deep sequenced in *OX40L*hi and *OX40L*lo individuals (n=100) from our UK cohort using the Next Generation 454 Titanium platform. **Results:** The association is strongly replicated in the Asian population (Pp=2 x 10^{-9}, OR=1.45, CI=1.27-1.63), with multiple SNPs in the 5' region of *OX40L* associated with SLE. The risk haplotype in East Asians is structured similarly to that in our UK Europeans (P_p=1.5 x 10^{-6}, OR=1.59, 95% CI=1.29-1.96). Recombination patterns differ most markedly between European and West African individuals, seen as greater haplotypic diversity in the African-American cohort. In this population, after correction for admixture using global major ancestry informative markers, the *OX40L* association with SLE is replicated (P_p=0.01, OR=1.30, CI-1.1.18-1.50) and haplotype structure delineates the association signal considerably, focusing it on 30kb in the 5' upstream region. The UK Afro-Caribbean data and our 454 sequencing results are currently in analysis. **Conclusions:** We have replicated and refined the *OX40L* association with SLE in multiple populations to establish *OX40L* as a global lupus susceptibility gene. The validity of trans-ethnic studies to map association signals in complex traits has been confirmed.

PO1.G.23

An association study of 8p23.1 region encoding *BLK, FAM167A, C8orf12* and *XKR6* with systemic lupus erythematosus in Japanese

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Objective: We previously reported that the genetic contribution of *FAM167A (C8orf13)* - B lymphoid tyrosine kinase (*BLK*) region to systemic lupus erythematosus (SLE) was considerably greater in Japanese as compared with Caucasians (Ito et al., Arthritis Rheum 2009). *XKR6* and *C8orf12*, also located at 8p23.1, have been reported to be associated with SLE in Caucasians, and it remains unclear whether these genes independently contribute to the genetic background of SLE, or these associations can be explained by linkage disequilibrium (LD) with other causative allele(s). To address this issue, we performed an association study in a Japanese population, which has a different LD structure from that of Caucasian populations. **Methods:**

A case-control association study was performed for 300 Japanese patients with SLE and 322 healthy controls. In addition to the 14 tag SNPs reported elsewhere (Ito et al., Arthritis Rheum 2009), 2 SNPs of *XKR6* (rs6985109 and rs6984496), previously shown to be associated with SLE in Caucasians, and 5 tag SNPs in *C8orf12-FAM167A* region selected based on the HapMap Phase II JPT data were examined in the same case-control set, and the results were compared. **Results:** Except for the previously reported *FAM167A-BLK* region SNPs, only rs2736282 and rs1594967 in *TDH-C8orf12* region showed marginal tendency towards association (Table 1). Evidence for the association of *XKR6* SNPs was not observed. These *XKR6* SNPs were found to be in moderate LD with the most strongly associated SNP in the *FAM167A-BLK* region, rs13277113, in Caucasians based on the HapMap Phase II CEU data (rs6985109: r2=0.32, D'=0.87, rs6984496: r2=0.29, D'=0.87). However, in Japanese, the LD between the *XKR6* SNPs with rs13277113 was weak (rs6985109: r2=0.005, D'=0.26, rs6984496: r2=0.003, D'=0.19), suggesting that the association of *XKR6* reported in Caucasians might be caused by LD with the causative allele(s) in the *FAM167A-BLK* region, which shows strong association in both populations. **Conclusion:** The genetic contribution of 8p23.1 region for SLE is largely explained by *FAM167A-BLK* region, but not by *XKR6* or *C8orf12*, in Japanese.

SNP	Gene	Position	Allelic association OR (95%CI)	Allelic association *P*
rs6985109	*XKR6*	intron 2	1.34 (0.79-2.27)	0.28
rs6984496	*XKR6*	intron 1	1.04 (0.60-1.78)	0.90
rs2736282	*TDH-C8orf12*	intergenic	1.41 (1.04-1.92)	0.029
rs1594967	*C8orf12*	intergenic	1.37 (1.03-1.81)	0.029
rs10105659	*C8orf12*	intergenic	0.87 (0.60-1.26)	0.46
rs9644737	*C8orf12-FAM167A*	intergenic	1.20 (0.92-1.60)	0.18
rs12548449	*C8orf12-FAM167A*	intergenic	0.83 (0.64-1.07)	0.15
rs10503423	*C8orf12-FAM167A*	intergenic	0.65 (0.52-0.83)	0.00034
rs6984212	*FAM167A*	intron 2	0.72 (0.56-0.91)	0.0065
rs10088323	*FAM167A*	intron 2	0.68 (0.54-0.87)	0.0016
rs2618431	*FAM167A*	intergenic	0.65 (0.51-0.83)	0.00047
rs12680762	*FAM167A*	intergenic	0.68 (0.48-0.98)	0.037
rs17799348	*FAM167A*	intergenic	0.99 (0.74-1.34)	0.97
rs2254891	*BLK*	intergenic	0.60 (0.47-0.78)	0.00013
rs13277113	*BLK*	intergenic	0.52 (0.41-0.67)	4.33X10^{-7}
rs2736354	*BLK*	intron 1	0.56 (0.43-0.72)	6.83X10^{-6}
rs2736360	*BLK*	intron 1	0.60 (0.46-0.78)	0.00013
rs1382566	*BLK*	intron 1	0.65 (0.50-0.84)	0.0010
rs11250144	*BLK*	intron 1	0.68 (0.54-0.87)	0.0019
rs12677843	*BLK*	intron 1	0.73 (0.58-0.93)	0.0094
rs2244931	*BLK*	intron 3	1.02 (0.79-1.33)	0.88

PO1.G.24

Independent association of variants in the class I region and the class II gene, HLA-DPB1, at the MHC in a Filipino SLE cohort

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Objectives: Variants within the MHC demonstrate the greatest genetic risk for lupus in European and Chinese populations. Recent high-density SNP genotyping studies have demonstrated multiple independent signals across the MHC in northern European cohorts. We therefore undertook a high-density SNP study of the MHC in a Filipino lupus cohort in order to further refine association signals in this haplotypically diverse South-East Asian population. **Methods:** We genotyped 11640 SNPs in 335 cases and 247 controls using a custom Illumina chip. 4743 SNPs were informative for major and European ancestry, 6045 SNPs were located within the MHC region (29 – 33.5 Mb) and 852 SNPs were lo-

cated in putative autoimmune loci outside the MHC region. *Results:* Following QC measures 217 cases and 176 controls were put forward for analysis. The overall pattern of association demonstrates that the major signal arises from the class II region of the MHC and therefore differs from that observed in European lupus cohorts where associations are seen in class II and class III. The top SNP is rs9270986 located between HLA-DRB1 and HLA-DQA1 (OR 2.7, CI 2.0-3.6; nominal p=1x10-10). The LD around this signal spans ~37 kb from HLA-DRB1 to the intergenic interval between HLA-DRB1 and HLA-DQA1. There is also a protective haplotype within the HLA-DRB1 and HLA-DQA1 intergenic interval. Conditional logistic regression analyses on the top SNP, rs9270986, reveals independent signals in the class I region of the MHC between HLA-G and HLA-H (conditional OR~ 0.48, CI~0.34-0.68, p~2.36x10-5) and HLA-DPB1 (conditional OR 0.42, CI 0.25-0.70, p=9.13x10-4). Interestingly, the class III SNPs which show association in European lupus cohorts are rare in this Filipino cohort. These latter data are similar to the recent findings of a Chinese case-control lupus genome-wide association study, where the minor allele frequencies for these class III SNPs were ~0.001. *Conclusion:* These observations support a significant role for variants within the MHC class II region but not the MHC class III region in South-East Asian SLE in marked contrast to associations seen in European lupus populations.

PO1.G.25

The regulation of the alternative splicing of BANK1, a gene associated with the autoimmune disease SLE

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Introduction: Recently, we discovered that the BANK1 gene, which plays a role in a B cell activation pathway, is associated with systemic lupus erythematosus through a nonsynonymous substitution G/A (rs10516487, R61H). We identified that the BANK 1 gene expresses two alternatively spliced isoforms, a full-length and a shorter isoform that lacks exon 2. The two isoforms were differentially expressed in susceptible lupus patients depending on the presence of a risk haplotype. *Objectives:* The question that we addressed in this study was to understand how BANK1 is spliced and what are the signals and the factors governing the splicing and expression of each isoform. *Methods:* We constructed minigenes with different genetic variants and tested the expression of the BANK1 isoforms in vitro using qPCR. The transfection and cotransfection experiments were conducted in HEK 293 cells and the analysis was performed after 24 hours post transfection. *Results:* QPCR analysis revealed that the G/A (rs10516487, R61H) SNP, which is located in exon 2, has a strong affect on the expression levels of BANK1 isoforms. Deletion of a polypyrimidine (PY) stretch downstream of the skipped exon, resulted in a dramatic drop in the full-length expression levels, probably due to the loss of the binding site for protein TIA1, which binds to T stretches and helps the selection of the correct splicing borders. Cotransfection of the minigene constructs that contains the G allele in the (rs10516487, R61H) SNP with hnRNPA1, a protein known to work as a splicing silencer, resulted in higher levels of the full-length isoform. On the other hand, hnRNPA1 did not have any affect on the BANK1 full-length expression when cotransfected with the minigene contains the A allele in the R61H SNP. *Conclusion:* Our findings indicate that, the R61H SNP has a functional role in the expression of BANK1 isoforms. Deletion of the T stretch located downstream of exon 2 resulted in down- regulation of the full-length isoform. HnRNPA1 is participating in the splicing regulation of exon 2 in BANK1 by enhancing the expression of the full-length isoform through directly or indirectly acting on the G allele in the R61H SNP.

PO1.G.26

Polymorphism of the immunoglobulin (Ig) enhancer element HS1,2A gene and systemic lupus erythematosus

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Objectives: To investigate the relationship of the polymorphic enhancer HS1,2 central to the 3' enhancer complex regulatory region (IgH3' EC) of the Ig heavy chain genes with SLE and its association with major organ involvement and Immunoglobulin (Ig) production. *Methods:* 168 (149 women and 19 men) Italian patients fulfilling at least four of the ACR criteria for SLE (Arthritis Rheum, 1997) and 573 controls, living in the same geographical area, were enrolled in the study. DNA from patients and controls was genotyped for the HS1,2A polymorphism by polymerase chain restriction fragment length polymorphism method (Giambra; Genes 2005). Clinical and immunological data were obtained from case records. Organ involvement and autoantibodies (ENA, aPL, anti-DNA and anti-nucleosome) positivity were evaluated for all the patients. Patients with renal involvement underwent renal biopsy and were classified according to WHO criteria. *Results:* The analysis of the four alleles of the HS1,2A enhancer showed an increased frequency of the allele*2 (53.9%) and more of the genotype 22 (31.5%) in the SLE cohort compared to controls (40.4% for allele *2, OR:1.72; 95%CIs: 1.35-2.20 and 15.7% for genotype 22, OR:2.47; 95%CIs: 1.67-3.67); moreover, we found a lower representation of the allele 1 (34.8%) and of the allele*3 (1.5%) in SLE patients when compared with controls (42.5% for allele*1, p=0.02 and 4.5% for allele*3, p=0.01, respectively). Among the 40 patients with lupus nephritis (LN), individuals with the classes III and IV of LN showed an increased frequency of the allele*2 of HS1,2A when compared to individuals with class II (55.7% vs. 20.0%, p = 0.03). SLE patients carrying the allele*2 had higher IgM levels than patients without allele*2 (129.8 ± 74.7 mg/dl vs 77.5 ± 47.9 mg/dl, p<0.001). There was an higher percentage of patients with IgM levels higher than 75 percentiles (155 mg/dl) in subjects carrying the allele*2 (28.7%) and more in patients with the 22 genotype (42.4%) compared to patients without allele*2 (7.7%, p=0.03; p=0.003 respectively). We failed to find any significant association between the HS1,2 A polymorphism and the other clinical manifestations and the presence of autoantibodies. *Conclusion:* These data confirm the hypothesis of an increased risk of developing SLE in carriers of the allele*2 of the HS1,2 enhancer polymorphism and a more aggressive renal disease occurring in allele*2 carriers.

PO1.G.27

Variants in the promoter region of osteopontin are associated with hematologic parameters in systemic lupus erythematosus patients

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Objective: It has been long established that genetic factors play a significant role in SLE. Variants of the osteopontin (OPN) gene have been previously associated with SLE susceptibility. OPN is a secreted extracellular matrix cell adhesion glycoprotein with various immunological functions. Although OPN gene variants have been linked to SLE susceptibility, it is unknown if they correlate with specific clinical sub-phenotypes of SLE. We hypothesized that the frequency of SLE susceptibility alleles of the osteopontin gene is increased in patients with specific clinical characteristics of SLE. *Methods:* ACR classification criteria for SLE and SLEDAI scores were collected for 168 SLE patients (118 African ancestry, 50 European ancestry). Patients were recruited at the University of Chicago and Rush University Medical Center. The following OPN SNPs were genotyped in the same patients using Taqman probe sets: rs11730582, rs28357094, rs6532040, and rs9138. Logis-

tic regression models were used to detect associations between OPN alleles and clinical manifestations within the SLE cohort. Clinical manifestations were recorded as binary variables representing the presence or absence of each of the 11 ACR classification criteria. Analyses were performed after stratifying the cohort by self-reported ancestry. *Results:* rs11730582 C was associated with thrombocytopenia in African American subjects (OR=2.63, 95% C.I. 1.20-5.78, p=.015). rs11730582 C also trended towards an association with the presence of hemolytic anemia in African Americans (OR=2.63, 95% C.I. 0.92-7.51, p= 0.07) and in European Americans (OR=12.45, 95% C.I. 0.68-228, p=0.09). In European-Americans, this same allele was associated with leukopenia (OR=4.16, 95% C.I. 1.15-15.07, p=0.03) and trended toward an association with thrombocytopenia in European American subjects (OR 9.12, 95% C.I. 0.85-95.0, p=.065). Additionally, in European Americans rs28357094 T was associated with lymphopenia (OR=4.02, 95% C.I 1.02-15.76, p=0.046). *Conclusions:* SNPs in the 5' promoter region of OPN are associated with multiple hematologic parameters in SLE patients of two different ancestral backgrounds. We have previously detected an association between these two SNPs and the presence of anti-RNP antibodies in SLE. Osteopontin is known to stimulate B cell immunoglobin production and proliferation. As the observed cytopenias are sometimes antibody mediated, our results could suggest a role for osteopontin in the pathogenesis of specific cytopenias via the production of autoantibodies in SLE.

PO1.G.28

Determination of Klinefelter's status using real time PCR in the study of an X dose effect for systemic lupus erythematosus

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Objectives: SLE is more common in women than in men. We recently demonstrated in patients with familial and nonfamilial SLE that Klinefelter's syndrome is increased in SLE men. We estimate 1 SLE patient among every 960 males with KS relative to 1 SLE patient in 1,324 women based on the ethnic distribution in our population. Thus, males with 47,XXY have a risk of SLE comparable with that in females with 46,XX. We therefore hypothesize that a gene-dose effect for lupus risk originating from the X chromosome confers a 10-fold higher risk than 46,XY. Consequently, we suggest that the rate of SLE among individuals with 45,XO (Turner's syndrome, TS) would be similar to the rate in males with 46,XY. We have successfully standardized a qPCR approach utilizing copy number analysis of the AR, SRY and GAPDh genes as a screening method for diagnosis of patients with KS and other chromosomal disorders involving an aberrant number of X-chromosomes. *Methods:* Patient samples were obtained from the Lupus Family Registry and Repository based at OMRF. Recruitment followed informed consent and protocols approved by IRB. FISH was performed with X and Y centromere specific probes. Typing was done at 256 SNPs from the non-pseudoautosomal regions of the X chromosomes, using the 10K GeneChip Array. Genomic DNA was isolated using standard protocols and DNA concentration was measured using the Nanodrop. qPCR was carried out on ABI 7300 using standard protocols and the following primers. AR-Fw 5'-CGACCAGATGGCTGTCATTC-3'; AR-Rev 5'-CTGGAGTT-GACATTGGTGAAGG-3'; GAPDh-Fw 5'-CTCCCCACACACATG-CACTTA-3' GAPDh-Rev 5'TTGCCAAGTTGCCTGTCCTT-3'; SRY-Fw 5'-AGCTCTTCCTTCCTTTGCACTG-3'; SRY-Rev 5'-ATCCTGGAC-GTTGCCTTTACTG-3'. *Results:* Our ongoing recruitment of males with SLE has over 240 male subjects with 7 confirmed KS cases. Using a qPCR approach we were able to identify X chromosome aneuplodies and then confirm 47,XXY, 45,XO and 47,XXX by FISH/karyotyping. There are no female SLE patients with TS among >2400. *Conclusions:* Investigation of X

chromosome polymorphisms does not always enable detection of 47,XXY. Karyotyping and FISH are the usual clinical modality for diagnosis for KS or TS, however, they require cell culture of WBC's arrested in the metaphase which are not always available. The process is expensive and requires specialized expertise. Using a qPCR approach we have streamlined and expedited the process of not only identifying SLE men with KS but also identifying X chromosome aneuplodies in general. Our results suggest a higher risk for SLE among KS men and a low risk of TS among SLE women.

PO1.G.29

Segmental duplication and gene conversion contribute to copy-number variations of human immunoglobulin Fcγ-receptors genes *FCGR3B*, *FCGR3A* and *FCGR2C*, and heat shock protein *HSP70B* and *HSP70B'* in SLE patients and healthy subjects

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Objectives: Immunoglobulin Fcγ-receptors FcγRIIA and IIB, FcγRIIIA and IIIB are important effectors of immune response. Gene copy-number variation (CNV) of *FCGR3B* has been suggested to be a risk factor for human SLE. However, how *FCGR3B*-CNV contributes to SLE remains controversial because the mechanisms leading to the CNV of *FCGR3B* has not been elucidated, and methodologies to determine and differentiate the CNVs of *FCGR3B*, *FCGR3A*, *FCGR2C*, *FCGR2A* and *FCGR2B* have not been firmly established. The objective is to investigate the genomic diversity of *FCGR2-FCGR3* region on chromosome 1q23, and to determine the CNVs of *FCGR2* and *FCGR3* in healthy subjects and SLE patients. *Methods:* The study population includes 281 SLE patients and 485 unrelated healthy controls of European ancestry, recruited from Ohio and Pennsylvania. Pulsed-field gel electrophoresis (PFGE), using intact genomic DNA in agarose-plugs prepared from mononuclear cells and digested with *Pme*I enzyme, was applied to determine the segmental duplication. *Taq*I restriction fragment length polymorphism (RFLP) employing three specific probes were used to elucidate the relative copy-numbers between *FCGR3A* and *FCGR3B*; among *FCGR2C*, *FCGR2A* and *FCGR2B*; and between *HSP70B* and *HSP70B'* (functional gene). *Results:* *Pme*I-PFGE and *Taq*I-RFLP revealed discrete 85-kb segmental duplications for contiguous genes *FCGR2*, *HSP70B* and *FCGR3*, which are termed an FRH module. One to four modules are detectable on a haplotype. Monomodular FRH usually has the structure *FCGR2A-HSP70B'-FCGR3A-FCGR2B*. Each additional FRH module is inserted between *FCGR3A* and *FCGR2B*, and mostly includes *FCGR2C-HSP70B-FCGR3B*. However, *HSP70B'* instead of *HSP70B*, and *FCGR3A* instead of *FCGR3B*, can be present in a duplicated module because of gene conversion-like events. A deficiency of *FCGR3B* is caused either by the presence of a monomodular structure with one copy of *FCGR3A*, or a bimodular structure with two copies *FCGR3A*. In our study population, homozygous and heterozygous deficiencies of *FCGR3B* had a frequency of 12.5% in SLE and 6.2% in healthy controls (odds ratio: 2.16, 95% CI: 1.29-3.60; p=0.0044). By contrast, high copy-numbers of *FCGR3B* had a frequency of 8.2% in SLE and 12.0% in healthy controls. Similar CNV phenomena were observable for *FCGR2C* and *HSP70B*. CNVs for *FCGR3A* and *HSP70B'* only occurred in 3-6% of the study population. *Conclusions:* Using a series of definitive genotyping assays, we have defined the 85-kb segmental duplications that contribute to the CNV of *FCGR3B*, and showed that homozygous and heterozygous deficiency of *FCGR3B* is a risk factor for SLE with an odds ratio of 2.2.

PO1.G.30

**Association of HIN200 gene polymorphisms
with susceptibility to SLE disease**

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The MNDA, IFIX, IFI16, and AIM2 genes whose products belong to hematopoietic interferon-inducible nuclear protein with 200 amino acid repeat (HIN200) gene family map on chromosome 1q21-23 which is the major SLE susceptible loci. We conducted an association study in 200 Thai SLE patients from King Chulalongkorn Memorial Hospital and 200 ethnically matched healthy donors from the Thai Red Cross Society using 11 putative functional SNPs according to computational prediction. The G13792T (rs856084) in IFIX and the C6771G (rs866484) and A23201G (rs1772414) in IFI16 showed significant differences with OR of 0.73, 95% CI of 0.54-0.98, empirical P = 0.033: OR of 1.33, 95% CI of 1.00-1.75, empirical P = 0.05 and OR of 1.37, 95% CI of 1.03-1.80, empirical P = 0.030, respectively. The presence of two G alleles (GG) conferred the significant P value of 0.009 (empirical P = 0.023) for rs866484 and P value of 0.013 (empirical P = 0.032) for rs1772414. The marginal significances are probably due to the limited sample size in this study. We performed haplotype analysis of significant SNP rs866484 and rs1772414 with strongest LD (D' = 0.814 and r2 = 0.643) by comparing each haplotype with another three haplotypes between SLE patients and controls. Our finding demonstrated that the GG was a risk haplotype with OR of 1.41 and P = 0.017 (empirical P = 0.031), whereas CA was a protective haplotype with OR of 0.73 and P = 0.032 (empirical P = 0.041). Since binding of IFI16 protein to the C-terminus of p53 has been reported to stimulate the transcription of p53-responsive reporter plasmids and leads to susceptibility to apoptosis of cells, the association of SNP rs866484 and apoptosis rate was studied using flow cytometry analysis. However, the present study could not reveal the association between SNP and apoptosis. In addition, we tested the association of the two SNPs within the intron of IFIX (rs856084) and IFI16 (rs1772414) genes with the expression of the isoforms (α and β for IFIX; A, B and C for IFI16) by RT-PCR. We did not find any difference in isoforms among genotypes. Our results suggest that these SNPs are not likely to be functional SNPs. We could not exclude the possibility that these SNPs are in linkage disequilibrium (LD) with a nearby causative SNPs. To clarify the role of IFIX and IFI16 gene, more extensive research using dense SNPs and an increased sample sizes are required in future study.

PO1.G.32

**Gene network analysis of bone marrow mononuclear
cells reveals activation of multiple kinase pathways in
patients with systemic lupus erythematosus**

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Objectives: Gene microarray studies provide important information for molecules relevant to a disease but are less informative of protein-protein interactions, post-translational modifications, and subcellular regulation. Integration of genomic data and construction of functional gene networks may provide additional insights into complex diseases such as systemic lupus erythematosus (SLE). *Methods:* We analyzed gene expression microarray data derived from bone marrow mononuclear cells (BMMCs) from 20 SLE patients (11 with active disease) and 10 control subjects (7 healthy individuals, 3 osteoarthritis patients). We used the bionformatic tool Ingenuity Gene Network Analysis to construct gene networks. Results were validated by western blot analysis in splenic B cells obtained from 5-month-old NZB/NZW F1 lupus mice. *Results:* Comparative analysis of BMMCs genes revealed a network in SLE patients with 18 central nodes as major gene regulators including ERK, JNK, and p38 MAP kinases, insulin, Ca2+ and STAT3. The most significant network (p = 10-35) is involved in cellular growth and with central nodes BCL3, JNK, insulin, p38 MAPK, MBP, PKC, NF-κB, ERK, MAPK and CCR5. Comparison between active and inactive SLE identified 31 central nodes associated with immune response, protein synthesis, and post-transcriptional modification. The most significant network (p = 10-50) consisted of proteins that were up-regulated in active patients with central nodes AKT, NF-κB, HSP90, proteosome, IER3 and HSPB1. A high degree of identity between gene networks in active SLE and non-Hodgkin's lymphoma (NHL) patients was observed, with overlapping central nodes including kinases (MAPK, ERK, JNK, PKC), transcription factors (NF-kB, STAT), and insulin. In western blot studies, splenic B cells from NZB/NZW F1 lupus mice showed activation of ERK, JNK, p38, and p32 kinases, compared to normal C57Bl/6 mice. *Conclusions:* Gene network analysis of SLE BMMCs identified central gene regulators implicated in disease pathogenesis which could represent targets of novel therapies. The high degree of similarity between active SLE and NHL networks provides a molecular basis for the association of the former with lymphoid malignancies.

PO1.G.33

**Association of variants in the TNFAIP3 region with
systemic lupus erythematosus in different populations**

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Objectives: Systemic lupus erythematosus (SLE) is a complex autoimmune disease with varied and potentially severe clinical manifestations affecting multiple organs. Prevalence of this disease varies between genders and among age-groups and ethnicities. SLE affects women nine times greater than men and is more common in non-Caucasians than in Caucasians. Recent studies indicate that genetic variants in the region of tumor necrosis factor alpha-induced protein 3 (TNFAIP3) are associated with SLE in subjects of European ancestry. TNFAIP3 encodes a zinc-finger protein called A20, a critical regulator of inflammatory signaling pathways. To further characterize and localize the effect of TNFAIP3, we genotyped and imputed single-nucleotide polymorphisms (SNPs) within and flanking TNFAIP3 in six populations: Europeans, including European Americans; African Americans; Asians, including Koreans; Hispanics, Native Americans, and Gullah. Using the SNPs described above and a panel of ancestry-informative markers (AIMs) we used transracial mapping to isolate a putative causal variant. *Methods:* Using a custom designed SNP panel for the Illumina system, we genotyped 127 SNPs in and around TNFAIP3. Then, using HapMap and 1000 genomes data we imputed a minimum of 456 additional SNPs for each of the populations (the number varied based on linkage disequilibrium structure). We assessed single marker association to SLE using logistic regression with sex, global and local adjustments. Global ancestry was calculated in ADMIXMAP using all AIMs across the genome. Local ancestry, used to partition an individual genome into distinct chromosomal segments of distinct ancestral origin, was estimated in ADMIXMAP using nearby AIMs. We also calculated the association between local ancestry and SLE case-control status to estimate the residual risk of SLE associated with admixture after controlling for each SNP in the TNFAIP3 region. *Results:* Association analysis identified a risk haplotype in Europeans and Asians likely to harbor a causal variant (p=1.25 x 10-9 and p=1.73 x 10-9, respectively). Further haplotype comparisons across populations revealed a putative causal variant in this haplotype with p=4.64 x 10-10, OR=1.80, 95%CI=1.50-2.17 in Europeans, p=8.38 x 10-9, OR= 2.22, 95%CI=1.69-2.91 in Asians, and p=3.70 x 10-9, OR=2.47, 95%CI=1.83-3.34 in Koreans alone. No significant association was found in other populations.

Conclusions: These results support a putative causal variant for SLE within TNFAIP3, unique to Europeans and Asians and further demonstrate the complexity of identifying associations across different populations. Sequencing and functional studies are necessary to validate this variant and determine the contribution of TNFAIP3 to SLE.

* Dr. Gaffney and Dr. Gray-McGuire are co-senior authors.

PO1.G.34

Identification of a novel systemic lupus erythematosus susceptibility locus at 11p13 near CD44

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Objective: Systemic lupus erythematosus (SLE) is a complex autoimmune disorder characterized by the loss of tolerance to self-antigens. Genetic studies to date have established >30 loci contributing to the pathogenesis of SLE. We previously performed a genome-wide association (GWA) study using the Affymetrix Genome-wide Human SNP array 5.0 on 431 cases and 2155 controls (Graham et al. Nat Genet, 2008). In this study, we sought to identify and confirm additional novel loci that had not yet met stringent criteria for genome-wide association (typically $p<5x10-8$). To this end, we performed a large-scale replication study based on our initial GWA results in an independent cohort. *Methods:* Genotyping was performed using Illumina iSelect technology. Stringent quality control measures were applied for Hardy-Weinberg proportions, proportion of missing genotypes and missingness between cases and controls. Both EIGENSTRAT and ADMIXMAP were used to identify population strata within the sample set. After quality control filtering, 3606 SLE cases and 3532 controls of European ancestry were included in the replication analysis. Logistic regression was implemented using PLINK under dominant, recessive and additive genetic models. Covariates for gender and population admixture were included. Stoffer's weighted Ztrend scores were calculated for a meta-analysis between the GWA and replication results. *Results:* In our original GWA scan, we identified 2 SNPs ~74kb upstream of CD44 with suggestive evidence of association with SLE (rs2732552 p=0.004 OR=0.78 and rs387619 p=0.003 OR=0.78). In the current study, association of these two SNPs with SLE was independently replicated with rs2732552 (p=1.49x10-7, OR=0.83, 95%CI=0.71-0.91) and rs387619 (p=1.09x10-6, OR=0.83, 95%CI=0.78-0.90). The meta-analysis of these results yielded a Ztrend=3.66x10-9 for rs2732552 and a Ztrend=2.4x10-8 for rs387619, thus surpassing genome-wide thresholds for significance. These two SNPs flank a common haplotype of tight linkage disequilibrium (r2>0.9) and are likely tagging the same effect. *Conclusions:* We have established genetic association with SLE to a haplotype near CD44. This haplotype is in a substantially conserved region with numerous transcription factor binding sites and other regulatory elements, suggesting potential functional effects on protein expression. CD44 is a cell-surface glycoprotein involved in immune cell-cell interactions, cell adhesion and migration. Numerous studies in SLE and other inflammatory conditions have shown differential expression and complex splicing of the CD44 protein associated with disease. Ongoing studies will determine if this novel genetic association explains changes in CD44 function that contribute to SLE.

PO1.G.35

Assessing the associations between early immune targets unique to systemic lupus erythematosus (SLE) and specific to certain clinical features

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Introduction: Recent immunochemistry studies have implicated immune targets of several antibodies specific to Systemic lupus erythematosus (SLE) as the first structures of Sm B', Ro, and Sm D1 and the earliest signs of autoimmunity. Further, viral infection has long been associated with SLE and its many, varied manifestations. In this study, we collected epitope, viral, clinical and laboratory data for 558 African-American SLE patients. We then performed association analysis to determine the extent to which early epitope production or viral load are associated with specific lupus manifestations. *Methods:* Our epitope and viral data included the following quantitative measures: immune targets of Anti-SmB', Anti SM D1 and anti 60kD R epitopes (PPPGMRPP, PPPGRPP, Ro169), Epstein-Barr Virus (EBV) – viral capsid antigen (EBV-VCA), cytomegalovirus (CMV), EBV nuclear antigen 1 and 58 (EBNA1 and EBNA58), herpes simplex virus type 1 (HSV1) and herpes simplex virus type 2 (HSV2). Our clinical data included severity scores for: malar rash (MR), discoid rash (DR), photosensitivity, oral ulcer (OU), arthritis, serositis, pericarditis, pleuritis, renal discord (RD), proteinuria, cellular cast (CC), neurological disorder (ND), seizures, psychosis, hematology, hemolytic anemia (HA), leukopenia, lymphopenia, thrombocytopenia, immunological disorder (ID), LE cell and false-positive VDRL. Laboratory data included: anti-dsDNA, anti-Sm, lupus anticoagulant, antinuclear antibody (antiNC), ANA, ANA titer, anti-dsDNA titer (dsDNAt), anti-ssDNA titer (ssDNAt) and normal (ssDNAn), anti-Smith, anti-ENA (Ro, La, P, Jo), complement CH50 titer (Comp50t) and normal (Comp50n), anticardiolipin and IgG/IgM/IgA. We assessed strength of association between epitope and/or viral data and the various clinical and laboratory measures using ASSOC (S.A.G.E. software suite). All analyses were adjusted for age and sex and Box-Cox transformation was performed when appropriate. *Results:* We identified 19 effects with a significance level ≤ 0.05, including: PPPGMRPP and anti-Sm, ssDNAn, lymphopenia (p = 3.66x10-6; p = 8.21x10-6; p = 1.78x10-5, respectively); PPPGRRP and Comp50t, ID (p = 0.003; p =0.008, respectively); EBNA-58 and RD, antiNC (p =0.014; p =0.035, respectively); EBV and Comp50t, pleuritis, RD, MR (p = 0.0002; p=0.005; p=0.013; p=0.014, respectively); CMV and MR (p = 0.023); HSV1 and MR, antiNC (p = 0.007; p=0.009, respectively); HSV2 and dsDNAt, anticardiolipin, anticardiolipin IgG, DR (p=0.0002; p=0.0006; p=0.002; p=0.032, respectively). *Conclusions:* This study reports associations between early immune targets unique to lupus and specific to certain clinical features. It further replicates previously reported association between viral load and certain clinical and laboratory variables as well as identifies new ones.

PO1.G.36

Identification of novel genes associated with viral seroconversion and early autoantibody production in lupus patients

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Objectives: Systemic lupus erythematosus (SLE) is a complex, variably debilitating disease affecting African Americans (AA) more frequently and more severely than European Americans (EA) in the US. Studies of SLE strongly support a combined environmental and genetic disease origin. Further, recent immunochemistry studies have identified select epitopes of Sm B' and 60kD against which the first lupus related autoantibodies appear in some patients. They appear prior to clinical diagnosis and are associated with SLE-patient specific immune responses to viral infection (Epstein Barr virus

(EBV) nuclear antigen 1 (EBNA1), specifically). We have performed the first genetic association study of antigenic epitopes specific to the early stages of SLE in a sample of 558 AA SLE patients for over 16,600 single nucleotide polymorphisms concentrated in regions containing previously identified SLE effects. We also assessed genetic association between these same SNPs and seroconversion against select viral phenotypes, including EBNA1, EBV nuclear antigen58 (EBNA58), EBV viral capsid antigen (VCA), cytomegalovirus (CMV) and Herpes Simplex Virus (HSV) 1 and 2. This approach to identifying genes associated with the earliest precursors of SLE and its likely environmental trigger is both unique and perfectly suited to SLE. **Methods:** We obtained the phenotype data using sera of 558 AA SLE patients. Antibodies against an early Sm B' epitope PPPGMRPP, cross-reactive EBNA-1 sequence PPPGRRP and early 60kD Ro epitope (aa 169-180) and cross-reactive EBNA-1 sequence (aa 58-72), EBV-VCA, EBNA1, EBNA58, CMV, HSV1 and HSV2 were detected by ELISA. We then performed genetic association analysis of these quantitative traits using16,603 SNPs collected as a part of the first Large Lupus Association Study (LLAS1). We assumed an additive model and allowed for non-independence of observations (as sample included relatives). **Results:** After Bonferroni correction for multiple testing of 2777 independent regions (p< 1.8 x 10^{-5}), SNPs within two gene regions remained significant: IL1RN (p=1.79 x 10^{-5}) and ACE (p=1.79 x 10^{-5}) for PPPGRRP and Ro169, respectively. An additional 4 suggestive effects (p< 3 x 10^{-4} were found at DLG2 for both PPPGMRPP and EBNA1, MAP3K7 for PPPGRRP and EBNA1, ITPR1 for EBNA1 and EBV-VCA, and ACE for HSV1. **Conclusions:** Our novel study design to explore the genetics of the first lupus autoimmune epitopes and humoral viral responses identifies novel genes related to earliest autoimmune phenomena in SLE and strengthens evidence for previously implicated lupus genes. These studies further define gene by environment interactions likely to participate in initiating specific lupus autoimmune responses.

PO1.G.37

Association of MYH9 and lupus nephritis in multiple populations

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Objectives: Systemic lupus erythematosus (SLE) has many debilitating or even fatal manifestations. Lupus nephritis is among the most severe of these and affects African Americans (AA) three times more than European Americans (EA) and women more than men. Genetic determinants of SLE susceptibility and specific SLE manifestations are supported by high twin concordance rates, linkage and association study findings and the identification of variants associated with disease. Given the threefold increased risk of lupus renal disease in certain populations we chose in this study to investigate a recently identified gene for non-diabetic end stage renal disease, MYH9 (Myosin, heavy Chain 9), shown to be associated in African Americans. To this end, we performed genetic association tests in and around MYH9 in six different populations of lupus cases shown to have renal disease. **Methods:** We investigated MYH9 in independent cases and controls respectively, from six population groups: AA (635 / 1734), EA (1119 / 3546), Asian (531 / 1270), Native American (111 / 193), Hispanic (548 / 619) and Gullah (70 / 122). Cases were defined, from among SLE cases as individuals reported as positive for the American College of Rheumatology renal criteria. Controls were defined as either SLE cases without renal involvement or healthy individuals. Results were similar and therefore only the latter presented. Seventy-three single nucleotide polymorphisms (SNPs) in moderate linkage disequilibrium (LD) (r^2 > 0.80) were selected. We then performed single SNP tests for association with renal disease status using logistic regression adjusted for global African,

European and Asian ancestry age and sex assuming either an additive or dominant genetic model. **Results:** In EA, the association peak centered around 35,040,000 base pairs with multiple SNPs showing association. We identified three SNPs rs5750250, rs2413396 and rs4820232 within MYH9 that were strongly associated with renal disease (1x $10^{-4} \leq p \leq 9$ x10^{-4}) with high LD (D' ≥ 0.99).The best associated SNP was rs5750250 (P = 2.32 x 10 [-4]). In the Gullah, significance of association centered at 22q35.05. No statistically significant associations were seen in populations of other ancestral backgrounds. **Conclusions:** While we did not replicate previously reported findings in AA, a significant association in the Gullah population, despite a sample size of only 192, confirms the elevated risk of lupus nephritis in populations of African ancestry. The presence of our strongest effect in EA is a novel finding and suggests the role of MYH9 in lupus nephritis in diverse populations.

PO1.G.38

Risk and protective alleles of the major histocompatibility complex class I chain – related gene A (MICA) in SLE pathogenesis

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Objective: Genetic- epigenetic and genetic-environmental interactions contribute to ethiopathogenesis of systemic lupus erythematosus (SLE) development. Genes of the major histocompatibility complex (HLA) and the major histocompatibility complex class I chain – related gene A (MICA) are located within the SLE risk locus 6p21 and their products are involved in antigen presentation to CD4+, CD8+ and δγ T lymphocytes, respectively. The risk haplotypes for autoimmune diseases development have been identified despite the huge variability of HLA alleles across populations. The MICA gene shows several polymorphisms; the alleles of the microsatellite polymorphism with (GCT)n repetition and G-insertion in the exon 5 of the transmembrane (TM) domain are in linkage disequilibrium with polymorphisms of the extracellular part and may thus influence antigen presentation and initiation of (auto)immune response. Our work identified the HLA and MICA alleles in SLE patients and healthy controls of Czech, central European population. **Methods:** All of 123 SLE patients fulfilled at least 4 of the ACR criteria. As control group we investigated 96 healthy Czech individuals. SSP PCR and PCR-fragment analyses were used for HLA class II and MICA allele detection. **Results:** (1) Of all five alleles (MICA – A4, A5, A5.1, A6, A9) detected in Czech population, the MICA-A5.1 was significantly more common in SLE than in controls (55.7% vs. 39.9%, Pcorrected 0.005, OR 1.88, CI95% 1.29-2.77), whereas the MICA-A6 was occurred only in 10.6% SLE unlike 19.7% in controls (Pcorrected 0.035, OR 0.48, CI95% 0.28-0.82). There were no statistical differences in genotype distribution among two groups. (2) The HLA - DRB1*03 allele was found in 22.8% SLE and 10.6% controls, pc 0.008, OR 2.5, CI95% 1.44-4.27. Similarly, the HLA - DRB1*03-DQB1*0201 haplotype showed higher frequency in SLE group (44.7%) in comparison to controls (15.2%), pc <0.0001, OR 4.54, CI95% 2.36-9.09. (3) The MICA-A5.1 allele together with HLA - DRB1*03 is strongly associated with SLE (pc <0.000001, OR 9.71, CI95% 3.4-27.7). No linkage disequilibrium between MICA and HLA class II (DRB and DQB) alleles was identified. (4) There were no differences in MICA and HLA class II allele distribution among SLE patients divided according to clinical and serological manifestation. **Conclusion:** The MICA-A5.1 allele increases the risk for SLE in HLA - DRB1*03 susceptible individuals. We confirm HLA - DRB1*03-DQB1*0201 haplotype as a risk for SLE in Czech population. *Supported by MHCR: Research Project MZO 00023728 and grant NS 10618 - 3.*

PO1.G.39

Variant in the prolactin gene in systemic lupus erythematosus patients modulates prolactin, DHEA and estradiol levels

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Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease involving multiple organ systems. SLE is often detected in women in their reproductive years at a ratio of 9:1 compared to men. African American and Hispanic women have five times the incidence when compared to Caucasian women. The objective of this study was to determine the relevance of the 1149G/T functional single-nucleotide polymorphism (SNP) in the extrapituitary promoter in a cohort of African American and Caucasian women with and without lupus. The -1149G/T functional single-nucleotide polymorphism (SNP) in the extrapituitary promoter has been associated with higher prolactin levels in a cohort of women with lupus; however, that cohort did not contain sufficient numbers of African American women. Using RFLP and DNA sequencing the SNP was evaluated and correlated with prolactin, DHEA, and estrogen levels. This study examined the frequency of the promoter 1149G/T SNP in both African American and Caucasian women with (84) and without (56) lupus. The frequency of this SNP was also examined in a small cohort of men. Results of the study revealed that the TT-1149 genotype in women with lupus correlated to lower levels of DHEA in serum, and higher levels of prolactin in serum (p<.0001). The GT-1149 and GG-1149 and genotypes correlated to higher DHEA levels and lower prolactin levels in women with lupus. Women with lupus with the TT-1149 genotype had significantly higher levels of estradiol (p<0.03) when compared to those with the GT or GG genotypes. The TT-1149 (34%) and GT (46%) genotypes were prevalent with women with lupus. In the small cohort of men with lupus ,a higher frequency of the TT-1149 genotype was noted. Preliminary results suggest that the TT-1149 genotype may be a risk factor for lupus. Also, it was noted that a higher percentage of African American women had the TT-1149 genotype.

PO1.G.40

The integrity of double strand break repair in pediatric systemic lupus erythematosus cells

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Objective: Our laboratory previously demonstrated a delay in single strand break (SSB) processing in cells from pediatric patients with systemic lupus erythematosus (SLE). In general, SSBs are converted to double strand breaks (DSBs) at replication forks. Polymorphisms in the non-homologous endjoining (NHEJ) DSB repair protein XRCC4 have recently been associated with neuropsychiatric lupus and antiphospholipid syndrome. This study assesses the integrity of DSB recognition, signaling, and repair mechanisms in B-lymphoblastoid cell lines (LCLs) derived from pediatric and adolescent patients with SLE. **Methods:** Eight assays were used to assess four major pathways of repair of DSBs in pediatric SLE LCLs. The assays included: (i) γ-H2AX and (ii) 53BP1 ionizing radiation (IR)-induced nuclear foci (IRIF); (iii) immunoblot analysis of the kinetics of IR-induced phosphorylation of Structural Maintenance of Chromosomes 1 (SMC1); (iv) a DNA ligation assay to evaluate NHEJ; (v) neutral comet assay; (vi) monoubiquitination of FANCD2 to test the Fanconi pathway; (vii) flow cytometry to assess the S-phase checkpoint; and (viii) colony survival assay (CSA) as a measure of radiation sensitivity. **Results:** Four of eight assays showed abnormal patterns of response to IR-induced DNA damage in some patients. The pSMC1 kinetics was decreased in two of the cell lines indicating a defect in DSB recognition and recovery. Delayed DSB repair was observed in five cell lines using the neutral comet assay Defective in-vitro NHEJ was abnormal in two cell lines. One cell lines exhibited increased radiation sensitivity by CSA when compared with wildtype controls **Conclusion:** A variety of abnormal DSB repair defects were observed in some but not all of the cell lines derived from patients with SLE in this study. These post-IR abnormalities provide a line of evidence that indicates DSB repair and oxidative stress are involved in the pathogenesis of SLE. Further, these abnormalities not only demonstrate the complexity of DSB repair post-IR but also the involvement of multiple factors responsible for the observed clinical and cellular phenotypes observed in patients with SLE . Future research examining DNA repair processes in SLE patients will be important to define the etiology of SLE and provide tools for future research endeavors.

PO1H Health Services

PO1.H.1

Primary care service for Thai adolescents with SLE : parent's perspective

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Objective of this qualitative study was to explore the perception of parents related to primary care service for adolescents with SLE in Thailand. Participants in this study were parents of adolescents with SLE. Thirteen participants were selected by purposive sampling. Ten mothers and three fathers were key informants. Age between 25-48 years old. Most of them were farmers. All of them lived in North-East region of Thailand. After they signed in consent forms, in-depth interview, tape recorder, and observation were used to data collection. Content analysis was used to analyze qualitative data. The results showed that parents' perspective on primary care services in Thailand were not appropriate to care patients with SLE. They thought that SLE disease was the complex and difficulty to treat and care. Especially in adolescence stage, who were in transitional period from children to adult. Adolescents with SLE patients need specialist and expertise to care them. In Thailand, all of SLE clinic and specialist must be meet in tertiary hospital. But some of SLE adolescents necessary went to primary care when they had recurrent phrase and need emergence service. Primary care service could not treat anything for SLE patients. They should be took information that they had not doctor specialist to care SLE patients and referred all of these patients to high level of service everyone. Some patients with SLE died between referral to tertiary care, while primary care provider thought that it was out of control. A father of SLE adolescent said that he actually did not send his daughter to the primary care. Because primary care had the murderers more than health providers. Quality of services in primary care will be discussed. The results indicated that primary care in Thailand should be developed and improved quality of services for adolescents with SLE.

PO1.H.2

Health care cost in a Canadian population of patients with & without lupus nephritis

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Objectives: To determine whether health-care cost differs for SLE patients with & without Nephritis (LN & LNN, based on renal biopsy or ACR criteria). **Method:** LN and LNN patients enrolled in the Lupus Nephritis New Emerging Team study in Canada, were classified into those with active (ALN & ALNN) and inactive disease (ILN & ILNN) based on SLE disease activity index (SLEDAI). Scores>6 were considered active disease. Patients reported

on health care resource utilization including: visits to health care professionals, use of: diagnostic tests, assistive devices, alternative treatments, hospital emergency visits, surgical procedures and hospitalization in the 4 weeks preceding enrollment. Annual cost, not including the medications was calculated. *Results:* 141 patients (121 female, 20 male), 79 with LN (ALN: 53, ILN: 26) and 62 with LNN (ALNN: 38, ILNN: 24) were enrolled. LN patients were significantly younger compared to LNN (36.5±13.6 vs. 43.8±15.1 years; P=0.003) and had a higher SLEDAI score (9.5±7.0 vs. 5.1±3.8; P=0.0001). Compared to LNN, patients with LN were more likely to visit health professionals (88.6% vs. 74.2%; P=0.026) and had a higher number of visits to rheumatologists (0.8±0.1 vs. 0.6±0.1; P=0.09); family physicians (0.9±0.2 vs. 0.5±0.1; P=0.041) and nephrologists (0.3±0.1 vs. 0.0±0.0; P=0.001). The cost of visits to medical doctors was higher for LN compared to LNN ($112 ±96 vs. $86±86; P=0.094). LN patients were also more likely to undergo diagnostic tests (81% vs. 62.9%; P=0.016), most commonly blood (1.7±0.2 vs. 1.1±0.2; P=0.036) and urine tests (1.2±0.2 vs. 0.5±0.1; P=0.001). The use of assistive devices, alternative treatments, hospital emergency visits and hospitalization periods were similar between the two groups. The annual health care cost averaged at $7,361±9,096 for LN and $7,688±13,864 for LNN (P=0.88). Compared to patients with ILN, those with ALN, used more diagnostic tests (4.3±3.8 vs. 1.8±2.0; P=0.003), associated with a higher monthly cost for these tests ($176±294 vs. $53±83; P=0.006) and performed more surgical procedures (9.6% of patients vs. 0.0%), with a difference in monthly cost of $77±239 (P=0.024). The annual health care cost was higher for ALN ($8,525±9,314) than ILN ($4,888±8,262; P=0.09). In LNN, there was no difference in annual cost between ALNN ($9,590±16,937) and ILNN ($ 4,915±6,865; P=0.15). The use of complementary therapies, assistive devices, emergency hospital visits and hospitalization were similar between all groups. *Conclusion:* Health care resource utilization is similar between LN and LNN but is much greater in ALN than ILN.

PO1.H.3

Contraceptive counseling and use among women with SLE

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Objectives: Pregnancy can be complicated for women with SLE by disease activity and medication use. We sought to examine contraceptive counseling and use in a cohort of women with SLE. *Methods:* Data derive from an ongoing telephone survey of persons with confirmed SLE, recruited from clinical and non-clinical sources. In the 2008 questionnaire, participants were queried about SLE activity (Systemic Lupus Activity Questionnaire; SLAQ), treatments, and healthcare utilization, as well as reproductive history, family planning, and receipt of contraceptive counseling in the past year. Pregnancy intentions were assessed using a validated version of the London Measure of Unplanned Pregnancy. Premenopausal women under age 45 who were not surgically sterilized and were sexually active with men were considered at risk of pregnancy. We compared rates of contraceptive counseling and use, stratified by treatment with teratogenic medications, using chi-square tests. *Results:* Among 206 women under age 45, 115 were not at risk for pregnancy and 6 were pregnant or attempting to become pregnant, leaving 86 at risk for unintended pregnancy (see table). The mean age in this latter group was 33±6 years; 42% were Caucasian; 65% college graduates; most had active disease (mean SLAQ 8.0±6.4). Although more than half (46/86) reported treatment with potentially teratogenic medications, only 37% of these women reported receiving contraceptive counseling; 26% (12/46) reported inconsistent use of contraception, and 61% depended on barrier methods. Women using teratogenic medications were no more likely than those not on such medications to have received contraceptive counseling, to use contraception consistently, or to use more effective contraceptives. Intrauterine contraception (IUDs) was used by only 13% (11/86). *Conclusions:* In this well-educated cohort, most women at risk for unintended pregnancy reported no contraceptive counseling in the past year, despite common use of potentially teratogenic medications. Most women relied upon contraceptive methods with relatively high failure

rates and few used IUDs, a method offering effective, reversible contraception without increasing vascular risk. These findings suggest the need for interventions to improve contraceptive services for women with SLE.

Women, age <45	Total (n=206)	On potentially teratogenic medications (n=116)
Not at risk for pregnancy	114 (55%)	67 (58%)
Post menopausal	23 (11%)	12 (10%)
Surgically sterilized	44 (21%)	25 (22%)
No male partner/partner sterilized	47 (23%)	30 (26%)
At risk for pregnancy	92 (45%)	49 (42%)
Pregnant	3 (1%)	0 (0%)
Trying to become pregnant	3 (1%)	3 (3%)
Not trying to become pregnant*	86 (42%)	46 (40%)
Frequency of contraceptive use		
Never	10 (12%)	6 (13%)
Sometimes	9 (10%)	6 (13%)
Always	67 (78%)	34 (74%)
Methods of contraception		
None	10 (12%)	6 (13%)
Barrier method only	46 (53%)	28 (61%)
Other methods**	30 (35%)	12 (26%)
Contraceptive counseling in past year		
Yes	35 (41%)	17 (37%)
No	51 (59%)	29 (63%)

* 11 women reported that 'they wouldn't mind' becoming pregnant.

** Includes 15 women on estrogen-based methods, 4 on progestin only methods, and 11 women using an intrauterine device (IUD).

PO1.H.4

A pilot study of dynastic balance status in patients with systemic lupus erythematosus

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Objective: The aim of the study was to evaluate and study how to improve dynastic balance status in SLE patients with psychoneurotic problem. *Method:* SLE patients who fulfilled ACR criteria were recruited and divided into 2 groups: psychoneurotic SLE patients group (n=8) and control group (n=8), excluding various factors that influence patients' balance ability: such as visual factor, abnormal vestibular function and so on. The dynastic balance ability of people in two groups were assessed at baseline. The patients were supervised to have exercise 3-4 times per week in order to improve their balance ability. Dynastic balance ability of patients in SLE group was reevaluated 3 months after this study. *Results:* The balance ability in psychoneurotic SLE patients is reduced and it can be improved through exercise (P<0.05). *Conclusion:* The data demonstrates that it is necessary to pay close attention to balance status in patients with psychoneurotic SLE and the balance exercise can improve patients' balance ability, thus reducing the risk of their fall, wrench and fracture.

PO1.H.5

Impact of individual, neighborhood, and healthcare delivery system characteristics

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Objectives: To assess the impact of overall health status, sociodemographics, nature of health insurance, characteristics of local community, and variation among local health care markets for hospital services (HSAs) on utilization

of physician services among persons with SLE (MDSLE). ***Methods:*** Data are derived from the Lupus Outcomes Study (LOS), a U.S. cohort of 755 persons with confirmed SLE diagnoses. Principal data collection is from an annual structured telephone interview covering demographics, SLE symptoms and activity, overall health status, and health care utilization. Present analysis includes 2926 person-years of observation from the 2003-2007 interviews. Using geocoded addresses, contextual data were appended for neighborhood socioeconomic characteristics (source: 2000 Census), subspecialists per capita in the county (source: Area Resource File), and HSAs (source: Dartmouth Health Care Atlas). A linear mixed model estimated the impact of fixed effects for education, health care access, and living in a neighborhood with a high proportion of persons below poverty and random effects for the HSA on MDSLE, after adjusting for other sociodemographic characteristics and SLE severity and overall health status. ***Results:*** LOS respondents reported a mean of 11.2 (95% CI 10.3-12.1) MDSLE. Persons with SLE with a high school education or less, living in areas of concentrated poverty and receiving care from HMOs and generalist physicians had significantly fewer MDSLE (Table). Those with a combination of ≤ high school education and who lived in poverty areas had only 74% as many MDSLE as the remainder; those with a combination of HMO and generalist MDs had only 63% as many as the remainder (both results were statistically significant). There was a statistically significant impact of HSAs on MD-SLE, indicating that healthcare markets have an effect on utilization beyond individual and neighborhood characteristics. ***Conclusion:*** Personal, health care system, and community characteristics each contribute independently to MDSLE. Low levels of education, living in areas of concentrated poverty, receiving care from a generalist and being in an HMO reduce MDSLE. The healthcare market also affects MDSLE.

Education	Mean (95% CI)	HMO	Mean (95% CI)
Less than HS	10 (9,11)*	No	12 (11,12)*
Some College	12 (11,12)	Yes	11 (10,11)
College Grad	12 (11, 13)		
Poverty Area		Main SLE MD	
No	12 (11,12)*	Generalist	8 (7,9)
Yes	10 (9, 11)	Specialist	12 (11,12)*

PO1.H.6

Current practices in the management and monitoring of moderately active and life threatening SLE in South Africa
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Introduction: South Africa (SA) is a middle-income country with a total population of 48.5 million, of whom about 85% depend on state-funded health services (health expenditure per capita of US$200). ***Methods:*** We describe our collective experience at 3 large public sector tertiary hospitals serving major metropolitan areas with a total catchment population of about 10 million. ***Results:*** The approximately 600 adult SLE patients that are followed-up annually, of whom about 80 are new patients, are served by 11 rheumatologists. The standard drugs that are available for treating SLE include chloroquine, parenteral and oral corticosteroids and immunosuppressive agents (methotrexate, cyclophosphamide, azathioprine). Intravenous immunoglobulin (IVIG) therapy and mycophenolate mofetil are available on a limited scale. In general patients with moderately active disease are reviewed at 3 monthly intervals. Laboratory investigations to assess disease activity at each visit include a full blood count, ESR, CRP, C3 and C4, anti-dsDNA titres and urine dipstix. Life-threatening disease is treated with pulse intravenous methylprednisolone and, where indicated, cyclophophamide and/or IVIG, followed by oral corticosteroids. In spite of the availability of these drugs, the prognosis of SLE in SA remains poor (best case scenario: 72% 5-year survival rate) with most deaths related to either infections, particularly tuberculosis, or renal failure. ***Conclusion:*** Addressing the unmet needs of early detection of SLE at primary care

level and additional dialysis and intensive care facilities should result in better outcome for the indigent SLE patient in SA.

PO1.H.7

Is rheumatologist based quality of care any different at university vs county hospital for SLE patients?
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Introduction: Systemic Lupus Erythematosus (SLE) adversely affects physical, as well as social and psychological aspects of the patient's life. Physicians have a unique opportunity to educate, intervene and support their patient through their chronic illness, and thus influence the patient's health outcomes. Patient's perceptions about overall satisfaction with their medical care, physician accessibility, patient education, explanation and monitoring side effects of medications used, and physician's understanding the effects of the disease on their daily life are some measures of physician quality of care (P-QOC). County clinics mostly provide high volume medical care to poor and uninsured patients, therefore they may be lacking in resources available. We aimed at comparing P-QOC between two centers (University and County based rheumatology clinics) providing longitudinal care to SLE patients. Care at both centers was provided by rheumatologists. ***Methods:*** Previously collected outpatient data pertaining to demographic, clinical and health outcomes data on forty-two SLE subjects from the University clinic was compared with forty-one SLE subjects from the County clinic. All patients met the American College of Rheumatology classification criteria for SLE. Groups were matched by insurance type, demographics (age, ethnicity, and sex), and disease measures (disease activity-SLEDAI and disease damage-SLICC/SDI). P-QOC data was collected using 5 items with Likert scale from the LupusPRO®. Data were analyzed using chi-square analysis and t-tests to compare P-QOC at the two centers. A p-value of ≤0.05 was considered statistically significant on two tailed tests. ***Results:*** Mean (SD) age of the subjects was 41.60 (13.66) years. The mean (SD) SLEDAI and SDI were 6.17 (6.76) and 1.55 (1.70) respectively. University and County patients rated their overall satisfaction with their medical care (Mean (SD) 3.28 (1.05) vs 3.00 (1.22), p=0.47) respectively. However, University patients ascribed higher P-QOC scores than County patients on "My doctor understands the impact of lupus on my life" (Mean (SD) 3.44 (1.04) vs 2.90 (1.26), p=0.04) and "My doctor provided me with information to understand my disease" (Mean (SD) 3.33 (1.28) vs 3.05 (1.23), p= 0.05) respectively. Regarding "Physician accessibility when I had a question" (Mean (SD) 3.36 (0.95) vs 3.20 (1.05), p=0.47) and "My doctor discussed/monitored the side effects of lupus medicine" (3.23±1.245 vs 3.03±1.23, p=0.48), no significant differences were observed between the two centers. ***Conclusions:*** Patient perceptions of physician-based quality of care differed across the two centers, even though the demographic, insurance type, and disease features were similar in the two SLE groups. This study reinforces the need to further optimize patient educational opportunities and physician-patient relationships at County Hospitals to improve overall quality of care.

PO1.H.8

Lupus awareness ad council campaign
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Objectives: Demonstrate how a public awareness campaign can increase awareness among young minority women of childbearing age (18 to 44), to identify early warning signs of Lupus so they can ask their doctor or community health center for a medical evaluation. Show how young minority women who visit HHS' Office on Women's Health (OWH) web sites (www.couldihavelupus.gov and www.podriayotenerlupus.gov) or call OWH's toll

free number1-800-994-9662, can learn more about the symptoms and treatment options for lupus and access local resources. *Methodology:* Develop a Public Awareness Campaign designed to raise public awareness and understanding about this chronic disease that affects young women in hopes of an early diagnosis. *Conducted exploratory research in late 2007 that found:* (i) Healthy women are passive regarding personal health care and are not concerned about most health conditions that may affect them; (ii) Many have not heard of lupus; those who have are limited in knowledge; (iii) Lupus patients, while extremely involved in their treatment and care, knew nothing about the disease before diagnosed. They considered this to be a serious disadvantage on several levels; (iv) Created strategy brief in March 2008 to define creative elements including television, radio, print, outdoor (billboard and bus shelters) and internet banners. All the materials available in Spanish; (v) Tested creative elements in 2008. *Tested issue awareness in early March 2009, which showed:* (i) Minimal awareness of Lupus messaging; (ii) Most women demonstrated a basic understanding of lupus; but reported a lack of knowledge about the disease; (iii) Launched campaign on March 31, 2009. *Results: March 31, 2009 – December 31, 2009.* (i) Reached an audience of more than 26 million people with public relations launched on March 31, 2009; (ii) Received more than 27.24 million dollars worth of donated media, exceeding the average Ad Council campaign. *www.couldihavelupus.gov results:* (i) 26 average number of pages viewed per visit; (ii) Over 7 minutes average (7.49) per visit; (iii) 27,589 average number of visitor sessions per month; (iv) 36-59% visitors per month coming from direct traffic; (v) Over 12,000 downloads. As of April 14, 2010, 1,830 postings and 641 diary entries uploaded from women who have either been diagnosed with lupus, think they have lupus or family members who have relations with lupus. *Conclusions:* Campaign messaging will continue to prompt women to take action by asking questions about their symptoms and finding answers.

PO1I Immunology – B Cells

PO1.I.3

Tolerance mechanism permitting autoreactivity by human CD5+ pre-naïve B cells

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Background: CD5+ pre-naïve B cell population is a newly identified unique intermediate between transitional and naïve B cells during human peripheral B cell development. Although developmentally immature, CD5+ pre-naive B cells, at the same time, have capacities of the mature naïve B cells to differentiate and present antigen. Because of functional immaturity, most of CD5+ pre-naïve B cells are predicted to be removed by negative selection at peripheral tolerance checkpoint. However, because they possess functions of mature naïve B cells, inefficient tolerance checkpoint regulation at CD5+ pre-naïve B cell stage, may result in systemic autoimmunity. **Objective:** To determine the tolerance mechanisms operating at CD5+ pre-naïve B cell stage that may contribute to development of autoimmunity. **Methods:** Comprehensive comparative analysis of Ig H gene repertoire at transitional, CD5+ pre-naïve, and naïve B cell human peripheral B cell developmental check point was performed. VH rearrangements were amplified from genomic DNA of individual B cells by polymerase chain reaction. Analysis included 133 VH rearrangements from the transitional B cells, 371 from the CD5+ pre-naïve B cells, and 185 from the naïve B cells. To predict the autoreactivity of a B cell receptor, presence of long IgH CDR3, and highly positively charged amino acids were analyzed. **Results:** By comparing the distribution between the productive and the nonproductive repertoires of transitional, pre-naïve, and the naïve B cells, we found that negative selection for long and highly positive charged CDR3H occur at all human peripheral B cell developmental stages. However, analysis of the productive repertoires revealed that CDR3H length of pre-naïve B cells (52.5±12.5bp) was significantly longer compared to that of the transitional (48.9±12.6bp) and the naïve (49.0±10.5bp) B cells

(p<0.01). This skewing for longer CDR3H length in pre-naïve B cells was due to maintenance of significantly longer germline D segment. Notably, an increased proportion of CD5+ pre-naïve B cells were found in peripheral blood of patients with systemic lupus erythematosus (SLE). **Conclusion:** Distinctive feature of longer CDR3H in the expressed repertoire of CD5+ pre-naïve B cells suggests auto/poly-reactivity of these cells which may perform beneficial functions in normal immune regulation. However, ineffective tolerance checkpoint regulation at CD5+pre-naïve B cells may contribute to autoimmune pathology in patients with SLE.

PO1.I.4

Evaluation of memory B cells phenotype markers in systemic lupus erythematosus patients

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Objective: phenotyping of memory B cells has been difficult, for that reason many strategies to find memory markers have been evaluated. For a long time, CD27 had been used as a universal marker of human memory B cells. However, in SLE patients, memory B cells populations CD27- have been described by means of rhodamine 123 (R123) stain. Additionally, as TLR9 is expressed constitutively in memory B cells, in this study, we evaluated the expression of these three markers to define memory B cell phenotype in patients with SLE and healthy controls. **Methods:** peripheral blood mononuclear cells (PBMC) from patients diagnosed with SLE according to the 1982 criteria of the American College of Rheumatology from Hospital Universitario San Vicente de Paul and healthy controls were studied. We used flow cytometry to analyze CD27, TLR9 and R123 as memory markers in CD19+ cells. **Results:** PBMC of 19 (18 women and 1 man) SLE patients and 18 (17 women and 1 man) healthy controls were analyzed. The medians of age (range) were 34 (21-59) and 34 (22-62) respectively. We found 45.2% of LB CD27+ and 59% of LB R123+ in healthy controls, while in SLE patients, the proportions of them were 28.8% and 63.4% respectively. The proportion of LB CD27+ R123+ in SLE patients was 34.6%, and 27.2% in healthy controls; however only a 4% of these cells CD27+ R123+ were TLR9+ in both groups. In healthy controls, the proportion of LB CD27- R123+ was 22.2% while in SLE patients it was 31.1%; only 1% of these cells CD27- R123+ were TLR9+ in both groups. **Conclusions:** The high proportion of LB R123+ found in SLE patients suggests the existence of a subpopulation of memory B cells CD27-R123+ which is in agreement with previously reports. The low percentages of CD27+ TLR9+ and CD27- TLR9+ B cells are in contrast with the high percentage of R123+ cells; this finding make doubtful the role of TLR9 as a memory marker. Our results and the previously described ones suggest that R123 is a good memory marker.

PO1.I.5

The effects of the anti-CD22 monoclonal antibody epratuzumab on B cell surface proteins

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Objectives: Epratuzumab is a monoclonal antibody against CD22 that is currently being evaluated clinically in Systemic Lupus Erythematosus (SLE). Epratuzumab is known to induce internalisation of CD22 on B cells (Jacobi *et al.* (2008) Ann Rheum Dis 67: 450). The aim of the current study was to investigate the kinetics of internalisation on B cells from healthy volunteers and to look at a range of other cell surface proteins. **Methods:** B cells were purified by negative selection from human blood and incubated with a concen-

tration range of epratuzumab or an isotype control antibody over time, washed and then stained with fluorescently-labeled antibodies specific to a range of B cell surface proteins and analysed by flow cytometry. In order to assess the expression of CD22 in the presence of epratuzumab, a non-competing anti-CD22 antibody (S-HCL-1-PE) was employed. CD22 internalisation was also evaluated in a qualitative manner in confocal microscopy experiments employing Alexa488-labeled epratuzumab. **Results:** Epratuzumab caused rapid internalisation of B cell surface CD22 in a range of experiments. Maximal internalisation occurred in 30-60 minutes at concentrations above 1-2 µg/mL but the level of internalisation typically did not exceed 50-70%, even at higher antibody concentrations, suggesting that some B cell surface CD22 is resistant to epratuzumab-induced internalisation. Internalisation was prevented by prior cell fixation with paraformaldehyde and was reduced at lower temperature. Internalisation of CD22 on B cells in response to epratuzumab treatment was also confirmed using confocal microscopy. Although epratuzumab was capable of modulating the expression of CD22 in B cell cultures, it had no consistent effect on several other B cell markers including IgM, IgD, CD19, CD20, CD27, CD32, CD38, CD69, CD79b, CD95, HLA-DR Class II and CD62L measured at a variety of time points up to 6 days. **Conclusions:** Epratuzumab stimulated rapid internalisation of its target, CD22, on human peripheral blood B cells *in vitro* but had no consistent effect on a range of other B cell markers. This could lead to modulation of B cell functional responses that are regulated specifically by CD22, which may be relevant in the context of SLE.

PO1.I.6

Danger zone of SLE and TB : alarming diagnostic, therapeutic dilemmas: novel B-cell targeted therapies to rescue?
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SLE patients, as such immunocompromised, conventionally treated with steroids and cytotoxics are easy targets for opportunistic infections: The riddle of TB, with its bizarre and baffling clinicopathology has defied early detection and effective treatment. TB prevalence in SLE seems underestimated, underreported : 1.3% in Spain, 1.5% in Turkey, 20% (40 times higher) in India. In free Arthritis Camps the author conducted in village setting in India (n=6450), 17% of all SLE patients had manifest, detectable TB. Inherent limitations in laboratory diagnosis of TB, equivocal Mantoux test presentations call for expeditious improvement of newer immunological tests such as PCR, quantiferon gold tests, reported "30 minute spot" screening test, made practical with acceptable specificity-sensitivity ratios. Four-drug anti-TB regime has been the only salvation over decades albeit the rise of 'MDR / XDR' resistant TB cases. The havoc of HIV virus further poses therapeutic challenges. SLE has not known new treatments approved by USFDA over last 25 years. TB & SLE, if not detected and treated aggressively in time, is almost certain to have long-term morbidity, even high mortality. Isoniazid prophylaxis is routinely prescribed to most SLE patients at our clinic. In flare-up cases, aggressive B-cell depletion offers promising option to induce remission of SLE minimizing complications. Rituximab currently seems the choice anti-CD20 biological with relative safety as reported, and as of our own case reports. Newer B-targeted therapies under evaluation are ocrelizumab (humanized anti-CD20), small modular immunopharmaceutical (SIMP) anti-CD20 and other anti-CD22, and belimumab. The key message is, TB detection should be mandatory in every SLE patient in high TB endemic areas, and a definite add-on option even elsewhere. Newer diagnostic nanotech kits, and biotech targeted therapies, together with judicious conventional treatments, are advocated / anticipated to reduce morbidity, and mortality of SLE ± TB patients with a promising scenario.

PO1.I.7

Peripheral blood B cells subsets in patients with systemic lupus erythematosus: correlation with disease activity and organ involvement.
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Objective: The aim of the study was to analyze the frequency and distribution of B cells subsets in a cohort of patients with SLE with different organ involvement and the possible correlation with disease activity. **Patients and methods:** 60 SLE patients (53 females; mean age 39.0±13.6 years; 29 with renal, 21 with articular, 4 with SNC, 4 with vascular and 2 with cutaneous involvement; 30 with an active disease-SLEDAI>10) and 30 healthy controls matched for age and sex were analyzed for the distribution of circulating PB B cell subpopulations by staining for CD19, CD38, and IgD in combination with the B cell memory marker CD27, by flow cytometry. **Results:** Considering the Bm1-Bm5 classification, there was no difference in the percentage of B cells subpopulations between SLE patients and controls. Instead, SLE patients showed an higher percentage of CD19+/ZAP70+ cells (6.1±6.2%) compared with controls (2.1±1.4, p=0.01). In SLE patients, disease activity index (SLEDAI) correlated positively with the percentage of CD19+ cells (r=0.43, p=0.001) and with the absolute number of CD19+/CD27+/IgD- (r= 0.42, p=0.004), CD19+/CD27+/CD38+ (r=0.37, p=0.01), CD19+/CD27-/IgD- (r=0.31, p=0.04) and CD19+/ZAP70+ (r=0.41, p=0.005). SLE patients with an active disease had an higher percentage of CD19+ cells compared with patients with inactive disease (11.0±7.9% vs 4.4±4.2% respectively, p<0.001). Patients with active disease had an higher absolute number of memory cells (eBm5+Bm5 34±36/ul, CD19+/CD27+/IgD- 16±17), CD19+/CD27+CD38+ (10±13) and CD19+/ZAP70+ (7±10) compared with patients with inactive disease (eBm5+Bm5: 12±12, p=0.001; CD19+/CD27+/IgD-: 5±5, p<0.001; CD19+/CD27+CD38+: 3±3, p=0.001; CD19+/ZAP70+: 2±3, p=0.04). The distribution of Bm1-Bm5 and IgD/CD27 subsets was similar in patients with renal and articular involvement, even though considering only the subgroup with active disease, patients with renal involvement showed an higher percentage of memory B cells (eBm5+Bm5 36.3±21.2%) and of CD19+/CD27+CD38+ (11.8±8.6%) compared with patients with articular involvement (eBm5+Bm5: 15.0±8.5%, p=0.003; CD19+/CD27+CD38+: 5.0±4.0%, p=0.03). This difference was also confirmed by the ratio Bm2+Bm2'/eBm5+Bm5 in active renal disease (2.1±2.9) compared to 6.5±6.0 in active articular disease (p=0.005). Moreover, SLE patients with active renal disease had an higher percentage of CD19+/ZAP70+ cells (7.1±5.0) compared with patients with active articular involvement (3.3±2.6, p=0.04). **Conclusions:** SLE patients showed a significant reduction of memory B cells compared with controls, regardless of the organ involvement. An increase of memory B cells appeared, however, in patients with renal active engagement, while an higher percentage of active cells was present in patients with active articular involvement.

PO1.I.8

IL-17 down-regulated p50 to sustain increased NF-κB signaling responses in splenic B cells of autoimmune BXD2 mice
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Purpose: Increased activation of NF-κB signaling is associated with many autoimmune diseases. BXD2 mice express high levels of IL-17 and spontaneously develop lupus and erosive arthritis. High IL-17 in these mice correlate with constitutively active NF-κB signaling and upregulated Rgs genes expression in splenic B cells, and result in suppression of B cell chemotactic responses to CXCL12, leading to the development of spontaneous germinal centers. This study is to explore the IL-17-mediated mechanism in sustaining increased NF-κB signaling and autoantibody production in BXD2 splenic

B cells. **Methods:** Purified splenic B cells from BXD2, BXD2-Il17ra-/- or B6 mice were incubated with or without IL-17 for varying time points. Cells were cytospun to detect phospho-p65 by immunofluorescence staining. The total cell lysates or the cytoplasmic and nuclear extracts were analyzed by Western blotting using antibodies against P-p65, p65, p50, p105, GAPDH and SP1. Expression of Rgs genes was determined by qPCR. **Results:** Splenic B cells from BXD2 mice exhibit dramatically higher protein levels of p50 and p105 than B6 B cells (>10.0-fold, p<0.001). Surprisingly, we also found both p50 and p105 in BXD2 B cells were significantly lower than those in B cells from BXD2-Il17ra-/- mice, indicating that IL-17 down-regulated p50 in B cells. Consistent with this finding, injection of AdIL-17RA:Fc to block IL-17/IL-17RA signaling in BXD2 mice led to significantly increased p50 and decreased phospho-p65 in splenic B cells, and decreased autoantibody production. In contrast, injection of AdIL-17A led to significantly decreased p50, increased phospho-p65 and autoantibody production. Stimulation of BXD2 B cells with IL-17 led to cytoplasmic translocation of p50 from the nucleus, while p65 was phosphorylated and translocated to the nucleus in 5 min. In BXD2-Il17ra-/- B cells, there was increased localization of p50 in the nucleus compared to the wild type BXD2 B cells. These increased nuclear p50 in BXD2-Il17ra-/- B cells were associated with decreased LPS and anti-CD40 induction of NF-κB response genes including Rgs which are essential for development of autoreactive germinal centers, indicating high p50 form homodimers in BXD2-Il17ra-/- B cells to inhibit these genes expression. **Conclusions:** Our finding suggests IL-17 down-regulate p50 and its nuclear localization to sustain efficient active classical NF-κB signaling responses in autoreactive BXD2 B cells. These results indicate a novel IL-17-mediated mechanism for resulting in production of pathogenic autoantibodies, lupus, and erosive arthritis, and will offer new therapeutic targets for prevention of autoantibodies-mediated autoimmune diseases.

PO1J Immunology – Innate

PO1.J.1

Cell surface S100A8/A9 on subpopulations of leukocytes in systemic lupus erythematosus

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Objectives: Systemic lupus erythematosus (SLE) is an autoimmune disease with immunological features such as ongoing inflammation, activation of leukocytes and production of pro-inflammatory cytokines. The heterodimer of the cytosolic calciumbinding proteins S100A8 and S100A9 (S100A8/A9) is secreted by activated neutrophils and monocytes and serves as a serum marker for several inflammatory diseases. The heterodimer is also expressed on the cell surface of monocytes and both S100A8 and S100A9 proteins are agonists of Toll like receptor 4 (TLR4). In the present investigation, we have studied the activation of leukocytes as well as cell surface S100A8/A9 on leukocytes in SLE patients and healthy controls. **Methods:** Isolated peripheral blood mononuclear cells were stained with antibodies and the cell surface S100A8/A9 determined on different cell populations. **Results:** SLE patients had a clearly increased percentage of activated leukocytes such as CD14+CD16+ pro-inflammatory monocytes (p=0.002), CD4+HLA-DR+ T-cells (0.0002) and decreased percentage of naïve CD19+CD27-IgD+ B-cells (p=0.006). Patients with nephritis or high levels of serum IFNα had decreased percentage of circulating plasmacytoid dendritic cells (pDCs) which could be compatible with redistribution of pDCs to tissue. Even though pDCs, monocytes and granulocytes were found to express high level of S100A8 and S100A9 mRNA, cell surface S100A8/A9 was detected on most cell populations in-

vestigated, including dendritic cells. No S100A8/A9 was detected on T-cells. Patients with active disease had increased cell surface S100A8/A9 on their pro-inflammatory monocytes which correlated to SLE disease activity index. SLE patients had also increased serum levels of S100A8/A9 heterodimers, and the S100A8/A9 serum concentration was increased particularly in patients with arthritis. **Conclusions:** Our findings in patients with SLE suggest that S100A8/A9 was increased both in serum and on cell surfaces and could be important in the amplification of inflammation. Thus, it could be regarded as a potent target for treatment of inflammatory diseases such as SLE.

PO1.J.2

Human keratinocytes actively contribute to skin inflammation in lupus erythematosus

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Cutaneous manifestations belong to the most common clinical features in Lupus erythematosus (LE). It was the aim of this study to further decipher the role of keratinocytes in the inflammatory response. In immunohistochemistry we found a high expression of inducible HSP70, IL-18R and IL-18 in lesional LE skin. Increased expression of TNFalpha and high mobility group box-1 (HMGB-1) has been described previously. Studies with cultured, patient derived keratinocytes showed that IL-18 stimulation results in significant TNFalpha production in LE derived but not in healthy keratinocytes. Keratinocytes from LE patients are more prone to die upon exposure to IL-18 and this increased apoptosis can be abrogated by blockade of endogenously produced TNFalpha. Furthermore, we could show that keratinocytes release high levels of HSP70. Here we provide evidence, that HSP70-peptide complexes are internalised by keratinocytes and that autoantigenic peptides induce the production of IFNgamma in T cells via this route. Of note, HMGB-1 as well as TNFalpha significantly enhanced the uptake of HSP70 into human primary keratinocytes. In conclusion, we provide evidence that skin resident cells play an active part in maintaining LE inflammation also by means of secretion of molecules which belong to the danger-associated molecular pattern (DAMP) family.

PO1.J.3

Increased numbers of CD14high CD16+ monocytes in patients with systemic lupus erythematosus (SLE)

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Objectives: In humans, three subpopulations of circulating monocytes have been described. These subpopulations were defined according to the expression of CD16 and CD14. In healthy individuals, it is estimated that approximately 90% of monocytes are CD14^{high}CD16- (classic monocytes) and approximately 2% of the monocytes had low expression of CD14^{low} and CD16^{high}. Last subpopulation has been associated with proinflammatory responses. The other subpopulation CD14^{high}CD16+ cells have been associated with IL-10 production. Alterations of these subpopulations have not been extensively studied in patients with Systemic Lupus Erythematosus (SLE). We compare the proportion of monocyte subpopulations in patients with SLE and healthy controls and determine the expression of HLA-DR in different monocyte subpopulations. **Methods:** Patients with SLE (n=17) diagnosed according to the American College of Rheumatology criteria and healthy controls (n=15) were evaluated. These groups were matched by gender and age. Twenty five

microliters of peripheral blood were stained with anti-CD14, anti-CD16, anti-HLA-DR. Samples were hemolyzed and analyzed by flow cytometry using a FACSCANTO II, Becton Dickinson. *Results:* There were not differences in the numbers of total CD14 cells in both SLE and healthy individuals; however we found statistically higher proportions and absolute numbers of CD14^{high} CD16+ monocytes (p ≤0.04). In patients with SLE, all subpopulations had a reduced expression of HLA-DR and CD16, suggesting possible functional alterations in this subpopulation. *Conclusion:* Our results indicated elevated proportion and numbers of CD14^{high}CD16+ cells which have been associated with IL-10 production. All mononuclear phagocytes displayed low expression of HLA-DR and CD14, indicating functional alterations in monocytes from SLE patients. The meaning of this finding needs to be explored studying the in vitro and in vivo function of these cells.

PO1.J.4

Genetically determined serum levels of mannose-binding lectin correlate negatively with common carotid intima-media thickness in systemic lupus erythematosus

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Objective: Patients with systemic lupus erythematosus (SLE) have excess cardiovascular morbidity and mortality due to accelerated atherosclerosis that cannot be attributed to traditional cardiovascular risk factors alone. Variant alleles of the mannose-binding lectin gene (MBL2), causing low serum levels of functional mannose-binding lectin (MBL) are associated with SLE as well as with severe atherosclerosis. Moreover, recent studies show that the variant alleles are associated with increased risk of arterial thrombosis and cardiovascular disease in patients with SLE. Intima-media thickness of the common carotid artery (ccIMT) is a validated non-invasive anatomic measure of subclinical atherosclerosis. In a cross-sectional study including 41 SLE patients we examined the relation between ccIMT, MBL2 genotypes and serum concentrations of MBL. *Methods:* The MBL2 extended genotypes (YA/YA, YA/XA, XA/XA, YA/O, XA/O, O/O) and serum concentrations of MBL were assessed in all patients. ccIMT was determined by means of ultrasonography. The following traditional and non-traditional cardiovascular risk modifiers were assessed: male sex, age, blood pressure, smoking, body mass index, serum cholesterol, insulin resistance, C-reactive protein, SLEDAI, treatment with glucocorticoids and DMARDs. *Results:* The median ccIMT was 0.57 mm (range: 0.37-1.5 mm). Seventeen patients had the low-expressing genotypes (XA/XA+YA/YO+XA/YO+YO/YO) and 24 patients had the high-expressing MBL2 genotypes (YA/YA+YA/XA). The median serum MBL concentration was 0.85 mg/L (range: 0-4.3 mg/L). Using non-parametric Mann-Whitney test we found a significant difference in ccIMT between low-expressing (XA/XA+YA/O+XA/O+O/O) and high-expressing (YA/YA+YA/XA) MBL2 genotypes (P-value = 0.035). The difference in ccIMT remained significant in a multivariable analysis adjusting for traditional and non-traditional cardiovascular risk modifiers (P-value = 0.032). ccIMT and serum MBL was shown to correlated negatively with a Spearman rho = -0.33 (P-value = 0.037). This negative association also remained significant in multivariate analysis (P-value = 0.023). *Conclusion:* MBL2 low-expressing genotypes and low serum levels of MBL are both correlated with ccIMT in SLE patients independently of the effects of traditional and non-traditional cardiovascular risk modifiers. These results provide further support to the notion that genetically determined low levels of MBL may be associated with atherosclerotic disease in SLE. Future studies should focus on the mechanisms by which MBL may influence development of atherosclerosis in SLE patients and if our findings may be applicable beyond SLE.

PO1.J.5

Regulation of VLA-4 in lupus monocytes

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Objectives: The development of atherosclerosis and subsequent increased risk of cardiovascular disease (CVD) is known to be associated with inflammation. Studies have shown an increased risk of CVD in systemic lupus erythematosus (SLE). Inflammation seen in atherosclerosis involves various immune mediators, including interactions between adhesion molecules on endothelial cells and on monocytes. Very late antigen-4 (VLA-4) is an adhesion molecule in the integrin family found on monocytes and has been implicated in monocyte recruitment during development of atherosclerosis. A recent study noted an increased expression of VLA-4 in SLE monocytes, but the role of VLA-4 and its regulation are poorly understood. We aimed to further define variables governing the expression of VLA-4 on monocytes in patients with SLE compared to healthy controls. *Methods:* CD14+ human monocytes were obtained from whole blood of healthy adult patients and SLE patients; flow cytometry was used to measure expression of VLA-4 at rest and upon exposure to various agents, including interferon gamma, interferon alpha, the TH2 cytokine interleukin-4 (IL-4) and the phosphoinositide 3-kinase (PI3K) inhibitor Wortmannin. *Results:* VLA-4 expression on control monocytes was $30.4 \pm 16\%$. In comparison, VLA-4 expression on lupus monocytes was $72.8 \pm 25\%$, a statistically significant increase (p<0.01, z-test). We next evaluated whether certain immunomodulators would affect expression of VLA-4. Treatment of control monocytes with IL-4 caused an up-regulation of VLA-4 expression ($7 \pm 4\%$, p<0.01, paired t-test). In the presence of Wortmannin, this effect of IL-4 up-regulation was inhibited. *Conclusions:* We established a baseline level of VLA-4 expression in monocytes in a healthy population. There was a significant up-regulation of expression of VLA-4 in SLE monocytes as compared to the healthy population. Although IL-4 has not been implicated in SLE pathogenesis, it has been suggested to play a role in VLA-4 mediated cell adhesion pathways during inflammatory processes in allergic diseases and multiple sclerosis. We noted the novel finding that IL-4 significantly up-regulated VLA-4 expression in monocytes. Using pharmacologic inhibitors, we determined that IL-4 regulation of VLA-4 in monocytes is mediated by PI3K signaling. This study confirms that there is elevated VLA-4 expression in SLE monocytes compared to healthy controls. In addition, it implicates a role for IL-4 in VLA-4 regulation in monocytes. These data provide a context for considering novel therapeutics to treat inflammation in SLE.

PO1.J.6

Expression of classical Fc-receptors and novel immune complex receptors on renal parenchymal cells

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Objectives: The goal of this study is to characterize the receptors for immune complexes (IC) expressed on parenchymal cells of the kidney. Kidney involvement is a major cause of morbidity and mortality in lupus. A critical step in the pathogenesis of kidney injury in lupus is the formation and deposition of IC in glomeruli. However, the mechanisms of IC deposition in the glomeruli are not well known. Despite efforts in the past to define the role of classical Fc-receptors (FcRs) on renal parenchymal cells in IC deposition, their expression remains debatable, and it is unclear whether renal IC receptor binding is pro- or anti-inflammatory. *Methods:* Glomeruli were isolated from non-immune mice, and mouse kidney cell lines were cultured: visceral epithelial cells (podocytes), endothelial cells, and pericytes (mesangial cells). IC receptor expression was systematically measured by quantitative RT-PCR, immunostaining, and Western blotting. In all, 27 genes encoding receptor subunits were tested, including 8 classical FcR, 6 immunoglobulin (Ig) transporter, and 13 candidate IC receptor molecules with highly conserved Ig domains and ITIM/ITAM motifs. In addition, individual cell types were treated with mouse IgG aggregates to study functional responses. *Results:* Mouse glomerular cells express significant numbers of both classical FcRs

and related proteins which have been considered candidate IC receptors. Classical FcR expression was largely restricted to mesangial cells, and overlapping subsets of candidate IC receptors were identified in each of the three glomerular cell lines. Mesangial cells and renal endothelial cells each bind heat aggregated mouse IgG (HA-IgG), and multi-plex antibody assays showed that cells respond to HA-IgG binding by production restricted combinations of cytokines and chemokines. Binding of HA-IgG to renal endothelial cells was not mediated by classical FcRs, and may be mediated by a unique member of the CD300 family, not expressed on leukocytes. *Conclusions:* IC specifically bind and activate glomerular parenchymal cell populations from non-immune mouse kidneys. Past difficulties in identification of IC receptors on resident kidney cells may be due to the predominance of "non-classical" IC receptors, including CD300 proteins, whose expression in some cases appear confined to parenchymal tissues.

PO1.J.7

Distinct mechanisms of granulocyte and monocyte migration in chronic inflammation associated with murine lupus

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Introduction: Chronic inflammation, characterized by the continuous recruitment and activation of immune cells such as monocytes and granulocytes in response to a persistent stimulus, is often associated with the development of autoimmune diseases. Although chemokine gradients play a prominent role in leukocyte migration, the mechanism(s) responsible for the sustained chemokine production and subsequent influx of granulocytes and monocytes in chronic inflammation are not well defined. Intraperitoneal administration of TMPD (2,6,10,14-tetramethylpentadecane; pristane) in mice potently induces chronic inflammation and potentiates the development of a lupus-like disease. In this study, we aimed to identify specific mediators and pathways responsible for the persistent recruitment of granulocytes and monocytes in the TMPD model of lupus. *Method:* Wild-type or cytokine-deficient mice were treated (i.p.) with TMPD. Surface markers on peripheral blood and peritoneal exudate cells were analyzed by flow cytometry. Chemokine expression in peritoneal exudate cells was analyzed by quantitative PCR and cytokine production in vitro was measured by ELISA. *Results:* TMPD treatment resulted in the persistent influx of granulocytes and Ly6C-hi "inflammatory" monocytes into the site of inflammation for more than two months. The innate adaptor MyD88, but not TRIF, was essential for this response as the migration of both cell subsets were abolished in MyD88-deficient mice. Interestingly, detailed analyses using cytokine-deficient animals and antibody blockade uncovered distinct mechanisms for the recruitment of granulocytes and monocytes. The migration of granulocytes was specifically initiated by IL-1α, but not other pro-inflammatory mediators including IL-1β, IL-6, TNFα, IFNγ and IFNα/β. IL-1 receptor signaling induced the production of CXCL5, a potent chemokines that promotes granulocyte chemotaxis via the receptor CXCR2. The persistent influx of inflammatory monocytes, on the other hand, was independent of IL-1 but depended on the production of IFNα/β downstream of Toll-like receptor 7 (TLR7) signaling. IFNα/β subsequently triggered the generation of monocyte chemoattractants CCL2/MCP-1, CCL7/MCP-3, and CCL12/MCP-5, leading to the recruitment of Ly6C-hi monocytes in a CCR2-dependent manner. While the deficiency of IL-1 receptor and TLR7 abrogated the migration of granulocytes and monocytes, respectively, MyD88 was required for the recruitment of both subsets due to its role as a shared adaptor for IL-1 and TLR pathways. *Conclusion:* Our findings reveal novel pathways responsible for the persistent recruitment of granulocytes and inflammatory monocytes in chronic inflammation. These findings may help us better understand the mechanism of chronic autoimmune inflammation in system lupus erythematosus.

PO1.J.8

CpG content in DNA-immune complexes is required for TLR9-dependent DC activation

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Objectives: Although Toll-like receptor-9 (TLR9) was originally thought to specifically recognize microbial DNA, it is now evident that mammalian DNA can be an effective TLR9 ligand, and this TLR9 activation may contribute to the pathogenesis of the autoimmune disease systemic lupus erythematosus (SLE). However, the DNA sequence required for TLR9 activation is controversial, as studies have shown conflicting results depending on the nature of the DNA backbone, the route of DNA uptake, and the cell type being studied. In SLE, a major route whereby DNA gains access to intracellular TLR9, and thereby activates dendritic cells (DCs), is through uptake as a DNA-containing immune complex. Our objective was to determine the DNA sequences required for TLR9 activation in this context. *Methods:* We used defined dsDNA fragments with a natural (phosphodiester) backbone and examined which DNA sequences are required for murine DC TLR9 activation induced by a DNA-containing immune complex. We measured cytokines IFN-alpha and IL-6 as well as upregulation of CD40 as markers of DC activation. *Results:* The strongest activation is seen with dsDNA fragments containing optimal CpG motifs (purine-purine-CpG-pyrimidine-pyrimidine) that are common in microbial DNA but rare in mammalian DNA. Importantly, however, activation can also be induced by CpG-rich DNA fragments that lack these optimal CpG motifs and that we show are plentiful in CpG islands within mammalian DNA. No activation is induced by DNA fragments lacking CpG dinucleotides, although this CpG-free DNA can induce DC activation if internalized by liposomal transfection instead of as an immune complex. *Conclusions:* Overall, the data suggest that the release of CpG-rich DNA but not CpG-free DNA from mammalian DNA may contribute to the pathogenesis of autoimmune diseases such as SLE and psoriasis in which activation of TLR9 in DCs by self DNA has been implicated in disease pathogenesis.

PO1K Immunology – T Cells

PO1.K.5

Targeted depletion of inducible co-stimulator (ICOS) bearing T cells prevents disease in a graft versus host mouse model of scleroderma

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Purpose: Inducible T-cell co-stimulator (ICOS) is a T cell-specific surface antigen that is expressed by recently activated CD4+ T cells, a subset of helper memory cells, and on T follicular helper (TFH) cells. Here, we investigated the function of ICOS-bearing T-cells in the pathogenesis of a graft-versus-host disease (GvHD) mouse model of scleroderma (SSc), utilizing a glycoengineered anti-mouse ICOS monoclonal antibody (MAb) with enhanced antibody-dependent cellular cytotoxicity (ADCC). *Methods:* GvH-SSc was induced by grafting B10.D2 splenocytes into Balb/c x Rag2 deficient mice, which recapitulates key aspects of human SSc, including inflammation, fibrosis, and vasculopathy. Mice were treated therapeutically with either the ADCC-enhanced depleting MAb or with a ligand-blocking anti-ICOS MAb. Development of skin pathology was monitored and scored for the entire duration of the study. In addition, FACS-based immunophenotype and gene array analysis was performed prior to ICOS MAb treatment (day 12) and at the end of the

study (day 26) to evaluate the autoimmunity and inflammatory components. *Results:* Administration of the depleting anti-ICOS MAb reduced severity and incidence of skin lesions when compared to isotype control MAb and control syngeneic graft, while treatment with the ligand-blocking anti-ICOS MAb was significantly less effective in controlling dermal disease progression. The depleting MAb prevented the disease-associated expansion of TFH cells and the associated generation of germinal center B cells in lymphoid tissues and inhibited the appearance of immunoglobulin secreting B cell gene transcripts in the skin. Further, the reduction in dermal fibrosis and inflammation by the depleting MAb correlated with complete inhibition of ICOS-bearing T helper- and inflammatory cell-associated cytokines, including IL-21, IFN-gamma, and IL-13 and a reduction in the levels of S100 family members. *Conclusions:* The results from this study indicate that ICOS positive T cells play an important role in the pathology of murine GvH-SSc, as selective depletion of the ICOS-expressing T-cell subset reduced the overall skin clinical disease score. The identification of dysregulated ICOS bearing TFH cells in GvH-SSc underscores their critical function in driving the generation and differentiation of pathogenic B cells into immunoglobulin secreting cells in the skin. MAb-mediated depletion of ICOS expressing T cells, including TFH cells, may have therapeutic potential for the treatment of systemic autoimmune diseases.

PO1.K.6

IL-17 in cutaneous lupus erythematosus

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Background: Lupus erythematosus (LE) is a heterogeneous disease with broad clinical spectrum from cutaneous to visceral and systemic inflammation. IL-17 isoforms (IL-17A and IL-17F) are proinflammatory cytokines with unclear implications in lupus erythematosus pathogenesis. In this study we focus upon IL-17 in normal and modified lupus skin with a correlative study between local and serological expression. **Material And Methods:** 89 patients were recruited divided in 5 groups-10 patients with psoriasis (disease control group), 13 healthy controls, 26 with discoid chronic lupus (DLE), 23 with systemic lupus erythematosus (SLE) and 17 with subacute lupus erythematosus (SCLE). Blood samples and skin punched-biopsy specimens were performed. Serum IL-17A, IL-17F, IL-23 concentration was assayed by ELISA. Skin IL-17A and CD4 expression were evaluated by immunohistochemistry. **Results:** Immunohistochemical expression of IL-17A was higher in DLE, SCLE and SLE patients than negative control subjects (all $p<0.05$). Serum IL-17A concentrations were higher in DLE and SLE patients than negative controls ($p<0.05$). Serum IL-17A concentration was similar in SCLE and negative controls ($p>0.05$). Serum IL-17F concentrations were higher in DLE, SCLE and SLE patients than healthy controls (all $p<0.05$). Serum IL-23 concentrations were similar between DLE, SCLE, SLE patients and healthy controls ($p>0.05$). Serum anti Ro antibodies correlates with IL-17A+ lymphocytes from SCLE lesion and SLE normal skin (all $p<0.05$). **Conclusion:** IL-17 isoforms (IL-17A and IL-17F) are implicated in SLE but also in DLE and SCLE pathogenesis.

Key-words: cutaneous lupus erythematosus, IL-17A, IL-17F, Th17

PO1.K.7

IIL-21/IL-21R interactions on host B cells but not on donor CD4 T cells are critical for the development of autoimmune features in chronic graft versus host disease

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Studies in murine models of lupus have indicated increased production of IL-21 and attenuation of autoimmune features following IL-21 blockade. IL-21 exerts an autocrine effect on T follicular B helper cells (TFH) cells and also stimulates B cell proliferation, plasma cell (PC) differentiation, isotype switching and germinal center (GC) expansion. To assess whether IL-21 promotes systemic lupus through effects on TFH cells or B cells, we assessed the effect of IL-21/IL-21R signaling on B cells independent from the effect on CD4 T helper cells. To this end we used IL-21R-/- or IL-21R+/+ mice as donor or hosts in the P-into-F1 and Bm12-into-B6 models of cGVHD. When induced by injection of IL-21R-/- CD4+ cells from B6 mice into B6D2F1 hosts, cG-VHD was characterized by a decrease in the expansion of donor CD4+ cells by 50%, of CD4+CXCR5+ICOS+ TFH cells by 30% and of IgD-PNA+GC cells by 37% compared to mice that were injected with IL-21R+/+ donor cells. However, other parameters of cGVHD such as host B cell expansion, MHC class II upregulation, PC differentiation and anti-ssDNA autoantibody production did not differ between groups. In contrast, when cGVHD was induced by injecting Bm12 spleen cells into IL-21R-/- B6 mice, parameters of cGVHD were markedly attenuated compared to IL-21R+/+ hosts. Specifically, MHC class II upregulation of host B cells decreased by 32%, PC differentiation by 66%, GC B cells by 60% and anti-ssDNA antibody production by 81%. These results suggest that IL-21R signaling on host B cells and possibly myeloid cells but not on Ag-specific donor CD4 T cells is critical for the initiation and progression of systemic autoimmunity in cGVHD.

PO1.K.8

Toll-like receptor signaling contributes to thymic abnormalities in MRL-Fas lpr/lpr lupus-prone mice

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Objectives: MRL-Fas lpr/lpr mice represent an excellent model for studying lymphoproliferation and autoimmunity. These mice carry a mutation in the Fas gene causing peripheral expansion of T cells with an unusual phenotype (B220+CD3+CD4-CD8-). B cells may have an important role by acting as antigen-presenting cells for self-antigens. Recently, a role for nucleic acid sensing TLR7/9 has been suggested. While the central tolerance in this strain appears to be grossly intact, subtle abnormalities in the T cell development have been observed. Presently, it is not known whether these abnormalities could be linked to abnormal TLR activation during thymic development. **Methods:** To determine whether TLR signaling contributes to thymic abnormalities in MRL-Fas lpr/lpr mice, we injected pre-diseased lupus mice with inhibitory oligonucleotides (ODNs), three times weekly at 1 mg/kg dosage, starting from week 4. Surviving mice were sacrificed at week 16 and their parenchymal organs were examined by histology. Single cell suspensions were prepared and analyzed by FACS. Sera were collected for autoantibody testing. **Results:** At the age of 4-5 weeks, central (BM and thymus) and peripheral lymphoid organs (spleen and lymph nodes) in the MRL-Fas lpr/lpr strain showed very little abnormalities compared to controls. However, starting with 6 weeks splenic MZ-B cells expanded in female mice. This coincided with decreased numbers of total B220+IgM+AA4.1+ cells in the bone marrow and follicular B cells in the spleen. Interestingly, MZ-B cell expansion antedated the accumulation of B220+CD3+ T cells in multiple lymphoid organs (e.g. spleen, LN and thymus). We have additionally observed an abnormal ratio between single-positive (e.g. CD4+, or CD8+) and double-positive (CD4+CD8+) thymocytes, which appeared to be unrelated to the thymic accumulation of B220+CD3+ cells. In mice treated with INH-ODNs, the ratio between single-positive to double-positive cells and the percentage of thymic

B220+CD3+ cells almost completely normalized. Similarly, the percentage of total splenic B cells returned to normal, while the relative and absolute numbers of abnormal B220+CD3+ T cells substantially decreased resulting in reduced splenomegaly and lymphadenopathy. *Conclusion:* By using a pharmacologic approach, we concluded that multiple lymphoid abnormalities in the MRL-Fas lpr/lpr strain could be linked to TLR-dependent activation of APCs. One of the earliest abnormalities detected was the expansion of splenic "innate-like" MZ-B cells, known to have a very low threshold for TLR7/9-dependent activation. Therefore, strategies aimed at targeting TLR signaling may prevent abnormal T cell maturation in the thymus resulting in reduced lymphoproliferation in MRL-Fas lpr/lpr mice.

PO1.K.9

Characterization of the T cell response in pristane-induced lupus (PIL)

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Objective: Murine PIL resembles human SLE in many respects such as presence of anti-chromatine antibodies (abs) and organ involvement. Unlike other experimental lupus models, lymphocytes are not genetically altered in PIL. Since PIL thus represents a suitable tool to study these cells in lupus, we analyzed the distribution of different lymphocyte subsets in lymphoid tissues. *Methods:* For disease induction, BALB/c mice were. injected i.p with either 0.5ml of pristane or PBS as control and sacrificed after 8 months. Cells were taken from (i) regional lymphnodes (LN), (ii) spleens, and (iii) intraperitoneal granulomas which are typical for PIL. We analyzed by FACS the proportions of CD4+, CD8+ and CD19+ lymphocytes, and within the CD4+ population, regulatory T cells (CD4+CD25+FoxP3+; Treg) and activated Teffector (CD4+CD25+FoxP3-; Teff). CD4+ cells were also assessed for Th1, Th2 and Th17 phenotypes by intracellular IFNγ, IL-4, IL-17 staining after in vitro re-stimulation with αCD3 and αCD28 antibodies. In addition, anti-chromatine and anti-histone serum-Abs levels were determined. *Results:* All mice injected with pristine developed anti-chromatine and anti-histone Abs after 4 and 6 months, respectively, whereas controls did not. All PIL mice but no control developed lupus pneumonitis. In spleens, CD19+ B cells was the major cell population, while CD4+ and CD8+ were less frequent. In LN and in granulomas, we found a similar pattern with frequencies of CD4 > CD19 > CD8 cells. In comparison to controls no significant differences in the distribution of lymphocyte subsets in spleens and LN was observed in PIL. Though the total percentage of CD4+ in the peritoneal granuloma was lowest, the relative percentage of activated Teff (13.5±12.9%) was significantly higher than in LN (5.2±4.6%, p<0.0001) or spleens (3.6±2.5%, p<0.0001), while activated Teff were similarly frequent in the respective regions of controls (p=n.s.). Percentages of Tregs did not differ among the analyzed sites. In PIL, we found more Th2, Th17 and particularly more Th1 cells in the peritoneal granulomas compared to other sites (26.8±18.0% vs. 16.3±13.2% among LN, vs. 11.0±7.9% among spleen lymphocytes, p<0.01 and p<0.0001, respectively), whereas percentages of Th1, Th2, and Th17 from LN and spleens closely mirrored the results found in controls. Functional assessment will have to complement these data. *Conclusion:* The most prominent T cell response to pristane was found in intraperitoneal granulomas which thus may constitute the most important site to study in PIL. The phenotypes of spleen or LN-derived lymphocytes may not sufficiently reflect the T cell response underlying experimental lupus. The increased percentages of Th1 cells at the major site of inflammation is in line with previous results showing that IFNγ-/- mice do not develop PIL.

PO1.K.10

Blockade of programmed death-1 (PD-1) in (New Zealand Black x New Zealand White) BWF1 lupus-prone mice induces suppressive capacity of CD8+ T cells in vivo and ex vivo

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Programmed death-1 (PD-1) has been regarded as a negative regulatory signal in T cells. Previously, we have induced immune tolerance by administration of an Ig-related artificial peptide, p-Cons, which induces Foxp3+ regulatory cells in both CD4+ and CD8+ T cells. We have published that PD-1 expression on CD8+ T cells is reduced when BWF1 lupus-prone mice are treated with pCons. These CD8+ Ti, upon adoptive transfer, decrease anti-DNA production and prolong survival. We have shown that young BWF1 mice injected with a neutralizing antibody against PD-1, also have delayed onset of proteinuria and improved survival, with development of CD8+ Ti. In contrast to naïve BWF1 mice, anti-PD1 treatment maintains numbers of suppressive Foxp3+CD8+ T cells over time. These CD8+ Ti suppress CD4+CD25- helper T cell proliferation and induce apoptosis in B cells, resulting in suppression of autoantibodies and nephritis. In contrast, administration of anti-PD-1 Ab to pCons-treated mice reverses tolerance and the suppressive capacity of regulatory T cells, including CD8+ Ti. Furthermore, silencing of PD-1 with siRNA in CD8+ Ti abrogates their suppressive capacity. Thus, regulation of signaling through PD-1 is required for maintenance of regulatory T cells and control of autoimmunity in BWF1 mice. Tight regulation of PD-1 expression on CD8+ T cells, and/or differences in the activation of PD-1-related molecular pathways in conditions of high, intermediate, or low expression of PD-1 are critical to the ability of these cells to regulate autoimmunity.

PO1.K.11

Activation via the CD3/CD28 pathway displaces adaptor protein LAT from the immunological synapse in T cells from lupus patients

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Objective: Abnormal assembly of supramolecular activation clusters (SMACs) in lipid rafts may impair downstream MAPK activation in lupus T cells. *Methods:* 1. T cells were stimulated for 5 min at 37ºC with OKT3 in multi well plates incubated with GAM. The cells were collected, lysed with 1% NP-40 and immunobloted with a polyclonal anti-LAT antibody. 2. Highly enriched T cells were adhered to pLL coated slides and activated for 5 and 15 min at 37°C, with 4.5 μm superparamagnetic polystyrene beads coated with antibodies against CD3ε and CD28. The cells were fixed, permeabilized and stained with antibodies recognizing LAT, Grb2 and PLCγ1. The cell-bead complexes were evaluated by confocal microscopy and the densitometries were obtained using ImageJ, v1.6, NIH, USA. *Results:* We have previously observed that activation via TCR/CD3 induces a significant decrease in the amount of LAT levels in total cell lysates from lupus T cells stimulated for 5 minutes compared with healthy control T cells (13.25±2.65 vs. 21.58±2.78 ; MFI ± SME, p= 0.038, n= 16). This finding was confirmed using confocal microscopy, a method that also revealed a disruption in the co-localization of LAT and GM1 in lipid rafts in SLE T cells stimulated for 5 min versus resting T cells (0.978±0.001 vs. 0.975±0.003; MFI ± SME, p= 0.008, n= 14). More recently, analysis with the confocal microscope revealed that activation via CD3/CD28 during 15 minutes induced a significant decrease in the amount of LAT at the synapse in lupus T cells in comparison with resting T cells (24.95±4.48 vs. 35.58±5.14; MFI ± SME, p=0.020, n= 8), a difference not found in healthy control cells. LAT levels out of the synapse were also diminished after activation of lupus T cells (18.39±4.05 vs. 27.53±5.08 ; MFI ± SME, p=0.022, n=8). PLCγ1 tends to translocate to the synapse and Grb2 recruited to the synapse remained unchanged upon activation in both SLE and healthy T cells. *Conclusions:* We conclude that activation via CD3/CD28 negatively regulates LAT expression both in and out of the synapse in SLE T cells. The mechanistic cues for the downregulation of LAT in response

to T cell activation in lupus T cells are currently unclear. The diminished expression of LAT after TCR-CD3 activation may potentially disrupt proximal signaling events and impair downstream activation of the MAPK cascade in human lupus T cells.

PO1.K.12

Nuclear expression of CXCR5 in spleen follicular T helper cells in autoimmune BXD2 mice

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Objectives: Autoimmune disease and autoantibody production in SLE is associated with B cells activation by CD4 T cells. Follicular T helper (Tfh) cells in germinal centers (GCs) provide survival signals to promote B-cell differentiation into antibody producing plasma cells and memory B cells. Interaction of cell surface CXCR5 with its ligand CXCL13 is an important mechanism to direct the migration of CD4 T and B cells to GC light zone area. The purpose of this study is to determine how CXCR5+ Tfh cells are involved in the pathogenic GC response in lupus-prone BXD2 mice. **Methods:** Single cell suspensions prepared from the spleens of BXD2 mice and normal B6 mice were subjected to FACS staining with some cells cytospinned for fluorescent imaging analysis. Frozen spleen sections were used for fluorescent confocal imaging analysis. Paraffin-embedded spleen sections were used for immunohistochemistry staining. Subcellular expression of CXCR5 in purified CD4 T cells with or without CXCL13 stimulation was determined by western blot analysis. **Results:** There was 2.5- and 1.5-fold increased expression of ICOS+CXCR5+ and IL-21+ CD4+ T cells, respectively, in the spleens of BXD2 compared to that in B6 spleens. ICOS+CXCR5+ CD4+ T cells mainly clustered in GCs and were located adjacent to CD21/CD35+ follicular dendritic cells. CXCR5 was rarely expressed by naïve CD4 T cells and was mainly detected inside the nucleus compared to cell membrane of CD4 T cells in GC light zone. Western blot further confirmed that there was increased expression of CXCR5 in the nucleus compared to the cytoplasm of CD4 T cells from BXD2 mice. CXCL13 stimulation on purified CD4 T cells from BXD2 mice caused a time-dependent increase of nuclear CXCR5 expression, increased proliferation and decreased apoptosis. CXCR5+CD4+ T cells from BXD2 mice also expressed higher levels of BrdU and lower levels of Fas, compared to those from B6 mice. Consistent with this, in vivo nuclear expression of CXCR5 co-localized with Ki67+ cells. **Conclusions:** The finding of increased expression of CXCR5 in the nucleus of Tfh cells in autoimmune BXD2 mice is novel. CXCL13 binding to CXCR5 induced increased levels of nuclear CXCR5 which may further play an important role in increasing proliferation or inhibiting apoptosis of Tfh leading to formation of autoreactive Tfhs and facilitating autoreactive GC responses in BXD2 mice. Therefore, CXCL13-CXCR5 may become a therapeutic target for autoimmune diseases including SLE.

PO1.K.13

Chimeric maternal cells as T lymphocyte targets in pediatric SLE

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Background: Maternal cells passing into the fetus can persist for decades after birth, creating a state of maternal microchimerism. Loss of tolerance to non-shared antigens from maternal cells could lead to chronic activation of host T lymphocytes, with subsequent "autoimmunity" similar to the graft-versus-host disease seen after parental to F1 stem cell transplantation. **Hypothesis:** Normal T lymphocytes are tolerant to chimeric maternal cells; in

SLE T cells specific for maternal cells are expanded. **Methods:** PBMC from 22 pediatric SLE patients and 27 age-matched controls were stimulated with maternal or HLA incompatible unrelated donor PBMC for 5 days. Cytokine expression and proliferation in T cell subsets was assayed by flow cytometry. Maternal microchimerism was assayed by Real-Time QPCR amplification of non-shared maternal alleles using >100,000 genome equivalents of genomic DNA isolated from the child's PBMC. SLE disease activity was assessed by the SELENA-SLEDAI. **Results:** Levels of maternal microchimerism were not increased in SLE patients with active disease, but rather tended to be lower (126 per million maternal cells in controls versus 5.2 in SLE, P>0.05). T lymphocytes in patients with SLE were hyperactive in response to maternal cells. Proliferation of CD4+ T lymphocytes in response to maternal cells was increased 2-fold in SLE patients compared to controls, and IFN-γ production by CD4+ lymphocytes was increased in patients with active SLE (SLEDAI>4), but not in those in remission or controls. TNF-α production by CD4+ T lymphocytes specific for maternal cells correlated with SLE disease activity. **Conclusions:** Elevated CD4+ T lymphocyte responses to maternal cells are consistent with a model of persistent T lymphocyte activation by chimeric maternal cells within target organs with subsequent T cell-mediated elimination of chimeric maternal cells in the periphery.

PO1.K.14

PRES in connective tissue disorders (CTD): a systematic review of outcome and treatment

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Posterior reversible encephalopathy syndrome (PRES) is a rare but well recognized syndrome that has been associated with both inflammatory disorders and immunosuppresive therapy. We reviewed the literature on a subset of patients with PRES who had connective tissue disorders (CTD) in order to identify factors that influenced outcome. **Methods:** We performed a PUBMED search of the English literature and found 72 cases of PRES in patients with CTD. 57 cases had SLE, one UCTD, one limited scleroderma, one overlap syndrome, and eight had vasculitis (Wegener's Granulomatosis, Henoch–Schönlein purpura, Microscopic polyangiitis and p-ANCA associated vasculitis). Finally, we report three cases of PRES associated with CTD seen at our institutions, two with SLE and one with RA. **Results:** Preexisting treatment could be identified in 61 out of 72 patients. 56 of 61 were on treatments previously associated with PRES. 46 out of these 56 patients had started or increased immunosuppressive therapy less than six weeks before the onset of PRES as follows: 54% corticosteroids, 25% cyclophosphamide, 6.5% cyclosporine, 3.2% Mycophenolate Mofetil, 3.2% Azathioprine, and 3% Rituximab. Treatment of PRES: Of the 72 patients, 14 were treated only with supportive treatment (antihypertensive and antiseizure agents), 12 patients continued the same immunosuppressive therapy, 20 patients switched to another immunosuppressive or added a new one, 8 patients discontinued the immunosuppressive treatment. In 13 patients it could not be determined if immunosuppressive therapy was continued or discontinued, in five patients no data was available regarding therapy used. 42 patients had a CTD flare, six had no flare and 24 patients had insufficient information to evaluate. Five of 70 patients had permanent damage (clinical or radiological) at the time of follow-up. Two patients had no information regarding permanent damage or lack thereof. There were several cases of recurrent PRES; none had permanent damage. In our review of 72 cases of PRES in CTD, 79% had SLE. Although 84% of patients started or increased immunosuppressive therapy within six weeks prior to onset, most recovered whether immunosuppressive therapy was continued or changed and 88% had CTD flare. In five cases with permanent damage, only one had received continuous immunosuppression, the other four had been treated with supportive care only. (O.R.=12.4; p=0.0248) **Conclusions:** This data suggests that essential therapy for the underlying CTD should not be withheld since permanent damage was rare, and not significantly associated with immunosuppressive therapy.

PO1L Immunology – Tregs

PO1.L.4

A77 1726, the active metabolite of leflunomide, attenuates lupus nephritis by promoting the development of regulatory T cells and inhibiting IL-17-producing double negative T cells

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Systemic lupus erythematosus (SLE) is a potentially fatal non-organ-specific autoimmune disease that predominantly affects young women. Lupus nephritis is a challenging problem that affects 30–60% of patients with SLE. Despite intervention, the rates of end-stage renal disease due to lupus nephritis are increasing worldwide. Several studies showed that leflunomide is a safe and probably efficacious treatment in patients with lupus nephritis who do not respond or cannot tolerate conventional treatments. As T cells play a central role in the orchestration of in both physiological and pathological immune responses, it has been proposed that the ability of leflunomide to suppress inappropriate and undesirable immunity is related to a functional inhibition of T cells. The purpose of this study is to study the effect of a reliably absorbed A77 1726, the active metabolite of leflunomide, on lupus nephritis and its potential effect on T cell functions using MRL-lpr mouse model. Here, we report that A77 1726 effectively inhibits development of lupus nephritis as revealed by histopathology and proteinuria by ELISA, and attenuates the generalized autoimmune features, including splenomegaly, lymphadenopathy, and serum autoantibodies. At the cellular level, A77 1726 treatment suppresses the expansion of double negative (DN) T cells, and inhibits both T and B cell activation as shown by surface activation markers. Intriguingly, A77 1726 treatment significantly increases CD4+Foxp3+ regulatory T cells but suppresses potential "pathogenic" IL-17-producing DN T cells in lymph nodes. In vitro experiment shows that A77 1726 potentiates the conversion of naïve CD4+CD25- T cells into CD4+CD25+Foxp3+ inducible regulatory T cells (iTregs). Further analysis reveals that A77 1726 inhibits TCR-induced activation of Akt, but not ERK and JNK. Taken together, the efficacy of A77 1726 for lupus nephritis may be mediated, at least in part, by augmenting iTregs which suppresses pathogenic IL-17-producing DN T cells through an Akt-dependent mechanism.

PO1M Pathogenesis

PO1.M.2

Annexin II on human mesangial cells mediates anti-dsDNA antibody binding

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Objectives: Cardinal features of lupus nephritis include the production of anti-dsDNA antibodies, mesangial proliferation and renal inflammation. The mechanism through which anti-dsDNA antibodies might interact with resident renal cells remains to be elucidated. We and others have previously demonstrated that anti-dsDNA antibodies could bind to human mesangial cells (HMC) independent of bridging chromatin material. We further characterized the cross-reactive antigen(s) on HMC which bind human polyclonal anti-dsDNA antibodies. ***Methods:*** HMC were isolated from renal cortical tissue using differential sieving followed by collagenase treatment. Human polyclonal anti-dsDNA antibodies were isolated from patients with lupus nephritis using Protein A-Sepharose followed by DNA-cellulose affinity chromatography. HMC surface proteins were removed by limited trypsin (10μg/ml) treatment and the binding of anti-dsDNA antibodies to cells assessed by flow cytometry and cellular ELISA. HMC plasma membrane fractions were subjected to Western blot analysis, then probed with purified anti-dsDNA antibodies and subjected to MALDI-TOF spectrometry to identify 'cross-reactive' membrane proteins. Renal biopsies were assessed by immunohistochemistry. ***Results:*** Limited trypsin but not DNAse treatment of HMC significantly reduced anti-dsDNA antibody binding (p<0.05). Anti-dsDNA antibodies predominantly bound to a cell surface antigen with a molecular weight of ~36kDa and this band was identified as annexin II by MALDI-TOF spectrometry. The binding activity between anti-dsDNA antibodies and annexin II correlated with disease activity. Glomerular annexin II expression was significantly increased in active lupus nephritis (p<0.05), compared with controls and non-lupus kidney diseases. ***Conclusions:*** Our data demonstrated that annexin II on the plasma membrane of HMC mediates anti-dsDNA antibody binding.

PO1.M.3

Mycophenolic acid ameliorates anti-dsDNA antibody binding to proximal tubular epithelial cells and the subsequent induction of inflammatory and fibrotic processes

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Objectives: Severe active proliferative lupus nephritis is associated with inflammation in the renal parenchyma and elevated levels of anti-dsDNA antibodies. Up to 70% of patients with lupus nephritis show immune deposits along the tubular basement membrane, and the severity of tubulo-interstitial inflammation and injury correlate with long-term renal prognosis. We investigated the interaction between polyclonal anti-dsDNA antibodies isolated from patients with lupus nephritis and cultured proximal tubular epithelial cells (PTEC), and the effect of mycophenolic acid (MPA). ***Methods:*** Human polyclonal anti-dsDNA antibodies were isolated from patients with lupus nephritis using affinity chromatography. Their interaction with PTEC in the presence or absence of MPA (5μg/ml) was assessed by flow cytometry, cellular ELISA and immunohistochemistry. Induction of IL-6, IL-8, MCP-1 and fibronectin was assessed by commercial ELISAs, and MAPK activation by Western blot analysis. ***Results:*** Anti-dsDNA antibodies bound directly to PTEC surface without the need for bridging chromatin material, and were then internalized, in a time- and temperature-dependent manner, into the cytoplasmic and nuclear compartments. Binding of anti-dsDNA antibodies to PTEC induced cell proliferation, IL-6 secretion through JNK activation, and MCP-1 through p38 MAPK activation. Activation of ERK, p38 MAPK and JNK contributed to the induction of IL-8 and fibronectin secretion by anti-dsDNA antibodies (p<0.05 for all compared to control IgG). MPA inhibited anti-dsDNA antibody binding to PTEC, its induction of cell proliferation, activation of ERK, p38 MAPK and JNK, and IL-6, IL-8, MCP-1 and fibronectin secretion. ***Conclusions:*** Our data demonstrate the significance of anti-dsDNA antibody interaction with PTEC in the pathogenesis of tubulo-interstitial inflammation and fibrosis in lupus nephritis, and the therapeutic effect of MPA in this regard.

PO1.M.4

Hyaluronan in the pathogenesis of lupus nephritis in NZB/W mice and the effect of 4-methylumbelliferone

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Objectives: Renal inflammation is a hallmark of lupus nephritis. Hyaluronan (HA) is a non-sulfated glycosaminoglycan ubiquitous in the extracellular environment of all cells. Tissue inflammation and fibrosis are associated with increased HA levels. Increased glomerular and tubular-interstitial HA expression has been shown in lupus nephritis, but its role in pathogenesis remains to be defined. Using 4-methylumbelliferone (MU), a specific inhibitor of HA synthesis, we examined the role of HA on renal histopathology and clinical manifestations in NZB/W mice with lupus nephritis. ***Methods:*** Twenty-four female NZB/W mice with active nephritis denoted by persistent proteinuria >300mg/dl were divided into 4 groups and administered saline or MU (0.5,

1.0 or 5.0 mg/kg/day) by oral gavage for 12 weeks. They were then sacrificed and the urine, blood and kidneys analysed. ***Results:*** MU significantly reduced serum HA levels in a dose-dependent manner compared to controls (p<0.05). This was associated with reductions in anti-dsDNA antibody titre (1316±718ng/ml vs 807±381ng/ml, control vs 5mg/kg/day MU, p<0.05), urine protein-to-creatinine ratio (23 vs 13 mg/mg creatinine for corresponding groups, p<0.05), and splenomegaly (0.16g vs 0.12 g for corresponding groups, p<0.05). Mice with active nephritis showed glomerular hypertrophy and increased intra-renal expression of CD4, CD8, CD19, CD23, CD45 and Mac-1. The intra-glomerular expression of HA and its receptor CD44, as well as that of IL-6 and MCP-1, was also increased. These changes were abrogated in mice treated with MU at 1 and 5 mg/kg/day. ***Conclusions:*** Our data suggest that HA participates in the pathogenesis of inflammation and histopathologic manifestations of renal injury in lupus nephritis, and thus may be a potential target for therapy.

PO1.M.6

Hyperprolactinemia: a cause of SLE-like disease and the effects of dopaminergic therapy
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Objective: Prolactin has multiple interactions with the immune system and has a role in the pathogenesis of autoimmune disorders like systemic lupus erythematosus. We present patients with SLE that have elevated prolactin levels and serological changes that respond to treatment with dopaminergic agents. ***Methods:*** We followed a subset of 5 patients (three females and two males) with hyperprolactinemia associated with SLE. For each patient, diagnosis was established by asking about breast tenderness, fatigue, headache, visual changes and arthralgias. Pituitary imaging validated the source of the prolactin. Antiphospholipid antibody titres and ESR correlated with prolactin levels and were followed serially at each visit to assess response to dopaminergic therapy. ***Results:*** Correlation between antiphospholipid antibody levels, other serological markers and disease activity occurred before and after therapy with dopamine agonists in both male and female patients. The sedimentation rate and other acute phase reactant levels like CRP varied with the serum levels of prolactin in two male patients with pituitary adenomas after treatment of hyperprolactinemia with a bromocriptine analogue. In one year ESR declined from 70 to 35 mmHg. In two months the anticardiolipin (ACA) IgM antibody decreased from 80 to 10 units and IgG ACA decreased from 60 to 35 units over the same time period. All of this occurred with dopaminergic therapy alone in the absence of any other medication. Both platelet counts and PTT improved with therapy, but in a very sporadic fashion over a period of 6 to 10 months; at first increasing and then precipitously decreasing. Upon discontinuation of therapy, all ACA titers increased as did acute phase reactants. ***Conclusion:*** Hyperprolactinemia in SLE patients is responsible for a constellation of symptoms that respond to therapy with dopaminergic agents and should be considered in certain patients. Prolactin levels along with antibody and inflammatory markers can be used to assess disease activity and follow response to therapy in this subset of patients.

PO1.M.7

The possible interplay between anti-ssDNA binding autoantibodies and parvoviral B19 DNA in the light of new hypothesis: wet lab and computational approach
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Objective: 1.To verify hypothesis that there is association of anti-ssDNA binding antibodies and human ParvovirusB19 ssDNA in patients with SLE.

2. To determine the spectrum of those anti-ssDNA autoantibodies, their hydrolytic activities, and possible reason(s) for binding and hydrolysis of Parvovirus B19. ***Methods:*** (i) Two step affinity purification magnetic bead method (Pavlovic et al., 2007); (ii) Lab-on chip method; (iii) ELISA test; (iv) Western blot; (v) Fluorescence original method for detection of antibodies' hydrolytic activity (Cavallo et al, 2005); (vi) Computational analysis of distribution and frequency of thymidine pentamers within Parvovirus B19 sequence, and symmetry features in the statistically dispersed nucleotide bases versus their complements, along the single strand of a genomic sequence. ***Results:*** WB confirmed the exclusive transfer of IgGs. Electrophoretic and Lab-on chip data analyses strongly suggested polyclonal nature of purified anti-ssDNA antibodies, showing their different patterns, and hydrolytic activity in lupus patients compared to controls. The ELISA results revealed a larger spectrum of antibodies and their subclasses in SLE versus normal population. IgG1 and IgG4 were the most abundant. Km and Vmax were different between two enzymatic categories used under same conditions. While DNAse I hydrolytic rate at the same concentration of the labeled substrate (1μM of 18-mer Gololobov`s modified oligo DNA) at 37°C is very fast (30 minutes), for anti-DNA antibodies, it is about 1x10^4 lower. Computational results confirmed a very dense distribution of oligo-T motifs within the Parvovirus B19 sequence. An entropic approach in ascertaining the statistical dispositions in nucleotide bases along the strand of a test B19-Au DNA: computing the Kullback-Lieber and Jenses-Shanon discrimination functions, determined with respect to a "junk" strand populated by uniformly distributed A, T, C and G bases. These measures indicate the site at which hairpin bands are perceived in the open reading frame. ***Conclusions:*** Within 5543 nt of B19 sequence, tymidine pentamers were very frequent. This strongly suggests the possible binding of SLE anti-ssDNA antibodies to those "hot spots" on viral ssDNA (Tanner, 2001). Binding, as prerequisite for hydrolysis, occurring under optimal substrate: abzyme ratio is confirmed in our laboratory settings. The finding of prevailed symmetry features of nucleotide bases suggests the possibility of a hairpin structure - toward stable and energy-minimized format, the additional condition for anti-ssDNA andibody binding. Hydrolytic activity of lupus anti-ssDNA antibody could be tightly associated with the antibody's pathogenicity by maintaining and perpetuating the "vicious cycle" through constant cleavage and exposure of viral ssDNA to the hyperactive immune system in SLE. It is highly likely that these "multipatterned" andibodies are the result of host antimicrobial attack, hydrolyzing Parvoviral ssDNA by binding to thymidine pentamers: mechanism suggested by Pavlovic et al, (2007, and 2009).

PO1.M.8

Expression of intercellular adhesion molecules is strongly correlated between brain and kidney of lupus-prone mice
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The central nervous system is frequently affected in systemic lupus erythematosus (SLE), but the pathophysiological mechanisms are largely unknown so far. It has been proposed that induction of cellular adhesion molecules such as ICAM-1, VCAM-1 and E-Selectin by circulating immune complexes leads to an increased migration of inflammatory cells through the blood vessels, contributing to the brain pathology seen in human SLE. Therefore, we analyzed the expression of VCAM-1, ICAM-1 and E-Selectin in the brain and kidney from young (4 weeks) and old (16 weeks) lupus-prone MRL/lpr mice and compared the data with findings in MRL and C57/B6 mice, respectively. Besides histopathological examination of brain and kidney, renal function was determined by measuring the level of proteinuria in these mice. Additional human autopsy brain tissue from SLE patients was studied for the expression of adhesion molecules. Neither young, nor old mice from all groups showed significant histopathological changes in the brain, but old MRL/lpr mice had significantly increased levels of proteinuria associated with showed an increased histopathological kidney score indicating severe kidney tissue damage. These changes were associated with significantly increased levels of E-selectin and ICAM-1 in the kidney. In mouse brain, tissue samples from the cerebrum and the cerebellum showed significantly increased expression levels for VCAM-1, E-selectin and ICAM-1 in old MRL/lpr mice. Surpris-

ingly, expression of cerebellar ICAM-1 and VCAM-1 in old MRL/lpr mice was strongly correlated with kidney expression of both adhesion molecules, while E-selectin levels were uncorrelated. Moreover, expression of VCAM-1 and ICAM-1 was significantly associated in kidney and brain tissue with each other, while E-selectin did not. In human brain, especially cerebellar tissue samples also displayed increased levels of adhesion molecule expression despite the absence of major pathology. These data provide direct evidence that a strong correlation exists between kidney pathology, renal adhesion molecule expression, and induction of adhesion molecules in the brain in aged MRL/lpr mice already in the absence of major brain pathology. These changes might contribute to the neurological dysfunction seen in MRL/lpr lupus mice and may indicate that based on kidney damage brain function is also altered despite the lack of neuropathological changes.

PO1.M.9

Evidence that decline in vitamin D status may be mechanistically related to SLE flare

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Objectives: Cross-sectional studies have shown lower vitamin D levels in SLE patients with increased disease activity. These studies suggest that decline in vitamin D status may induce SLE flare (reviewed in Curr Opin Rheumatol, 2008; 20:532). This hypothesis, however, has never been definitively tested through serial measurements of vitamin D at regular intervals leading up to SLE flare. The purpose of this study was to do so. **Methods:** All flares (N=91, in 48 patients) identified in the Ohio SLE Study (OSS) for which serum was available from 4 and 2 months before flare, and at flare, were tested. The OSS is a longitudinal study of flare pathogenesis comprised of chronically active SLE patients followed regularly at bimonthly intervals. The tests included serum 25-OH vitamin D, intact parathyroid hormone (iPTH), calcium, and phosphate levels. Additionally, serum 1,25-OH vitamin D was measured in 53 of the 98 flares. The data were analyzed by Friedman's repeated measures ANOVA. **Results:** No serial differences were identified among -4, -2, or at flare values for 25-OH vitamin D, or any other variable. However, when only the flares that occurred during 6 months that annually have the lowest daylight hours (October through March, N = 56) were analyzed, significant decreases occurred at flare, both renal and nonrenal, as shown in the table. For these low-daylight months, no significant changes occurred in serial levels of 1,25-OH vitamin D, iPTH, or phosphate. Minor calcium changes did not reflect the trends in vitamin D status.

	-4	-2	0	P value
ALL MONTHS				
Renal flares (n=37)	59.9	62.9	49.5	0.1390
Nonrenal flares (n=54)	57.3	55.6	52.1	0.8419
All flares (n=91)	58.4	57.8	50.3	0.2354
LOW-DAYLIGHT MONTHS				
Renal flares (n=26)	60.0	61.7	47.5	0.0006
Nonrenal flares (n=30)	60.4	58.5	47.9	0.0140
All flares (n=56)	60.0	59.8	47.7	0.0001

Conclusions: Our data suggests that declining serum 25-OH vitamin D levels are mechanistically involved in SLE flare, but only during months of relatively low daylight periods. This seasonal effect suggests a delicate balance in the relationship between vitamin D levels and onset of lupus flare. The lack of corresponding change in serum levels of 1,25-OH vitamin D or iPTH lsuggests that the 25-OH vitamin D main effect is through local generation of 1,25-OH vitamin D.

PO1.M.10

Novel peptides as potential treatment of systemic lupus erythematosus

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Objective: Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by a loss of immunologic tolerance, production of auto-antibodies, and inflammatory damage in multiple organs. Since the currently available drugs for treatment of SLE are often of limited efficacy and may cause adverse effects that reduce patient compliance, the aim of the present study was to develop novel peptides for treatment of SLE. **Methods:** Peptides were administered into MRL/lpr mice, an animal model of SLE. Proteinuria and hematuria were determined by commercial kits. Autoantibodies and cytokines were examined by ELISA and, CD4-CD8- cells and B220+ cells by FACS analysis. **Results:** Oral administration of the anti-inflammatory IIIM1 peptide (H2A histone fragment 36-44) at early stage of disease caused reduction in proteinuria and serum anti-dsDNA antibodies. Starting the treatment at advanced stage of disease resulted in prolonged animal survival, decreased lymphadenosis and reduced levels of pathogenic or abnormal double negative CD4-CD8- cells and B220+ cells in lymph nodes and spleen. We discovered that IIIM1 induces the production of an additional peptide, a fragment of alpha-1-antitrypsin, termed UBE. Relatively low dose (1μg/kg) of UBE reduced proteinuria and hematuria in MRL/lpr mice. The beneficial effect of the peptide was corroborated by histological examination. Furthermore, a significant reduction in serum IL17, IL12 and anti dsDNA antibodies was observed in the UBE-treated mice. Isolated CD4 cells incubated with the peptide showed similar cytokine profile. Decreased levels of double negative CD4 CD8 and B220+ cells were determined in lymph organs of UBE-treated animals. **Conclusions:** The beneficial effects of both UBE and IIIM1 suggest these peptides as potential drugs for SLE.

PO1.M.11

Netting neutrophils are major inducers of type I IFN production in SLE

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Objective: Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease characterized by a breakdown of tolerance to nuclear antigens. Genomic approaches led to the identification of prominent interferon (IFN) and granulopoiesis-related signatures in SLE leukocytes. Early release of neutrophil precursors could be due to accelerated mature neutrophil death, but whether IFN plays a role in this process is not known. A particular type of neutrophils cell death characterized by the release of a network of DNA and antimicrobial peptides that trap pathogens (Neutrophil Extracellular Traps or NETs) has been recently described. Because NETs contain DNA and DNA-modifying factors, we asked whether SLE neutrophils, in synergy with IC, would contribute to the hyperactivation of pDCs and production of IFN through the release of NETs. **Methods:** SLE and healthy neutrophils were stimulated with media or IC. After 6 hrs the resulting supernatants were collected and used to stimulate pDC at 20% final concentration. The pDC maturation markers and the IFN production was analyzed by flow cytometry and LUMINEX respectively. Alternatively, healthy neutrophils were primed for 6 hrs with 1000 U/ml of recombinant human IFN before being stimulated as described above. **Results:** We show that mature SLE neutrophils are primed in vivo by type I IFN and die by NETosis upon exposure to anti-RNP antibodies. This is accompanied by the release of LL37 and HMGB1, two proteins that facilitate the uptake and recognition of mammalian DNA by plasmacytoid dendritic cells (pDCs). Indeed, SLE NETs activate pDCs to produce large amounts of IFNa in a DNA-dependent manner, as this effect is abrogated by DNase treatment. Moreover, priming of healthy neutrophils with IFN recapitulates the NETotit effect of anti-RNA antibodies. **Conclusion:** SLE serum contains potential interferogenic factors in the form of IC. However,

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IC alone are not efficient activators of pDC unless combined with nucleic acids from dying cells and/or in the presence of other mononuclear cells that act as adjuvants. Here we demonstrate that SLE IC induce SLE neutrophils to die by NETosis and that SLE NETs are potent pDC activators. This leads to a self-amplifying loop, as IFN in turn is a priming factor for anti RNP-mediated neutrophil activation-induced death. Our results reveal an unsuspected role for neutrophils in SLE pathogenesis and identify a novel link between nucleic acid-recognizing antibodies and type I IFN production.

PO1.M.12

What defines the different forms of cutaneous lupus erythematosus: an immunofluorescent pilot study

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Objectives: Cutaneous involvement is common in the course of systemic lupus erythematosus (SLE). Not only acute (ACLE), but also subacute (SCLE) and chronic (CCLE) cutaneous LE can affect SLE patients. In this study, we used immunohistology to characterize the inflammatory infiltrate in different forms of cutaneous LE (CLE) aiming at identifying subtype-specific signatures. *Methods:* Sections from lesional skin of LE patients (3 ACLE, 3 SCLE, 2 CCLE) and 4 healthy controls were stained with mouse anti-human antibodies against CD3 (FITC-labeled), CD4 and CD8 (each purified, 2nd step goat anti-mouse-TRITC), CD123 (purified; amplified with Zenon®-technology), BDCA2 (purified; 2nd step goat anti-mouse biotinylated, 3rd step streptavidin-TRITC) and isotypes. Populations were defined as CD4 T-cells (CD3+CD4+), CD8 T-cells (CD3+CD8+) and plasmacytoid dendritic cells (pDCs; CD123+BDCA2+). *Results:* As expected, lesional epidermis and dermis were infiltrated with CD4+ and CD8+ cells. ACLE showed a sparse dermal infiltrate as compared to SCLE and CCLE. The epidermal infiltrate was most prominent in CCLE. Remarkably elevated numbers of pDCs were found in SCLE and CCLE but not in ACLE. The epidermis was almost devoid of pDCs. *Conclusions:* At least on a numerical basis, T cells of the CD4 and CD8 lineage are apparently major players in LE tissue inflammation. The mechanisms leading to their activation (pDC?) remain to be determined.

PO1N Sjogrens

PO1.N.1

Lupus susceptible gene polymorphisms in patients with Sjögren's syndrome in Japanese population

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Genetic background of Sjögren's syndrome is still unknown. Sjögren's syndrome can occur alone (primary Sjögren's syndrome) or in conjunction with other autoimmune diseases such as systemic lupus erythematosus or rheumatoid arthritis (secondary Sjögren's syndrome). Several novel lupus susceptible genes were reported in the recent candidate gene approach and genome wide association studies in Caucasian population. In this study, we analyzed these lupus susceptible gene polymorphisms in patients with Sjögren's syndrome in Japanese population. This study comprised 190 Sjögren's syndrome patients and ethnically matched 428 healthy controls. Patients with systemic lupus erythematosus were excluded. Signal transducer and activator of transcription 4 (STAT4) rs7574865, B lymphocyte specific tyrosine kinase (BLK) rs13277113, B-cell scaffold protein with ankyrin repeats 1 (BANK1) rs10516487, tumor necrosis factor alpha induced protein 3 (TNFAIP3) rs2230926, tumor necrosis factor ligand superfamily member 4 (TNFSF4) rs844644, rs10798269 in

1q25.1 region and so on were evaluated using TaqMan Genotyping Assay. Allele frequencies in each polymorphism were compared using chi-square test and the related risk was approximated by the odds ratios. The allele frequencies of lupus risk in Sjögren's syndrome were significantly higher in STAT4 (odds ratio (OR) =1.83 , 95% confidence interval (CI):1.43-2.36), BLK (OR=1.61, [95%CI:1.12-2.15]) and rs10798269 in 1q25.1 region (OR=1.62, [95%CI:1.20-2.18]), compared with those in healthy controls. No significant associations were found in BANK1, TNFAIP3 and TNFSF4. Sjögren's syndrome may share, in part, common genetic background with systemic lupus erythematousus.

PO1.N.2

Secondary and primary Sjögren's syndrome in lupus families

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Purpose: Systemic lupus erythematosus (SLE) and Sjögren's syndrome are closely related diseases, but the relationship of primary and secondary Sjögren's syndrome within families with SLE is not known. We undertook this study to determine whether primary Sjögren's syndrome is increased among SLE-unaffected family members, and whether primary and secondary Sjögren's syndrome are associated within SLE families. *Methods:* Families were collected through the Lupus Family Registry and Repository. All SLE patients met the 1982 revised classification criteria for SLE, and all families had at least one member with SLE. All subjects completed a standardized questionnaire, and had autoantibodies determined by ELISA and double immundiffusion. Sjögren's syndrome was considered present when subjects met the combined US-European Sjögren's classification criteria for dry mouth as well as dry eyes and had anti-Ro (or SSA). *Results:* Among a total of 2537 SLE patients, there were 505 (19.9%) with secondary Sjögren's syndrome. There were 65 SLE-unaffected relatives of SLE patients who had primary Sjögren's syndrome, while none of 1304 age and sex matched healthy controls had Sjögren's ($\chi2$=12.7, p=0.02). Seventeen (26.2%) of the 65 subjects with primary Sjögren's had an SLE-affected relative with secondary Sjögren's syndrome, compared to 493 (7.1%) of 6922 SLE-unaffected family members without primary Sjögren's ($\chi2$=34.8, p<0.00001, odds ratio=5.0). *Conclusion:* We find that primary Sjögren's is found among relatives of SLE patients statistically more often than in healthy controls. Furthermore, within these SLE families primary Sjögren's syndrome in SLE-unaffected members was associated with secondary Sjögren's. That is, there was a familial distribution of primary and secondary Sjögren's. Primary Sjögren's affected family members were about 5 times more likely to have an SLE-affected relative with secondary Sjögren's than family members without primary Sjögren's.. These data suggest common susceptibility factors, possibly genetic, for primary and secondary Sjögren's syndrome

PO2A Autoantibodies

PO2.A.3

The effects of hydroxychloroquine on antiphospholipid antibody titers in SLE

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Purpose: Treatment with hydroxychloroquine (HCQ) is associated with a decreased risk of thrombosis in APLS. In vitro, HCQ decreases the binding of

aPL-beta2 glycoprotein I complexes to phospholipid bilayers and reduces aPL levels in clinical assays. We investigated the association between HCQ therapy and changes in aPL titers over time. *Method:* We identified all SLE patients followed at a tertiary care center with aPL or lupus anticoagulant (Lac) measured at least twice between 2002 and 2009. At baseline, patients were defined as aPL/Lac- if they tested negative for both aPL and Lac, and as aPL/Lac+ if they tested positive for either aPL or Lac, using standard laboratory cut-offs. An "event" was defined as aPL and/or Lac conversion from positive to negative in the aPL/Lac+ group, and aPL or Lac conversion from negative to positive in the aPL/Lac- group. "Time to event" was defined as months from baseline to censoring or conversion. Patients were defined as HCQ+ if they were being treated with HCQ at the time of the event, and HCQ- if they were not. Kaplan-Meier graphs were generated separately for the aPL/Lac+ and aPL/Lac- groups. *Results:* Eighty four patients met the above inclusion criteria: 31 HCQ-, 27 HCQ+, and 26 missing HCQ data. HCQ+ and HCQ- groups had similar demographic and laboratory characteristics at the event time. During the course of follow-up, HCQ+ patients were more likely to be prescribed prednisone (p=0.004) and/or other DMARDs (p=0.02). Twenty (64%) patients in the HCQ- group and 9 (33%) in the HCQ+ group (p=0.02) were placed on warfarin during the course of follow-up, suggesting that there was a higher rate of thrombotic events in the HCQ- group. Thirty two patients, 16 (50%) HCQ+ and 16 (50%) HCQ-, were APL/Lac- at baseline. Three (9%) patients became APL/Lac+, all in the HCQ- group. The differences were statistically significant by the log-rank test (p=0.0026). Twenty six patients, 11 (42%) HCQ+ and 15 (58%) HCQ-, had at least one APL/Lac+ at baseline. Six (55%) were on HCQ and 3 (20%) were not on HCQ at the time of conversion to all APL/Lac-. The differences were statistically significant by the log-rank test (p=0.0009). The mean decrease of APL antibody levels was 10.9 GPL in HCQ- group and 20.25 GPL in the HCQ+ group. *Conclusion:* Despite of several limitations associated with this retrospective analysis, our data indicate that HCQ may play a role in decreasing APL antibody levels in APL positive patients and maintaining low APL levels in APL negative patients. This observation is consistent with previously published laboratory data. If confirmed this observation is confirmed in long-term prospective studies, HCQ may be used to treat APLS in the future.

PO2.A.4

Coxiella burnetii infection (Q fever)-induced antiphospholipid antibodies
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Objectives: A variety of autoantibodies have been described in patients with Q fever. There are only a few reports of patients with Coxiella burnetii infection who developed antiphospholipid (aPL) antibodies. Rarely, these patients may present thrombotic events. We report two cases of Coxiella burnetii infection which developed aPL antibodies. *Methods:* Two cases were investigated retrospectively and literature was reviewed *Results:* Case 1: A 61-year-old man presented with acute fever, night sweats, malaise, arthralgias, and a skin rash. Physical examination revealed fever, hepatomegaly, and erythematous nodules on the anterior legs. Laboratory studies revealed a high erythrocyte sedimentation rate, and C-reactive protein level. Cytolytic hepatitis was observed. Cultures of blood, urine, and stools were negative for bacteria and fungi. High titres of anticardiolipin (IgM and IgG) and antibeta-2 glycoprotein I (IgM and IgG) antibodies, were observed. Other serologic tests for autoimmune disorders, and common viral and bacterial infections were negative or normal. Abdominal CT scan showed hepatomegaly. Liver biopsy showed granulomatous hepatitis. Histologic examination of skin biopsy specimens disclosed granulomatous panniculitis. Coxiella burnetii phase II antibodies were increased. Q fever was diagnosed and the patient improved in response to doxycycline 200 mg daily. After therapy, progressive disappearance of aPL antibodies was observed. Case 2: A 20-year-old man presented with a 7-day history of fever, night sweats, headache, malaise, and weight loss. Physical examination revealed fever, and hepatomegaly. Laboratory data showed anemia, thrombocytopenia, high erythrocyte sedimentation rate, and C-reactive protein level, and cytolytic hepatitis. Cultures of blood, urine, and stools were negative for bacteria and fungi. Lupus anticoagulant was identified by activated partial thromboplastin time more than 120 s with failure to correct after the addition of normal plasma. High titres of anticardiolipin (IgM and IgG) and antibeta-2 glycoprotein I (IgM and IgG) antibodies, were observed. Antinuclear antibodies and anti-double-stranded DNA antibodies tests were positive. Other serologic tests for autoimmune disorders were negative. Liver biopsy showed granulomatous hepatitis. Coxiella burnetii serology was positive for phase II antibodies, consistent with a diagnosis of acute Q fever. Administration of doxycycline 200 mg per day for 20 days resulted in progressive disappearance of aPL antibodies. *Conclusions:* Clinicians should considerer the diagnosis of Coxiella burnetii infection in patients with fever and antiphospholipid antibodies. The relationship between induced aPL antibodies and Q fever was confirmed by the disappearance of the aPL antibodies after treatment of the Coxiella burnetii infection.

PO2.A.5

Proteomic microarray for autoantibody profiling and for the discovery of new biomarkers in systemic lupus erythematosus
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High-throughput Proteomic microarray technology has been established in our laboratory and used for autoantibody profiling of the autoimmune diseases. The proteomic array contains over 100 autoantigens which were spotted on the nitrocellulose-coated multi-section slides. The protocol was optimized for simultaneous detection of IgG, IgM and IgA autoantibodies with only 1ul of serum. The autoantigen arrays have been used to screen 373 samples from patients of systemic lupus erythematosus (SLE, n=125), the Incomplete lupus (ILE, n=75), the first degree relative of SLE (FDR, n=50) and the health controls (NC, n=123) with the purpose of identifying the unique autoantibody profiles responsible for the transition of disease situation. Our result shows that over 50% of the IgG autoantibodies increased dramatically in both ILE and SLE patients, comparing with NC and FDR (P<0.05). Two IgG autoantibody clusters were identified to be highly related with the disease criteria and ANA titers, the cluster 1 contains mainly the DNA fragments (e.g., dsDNA, ssDNA, chromatin, dsRNA, glomerular extract, histons) and cluster 2 contains some nuclear antigens (Ro, La, RNP, Sm, Scl-70, Ribo P0), indicating that these IgG autoantibodies are possibly pathogenic antibodies in SLE. Interestingly, most of the IgM autoantibodies showed an increased expression in ILE patients but decreased in SLE, implying that an autoantibody transition from IgM to IgG is required for the development of SLE. Furthermore, using the modified polypeptide, we have developed peptide arrays in order to distinguish the specific epitopes and the amino acid modification in the autoantigens that will bind the autoantibodies. With the combination of proteomic and gene expression microarrays, we have identified a cluster of interferon signature genes which are highly correlated with the autoantibody production in SLE patients. In conclusion, the proteomic microarray is becoming a useful tool for discovering new biomarkers and for early diagnosis of autoimmune diseases.

PO2.A.6

Comparison between multiplex assays for autoantibody detection in systemic lupus erythematosus
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Objective: To compare three multiplexed immunoassays for measurement of multiple autoantibodies and to examine their association with global disease activity, active nephritis and cumulative organ damage in systemic lupus erythematosus (SLE). **Methods:** Stored sera, clinical and laboratory data from the enrollment visit of a long-term lupus registry were used. Autoantibodies

were measured using the BioPlex 2200 ANA screen (Bio-Rad Laboratories), QuantaPlex ENA8 (INOVA Diagnostics) and recomLine ANA/ENA (Mikrogen). The analytes included dsDNA, chromatin, ribosomal P protein, SS-A/Ro60, Ro52, SS-B/La, Sm, U1-RNP, centromere B, topoisomerase 1 and Jo-1 (histidyl tRNA synthetase). Global SLE disease activity was measured by the SLE disease activity index (SLEDAI) and cumulative organ damage by the SLICC/ACR damage index (SDI). *Results:* One hundred ninety two patients (87% female; 91% Caucasian; mean disease duration 8.8 years) were studied. Agreement between the 3 assays varied from 70 - 99% (Cohen's kappa: 0.04-0.88). There were significant associations between SLEDAI scores (excluding anti-dsDNA variable from the index) and ANA (INOVA, Mikrogen), anti-dsDNA (Bio-Rad, Mikrogen), anti-chromatin (Bio-Rad, INOVA), anti-Ro (Mikrogen), anti-Sm and anti-U1-RNP (all 3 immunoassays) (p = 0.002 – 0.05). Concurrent lupus nephritis was associated with anti-dsDNA (Bio-Rad (p=0.017) or Bio-Rad and Mikrogen together (p=0.015). There was no significant association between autoantibodies and SDI scores. *Conclusion:* The overall agreement between the 3 immunoassays for the detection of autoantibodies was reasonable. The greatest discordance (70-83%) occurred with those autoantibodies which were most strongly associated with global SLE disease activity (ANA, anti-dsDNA, anti-chromatin and anti-Sm). Furthermore, there were differences between assays in their associations with global SLE disease activity and lupus nephritis.

PO2.A.7

Autoantibody production in Latin-American lupus patients: data from the multinational Latin-American GLADEL cohort

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We have previously observed potential differences in the prevalence of antinuclear antibodies (ANA's) in the major Latin-American ethnic groups of the GLADEL cohort, but the lack of inter-laboratory control precludes a definitive conclusion. We therefore, have evaluated, in a single center, the prevalence of ANAs and anti-dsDNA in SLE patients enrolled in this cohort derived from 5 Latin-American countries in order to determine if antibody production may vary between Latin-American countries. All the serological studies were performed in a single laboratory to avoid the use of different techniques. *Patients and Methods:* We evaluated 445 serum samples from SLE patients (ACR criteria) from: Argentina, Brazil, Mexico, Peru, and Colombia. As a control group we studied 110 serum samples from healthy subjects. ANAs were detected by indirect immunofluorescence (IIF) assay on HEp-2 cells (The Binding Site Ltd. U.K.). A positive result was considered when ANAs were present at ≥1:160. An equivalent technique (IIF) was employed for anti-dsDNA antibodies detection using Crithidia luciliae (CL) (The Binding Site Ltd., U.K.). The statistical analysis included X2 test for independence. *Results:* ANAs were positive in 81% of SLE patients (361/445) and 9% (10/110) of the control samples (p<0.0001). When comparing these results between the 5 countries, the highest frequency was observed in patients from Brazil and Argentina (p<0.001). In general, the speckled pattern was observed in almost half of the patients 48% (214/445) at ≥1:160 dilution. When comparing the three most frequent patterns observed in this study, there were also some significant differences as it is shown in the Table. Finally, positive anti-dsDNA antibodies were found in 33% (147/445) of the patients, with similar frequencies in all countries. None of the controls had this antibody reactivity.

	Argentina N=178	Brazil N=85	Mexico N=79	Peru N=64	Colombia N=39	P value
ANA's	155 (87%)	77 (93%)	59 (75%)	39 (61%)	29 (74%)	<0.001
Speckled	82 (46%)	40 (47%)	51 (64%)	29 (46%)	16 (41%)	<0.05
2 IF patterns	62 (35%)	26 (30%)	7 (9%)	20 (31%)	15 (38%)	<0.001
Homogeneous	18 (19%)	19 (22%)	21 (27%)	15 (23%)	8 (21%)	<0.01
Anti-dsDNA	58 (33%)	26 (30%)	33 (42%)	18 (28%)	11 (28%)	NS

Conclusions: The differences found in this study suggest that more studies are needed to further evaluate the distinct ethnicity and geographical localization that may have a role in the overall autoimmune response in lupus patients.

PO2.A.8

Immunodominant specificities of circulating autoantibodies in patients with systemic lupus erythematosus. Data from the multinational Latin-American GLADEL cohort.

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The Latin-American population is a unique group with a diverse ethnic background with scarce data in the literature regarding autoantibody profile in lupus patients. In the present study we investigated the specificity of autoantibodies in a cohort of systemic lupus erythematosus (SLE) patients enrolled in the GLADEL cohort that were derived from 5 Latin-American countries with a focus on regional differences. *Patients and Methods:* We evaluated 445 serum samples from SLE patients (ACR criteria) from: Argentina, Brazil, Mexico, Peru, and Colombia. As a control group we studied 110 serum samples from healthy subjects. The immunospecificity was investigated using a line immunoassay (LIA) system (ANA line Blot, DPC, Los Angeles, CA., USA) in a single laboratory. The statistical analysis included X2 test for independence. *Results:* The frequency of each specificity in the table is given in percentage (%):

Autoantibody	Global	Argentina N=178	Brazil N=85	Mexico N=79	Peru N=64	Colombia N=39	P value
dsDNA	20	20	14	21	19	49	NS
Histones	41	47	36	48	36	44	NS
SmB	53	57	59	47	45	44	NS
SmD	20	22	8	26	19	26	NS
RNPr	21	21	18	26	23	13	NS
RNP70	19	22	10	14	23	26	NS
RNP-A	37	42	35	34	28	36	NS
RNP-C	20	25	10	14	19	26	NS
Ro52	40	41	61	38	17	26	<0.0001
SS-A/Ro60	23	27	30	16	14	13	NS
SS-B/La	13	15	24	9	2	5	<0.001

The autoantibody profile of lupus patients of this cohort was within the expected range described for other population using the various methodologies. The overall frequency of autoantibodies to dsDNA, histone, SmB, SmD, RNPr, RNP70, RNP-A, RNP-C and SSA/Ro60 was also comparable among the five analyzed countries (p>0.05). A significant higher frequency of anti-Ro52 (p<0.0001) and anti-SS-B/La (p<0.001) was observed in patients from Brazil and Argentina when compared with the other 3 countries. *Conclusions:* Our finding suggests that regional differences and ethnicity do not seem to play a major role in the pattern of autoantibody secretion for most lupus autoantibodies. In contrast, the distinct frequencies of anti-Ro52 and anti-SS-B/La antibodies may suggest a genetic susceptibility for these particular specificities.

PO2.A.9

Variable effects of antiphospholipid antibodies on in vitro platelet aggregation: possible pro- and anticoagulant mechanisms?

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Objectives: Antiphospholipid antibodies (APA), e.g., anti-beta-2-glycoprotein-1 (anti-β2GP1) and anti-prothrombin (anti-PT), are thought to contribute to the development of thrombosis in systemic lupus erythematosus (SLE), although the precise mechanisms have not been elucidated. The effect(s) of anti-β2GP1 and anti-PT antibodies on platelet activation and aggregation have yet to be extensively investigated and were therefore evaluated in this study. **Methods:** Platelet rich plasma (PRP) was collected from normal donors and standardised to 250 x 109/L for all experiments. ADP (5 and 2.5 µM) and collagen (5 and 2.5 µg/mL) induced platelet aggregation was performed using an AggRAM platelet aggregometer (Helena Laboratories). PRP (200 µL) was incubated with anti-β2GP1 or anti-PT antibodies (25 µL) to final concentrations of 1.25-10 µg/mL for 10 minutes at 37oC. Data, including %Maximum aggregation (%Max) and Slope, was generated and compared to a baseline control (PRP and saline). Results for all parameters were determined as the difference between test and baseline control. **Results:** Anti-β2GP1antibodies significantly reduced platelet aggregation (%Max) in a concentration dependent manner using both 5 µM (P=0.0007) and 2.5 µM (P=0.038) ADP, but did not significantly affect the primary slope (rate) of aggregation. In contrast, the same antibodies significantly enhanced platelet aggregation (% Max) in a concentration dependent manner using 2.5 µg/mL (P = 0.0018), but not 5 µg/mL collagen. Anti-PT antibodies significantly enhanced 5 µg/mL collagen-induced platelet aggregation (% Max) in a concentration dependent manner (P=0.034), but did not affect ADP-induced platelet aggregation. **Conclusions:** Purified anti-β2GP1 antibodies reduced in vitro ADP-induced aggregation, but not the slope/rate, suggesting these antibodies interfere with secondary platelet aggregation. Potential mechanisms may include interference to the G-protein coupled membrane ADP receptors, P2Y1 and P2TAC, or by binding to anionic phospholipids associated with platelet membranes to reduce the release of arachadonic acid and subsequent thromboxane A2 production. In contrast, both anti-β2GP1 and anti-PT antibodies significantly increased collagen-induced platelet aggregation. The results of this study suggest interactions between anti-β2GP1 and/or anti-PT antibodies and platelets are complex, but potentially have a significant effect on the overall balance of haemostasis in patients with SLE.

PO2.A.10

Effects of anti-modified C-reactive protein autoantibodies from patients with lupus nephritis on the interaction between modified C-reactive protein and complements

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Objectives: Previous studies showed high prevalence of mCRP autoantibodies in patients with lupus nephritis, and indicated their association with disease activity. Moreover, the levels of mCRP autoantibodies were correlated with tubulointerstitial lesions in the kidney. The mCRP autoantibodies may be not only a biomarker for disease activity, but also an important contributor in the pathogenesis of SLE, especially lupus nephritis. An important bioactivity of CRP is to activate the classical complement pathway when bound to complement 1q (C1q) (composed of globular region (C1qGR) and collagen-like region (C1qCLF)) and regulate the alternative complement pathway via recruitment of factor H. This study aims to detect whether mCRP autoantibodies could interfere the binding between mCRP and C1q or FH. **Methods:** Autoantibodies against mCRP were purified from plasma exchange from 3 patients with lupus nephritis. Interactions between mCRP and C1q, C1qGR, C1qCLF were analyzed by enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance tests (SPR), respectively. Interactions between mCRP and factor H was performed by ELISA. Inhibition test were performed by incubating mCRP with autoantibodies against mCRP or IgG from normal controls in the former binding assays, respectively. **Results:** mCRP could bind to C1q (KD= 0.52nM). mCRP showed stronger binding activity with C1qCLF than that with C1qGR in ELISA assay and SPR (KD= 15.7nM, KD= 133nM, respectively). We defined the inhibition as the OD value of mCRP incubating with autoantibodies against mCRP decrease over 30% compared with that of mCRP incubating with IgG from normal controls. When the levels of autoantibodies against mCRP went above 5.27ug/mL, the inhibition for the binding between mCRP and C1q occurred. The binding between FH and mCRP was dose-dependent. When the levels of autoantibodies against mCRP raised above 4.45ug/mL, the inhibition for the binding between mCRP and C1q occurred. **Conclusions:** Autoantibodies against mCRP could inhibit the binding acitivity between mCRP and C1q, mCRP and FH, respectively. mCRP mainly binds to C1qCLF rather than C1qGR.

PO2.A.11

Antiphospholipid antibodies and systemic lupus erythematosus: risk scale for the diagnosis of antiphospholipid syndrome

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Background: The antiphospholipid syndrome (APS) is a clinical syndrome consisting of vascular thrombosis and/or pregnancy morbidity in the presence of antiphospholipid antibodies (aPL): lupus anticoagulant (LAC), anticardiolipin antibodies(aCL) and/or anti- β2 glycoprotein-I (antiβ2GPI). APS occurs either as a primary condition or in the setting of an underlying disease, particulary systemic lupus erythematosus (SLE). The presence of LAC and the positivity of multiple aPL seem to be closely involved in the diagnosis of APS, while the association of different tests used to detect LAC with the diagnosis of APS is still unknown, especially in patients with co-existent diagnosis of SLE. **Purpose and Methods:** To develop a chart (graphic representation of classes of risk) "APS Risk Scale (ARS) for the diagnosis of APS in SLE, considering the association of the available tests for LAC, the multiple positivity of aPL and their titer with the diagnosis of APS in a retrospective study involving 124 patients affected by SLE in whom the presence of aPL (LAC by DRVVT, KCT, SCT and STACLOT-LA; antiβ2GPI and aCL was tested. Relative risk (OR) for APS was calculated. Chosen factors for chart construction were: aPL titer and methods used for LAC detection. **Results:** In 58 SLE patients (46,77%) at least one aPL was positive on 2 occasions, and of these, 18 (31 %) patients were diagnosed with APS. The OR for the diagnosis of APS were calculated for each method LAC: STATCLOT-LA 7.1, DRVVT 6.3, SCT 1.25, KCT 1.7. The OR for the diagnosis of APS calculated for each level of antibody titer (low, medium, high) of aCL and anti antiβ2GPI were: aCL-IgM 2.06, aCL-IgG 3.18; antiβ2GPI-IgM 3.22; antiβ2GPI-IgG ns. **Conclusions:** Among the aPL, LAC is more strongly associated with the diagnosis of APS in SLE, particularly if detected with STACLOT-LA and DRVVT, being the most specific for diagnosis of APS. Moreover, multiple aPL positivity, particularly the association LAC+ aCL IgM and IgG and anti B2GPI IgM further increases the risk of APS.

Table 1. APS risk in patients with SLE and aPL

	Titer aCL/anti B2GPI IgG/IgM (kU/l)			
	< 10	10 - 30	30-50	> 50
Methods for LA detection				
SCT	Low Risk	Low Risk	Medium Risk	High Risk
KCT	Medium Risk	Medium Risk	High Risk	High Risk
DRVVT	Medium Risk	High Risk	High Risk	High Risk
STACLOT LA	High Risk	High Risk	High Risk	High Risk

PO2.A.12

Atacicept reduces immunoglobulin and B-cell levels in patients with rheumatoid arthritis

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Background: Atacicept is a soluble, fully human, recombinant protein comprising the extracellular domain of the TACI receptor and a modified human immunoglobulin (Ig) Fc domain, and thus targets the B-cell growth factors BLyS and APRIL. Atacicept is currently being investigated in Phase II/III studies in patients with systemic lupus erythematosus. ***Objectives:*** To report the effects of atacicept on B-cell populations and Igs in patients with rheumatoid arthritis. ***Methods:*** AUGUST I was a Phase II dose-finding study comparing the effects of placebo and atacicept 25, 75 or 150 mg, given twice weekly for the first 4 weeks (loading phase [LP]) and then weekly for 21 weeks. The Phase II AUGUST II study compared placebo, atacicept 150 mg with a LP, atacicept 150 mg weekly without a LP, and open-label adalimumab 40 mg weekly, given for a total of 25 weeks. ***Results:*** At week 26, reductions were seen in total Ig, rheumatoid factor (RF) and anti-CCP antibody titres (see table). Total Ig and RF levels returned towards baseline during a 12-week, treatment-free, follow-up period. Mature B-cell levels were also reduced. ***Conclusions:*** Atacicept had strong effects on all three classes of Ig and RF (~60% reduction in Ig-RF levels with a dose of 150 mg), and also reduced mature B-cell levels. Less marked effects were seen on anti-CCP titres.

Median % change (baseline to week 26)

| | AUGUST I | | | | AUGUST II | | | |
| | | Atacicept | | | | | Atacicept | | Adalimumab |
Dose, mg	Placebo	25	75	150	Placebo	150	150+LP	40
n	62	66	62	64	76	78	78	79
IgG	-1	-16	-23	-30	-2	-30	-31	-12
IgA	-3	-22	-39	-53	-3	-55	-56	-0.4
IgM	-0.1	-33	-59	-70	-5	-72	-66	-15
IgG-RF	0	-27	-57	-55	0	-57	-54	-15
IgA-RF	11	-32	-56	-66	-14	-67	-67	-26
IgM-RF	10	-21	-56	-56	0	-62	-60	-4
Anti-CCP	-7	-12	-23	0	-16	-30	-16	-5

PO2.A.13

Antiphospholipid antibodies and antiphospholipid antibodies: risk scale for the diagnosis of antiphospholipid syndrome

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Background: The antiphospholipid syndrome (APS) is a clinical syndrome consisting of vascular thrombosis and/or pregnancy morbidity in the presence of antiphospholipid antibodies (aPL): lupus anticoagulant(LAC), anticardiolipin antibodies(aCL) and/or anti- β2 glycoprotein-I(antiβ2GPI). APS occurs either as a primary condition or in the setting of an underlying disease, particularly systemic lupus erythematosus (SLE). The presence of LAC and the positivity of multiple aPL seem to be closely involved in the diagnosis of APS, while the association of different tests used to detect LAC with the diagnosis of APS is still unknown. ***Purpose and Methods:*** To develop a chart (graphic representation of classes of risk)"APS Risk Scale (ARS)"for the diagnosis of APS, considering the association of the available tests for LAC, the multiple positivity of aPL and their titer with the diagnosis of APS in a retrospective study involving 3088 subjects in whom the presence of aPL (LAC by DRVVT, KCT, SCT and STACLOT-LA; antiβ2GPI and aCL-IgG/IgM)was tested. Chosen factors for chart construction were aPL titer and methods used for research in LAC. ***Results:*** In 200 subjects (6.5%) LAC was positive in 2 occasions, and of these, 72(36%) patients were diagnosed with APS(in 27 patients APS was associated to SLE). In 425 subjects(13.8%)aPL (aCL and / or antiβ2GPI)medium to high titer were present in 2 occasions, in the absence of LAC and of these only 4(0.9%) receiving the diagnosis of APS. The OR for the diagnosis of APS were calculated for each method LAC: STATCLOT-LA 6.6, DRVVT 5.7, SCT 0.93, KCT 1.24. The OR for the diagnosis of APS calculated for each level of antibody titer(low, medium, high)of aCL and anti antiβ2GPI were: aCL-IgM 1.66, aCL-IgG 2.68; antiβ2GPI-IgM 2.33; antiβ2GPI-IgG ns. (see scale legend). ***Conclusions:*** Among the aPL, LAC is more strongly associated with the diagnosis of APS, particularly if detected with STACLOT-LA and DRVVT. Moreover, multiple aPL positivity, particularly the association LAC + aCL IgM and IgG and antiβ2GPI IgM further increases the risk of APS.

Table 1. APS RISK in aPL patients

| | Titer aCL/anti B2GPI IgG/IgM (kU/l) | | | |
	< 10	10 - 30	30-50	> 50
Methods for LA detection				
SCT	Very Low Risk	Low Risk	Medium Risk	Medium Risk
KCT	Low Risk	Low Risk	Medium Risk	High Risk
DRVVT	Medium Risk	Medium Risk	High Risk	High Risk
STACLOT LA	Medium Risk	High Risk	High Risk	High Risk

PO2.A.14

Clinical characteristics and quality of life of patients with primary and secondary antiphospholipid syndrome

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Objectives: To describe and compare the clinical characteristics, general health and quality of life (QOL) of patients with previous thrombovascular events (TE) followed at the systemic lupus erythematosus (SLE) and antiphospholipid (APS) clinics. ***Methods:*** Clinical data including type and numbers of TE and demographic characteristics, documented prospectively following a standard protocol were retrieved from the University of Toronto Lupus and APS clinic databases. TE was further categorized into arterial (ATE) and venous (VTE).QOL was determined using the Medical Outcomes Study Short Form (SF-36) at the most recent visit. Age and gender standardized Physical (PCS) and Mental component scores (MCS) were calculated. A group of SLE patients who did not meet criteria for APS and without past history of TE (SLE-TE) were included as controls for assessment of QOL. Data were analyzed and compared using descriptive statistics, Chi-square and analysis of variance (ANOVA). ***Results:*** The majority of the subjects in all groups were female: PAPS: 63.5%, SAPS: 87.7%, SLE+ TE: 60.4% and SLE-TE: 87.8%, with mean (SD) age at the time of analysis of 48.3(15.7), 53.2(13.1), 50.5(17.4), 43.3(15.7), respectively. Total number of TE by 100 person-year for ATE/VTE was 8/15, 10/5 and 16/16 for PAPS, SAPS and SLE+TE, respectively. Analysis of the first TE showed that ATE was less prevalent in patients with PAPS (40.4%) compared to those with SAPS (67.7%, P=0.0031) and SLE+TE (52.7%, P=0.14). The most common ATE location was: 1) cerebral for PAPS (n=17); coronary and cerebral for SAPS (n=17), coronary for SLE+TE (n=31). The most common VTE location was deep vein thrombosis in all 3 groups. PCS in PAPS [mean (SD) = 42.5(11.0)] was below the general population norm but was significantly higher than those with SAPS [36.8(12.2)] and SLE+TE [34.8(11.8), P=0.0076], but not different from SLE-TE [39.2(11.7)]. MCS was similar in all groups: PAPS: 47.4(11.4), SAPS:

44.2(11.6), SLE+TE: 46.1(11.2) and SLE-TE: 47.5(11.8). *Conclusions:* ATE is less prevalent in PAPS compared to the other groups. The physical health reported by patients with TE was better in patients with PAPS than those with SLE with and without APS regardless of their gender and age.

PO2.A.15

Detection of anti-Argonaute2 (Ago2/Su) and -Ro antibodies by immunoprecipitation, in primary anti-phospholipid syndrome (PAPS) patients: do they have a predictive role for evolution in systemic lupus erythematosus?

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Purpose: Primary antiphospholipid syndrome (PAPS) is defined as patients with anti-phospholipid antibodies and thrombotic or obstetric symptoms, without systemic lupus erythematosus (SLE) or other systemic rheumatic diseases. PAPS patients can have incomplete SLE-like features and some can evolve into SLE during follow-up. However, few studies systematically examined lupus autoantibodies and their clinical significance in PAPS. The aim of our study is to characterize lupus autoantibodies in PAPS to analyze their clinical and laboratory correlations and their predictive role for the evolution of PAPS into SLE. *Methods:* Sera from 52 PAPS patients were screened by indirect immunofluorescence (IIF) antinuclear antibodies (ANA), immunoprecipitation (IP) of 35S-labeled K562 cell extract, and ELISA [anti-Argonaute2 (Ago2, Su), 60kRo, 52kRo, La, dsDNA)]. Anti-Ago2/Su positive sera were also tested for anti-GW body (GWBs) by IIF double staining using rabbit anti-GWBs serum. *Results:* ANA were positive in 56% of PAPS patients. Anti-Ago2/Su antibodies were found in 13% (7/52) and anti-Ro/SSA in 10% (5/52) by IP. Interestingly, 79% (41/52) were negative by IP, thus anti-Ago2/Su and anti-Ro/SSA were the only identifiable specificities by IP in PAPS patients. No other lupus-related autoantibody was detected. Positive samples had a single specificity except one case that had both. No particular association with clinical features of anti-phospholipid syndrome was found, except that IgG anti-beta2glycoproteinI (beta2GPI) antibodies were less frequent in anti-Ago2/Su positive patients (p = 0.02). Although 2/7 anti-Ago2/Su positive patients had malar rash, there was no significant association between the presence of anti-Ago2/Su or Ro/SSA and SLE-like features in PAPS. None of anti-Ago2/Su or –Ro positive patients developed SLE during follow-up. Although Ago2 is a key component of GWBs and IIF staining of Ago2 localizes to GWBs, only 1/7 anti-Ago2/Su serum showed a typical cytoplasmic GWBs staining. *Conclusions:* Anti-Ago2/Su (13%) and –Ro (10%) antibodies were found in sera from PAPS, but no other lupus-related autoantibodies were identified by IP. Ago2, a microRNA binding protein, is a key component in RNA silencing complex (RISC) but the link with PAPS remains to be clarified. A recent report on specific interaction of Ro60 and beta2GPI in apoptotic cells may be relevant to our finding. Clarifying why Ago2/Su and Ro are specific targets of autoimmune response in PAPS may help to understand the mechanisms of autoantibody production.

PO2.A.16

Detection of antibodies to dsDNA and Ro in serum of animals immunized with beta 2-microglobulin: a possible new animal model of SLE

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Beta 2-microglobulin (B2-M) is a polypeptide (MW 117000), mainly secreted by B and T lymphocytes and is normally found in serum, urine, and other body

fluids. The serum B2-M levels are constant in both sexes except for a slight increase with age. Antibodies to B2-M have been reported in many rheumatic diseases such as systemic lupus erythematosus (SLE), sjogren syndrome (SS) and ankylosing spondylitis. Anti B2-M is found in 71% of SLE, 68% of ankylosing spondylitis and 27% of patients with rheumatoid arthritis. B2-M are also found in the saliva of SLE and SS. One study also reports the presence of Beta 2 microglobulin in tear fluid of patients with primary SS. Elevated B2-M levels have not been shown to be related to any particular clinical subset of the disease(s) but are rather due to an increased synthesis of B2-M, presumably as a result of an activation of the immune system. Beta 2 microglobulin is known to have important role in lymphocytoxic reactions in these patients.
Here we have detected antibodies to dsDNA in animals, immunized with B2-M. We also tested for the presence of anti-dsDNA in the sera of animals immunized with spectrin however, results were negative. Additionally, we found anti-Ro in the sera of animals immunized with this B2-M, but not in the sera of spectrin immunized animals. Considering that deposition of circulating soluble DNA-anti-DNA immune complexes is a major pathogenetic mechanism in lupus nephritis, we propose that the eventual glomerulonephritis observed in SLE could be due to a decreased renal elimination of B2- M caused by complexing of B2-M and autoantibody. In addition, it has been reported that Beta 2 microglobulin deficient mice have been protected from SLE. Therefore, we suggest Beta 2 microglobulin immunization of rabbits or mice, can lead to SLE and perhaps a good model of SLE.

PO2.A.17

Ethnic variations in autoantibody prevalence in lupus

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Ethnic differences in the clinical and serological expression of systemic lupus erythematosus (SLE) have previously been evaluated among African Americans and Caucasians or African Americans and Latin Americans. However, there is little literature that compares African American, Caucasian and Hispanic SLE patients simultaneously. *Aims:* To compare prevalence of serological auto-antibodies by ethnicity among SLE patients. *Methods:* We obtained demographic, clinical and serological data from 271 SLE patients. These data included demographic (age, ethnicity); Clinical (age at disease diagnosis, serological autoimmune antibodies). ANOVA and Fischer exact test were utilized to compare continuous and discrete variables by ethnicity of the subjects. A p value of ≤ 0.05 was considered significant. *Results:* Ninety three percent of the cohort was comprised of women. 56% were African American, 27% Caucasian, and 17% Hispanic. The median (IQR) age were as follows: African American 44.0 (21.0), Caucasian 48.0 (19.0) and Hispanic 31.0 (18.0) yrs (p=0.001). The median (IQR) age at diagnosis were: African American 32.0 (21.0), Caucasian 34.0 (17.0) and Hispanic 26.0 (16.0) yrs (p=0.005). Prevalence of serum auto-antibodies are shown in table 1. *Conclusions:* Ethnic minority patients were younger at disease diagnosis, and had significantly greater prevalence of Anti Smith, anti RNP and ant SS-A antibodies. Recognition of these ethnic differences in prevalence of auto antibodies may lead to greater understanding of the etio-pathogenesis, clinical and health outcomes variations in SLE.

Variables	African-American	Caucasian	Hispanic	P values
DS-DNA	58%	57%	48%	NS
Anti-Smith	61%	17%	41%	<0.001
Anti-RNP	65%	29%	38%	<0.001
SSA	68%	43%	55%	0.005
SSB	30.3%	30%	24%	NS

PO2.A.18

Vitamin D deficiency is associated with autoantibody production and B cell hyperactivity in systemic lupus erythematosus

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Objective: Autoimmune diseases have complex etiologies, likely including variable interactions between genetic predispositions and environmental risks. Vitamin D (Vit D) has recently been linked with many autoimmune disorders, including systemic lupus erythematosus (SLE). The influence of Vit D deficiency on autoantibody production and antigen specific immune responses in SLE patients is an area that remains to be explored. Our study tests whether Vit D deficiency is associated with SLE B cell hyperreactivity or improved antigen-specific antibody responses. **Methods:** This study included 32 European-American SLE patients who met ACR criteria and matched controls. 25-hydroxyvitamin D levels, lupus-associated autoantibodies (Ro, La, Sm, nRNP, ribosomal P, dsDNA AnAs and phospholipid antibodies), and influenza humoral immune response parameters including Bmax (relative amounts of anti-influenza antibodies against native or denatured antigen, measured separately), Ka (relative affinity), and hemagglutination inhibition (relative protective antibody response) were measured. Isolated PBMCs were used for multiparameter PhosFlow analysis of downstream B cell activation markers such as phopsho-LYN (pY508) and phosphor-ERK1/2 (pERK1/2). **Results:** SLE patients and autoantibody-positive controls were significantly more likely to be Vit D deficient than autoantibody-negative controls, 78% vs 61% vs 17%, respectively (p=0.0003 and p=0.026). In SLE patients we saw a significant correlation between Vit D deficiency and an increased humoral immune response to influenza vaccination (p=0.032, r2 =0.149). Furthermore, decreased vitamin D levels in SLE patients were correlated with hyperreactive B cells as measured by pY508LYN (p=0.019) and pERK1/2 (p=0.029). **Conclusion:** Our evidence suggests a role for Vit D deficiency in human B cell hyperreactivity and autoantibody production. We saw an increase in Vit D deficiency in autoantibody-positive controls that is similar to what is seen in SLE patients. Supporting the idea that Vit D deficiency leads to increased B cell activity, we saw a correlation between decreased Vit D levels and an improved humoral immune response to influenza vaccination. The icrease in B cell activity was corroborated by flowcytometric data indicating increased phosphorylation of LYN and ERK1/2 in Vit D deficient patients.

PO2.A.19

The prevalence of antiphospholipid antibody (aPL Ab) subtypes in SLE and CVA patients

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Background: The relative prevalence of aPL Ab subtypes in different diseases has not been well studied. We compared the prevalence of anticardiolipin (aCL), antiphosphatidylserine (aPS), and beta2 glycoprotein I (anti-b2GPI) IgA, IgG, and IgM (9 subtypes) in SLE and CVA. We evaluated an association of aPL Abs with age, gender, ethnicity, and seasons. **Methods:** We identified patients with ICD9 codes for SLE or CVA diagnosed between 1/2006 and 1/2009. We excluded patients with both SLE and CVA. APL Ab positivity (aPL Ab+) was defined as >= 40 GPL. **Results:** 217/1136 (19%) of SLE and 97/5827 (1.6%) of CVA patients had all 9 aPL subtypes measured. 185 SLE and 79 CVA patients were included. Compared with patients without aPL Abs, both SLE and CVA patients with aPL Abs were younger, and included a higher percentage of women. The median age was 38 y.o. IQR (23.7, 48.4) in SLE vs. 49.7 y.o. IQR (30.9, 59.1) in CVA. There were 92% women in SLE vs. 65% in CVA, p<0.001. There was no difference in the time to aPL measurement between the 2 groups (257 days IQR (59, 591) vs. 279 days IQR(15, 727).

The prevalence of aPL Ab+ subtypes, shown in Table 1, was between 0% and 8.7%. Only the prevalence of anti-b2GPI IgG+ was statistically significantly higher in SLE compared with CVA (7.6% vs. 1.3%, P=0.045).

Table 1. The prevalence of various aPL Ab+ subtypes in SLE and CVA

N(%) aPL Ab+	SLE n=185	CVA n=97	p-value (Fisher exact test)
aPS IgA	0 (0)	1 (1.3)	0.3
aPS IgG	4 (2.2)	1 (1.3)	>0.999
aPS IgM	4 (2.2)	3 (3.8)	0.4
aCL IgA	3 (1.6)	2 (2.5)	0.6
aCL IgG	10 (5.4)	4 (5.1)	>0.999
aCL IgM	3 (1.6)	3 (3.8)	0.37
anti-b2GPI IgA	16 (8.7)	2 (2.6)	0.11
anti-b2GPI IgG	14 (7.6)	1 (1.3)	0.045
anti-b2	5 (2.7)	4 (5.1)	0.46

Among patients with at least one aPL Ab+, the prevalence of anti-b2GPI IgG+ and anti-b2GPI IgA+ was higher in SLE vs. CVA: 48.3% vs. 14.3%, p=0.1; 55.2% vs. 28.6%, p=0.2. The prevalence of aCL IgM and aPS IgM was lower in the SLE vs. CVA: 10.3% vs. 42.9%, p=0.07; 13.8% vs. 42.9%, p=0.12. We found no statistically significant associations between aPL Ab+ and age, gender, ethnicity or season. **Conclusion:** The prevalence of clinically significant aPL Ab+ is very low even in this highly selected subgroup of SLE patients and CVA patients. Different aPL Ab subtypes may be associated with different diseases. Several clinically significant differences did not reach statistical significance because of the relatively small sample size. We plan to explore these differences in large prospective studies.

PO2.A.20

TNF-a induces 52 kD Ro/SSA dependent ADCC in primary human keratinocytes

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Introduction: Autoantibodies against the intracellular antigen 52 kD Ro/SSA are associated with photosensitive skin lesions in SLE. UVB induces surface expression of Ro/SSA autoantigens in keratinocytes resulting in ADCC and complement dependent cell lysis contributing to autoimmunity in SLE. The UVB-induced proinflammatory cytokine TNF-α induces intracellular and surface 52 kD Ro/SSA in human keratinocytes is suggested to play a role as mediator in photosensitive skin lesions in SLE. **Objective:** To study the role of TNF-α in 52 kDa Ro/SSA-dependent ADCC in human keratinocytes. **Methods:** Primary human keratinocytes of six donors were cultured for 20 h with/without TNF-α. Viable cells were stained with fluorescent calcein-AM before induction of ADCC with autologous PBMCs ± 52 kD Ro/SSA specific autoantibodies purified from positive tested patient's serum. Cell lysis was assessed in situ by fluorescence microscopy adding ethidiumbromide which penetrates disintegrated cell membranes and photometrically measuring calcein in the supernatants released from dying cells. **Results:** No significant ADCC was induced by addition of 52 kD Ro/SSA and autologous PBMCs in untreated keratinocytes. A significant enhancement of calcein release by TNF-α was observed in the photometric analysis in the six donors (p=0.04). Fluorescence microscopy revealed higher ethidiumbromid uptake in TNF-α treated keratinocytes. **Conclusions:** the TNF-α mediated induction of 52 kD Ro/SSA dependent ADCC suggests a critical role of TNF-α in the pathogenesis of photosensitive cutaneous SLE.

PO2.A.21

Mechanisms of tolerance abrogation by human parvovirus B19

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Objective: Synergistic interactions between viral infections and the immune system are increasingly recognized as important factors in autoimmune disease with significant consequences for the host. An important characteristic of SLE is the formation of anti-nuclear antibodies, with nucleosomal components as key autoantigens. Excess nucleosomes and apoptotic bodies are seen in the circulation in SLE patients, owing to excess apoptosis and/or inefficient clearance of apoptotic products. The model virus human parvovirus B19 is a common and geographically widespread infectious agent known to cause the common childhood exanthema erythema infectiosum (fifth disease) and is associated with a wide array of idiopathic autoimmune disease-like presentations including rheumatoid-like arthritis, vasculitis, and SLE. Infected patients may have circulating autoantibodies including antinuclear antibody, dsDNA antibody, cardiolipin antibody, and rheumatoid factor. Our previous work implicated the non-structural protein NS1 of B19 as the mediator of apoptotic death in non-permissive cells. As a consequence of this programmed cell death, apoptotic debris is created. The objective of this study was to identify cell constituents associated with B19-induced apoptosis that could be candidate autoantigens in SLE. We previously demonstrated that B19 NS1 modifies native DNA through bulky adduct formation. We hypothesized that B19-induced apoptotic bodies contain NS1-modified host cell DNA, as well as self-constituents such as nucleosomes and phosphatidylserine, and that the virally modified host DNA could break tolerance to associated proteins that could result in autoantibody production seen in SLE patients. **Methods:** Recombinant baculoviruses hosting B19 NS1 were used to transduce non-permissive hepatocytes. After 48 hr post-transduction, apoptotic cells and debris were purified and analyzed for potential self-antigens with scanning electron microscopy, flow cytometry and SDS-PAGE. **Results:** Investigation of apoptotic cells indicated that there was an accumulation of DNA damage due to NS1 interaction with cellular DNA, and that apoptotic blebbing was clearly underway 48 hr post-transduction. Direct immunofluorescent studies of apoptotic bodies demonstrated the presence NS1 as well as various self-antigens including DNA, histones, and phosphatidylserine. **Conclusions:** This research offers a novel mechanism to explain immune tolerance breakdown utilizing human parvovirus B19 as a model viral insult. Taken together, our data support our hypothesis that NS1 modifies native DNA and that apoptotic debris created during a B19 infection contains NS1, cellular DNA, and other cell constituents that are frequently targeted as autoantigens in SLE, possibly through epitope spreading from the anti-NS1 response. Our results suggest an autoimmune mechanism for viral induction and/or exacerbation of SLE.

PO2.A.22

Correlates of ANA positivity in healthy individuals

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Objectives: To determine demographic, immunologic and molecular correlates of significant ANA positivity in clinically normal individuals. **Methods:** ANA by ELISA (normal <20 EU) was measured in 401 healthy controls (HC) in the Dallas Regional Autoimmune Disease Registry (DRADR). Detailed studies were carried out on a subset of 18 HC with ANA > 40 EU, 18 HC with negative ANA and 14 SLE patients. Serum antibodies to ~100 autoantigens were detected by protein arrays. Gene expression was analyzed in peripheral blood RNA using the Illumina Human-6 V3 Bead Chip and Bead Studio software. **Results:** ANA positivity was present in 25% of HC and was strongly associated with female (F) gender (P<0.001) but not with age (R^2=0.01) or with total IgG (P=0.2). The high ANA HC subgroup showed significantly (P<0.04)

higher levels of IgG autoantibodies to thyroglobulin, E. Coli, LPS, fibrinogen IV, HSC70, phosphotidyl inositol and Jo-1 compared than the low ANA HC subgroup. Gene expression analysis comparing HC ANA high vs low groups identified 95 specificities at P<0.01. Only 5 specificities were increased in the ANA high group and the most over-expressed was TGM2 which encodes a celiac disease-associated autoantigen. Further analyses demonstrated a stepwise increase in expression of some IFN-alpha signature genes, including IFITM3, IFITM1, IFIT3 and MX1, going from HC Low ANA to HC High ANA to SLE, while other IFN-signature genes, including IFI27, were elevated only in SLE. **Conclusions:** ANA positivity in some HC individuals reflects preclinical autoimmune disease. Determination of risks for progression to SLE in demographic subgroups would be facilitated by identification of biomarkers which are likely to include other autoantibodies and pathogenic gene expression profiles.

PO2B Causation - Environmental

PO2.B.2

Drug-induced lupus caused by contraceptive pills

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Objective: We report a case of SLE induced by contraceptive pills. **Clinical presentation:** Mrs R is a 35-year-old lady presented with one year history of progressive pleuritic chest pain, joint pain, fatigue and excessive hair fall. The patient gave history of being on a regular dose of Diane-35 (a combination of cyprotereone acetate and ethinylestradiol) which was used as a contraceptive method for three years prior to admission. Hemoglobin was 96 g/L (normocytic normochromic with normal reticulocyte count), ESR 120, ANA 1:640, Anti-ds-DNA negative, anti-histones antibodies positive, anti-Ro and anti-La antibodies positive. Complements were normal and ANCA was negative. A work up for antiphospholipid syndrome was negative. Echocardiogram showed 7 millimeters of pericardial effusion and CT chest showed a minimal pleural effusion and pleural thickening. Viral screen including parvovirus serology was negative. All bacterial cultures were negative and ASOT titer was negative. There was no evidence of any other organ involvement including the kidneys. Diane-35 was discontinued. Methylprednisolone pulse therapy was initiated for three days followed by 30 mg of daily prednisolone. **Results:** The patient markedly improved after starting steroid therapy. Chest pain and joint pain subsided, ESR decreased to 40, Hgb increase to 132, A repeated echocardiogram showed disappearance of pericardial effusion. Chest X-Ray showed disappearance of pleural effusion. ANA became weakly positive and anti-histones, anti-Ro and anti-La antibodies became negative. Prednisolone was gradually tapered down and successfully discontinued after a total of six months. The patient has been followed up since then and she has been completely disease free and off medications for about four years. **Conclusion:** Contraceptive pills should be considered as one of the drugs that can induce SLE. Symptoms of SLE should be carefully looked for and diagnosed in patients on contraceptive pills. The disease is completely irreversible and treatable in our case.

PO2.B.3

Adverse effects of helplessness, internality and depression on disease specific health related auality of life in lupus

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Objective: Helplessness has been shown to be predictive of disease activity and health related quality of life in patients with Systemic Lupus Erythematosus (SLE). Generic health related quality of life (HRQOL) tools or preference based health indices have been previously utilized in such studies. Recent studies indicate that generic HRQOL tools do not include all domains pertinent to SLE patients and that they may not be sensitive to changes in the disease status. LupusPRO is a disease specific quality of life tool that includes both HRQOL and non-HRQOL constructs. Our objective was to determine the relationship of helplessness and Internality with disease activity as well as disease specific patient reported quality of life outcomes in SLE. *Methods:* Cross sectional Data from 98 SLE subjects collected for a health outcomes study was analyzed. Arthritis Helplessness Index was utilized to obtain helplessness and Internality scores. Patient Health Questionnaire-8 was used to measure depression. Descriptive, Spearman correlation coefficients were obtained between a) Helplessness, Internality and disease activity measure (SELENA-SLEDAI) b) Adjusted HRQOL (HRQOL scores excluding the emotional health domain) and non-HRQOL domains of the LupusPRO. Linear regression analysis with adjusted HRQOL as the dependent and helplessness and depression as independent variables was performed. P value of ≤ 0.05 on two tailed tests was considered significant. *Results:* 96% of SLE patients were women. 48% were Caucasians and 52% were Hispanics. Mean (SD) age was 44.2 (13.4) yrs. Median (IQR) disease activity score was SLEDAI 2.0 (4). Median (IQR) helplessness and Internality scores were 15 (5) and 24.5 (6.3). Correlation between helplessness, Internality and SLEDAI were -0.09 (p=0.4) and - 0.07 (p=0.5) respectively. Helplessness correlated with the following LupusPRO HRQOL domains: Lupus Symptoms (0.28, p=0.01), Cognition (0.34, p=0.00), Physical Health (0.30, p=0.00), Pain-Vitality (0.21, p=0.05), Emotional Health (0.32, p=0.00). The correlation with the summarized adjusted HRQOL score was 0.30 (p=0.006). Helplessness did not correlate with any of the non-HRQOL domains. Similar results were noted with internality, except the direction of association was reversed. On Linear regression analysis, helplessness could explain 10% of variance in the adjusted HRQOL (β 0.31, 95%CI 0.38, 1.92, P=0.004). However when depression was added to the model, helplessness did not retain significance. Similarly, Internality explained 8% of HRQOL variance (β -0.28, 95%CI -1.2, -0.17, P=0.01), but this association was lost by adding depression variable to the model. *Conclusions:* Helplessness and Internality are not associated with disease activity. Weak correlation with HRQOL, but not the non-HRQOL domains of the LupusPRO were observed with helplessness and internality. These associations however were due to confounding by depression.

PO2.B.4

Secondary autoimmune diseases after immunoablation and hematopoietic stem cell transplantation for SLE develop through occupation of empty plasma cell niches by newly generated autoantigen-specific plasma cells

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Introduction: Stem cell therapy for autoimmune diseases is rapidly developing with promising clinical results. It is increasingly recognized, however, that these patients have an added propensity to develop secondary autoimmune disorders, distinct from the underlying autoimmune disease. *Methods:* As part of a monocentric phase I/II clinical trial, eight patients received immunoablation with CYC and rabbit-ATG and CD34-ASCT for refractory SLE. Patients were evaluated at 3, 6, 9 and 12 months post-transplant and yearly thereafter, including clinical examinations, laboratory and serologic analyses and immunophenotyping of peripheral blood lymphocyte subsets. *Results:* With a median follow-up of 96 months, clinical remission (SLEDAI ≤ 3) could be achieved in all patients accompanied by the disappearance of anti-dsDNA antibodies and protective antibody titers in serum. Two patients died due to transplant related infectious complications. From the other 6 patients, three developed secondary autoimmune disorders while being in stable clinical remission of SLE: Graves' disease (p#1, +20mo), autoimmune thyreoiditis (p#2 +23mo) and acquired factor VIII hemophilia (FVIII AH, p#7, +9mo) with autoantibodies directed against TSH-receptor, thyroid-peroxidase and factor VIII, respectively. Flow cytometric analyses revealed a significant expansion of peripheral blood CD27++ CD20- plasma blasts during development of the secondary autoimmune diseases only in case of the FVIII AH. FVIII AH was refractory to treatment with rituximab and immunoadsorption, cyclophosphamide and intravenous immunoglobulin according to the modified Bonn-Malmö protocol, but disappeared completely during SLE-reactivation accompanied by high titers of anti-dsDNA antibodies +36mo after ASCT. *Conclusion:* We propose that the development of these primarily antibody-mediated secondary autoimmune diseases early after immunoablation may be promoted in SLE on a genetic background through occupation of empty plasma cell niches due to effective plasma cell depletion by newly generated autoantigen-specific plasma cells. This may happen when autoreactive B cells clones appear by chance at that time based on the genetic background. The spontaneous disappearance of the FVIII-inhibitor during the lupus-flare may illustrate a direct competition for plasma cell survival niches in the bone marrow with recurring "lupus-specific" plasma cells outcompeting the FVIII-inhibitor.

PO2.B.5

Prevalence of antibodies against hepatitis C virus in patients with systemic lupus erythematosus

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Objective: Viruses might be one of the environmental triggers of Systemic lupus erythematosus (SLE). Accordingly, the aim of the present study was to determine the prevalence of hepatitis C virus (HCV) antibodies in SLE patients monitored in the out-patient clinic of our institution. *Methods:* Eighty-eight SLE patients (83 women and 5 men, mean age 33.5 years) diagnosed according to ACR criteria for the diagnosis of SLE were recruited in this case-control study. Patients were evaluated clinically and disease activity was assessed by SLEDAI (Systemic Lupus Erythematosus Disease Activity Index) and laboratory investigations (CBC, anti-dsDNA, C3, urine analysis, urine albumin ratio). Serodiagnosis was conducted in all patients to examine the antibodies of HCV with a third generation ELISA (ELISA-3). Positive results for anti-HCV were confirmed by a third generation recombinant immunoblot assay (RIBA-3) and polymerase chain reaction (PCR). Three hundred and fifty

healthy blood donors presenting to the regional blood bank and transfusion centre served as controls. *Results:* The results were compared with the prevalence of HCV viraemia in the control group of blood donors recruited from the regional blood bank and transfusion centre in the same geographic area as the patients. The results showed no significant difference between the healthy blood donors and SLE patients in terms of prevalence of anti-HCV (p > 0.05). Antibodies to HCV were present in (10/88)11% of the SLE patients versus (34/350)10% in the control group. Among the anti-HCV positive group, HCV was confirmed by PCR in 7% (6/88) which was not statistically different from the prevalence of the control group 6.6% (23/350), p>0.05. *Conclusion:* Our findings revealed that the prevalence of anti-HCV in SLE patients was not higher than that of the general population. Consequently a causal relationship between HCV and SLE could not be established.

PO2C Clinical Measurement

PO2.C.1

Assessment of the LupusQoL questionnaire in a longitudinal evaluation

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The LupusQoL questionnaire has been developed in the United Kingdom for adults with lupus. We aimed to assess whether the LupusQoL contributed additional information not obtained using the SF-36. *Methods:* 41 patients seen at a single centre were followed monthly for 12 months. Both questionnaires were co-administered to the patient on each visit. For both questionnaires, 0 reflects worst and 100 best. We determined the correlation of the 4 similar domains of both questionnaires; Physical Health and Physical Functioning, Emotional Health and Mental Health, Pain and Bodily Pain, and Fatigue and Vitality. We determined the correlation between each domain of the LupusQoL with both the physical component score (PCS) and the mental component score (MCS) of the SF-36. We analyzed the mean score for all comparable and non-comparable domains in both questionnaires in 351 patient-visits and 41 patients' average scores. *Results:* Among the 41 patients 37 were female and 4 were male. 351 patients-visits were recorded for both questionnaires. Patients were 56% Caucasian, 17% Black, 7% Asians, and 20% other. The age at study start 54±13.2 and disease duration at study start 14.9±10.3 years. We found that related domains of the LupusQoL correlated with the SF-36 domain; Physical Health and Physical Functioning r=0.74, Emotional Health and Mental Health r=0.83, Pain and Bodily Pain r=0.75, and Fatigue and Vitality r=0.75 (all with p<0.0001). For the 4 non-comparable domains of the LupusQoL, there was a correlation between Body Image and MCS SF-36, Planning and MCS SF-36 , Intimate Relationships and PCS SF-36 , and Burden to Others and MCS SF-36 (Table1).

Table1. Correlation Coefficient between Summary scales of SF-36 and domains of LupusQoL in all 351 patients-visits (all p<0.0001)

	SF-36: PCS	SF-36: MCS
Domains	R	r
Physical	0.80	0.54
Emotional	0.48	0.81
Body Image	0.49	0.61
Pain	0.73	0.51
Planning	0.59	0.68
Fatigue	0.70	0.66
Intimate	0.73	0.41
Burden	0.44	0.70

The mean score for comparable domains in LupusQoL and SF-36 in 351 patient-visits was always higher in LupusQoL; (Mean score in 351 patients-visits: Physical Health and Physical Functioning 70.7±22.8/63.6±27.5, Emotional Health and Mental Health 76.8±22.9/67.9±22.3, Pain and Bodily Pain 73.8±23.9/64.5±27.2, Fatigue and Vitality 63.8±26.6/50.0±26.2). The same

was seen with mean of patient's averages. *Conclusions:* LupusQoL correlated with the comparable domains of the SF-36. Non-comparable domains of LupusQoL offer no additional information to SF-36. There is no superiority of LupusQoL over SF-36 in assessing lupus patient's quality of life. The utility of LupusQoL change over time and with disease activity needs to be evaluated in future studies.

PO2.C.2

Serum levels of RANTES in systemic lupus erythematosus

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Objective: RANTES or CCL5 has function as chemotactic cytokine for white cells (esinophils, basophils, T-cells), which plays active role in inflammatory process. IL-2 and IFN-γ released by T-cells facilitate with RANTES proliferation and of natural-killer (NK) cells, which form CHAK (CC-Chemokine-activated killer) cells. The local production of RANTES was reported in various inflammatory sites in autoimmune diseases like joints in rheumatoid arthritis or in murine models of lupus nephritis.It was therefore hypothesized to play a role in immune-inflammatory systemic lupus erythematosus (SLE). *Aim and Methods:* Soluble serum RANTES was measured using ELISA in SLE (n=20) in comparison to rheumatoid arthritis (RA, n=15), kidney transplant patients (n=15) and healthy controls (=15). *Results:* The mean level of RANTES (pg/ml) was 73356.25±40545.37 in SLE. There was found correlation with anti-dsDNA antibodies (r=0.604,p <0.01) of all used SLE tests. The corresponding levels of RANTES in RA patients were 71191.33±40067.80, 42190.0±20433.78 in transplant patients and 45558.33±13656.33 in healthy controls. There was found difference between SLE patients and healthy controls, between SLE and transplant patients. There was also found difference between RA and transplant patients as well as RA and healthy controls (p<0.01 for all). *Conclusion:* RANTES serum levels were increased in patients with autoimmune diseases (RA, SLE) known for variety of T and B cells impairment leading directly to excessive cytokine production, thus making possible asset in diagnostic tests. *Key words:* RANTES, CCL5, systemic lupus erythematodes, kidney transplantation, rheumatoid arthritis. *Supported by grant n. RP 164*

PO2.C.3

Evidence that the criteria used to define a proteinuria flare (PF) are set too high: an analysis based on quantifying the spontaneous variation in urine protein(P)/creatinine (C) ratio in SLE GN patients who are clinically stable (not flaring)

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Objectives: The published thresholds for PF can be categorized as low (24-hr P/C ratio increase from ≤ 0.5 to > 1.0), medium (24-hr P increase from < 200 mg to > 1,000 mg, or high (> 2,000 mg increase if baseline 24-hr P < 2,000 mg) (CJASN 3:1028-1033, 2008). These are based on expert opinion. Ideally, the PF threshold should be high enough to reasonably exclude spontaneous variation in P but not so high as to excessively expose the kidneys to heavy P. The Ohio SLE Study (OSS) database was used to examine this issue. *Methods:* SLE GN patients (ISN/RPS III, IV, and V, N = 73) followed prospectively in the OSS for a median of 3.6 years provided 1169 urine collections (UC) on which P/C was measured (89% 24-hr UC, 11% random spot UC). 91% of the UC were bimonthly. Excluded were the UC collected 2 months before PF, at PF, and until P/C ratio returned to baseline or to a new steady state. The included P/C values (N=894) were consecutive measures stratified

into groups (Gp) according to the mean value, as follows: Group 1: P/C ≤ 0.15 (this corresponds to P ≤ 200 mg/d), Gp 2: mean P/C > 0.15 to ≤ 0.38, Gp 3, 4, 5, and 6 mean P/C > 0.38 to ≤ 0.77; P/C > 0.77 to ≤ 1.54 to ≤ 3.1 and P/C > 3.1, respectively. The rationale for stratification is that most PF criteria specify P change from a given baseline level. To quantify spontaneous variation, we determined for each Gp how many standard deviations (SD) above the mean were needed to reach the P/C level that constituted a PF, with a larger number of SD indicating less spontaneous variation: **Results:** See table.

	Gp number (N)[1]	# of SD above the mean P/C to meet PF criteria[2]
Low threshold criteria		
LJP 394	1 (272)	10.5
OSS	1 + 2 (511)	8.5
Intermediate threshold criteria		
BILAG A	1 (272)	10.5
High threshold criteria		
See CJASN reference (above)	All Gp (894)	6.6

1 The parentheses show the number of individual P/C measures.
2 ±2.58 SD = 99% CI.

Conclusions: The high number of SDs required to reach the P/C criteria for PF reveals that the current SLE PF criteria are set well above the 99% CI for spontaneous variation in proteinuria. Using these criteria may lead to chronic kidney injury due to excessive exposure to proteinuria before therapy is instituted. A randomized trial is needed to test this hypothesis.

PO2.C.4

A comparison of vitamin D status in Hispanics and African Americans with lupus and rheumatoid arthritis

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Objective: Vitamin D is important for bone health, and possibly for the pathogenesis of autoimmune diseases. Previous studies demonstrated that vitamin D deficiency is highly prevalent in people with autoimmune diseases. While it is well established that African-Americans have higher rates of vitamin D deficiency compared with Caucasians, the rates of vitamin D deficiency have not been described in Hispanic patients with SLE or RA. Several studies showed that SLE patients had lower vitamin D levels compared with RA patients in the univariate analysis. However, there are no studies to date comparing vitamin D levels in SLE and RA using multivariate analysis adjusting for demographics, laboratory data, and medications. **Method:** We identified all patients followed at Montefiore Medical Center rheumatology clinics with confirmed diagnoses of SLE and RA who had vitamin D measured between 2000 and 2009. Data were analyzed by disease category and by ethnicity using multivariate logistic regression. **Results:** Over 90% of SLE patients and over 75% of RA patients were vitamin D deficient (<20 ng/ml). Median (IQR) vitamin D levels were higher in RA patients compared with SLE patients (20.9 (16.1, 29.9) vs. 18 (11.3, 25.4) respectively, p=0.004) consistent with previous reports. However, in the multivariate analysis, the odds of having vitamin D levels in the highest quartile vs. the lowest quartile in RA patients were 1.1, 95%CI (0.59, 1.93) compared with SLE patients. Hispanic patients had similar rates of vitamin D deficiency compared with African-Americans in both disease categories. **Conclusion:** Vitamin D deficiency is highly prevalent in our patient population with SLE and RA. However, the odds of low vitamin D are similar in SLE and RA after adjusting for medications, and demographic and laboratory parameters. Hispanic patients with SLE, like African-American patients, are at an increased risk for vitamin D deficiency compared with other ethnic groups.

PO2.C.5

Vitamin D deficiency in ethnically diverse population with systemic lupus erythematosus (SLE) and type 2 diabetes (DM2)

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Objective: Over 60% of SLE patients are vitamin D deficient or insufficient. Several small studies have compared vitamin D levels in SLE and other chronic diseases including fibromyalgia, rheumatoid arthritis (RA), and osteoarthritis (OA), using univariate analysis, demonstrating that SLE patients have significantly lower vitamin D levels compared with RA and OA, but not compared with fibromyalgia patients. Our hypothesis was that vitamin D levels may be influenced by medications, as well as demographic and laboratory parameters, and may serve as a marker of chronic disease state. Therefore, we compared vitamin D (25-hydroxyvitamin D, or 25OHD) levels in two chronic diseases, SLE vs. DM2. We also compared the rates of vitamin D deficiency in our patients with the rates from the 2001 - 2004 NHANES (The National Health and Nutrition Examination Survey). **Method:** We identified all patients followed at Montefiore Medical Center (Bronx, NY) with confirmed diagnoses of SLE or DM2 who had 25OHD measured between 2000 and 2009. Patients with both diseases, or with coexisting diagnoses of other autoimmune diseases, were excluded. **Results:** We included 123 SLE and 3691 DM2 patients with confirmed diagnoses in our final analysis. Almost 70% were African-American or Hispanic. Compared with SLE patients, the odd ratio of having low 25OHD was 2.0, 95%CI (1.3, 3.1) in the DM2 group, p = 0.003. However, based on the results of the most recent NHANES survey, the prevalence of 25OHD deficiency was lower in our DM2 and SLE patients than in the general population. Furthermore, we found that different multivariate models apply to different ethnic groups. In 1132 African-Americans, younger age, low serum calcium, high serum creatinine, and low light season (October to March) were associated with vitamin D deficiency. In 927 non-African-Americans/non-Hispanics, DM2, younger age, and low serum calcium were associated with 25OHD < 20 ng/ml, while bisphosphonate therapy and vitamin D supplementation were associated with 25OHD > 20 ng/ml. In Hispanics (n=1030), only younger age and low serum calcium were associated with the higher odds of 25OHD deficiency. **Conclusion:** 25OHD deficiency is highly prevalent in our patient population with SLE and DM2. However, the prevalence of low vitamin D levels is surprisingly not higher than in the general population based on the most recent NHANES report. Furthermore, in the multivariate analysis, SLE patients were less likely to be 25OHD deficient compared with DM2 patients. Importantly, different demographic and laboratory factors predicted vitamin D deficiency within different ethnic groups. Therefore, disease-specific and ethnicity-specific definitions may need to be established in future studies.

PO2.C.6

Prevalence of alopecia in rheumatic disease

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Objectives: Although alopecia is a common finding in patients with systemic lupus erythematosus (SLE), there is a paucity of data in the literature regarding the prevalence of alopecia in other rheumatic disorders. The primary objective of this study was to provide novel data on the prevalence of alopecia in rheumatic disease. The secondary objective was to explore how alopecia affects quality of life in SLE patients. **Methods:** We analyzed data from a longitudinal outcome study of patients with rheumatic diseases including rheumatoid arthritis (RA), fibromyalgia, non-inflammatory rheumatic diseases (NIRD), and SLE. Patients were sent comprehensive biannual questionnaires. In July 2008, the survey was updated to include additional questions about alopecia. **Results:** 6760 patients responded to the July 2008 survey. The overall prevalence of alopecia among survey respondents was 23.56%, and in patients with

SLE was 38.88%. Patchy alopecia was reported in 9.27% of SLE patients compared to a range of 1.31-1.82% in all other patients.(Table 1) When controlling for SLE damage, alopecia was a significant predictor of QOL reducing the visual analog scale (VAS) coefficient by 0.024 units (p=0.01) and the EQ-5D coefficient by 0.63 units. (p=0.002). **Conclusions:** Although alopecia is seen in many rheumatic diseases, it is almost twice as prevalent in SLE and SLE patients report more patchy alopecia. In SLE patients, alopecia decreased QOL as measured by both the EQ-5D and the VAS QOL scale. Although illness severity was controlled for in this analysis, some of the decrease in quality of life may be attributed to confounding variables that were not accounted for in this analysis such as medications or other illnesses.

Variable	Fibromyalgia, mean (SD)	SLE, mean (SD)	RA, mean (SD)	NIRD, mean (SD)
N	605	474	4839	842
Hair loss in last 6 months (%)	25.17	38.88	22.44	20.33
Visibility of hair loss	1.64 (0.69)	1.75 (0.71)	1.76 (0.75)	1.87 (0.76)
Not visible (%)	48.03	40.56	42.94	36.11
Slightly visible (%)	40.13	43.89	38.17	41.11
Obviously visible (%)	11.84	15.56	18.9	22.78
Hair loss - patches (%)	1.83	9.27	1.46	1.31
Hair loss caused by medication (%)	7.32	11.23	12.15	5.02
Hair loss caused by illness (%)	5.67	24.19	5.77	2.15
Hair loss severity (0-10)	2.72 (2.45)	3.06 (2.47)	2.22 (2.43)	2.40 (2.69)

PO2.C.7

Cardiorespiratory abnormalities during graded exercise in woman with systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disorder that affects all organs including the cardiorespiratory system. It is well-known that its parenchymal and vascular pulmonary lesions can lead to ventilatory restriction. Moreover, alterations in left ventricular function or structure have been identified in the disease. There are however, scarce information about cardiorespiratory responses (CR) in SLE patients. **Purpose:** To identify cardiorespiratory responses (CR) during graded exercise in SLE patients. **Methods:** Sixteen consecutive SLE women (SLE) with systemic lupus erythematosus activity score (SLEDAI) <4 ,without cardiopulmonary involvement were selected and compared to 13 healthy women (CONTROL). All subjects performed a progressive treadmill cardiopulmonary test until exhaustion to determine the maximal aerobic capacity, ventilatory anaerobic threshold (VAT) and respiratory compensation point (RCP). **Results:** Age (29.4 ± 1.4 vs. 25.8 ± 1.6 years, p=0.11) and body mass index (23.9 ± 0.9 vs. 21.9 ± 0.5 Kg/m2, p=0.09) were alike between SLE and CONTROL groups. SLE had significant lower peak workload (4.6 ± 0.2 vs. 5.6 ± 0.1 mph, P<0.05) and relative aerobic fitness [VO2peak] (28.1 ± 1.1 vs. 37.6 ± 1.4 mL.kg-1.min-1, P<0.05). In addition, HR, O2 pulse, VE/VCO2 and Pet CO2 were significantly altered in SLE compared to CONTROL (Table).

	VAT		RCP		PEAK	
	SLE	Control	SLE	Control	SLE	Control
HR (bpm)	$124.7 \pm 4.6^*$	140.5 ± 3.9	$155.1 \pm 4.0^*$	172.8 ± 3.3	$173.8 \pm 3.5^*$	188.2 ± 2.6
O2 pulse (ml/bpm)	8.2 ± 0.2	9.6 ± 0.5	$9.3 \pm 0.5^*$	11.1 ± 0.5	$9.7 \pm 0.6^*$	11.7 ± 0.4
VE/VCO2	$30.1 \pm 1.0^*$	26.0 ± 0.7	$31.0 \pm 0.9^*$	27.2 ± 1.0	33.8 ± 1.3	31.0 ± 1.0
PetCO2 (mmHg)	$32.5 \pm 0.8^*$	38.0 ± 1.1	$31.9 \pm 0.9^*$	37.0 ± 1.7	$30.0 \pm 1.2^*$	33.0 ± 1.5

Values are means ± SE. VAT- ventilatory anaerobic threshold. RCP- respiratory compensation point. VE/VCO2- ventilatory equivalent for carbon dioxide. PetCO2- end-tidal pressure for carbon dioxide. * P< 0.05 vs. Control (Two-way ANOVA).

Conclusion: The lowest value of O2 pulse during graded exercise observed in SLE may be associated to a low inotropic response and also to an impaired oxygen extraction in the peripheral muscles. Moreover, the increase in ventilatory demand during graded exercise is related to a lower ventilatory efficiency in SLE. The novel finding of a decreased cardiorespiratory response in SLE probably associated to abnormalities in oxygen delivery system may be a relevant contributing factor to aggravate the known decreased aerobic capacity observed in these patients.

PO2.C.8

Discordance between self-report/perception and physician-assessed disease activity in SLE patients: implications for clinical trial design

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Background: Indices of disease activity in systemic lupus erythematosus (SLE), including SLEDAI (SLE Disease Activity Index), BILAG (British Isles Lupus Assessment Group index), SLAM (SLE Activity Measure) and ECLAM (European Consensus Lupus Activity Measurement), are rated by a physician-assessor based on history, objective signs and laboratory data. Discordance between the physician and patient estimate of disease activity has been described previously in SLE. **Objectives:** To analyze agreement levels between patient and physician assessments. **Methods:** A cross-sectional study was performed in 50 consecutive SLE patients in a rheumatology practice. Patients completed the SLAQ (Systemic Lupus Activity Questionnaire) and MDHAQ (Multidimensional Health Assessment Questionnaire), which includes scales for physical function (FN); 0-10 visual analog scales for pain (PN), global estimate (PTGL), and fatigue (FT); RAPID3 (FN, PN, and PTGL); review of systems checklist (SX). The SLEDAI 2K, BILAG, SLAM, ECLAM, and physician global assessment (PGA) 0-3, were recorded by the rheumatologist. The level of agreement was determined by the strength of Spearman correlations. **Results:** The study included 45 women and 5 men, mean age 38.7 years, disease duration 7.3 years, 36% Caucasian, 18% Black, 26% Hispanic, 18% Asian. The mean PGA (1.10 ± 0.62), PTGL (3.11 ± 2.81) and SLE indices (SLEDAI 5.02 ± 3.75; BILAG 4.60 ± 4.31; SLAM 3.86 ± 2.92; ECLAM 1.97 ± 1.37) indicated mild/moderate lupus activity. Correlation between PGA and SLE indices was 0.60-0.72 and correlation between PTGL and patient measures 0.58-0.87. However, PTGL was correlated at lower levels (0.35-0.40) with BILAG and SLAM and not significantly correlated with SLEDAI or ECLAM. None of the patient measures significantly correlate with PGA.

		PTGL	PGA
Patient Measures	SLAQ total score	0.60*	-0.08
	SLAQ No positives	0.58*	-0.06
	SLAQ VAS activity	0.69*	0.34
	MDHAQ - FN	0.68*	0.08
	MDHAQ - PN	0.87*	0.08
	MDHAQ - FT	0.79*	-0.02
	MDHAQ - PTGL	---	0.14
	MDHAQ - Sx	0.66*	0.025
Physician Measures	SLEDAI	0.16	0.72*
	BILAG	0.35**	0.70*
	SLAM	0.40*	0.64*
	ECLAM	0.26	0.60*

* p<0.0001 **p<0.01

Conclusions: All physician-utilized instruments correlated well with each other. Similar results were observed for the patient-utilized instruments. Despite these correlations, there was a near absence of agreement between physician and patient's ratings. Further understanding of this discordance is required to gauge the clinical relevance of outcomes in trials and improve care and compliance in patients with SLE.

PO2.C.9

A multi-dimensional health assessment questionnaire (MDHAQ) provides clues to differentiate non-inflammatory from inflammatory symptoms in patients with SLE

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Background: Many patients with systemic lupus erythematosus (SLE) have non-inflammatory symptoms (NIS) and concomitant fibromyalgia. The clinicians' challenge is differentiating NIS from inflammation since treatment of NIS with anti-inflammatory therapy is unlikely to result in clinical benefit. **Objective:** To analyze if quantitative scores on a multidimensional health assessment questionnaire (MDHAQ) provide clues to the likelihood of NIS in SLE. **Methods:** A cross-sectional study was performed in usual care of 50 consecutive SLE patients. Patients completed an MDHAQ [including scales for physical function (FN), 0-10 visual analog scales for pain (PN), global estimate (PTGL), and fatigue (FT), review of systems checklist (SX)] and also completed a SLAQ (SLE assessment questionnaire). The rheumatologist (absent knowledge of the MDHAQ) recorded the SLEDAI-2K (SLE Disease Activity Index), BILAG (British Isles Lupus Assessment Group index), SLAM (SLE Activity Measure), ECLAM (European Consensus Lupus Activity Measurement), and physician global assessment (PGA) 0-3. In addition the same physician estimated NIS level on a 0-3 scale. Differences between SLE patients with no/minimal NIS (<0.5) and those with NIS (>=0.5) were analyzed. **Results:** The study included 45 women/5 men, age 38.7 years, disease duration 7.3 years, 34 noNIS and 16 NIS. NIS patients had significantly higher FN, PN, FT, PTGL, SX, SLAQ, SLAM without labs, and lower CRP; however, no significant differences between the groups in SLEDAI, BILAG, SLAM, ECLAM, C3, C4, antiDsDNA, or ESR. Fewer than 50% of noNIS patients had FN, PN, PTGL, or FT >= 2, while 100% of NIS had FT and 94% PTGL>2. More than 5 SX were reported by 15/34 of noNIS vs 16/16 NIS patients, > 10SX for 6/34 noNIS vs 12/16 NIS patients.

	SLEnoNIS		SLE+NIS		p Mann-Whitney
	Mean	Median	Mean	Median	
MDHAQ Physical funct	0	0.74	2.7	2.7	0.01
MDHAQ Pain VAS	1.8	2.5	5.0	4.9	0.02
MDHAQ Fatigue VAS	2.0	2.8	6.5	6.3	0.01
MDHAQ Global VAS	1.3	2.2	5.5	5.4	0.01
MDHAQ # Symptoms	5	6.9	17	16.9	0.001
SLEDAI	4.0	5.1	4.0	4.8	0.83
BILAG	2.5	4.2	4.0	5.4	0.27
ECLAM	1.5	1.9	2.0	2.2	0.65
SLAM	3.0	3.7	4.0	4.2	0.32
SLAM-no lab	1.0	1.6	3.0	3.3	0.01
SLAQ-total	6.5	7	16	16.8	0.001
C3	103	102.1	110	123	0.67
C4	17.5	20.1	22	21	0.37
DNA+	17		4		0.129
CRP	3.1	5.4	0.2	0.7	0.015
ESR	12.5	27.1	10	11.2	0.27

Conclusion: High scores for dysfunction, pain, fatigue, global estimates, and symptoms are common in SLE with NIS. SLE indices do not distinguish patients with NIS from those assigned noNIS. Collection of a 0-3 scale to estimate NIS may be informative in therapeutic decisions, particularly if reinforced by patient questionnaire patterns.

PO2.C.10

Low bone mass in female patients with systemic lupus erythematosus (SLE): a Canadian study

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Objective: To determine the prevalence and risk factors for low bone mass in a Canadian population of SLE patients enrolled in the Health Improvement & Prevention Program (HIPP). **Method:** 270 women with SLE without history of osteoporosis were recruited from three Canadian lupus centers and underwent bone mineral densitometry (BMD) of the lumbar spine and femoral neck by DXA scans. Osteoporosis was defined as T-score < -2.5 for those > 50 years, and low bone mass as z-score < -2 for those < 50 years of age. Ten year fracture risk was calculated using the Osteoporosis Canada tool. Data on age, BMI, SLE duration, menopausal status, tobacco use, physical activity, falls, previous fractures, and use of calcium, vitamin D and corticosteroids at the time of enrolment were collected. Descriptive statistics are presented. **Results:** Baseline characteristics were: mean (SD) age: 44.0 (12.9) years, 67.4% were < 50 years of age, SLE duration: 11.6 (10.4) years, BMI (kg/m2): 25.7 (5.9), current smoking: 25.9% (n=70), sedentary lifestyle: 48.7% (n=131), postmenopausal: 36.3% (n=98) and; 69.3% (n=187) were taking prednisone at the time of enrolment. Calcium and vitamin D were used by 53.0% (n=143) and 40.4% (n=109) respectively, 28.8% had falls in the preceding year and 25.9% had previous fractures. In women < 50 (n=182), 74.2% were on prednisone and 42.3% were taking calcium/vitamin D supplement. Low lumbar spine, femoral neck BMD was noted in 5.9% (n=10) and1.8% (n=3) respectively. In those ≥ 50 (n=88), 59.1% were on prednisone and 56.8% were taking calcium/vitamin D supplement. Osteoporosis was documented in 11.8% and 4.7% in lumbar spine and femoral neck respectively, 52.9%, 33.3% and 13.7% had low (<10%), moderate (10-20%) and high(>20%) 10-year fracture risk. Including: smoking, sedentary lifestyle, post menopausal status, BMI>25 kg/m2 and the use of prednisone, as common risk factors for low BMD, there were only 10 patients (3.7%) with no risk factors, 152 (52.9%) had 1-2 and 106 patients (43.4%) had 3 and more risk factors. **Conclusion:** The majority (>75%) of women with SLE have multiple risk factors for osteoporosis. Among women over the age of 50, the 10-year fracture risk is moderate to high in more than 50% of patients. Osteoporosis is under recognized in women with SLE in both current steroid users and non users. These findings highlight the importance of routine BMD for patients with SLE.

PO2.C.11

Danish SLE patients have reduced mental and physical health as measured by SF-36

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Objective: To study self-reported general health status by means of the SF-36 questionnaires in a population based cohort of Danish SLE patients. **Method:** Seventy-three consecutive out-patients with criteria SLE were recruited from an unselected lupus population comprising a total of 109 individuals of predominantly Caucasian ancestry (1). Patients were invited to fill in a validated version in Danish of the SF-36 questionnaire with particular emphasis on physical (PCS) and mental (MCS) score. SLICC, SLEDAI, HAQ and a patient fatigue VAS scale were recorded concomitantly. **Results:** Male/female ratio was 4/69. Mean age was 45.9 (42.9-48-9), disease duration 14.6 (12.7-16.7). Median SLEDAI amounted to 2 (range 0-8) and median SLICC 1 (range 0-9). Mean physical score, PCS, was reduced to 42.0 (39.5-44.5) and mean mental score, MCS, 48.9 (46.6-51.3) as compared to 45-54 year-old

females from the general Danish population with mean PCS 49.9 and MCS 53.1. Mean fatigue VAS was 4.4 (3.6-5.2). There was no correlation between PCS, MCS and SLICC/SLEDAI. On the other hand PCS correlated inversely with HAQ (Spearman's rho -0.68, p<0.001) and VAS fatigue (Spearman's rho -0.64, p<0.001). MCS correlated inversely with VAS fatigue value (Spearman's rho -0.41, p<0.001). *Conclusion:* Danish lupus patients have reduced quality of physical and mental life as measured by SF-36. The lack of correlation with SLEDAI and SLICC scores may partly be due to rather low disease activity and damage accrual in this subset of outpatients. The association of self reported physical and mental scores with self reported fatigue, underscores the importance of adding patient perception aspects of disease impact to traditional activity and damage scores. It is noteworthy that lupus patients in spite of better physical status report lower mental scores, MCS, when compared to arthritis patients (2). This suggests that mental well-being is probably only loosely associated with physical capability in Scandinavian patients with systemic lupus erythematosus.

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PO2.C.12

Cardiac involvement in patients with systemic lupus erythematosus

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Introduction: Systemic lupus erythematosus (SLE) is a chronic, relapsing-remitting autoimmune disease that can take several forms. It can affect any part of the body, but in most cases involves problems with the skin, heart, lungs, kidneys, joints and nervous system. In the following study we focus on the cardiac involvement in SLE. *Aim:* The objective of our study was to evaluate the frequency and type of cardiac disorders in patients with SLE. *Material And Methods:* The study involved twenty three patients with systemic lupus erythematosus. The evaluation of heart was established, based on physical examination, basic laboratory analysis, resting 12-lead ECG, 24-hour Holter ECG and RR, echocardiography and ergospirometry. *Results:* The female/male ratio was 18/5, the mean age at recruitment was 53 (range 29-77).The duration of the disease was 5-6 years (range 4 mounts – 9 years). 2 patients had a history of MI, one patient suffers from dilatated cardiomiopathy. Only 4 patients (17%) had cardiac symptoms at the time of the admission. The high blood pressure was presented in 8 patients (35%), hyperlipidaemia in 13 (56%), diabetes mellitus (type 2) in 3 (13%). Abnormal ECG was seen in 10 (43%) and abnormal echocardiography in 8 (34%). Holter ECG shows atrial premature beats in 6 (26%), simple ventricular premature beats in 9 (39%), Holter RR shows abnormalities in 7 patients (30%). In ergospirometry test average VO2 peek was 24,0 ml/kg/min, *Conclusion:* Our center's experience suggests that the prevalence of cardiac disorders in patients with SLE may be higher than expected compared to the general population. Patients with SLE , may have cardiac involvement, but no cardiac symptoms. Our study has shown of the great importance of cardiac screening in patients with SLE.

PO2.C.13

Discordance between BILAG adjudication-defined and physician-defined flare: results from an exploratory study of abatacept in systemic lupus erythematosus (SLE)

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Objectives: The primary endpoint of this Phase II exploratory trial was new BILAG A/B adjudicated flare (as BILAG A/B disease activity scores may reflect flare or persistent activity, events were adjudicated according to a pre-defined protocol). Post-hoc analyses examined different aspects of BILAG and physician-assessed flare evaluation. Here we examine concordance between these flare measures. *Methods:* SLE patients with active polyarthritis, serositis and/or discoid lesions were randomized to abatacept (~10 mg/kg) or placebo for 1 year. Prednisone (30 mg/day or equivalent) was given for 1 month, then tapered by protocol. The primary endpoint was the proportion of patients with new adjudicated BILAG A/B flare over 1 year after the start of steroid tapering. Post-hoc assessments included flare defined by BILAG A only or physician-defined flare in response to the question 'Since the last visit, does the patient exhibit symptoms of an acute SLE flare?' (yes/no). *Results:* 118 abatacept and 57 placebo patients were evaluated. The proportion of patients with ≥1 adjudicated BILAG A/B flare (95% CI) was 79.7% (72.4, 86.9) for abatacept versus 82.5% (72.6, 92.3) for placebo. Adjudicated BILAG A flare only occurred in 40.7% (31.8, 49.5) of the abatacept versus 54.4% (41.5, 67.3) of the placebo group. The proportion of patients with physician-assessed flare was 63.6% (54.9, 72.2) for abatacept versus 82.5% (72.6, 92.3) for placebo. The rate of agreement between adjudicated BILAG flares and either total BILAG A/B scores or physician-assessed flares is shown (Table). Most adjudicated BILAG-defined flares that physicians did not rate as flares were B events (84/94 in abatacept-treated patients), supporting increased robustness of the rarer A flares to define events that physicians considered significant.

Table. Cross-tabulation of presence/absence of flare according to different BILAG and physician-defined measures

		Abatacept (N=118)			Placebo (N=57)		
		Total, N	Yes, n (%)	No, n (%)	Total, N	Yes, n (%)	No, n (%)
		Adjudicated BILAG A or B flare					
BILAG A or B flare	Yes	611	162 (26.5)	449 (73.5)	263	79 (30.0)	184 (70.0)
BILAG A or B flare	No	698	0	698 (100.0)	353	0	353 (100.0)
		Adjudicated BILAG A or B flare					
Physician assessment of flare	Yes	131	71 (54.2)	60 (45.8)	82	47 (57.3)	35 (42.7)
Physician assessment of flare	No	1178	94* (8.0)	1084 (92.0)	533	32 (6.0)	501 (94.0)
		Adjudicated BILAG A flare					
Physician assessment of flare	Yes	131	23 (17.6)	108 (82.4)	82	17 (20.7)	65 (79.3)
Physician assessment of flare	No	1178	10 (0.8)	1168 (99.2)	534	1 (0.2)	533 (99.8)

*10 were A flares; the remaining 84 were B flares

Conclusions: The use of the BILAG index for flare assessment was refined when events were both adjudicated to determine a true increase in disease activity and restricted to the more severe BILAG A category. BILAG B flares can be variable in severity and were more frequently discounted by the assessing physician. Although A flares are rarer, their improved specificity for detecting clinically significant events might be a useful endpoint for clinical trials.

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PO2.C.14

Intima-media wall thickness and other atherosclerosis risk factors in patients with systemic lupus erythematosus

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Objectives: Accelerated atherosclerosis in systemic lupus erythematosus is an important cause of morbidity and mortality. In this context it is important to find tools for better prediction of individual risk and increase the possibility of early intervention. The objectives of this study was the evaluation of intima media thickness (IMT) and other atherosclerotic risk factor in a group of lupus patients in comparison with disease activity and presence of lupus nephritis. **Methods:** The study enrolled 63 patients with SLE (female/male 54/9, mean age 38,4±12,7 years, mean disease duration 143±173,5 months, BMI 24,74±5,06, waist circumference 83,38±16,58). Twenty three patients had lupus nephritis proved by biopsy or typical clinical manifestation. The control group comprised 24 individuals (female/male 20/4, mean age 31,04±8,59). IMT was measured by ultrasound at 10 mm proximal point from bifurcation of common carotid artery on both sides. Serum levels of total cholesterol (CH), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), anti ds-DNA, anti-nucleosome antibodies and complement components (C3, C4) were measured by commercially available ELISA kits. Clinical disease activity was evaluated by SLEDAI and damage by SLICC indices. **Results:** Mean IMT in SLE patients was 0,57±0,079mm on left side, 0,56±0,062mm on right side, in control group 0,50 mm on left and 0,49mm on right. A significant difference of IMT was found between the lupus patients and age matched healthy controls, as well as between controls and both subgroups (with and without lupus nephritis), p≤0,05. There was a trend to higher IMT in lupus nephritis, which did not reach the statistical significance. The mean levels of cholesterol in SLE patients were 6,03 mmol/l, TG 1,72mmol/l, LDL 3,19±1,104mmol/l, HDL 1,38mmol/l, C3 0,99g/l, C4 0,15g/l, anti ds-DNA 98,38 IU/ml, anti-nucleosome antibodies 124,53 IU/ml and mean values of SLEDAI was 7,22 and of SLICC 1,17. The mean waist circumference was 83,38 cm and BMI 24,88. The IMT correlated with age (r= 0,76, p≤0,001), waist circumference (r= 0,33, p≤0,007) and C3, C4 complement component (r=0,24, 0,31, p≤0,05). Borderline correlation was found between IMT and BMI (r=0,28, p=0,06), disease duration (r=0,17, p=0,077) and anti-nucleosome antibodies (r=0,21, p=0,069). **Conclusion:** Lupus patients present commonly dyslipoproteinemia. Lupus patients have significantly higher IMT in comparison with controls. IMT correlates significantly with age, waist circumference and with levels of C3 and C4 complement components. Presence of lupus nephritis might be associated with higher IMT value and increased risk of atherosclerosis.

PO2.C.15

Physical and mental health related quality of life (QoL) components associate with disease activity and major depression in Chilean women with systemic lupus erythematosus (SLE)

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Objective: Psychosocial factors play an important role in the course of SLE. The aim of this study is to characterize the health-related quality of life (QoL) components in Chilean women with SLE and their relationship with disease activity, damage and mental health status. **Methods:** In this cross sectional study 51 non selected women with SLE, recruited in an 18 months period, were evaluated: (i) QoL using the Short Form-12 questionnaire (assessing general health status [GHS], physical component summary [PCS] and mental component summary [MCS], (ii) SLE disease activity (SLEDAI-2K), (iii) damage (SLICC-ACR), and (iv) 26 common mental disorders, according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), using the Mini-International Neuropsychiatric Interview (MINI-plus). **Results:** Characteristics of participants: median age: 33 years (range= 17-64); median disease duration from diagnosis: 2 years (0.1-26); stable couple: 37.3%; active working/students: 64.7%; median years of formal education: 14 (9-19); median SLEDAI-2K: 7 (0-25); median SLICC: 0 (0-4). At least one common mental disorder (CMD) was present in 27 (52.9%) patients, 14 (27.5%) major depressive episode (MDE), 15 (29.5%) at suicidal risk (29.4%): four were at high level, 5 at moderate and 6 at low risk. QoL: patients considered their GHS: poor 8 (15.7%), regular 24 (47.1%), good or very good 19 (37.3%). PCS median was 39.1 (range 24.28-57.58) and MCS median was 35.8 (16.2–59.4). PCS and MCS inversely correlated with disease activity (Spearman Rho= -0.327; P= 0.023; R= -0.349; P= 0.015, respectively). Accrued disease damage inversely correlated with PCS (R= -0.347; P=0.015). The 14 patients affected with MDE had lower PCS and MCS than those without MDE (P=0.045; P=0.00, respectively). Disease activity was similar in both groups. **Conclusions:** CMD are higher than the general Chilean population living in Santiago, Chile (52.9% versus 17.5%) moreover the number of patients at suicidal risk is high. QoL components PCS and MCS were > 1 SD lower compared to Chilean general population (score 50 ± 10). MDE affected both components of QoL. Disease activity related to a worse QoL in both components while accrued damage associated only with lower PCS score. Overall, 62.7% of patients considered their general health status as poor or regular implying a challenge for the rheumatology staff to provide a better health care involving transdisciplinary teamwork.

PO2.C.16

Quantification of cerebral blood flow in lupus patients with acute neurological syndromes

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Background: Major neuropsychiatric systemic lupus derythematosus (NPSLE) manifests as stroke, transient ischemic attack (TIA), cognitive dysfunction, acute confusional state, seizures, or psychosis is highly prevalent and contributes significantly to patient's morbidity and mortality. In these patients, relative, focal decrease in cerebral perfusion has been demonstrated by radionuclide studies (SPECT and PET) of the brain. However, there are limited studies assessing absolute cerebral blood flow (CBF) in these patients. Thus, we sought to determine hemispheric and whole cerebral blood flow using carotid Duplex in patients with SLE with or without major NPSLE as compared with age matched healthy controls. **Methods Used:** Thirty three patients with SLE, 94% women, with a mean age of 39, ± 12 years (range, 22-60), and 21 healthy volunteers, 91% women, with a mean age of 36, ± 12 years (range, 21-59), underwent clinical evaluations and complete carotid Duplex to determine right and left internal carotid and vertebral arteries diameters and corresponding velocity time integrals (VTI). Hemispheric and whole CBF were calculated using an established method (π x vessel radius 2 x VTI). **Summary of Results:** Patients and controls had similar whole CBF(1087 ml/min in SLE and 1038 ml/min in controls, p = 0.48). Of clinical relevance, whole CBF was also similar in SLE patients with and without major acute NPSLE as compared to controls (1046, 1071, and 1038 ml/min, respectively, p = 0.71). Also, whole CBF was similar in SLE patients with or without acute stroke/TIA as compared to controls (p = 0.29). Significantly higher heart rate and double product (HR x SBP) may explain the preservation of CBF in SLE patients with acute neurologic syndromes (both p<0.003). **Conclusions:** (1) Assessment of CBF in patients with SLE is easily performed with carotid Duplex and it offers assessment of both functional and anatomical data. (2) Of clinical relevance, CBF is preserved in SLE patients with acute neurologic syndromes. (3) To our knowledge, this is the first controlled study done in SLE patients using carotid Doppler to assess absolute CBF.

PO2.C.17

Isolated, persistently low complement C3 levels defines a subgroup of patients with less active systemic lupus erythematosus (SLE)

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Objective: In SLE, low levels of both C3 and C4 reflect immune mediated processes and correlate with active disease. This preliminary study was to evaluate whether patients with isolated persistently low C3 levels and normal C4 levels might represent a unique clinical and immunological phenotype. *Methods:* In this retrospective study, the medical records of 500 SLE patients (ACR criteria) in the Rheumatology Division between 2000 and 2007 were examined. Demographic, clinical and laboratory information was collected on those patients who had sufficient data on consecutive visits to document C3 and C4 status and disease activity measured by ECLAM, which has been validated for chart review. An ECLAM-E (ECLAM without ESR) was also calculated because of missing ESR data. Patients were categorized into three groups: A: low C3/ normal C4 (C3 >10% below normal range for 3 or more consecutive visits over at least 3 months or 2 consecutive visits over at least 1 year); B: low C3/ low C4; C: normal C3/ normal C4. We hypothesized that B would have a mean ECLAM score of >3 (active disease), C would have a score of <1.6 and A would be intermediate between B and C. Mean ECLAM scores per subject were compared among groups by ANOVA or Kruskal-Wallis with a Bonferroni correction as appropriate. *Results:* There were 15 patients in A, 14 in B and 15 in C and multiple visits per patient were recorded (mean=5). There were fewer African Americans in A. Differences in mean ECLAM and ECLAM-E scores as expected were higher in B than C and scores in A as hypothesized were intermediate between the two (Table). *Conclusion:* This preliminary study suggests that SLE patients with persistently low C3 and normal C4 levels appear to have less active disease than those with low C3 and low C4 levels. We are further characterizing this subgroup clinically. The immunological pathophysiology underlying the C3 hypocomplementemia in these patients is unknown.

GROUP	MEAN AGE (mean yrs)	SLE DURATION (median yrs)	% AA	ECLAM (mean)	ECLAM-E (mean)	ECLAM>3 (Odds Ratio compared to C)
A 15 (low C3, nl C4)	43.1	11	67	3.02	2.58+	2.7+
B 14 (low C3, low C4)	32.6	9.0	86	3.85 *	3.21*	7.4*
C 15 (nl C3, nl C4)	36.5	9.0	87	2.04 *	1.38+*	-
Statistical Significance	ns	ns	P=0.05	*p=0.006	+p=0.005 *p<0.0001	+p=0.04 *p<0.0002

PO2.C.18

Quality of life in patients affected by systemic lupus erythematosus: correlation with disease activity and chronic damage indexes

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The evaluation of quality of life (QoL) is considered a fundamental indicator during clinical assessment of patients affected by systemic lupus erythematosus (SLE). The systemic lupus international collaborating clinics (SLICC) group recommends the analysis of three domains while evaluating SLE patients: the disease activity, the chronic damage and the QoL itself. In particular the usage of the medical outcomes study short form 36 (SF-36) has been proved a reliable indicator of the QoL of SLE patients. We evaluated the QoL in patients affected with SLE and we correlated these scores with the disease activity and chronic damage indexes. *Patients and Methods:* Patients affect-

ed with SLE diagnosed according to the 1997 ACR criteria followed by the "Lupus Clinic", Rheumatology Unit, Sapienza Università di Roma, have been enrolled. The patients were evaluated every 3 to 4 months (in selected cases it could be shorter) and provided written informed consent at the time of first visit. At each visit, the patients underwent a complete physical examination. The clinical and laboratory data were collected into a standardized computerized electronically-filled form including demographics, past medical history with date of diagnosis, co-morbidities, previous and concomitant treatments. The disease activity was assessed using the SLEDAI and the ECLAM score, while chronic damage was measured by SLICC score. SF-36, functional assessment of chronic illness therapy-fatigue (FACIT-F), EuroQoL-5D health questionnaire, workplace productivity, were used to assess the QoL and the general health state. *Results:* Ninety-four patients affected with SLE were enrolled (M/F 10/84, mean age 41.3±10.6 years, mean disease duration 147±99.1 months). The results are summarized in Table 1. SLICC index showed a positive correlation with physical items of SF-36 (P=0.018). Moreover, when stratifying patients between those who had any chronic damage (SLICC≥1) and those who had none (SLICC=0), the difference in the physical items of SF-36 was even more evident with poorer scores in the group with chronic damages (table 1). Parallely, EuroQol-5D scores were significantly higher in patients with chronic damages. No statistically significant results were obtained when SLEDAI or ECLAM scores were used. *Conclusions:* The presence of chronic damage evaluated by SLICC index appears to worsen the QoL measured with SF-36 and Euro-QoL in SLE patients. The physical disability items are the most affected by chronic damage. The usage of questionnaire to evaluate QoL may be suggested of relevant usage not only in clinical trials but also in routinary clinical assessment of SLE patients.

	SLICC=0	SLICC≥ 1	P value
	(N=67)	(N=27)	
SF36 (physical items, mean±SD)			
Physical function	75.9±23.7	53.7±25.5	P=0.0004
Role Physical	48.7±43.0	21.9±37.1	P=0.009
Body pain	52.6±26.7	41.7±20.6	P=N.S.
General health	43.1±17.7	31.7±16.2	P=0.004
Total physical items	220.4±90.4	149.1±76.7	P=0.0007
EuroQoL (mean±SD)	7.3±1.9	8.7±2.1	P=0.007

PO2.C.19

The impact of disease activity and damage on quality of life in systemic lupus erythematosus (SLE) assessed by the LupusQoL questionnaire

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Objectives: The LupusQoL is a validated SLE-specific health-related quality of life (HRQoL) instrument with 34 items across 8 domains1. We studied the relationship between disease activity, organ damage and HRQoL as assessed by the Chinese version of the LupusQoL in patients with SLE. *Patients and methods:* Consecutive patients who fulfilled >=4 ACR criteria for SLE were invited for a HRQoL study using the LupusQoL questionnaire between February and August 2008. Disease activity and intercurrent disease flare of the participants were assessed by using the SLEDAI-2K, physicians' global assessment (PGA) (0-3) and SELENA-SLEDAI flare instrument. Organ damage was scored according to the SLICC/ACR damage index (SDI). Correlation among these variables was studied by the Spearman's rank correlation method. *Results:* 230 patients were studied (220 women). The mean age was 40.0±12.0 years and SLE duration was 8.9±7.5 years at the time of questionnaire study. 111(48.3%) patients had SLEDAI score of >=3. 20(8.7%) patients had mild/moderate flares and 16(7%) patients had severe flares. The mean PGA score was 0.49±0.71. The mean SDI score was 0.78±1.2. SLEDAI scores correlated well with PGA scores and the severity of disease flares. The mean scores (out of 100) of the 8 domains of the LupusQoL were 73.1±22 (physical health), 67.5±27 (pain), 72.6±27 (planning), 66.8±33 (intimate relationship), 57.3±29 (burden to others), 70.3±24 (emotional health), 64.7±28 (body image) and

61.5±25 (fatigue). The SLEDAI scores correlated inversely with the scores of the physical health (p=0.02), pain (p=0.009), planning (p=0.03), burden to others (p=0.01) and fatigue (p=0.001) domains of the LupusQoL. Patients who experienced recent disease flares had significant lower scores in the pain (p=0.01), planning (p=0.02), burden to others (p=0.02) and fatigue (p=0.02) domains. Patients with pre-existing organ damage (SDI score >=1) also had significantly lower scores in the physical health (p<0.001), pain (p=0.007), planning (p=0.001), intimate relationship (p=0.01), image (p=0.003) and fatigue (p=0.05) domains. Similar findings were observed in those patients with organ damage but no intercurrent disease flares. *Conclusions:* The HRQoL of SLE patients as assessed by the new LupusQoL is adversely affected by intercurrent disease activity and pre-existing organ damage.

1McElhone K, Abbott J, Shelmeridine et al. *Arthritis Care and Research.* 2007;57:972-9

PO2.C.20

Disease specific patient reported outcome benchmarks for US lupus patients: LupusPRO and LupusQoL-US

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Objective: Systemic Lupus Erythematosus (SLE) affects Quality of Life (QOL). This study pertains to measurement of QOL among our US patients with SLE using recently validated disease specific patient-reported outcome measures: LupusPRO and LupusQoL-US. *Methods:* LupusPRO (version 1.4) is derived from US patients with SLE (1;2) and has seven health related quality of life (HRQOL) domains (1) Lupus Symptoms (2) Physical Health (3) Emotional Health (4) Body image (5) Cognition (6) Procreation (7) Lupus Medications. Four Non-HRQOL domains are (1) Social Support (2) Coping (3) Desires-Goals, and (4) Satisfaction with care. LupusQoL-US is derived from UK patients with SLE, has been modified and validated in the US, and has 8 domains: Physical Health, Pain, Planning, Intimate relationships, Burden to others, Emotional health, Body Image and Fatigue. Both tools correlate with each other in the corresponding domains. Higher scores=Better QOL. Data from ongoing health outcomes study from SLE patients was utilized. Disease activity (SLEDAI) and damage (SLICC-ACR) were determined. Descriptive statistics based on data distribution were obtained. *Results:* 144 SLE patients with mean (SD) age 44.6 (14.5) yrs were assessed for LupusPRO. 92% were women. Ethnic makeup: African American 40%, 47% Caucasian, 12% Hispanic and 3% other. Median (IQR) physician Global Assessment and SLEDAI were 0(1) and 4(2) respectively. Median (IQR) HRQOL domain scores were: Lupus Symptoms 58.3(58.3), Physical Health 60 (45), Emotional Health 50(55), Body image 70(68.8), Cognition 62.5(59.4) (6) Procreation 100 (100) and Lupus Medications 62.5 (75). The median HRQOL (IQR) score was 67.1(46.1). Non-HRQOL domains scores median (IQR) were: Social Support 75(50), Coping 75(41.7), Desires-Goals 62.5(67.2), and Satisfaction with care 93.8(31.3). The median (IQR) Non-HRQOL score was 71.9 (25.6). 176 SLE patients with mean (SD) age 42.2 (12.8) yrs and median (IQR) disease duration of 30 (21) yrs were assessed for LupusQoL-US. 94% were women. Ethnic makeup: African American 60%, 23% Caucasian, 12% Hispanic and 6% other. Median (IQR) SLEDAI and SDI were 4(7) and 1(3) respectively. Median (IQR) domain scores were: Physical Health 46.4 (30.2), Pain 50 (33.3), Planning 50 (41.7), Intimate relationships 62.5 (37.5), Burden to others 50 (34.4), Emotional health 56.3 (29.2), Body Image 56.3 (33.3) and Fatigue 41.7 (21.6). *Conclusions:* This is the first patient reported outcomes study utilizing disease specific tools for SLE in the US. These results can be utilized as benchmarks for comparing health outcomes in SLE across centers.

PO2.C.21

The LupusPRO©: a disease-specific patient reported outcome measure for systemic lupus erythematosus in the USA

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Objective: Systemic Lupus Erythematosus (SLE) affects both health-related (HRQOL) and non health-related quality of life (N-HRQOL). We sought to develop and validate a disease specific patient-reported outcome measure (LupusPRO©) for SLE patients in the USA. *Methods:* The instrument was developed and validated in stages, where items were generated with input from patients and clinicians and analyzed using clinimetric and psychometric techniques. Construct validity was examined using generic HRQOL measures (SF-36, EQ5D), lupus specific HRQOL measure (LupusQoL-UK), Body Image quality of Life inventory, and Situational inventory of body image dysphoria. Internal consistency reliability (ICR), test-retest reliability, responsiveness and Confirmatory Factor Analysis were tested for the final version of LupusPRO v1.4. *Results:* Two constructs HRQOL and N-HRQOL were identified for overall QOL. Seven HRQOL domains emerged (1) Lupus Symptoms (2) Physical Health (3) Emotional Health (4) Body image (5) Cognition (6) Procreation (7) Lupus Medications. Four N-HRQOL domains were (1) Social Support (2) Coping (3) Desires-Goals, and (4) Satisfaction with care. ICR for each domain ranged between 0.82-0.96. The LupusPRO domains correlated with corresponding SF36, EQ5D and LupusQoL-US. LupusPRO was responsive to changes in health over time. *Conclusions:* LupusPRO© is Lupus specific health outcome tool derived from USA patients that is inclusive of all (HRQOL and N-HRQOL) domains pertinent to especially USA patients with SLE. It is valid, reliable and responsive to changes in SLE patient's health status. LupusPRO captures unique domains (Lupus Symptoms, Cognition, Procreation, Lupus Medication, Desires-Goals, Coping and Satisfaction with care) not captured by existent generic or disease specific patient reported outcome measures.

PO2.C.22

Analysis of different phenotypes in systemic lupus erythematosus

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Objective: To analyse different kinds of phenotype in lupus patients. *Patients and methods:* A total of 433 lupus patients were followed up for 5 years during which the presence of clinical manifestation was collected and the activity of lupus was determined by BILAG index. . Patients were assigned to four different groups based on their case history and disease activity: a mucocutanous group with 119 patients, a musculosceletal group with 177 patients, neuropsychiatric group with 28 patients and 109 lupus nephritis patients. *Results:* Serositis, carditis, alveolitis, pulmonary fibrosis were significantly higher in the musculosceletal group (p<0.05) than in the other groups, and other systemic autoimmun disease can coexist with lupus more often in this group. Scleroderma associates with mucocutanous group and the antiphospholipid syndrome as more often in patients with neuropsichiatric symptoms (p<0.05). Antibodies against nucleosoma correlated with the presence of lupus nephritis. In the follow up period, the following observation could be detected: patients once having a certain systemic involvement are likely to have progression or relapse in the same organ, whenever their disease is relapsing. *Conclusion:* In systemic lupus erythematosus different kinds of phenotype can be detected, and the previous disease course and or-

gan involvement determines a patient's likehood of developing further episodes of active disease. Our results need to be confirmed with longitudinal prospective studies.

PO2.C.23

Preliminary validation of Spanish version of the Lupus-PRO V1.4.

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Objective: LupusPRO is a disease specific patient reported health outcome measure derived from US patients with Systemic Lupus Erythematosus (SLE) (1;2) with seven HRQOL domains (1) Lupus Symptoms (2) Physical Health (3) Emotional Health (4) Body image (5) Cognition (6) Procreation (7) Lupus Medications. Four N-HRQOL domains are (1) Social Support (2) Coping (3) Desires-Goals, and (4) Satisfaction with care. Here we report the cross-cultural validation of Spanish translation version of the LupusPRO v1.4. *Methods:* Using standard guidelines for cross cultural validation of patient reported outcome measures; Spanish translation of the LupusPRO was achieved using forwards and backwards translation techniques. Minor modifications in the language were made to incorporate spoken Spanish over written Spanish. The measure was then administered, along with the SF-36 health status measure; to 59 Spanish- speaking SLE subjects in the Southern California area. The SF-36 has 8 domains: Physical Function (PF), Role Physical (RP), Bodily Pain (BP), General Health (GH), Vitality (VT), Social Function (SF), Role Emotional (RE) and Mental Health (MH). Internal consistency reliability (ICR) of the LupusPro domains was evaluated using Cronbach's alpha, and construct validity was examined by testing whether moderate to strong correlations were observed between related domains on the LupusPro and SF-36. Differential function analysis was conducted after matching for educational level for the English vs Spanish version for the items. *Results:* All the participants were Hispanics, and only 1 subject was male (2%). The mean age ±SD was 42 ±11.5 years (range 24-66 yrs). The mean SLEDAI score ±SD was 3.2 ± 3.5 (range 0-17). The ICR for the LupusPRO domains were as follows: Lupus Symptoms 0.62, Cognition 0.95, Lupus Medications 0.72, Physical Health 0.70, Pain-Vitality 0.86, Body Image 0.88, Emotional Health 0.90, Procreation 0.68, Desires-Goals 0.83, Social support 0.79, Coping 0.90 and Satisfaction with care 0.89. Moderate to strong correlations were observed between related domains of the LupusPRO and SF36: Physical Health domain with PF (r -0.44, p=0.001); Emotional Health with MH (r -0.54, p=0.001) ; Pain-Vitality with BP (r -0.83, p=0.001) and VT (r -0.76, p=0.001);Social Support with SF (0.35, P=0.01). No significant differences between items were noted when matched for educational level. *Conclusion:* These interim results support the reliability and validity of the Spanish translation of the LupusPRO v1.4.

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PO2.C.24

Validation of the Portuguese systemic lupus activity questionnaire (SLAQ) for population studies

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Objective: The Systemic Lupus Activity Questionnaire (SLAQ) is a patient self-report instrument to screen for disease activity in clinical studies of SLE. The goal of this work was to develop and validate the Portuguese version (P-SLAQ). *Methods:* The original English SLAQ (24 items) was translated and adapted into Portuguese. The P-SLAQ was completed by consecutive SLE patients fulfilling the 1997 ACR classification criteria just prior to a scheduled visit. One rheumatologist, blinded to P-SLAQ results, examined each patient and completed the Systemic Lupus Activity Measure (SLAM-R). Disease activity was also assessed with SLEDAI-2K and Physician Global Assessment (PGA) (VAS 0-100) at the same visit. The P-SLAQ internal consistency was evaluated using the Cronbach Alpha. Correlations between P-SLAQ and the SLAM without laboratory items (SLAM-nolab) as well as the other instruments were assessed with Pearson correlations. We evaluated the sensitivity, specificity, positive and negative predictive value of a series of dichotomous P-SLAQ cut-off points (ranging from 0 to 20) for clinically significant disease activity (SLAM-nolab ≥3). *Results:* The study included 122 SLE patients [89.3% female, mean age =41 years (range 16-84)]. The mean score of P-SLAQ was 10.3 (range 0-31); mean SLAM-nolab was 2.1 (range 0-9). Mean SLAM-R was 4.0 (0-15), mean SLEDAI-2K was 4.6 (range 0-22) and mean PGA was 12.6 (0-57). The P-SLAQ showed a high internal consistency with Cronbach alpha =0.9 (individual item ranges between 0.89-0.90). The P-SLAQ had a moderately high correlation with SLAM-nolab (r=0.58, p<0.001). The SLAM-nolab correlated with SLAM-R (r=0.78, p<0.001). SLAM-R correlated with SLEDAI-2K and PGA (p<0.001). Positive predictive values for the SLAQ ranged from 38% to 87% for detecting SLAM-nolab ≥ 3. Using a cutoff score of ten points or more on the P-SLAQ resulted in a sensitivity of 70%, specificity of 63%, positive predictive value of 48% and 81% negative predictive value to predict SLE disease activity. *Conclusion:* The P-SLAQ showed a good correlation with physician rating of SLE disease activity, very similar with the original instrument. P-SLAQ is now validated and is an adequate instrument for patient self-report screening of SLE disease activity, which can now be used in clinical studies.

PO2.C.25

A brief body image screening tool for systemic lupus erythematosus

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Systemic Lupus Erythematosus (SLE) affects young women, and may significantly affect body image. Currently there are no BI tools suitable for this multi-organ systemic disease with appearance altering potential. *Aims:* To develop and validate a patient derived body image tool specifically relevant to SLE patients. *Methods:* 18 SLE patients, both males and females, underwent semi structured cognitive interviewing to identify relevant body image issues. 11 items pertaining to body image and self image distress were identified for use in developing a BI Lupus Scale (BILS v 1.0). This was administered to 71 SLE patients. Exploratory factor analysis revealed one factor. The internal consistency reliability (ICR) of the 11 items scale was 0.89. Based on Rasch analysis, and feedback from patients and experts, 6 items were removed. The items were revised to enhance comprehension. The 5 item BILS v1.1 was administered to 165 SLE subjects along with a health status measure (EQ5D). Exploratory and confirmatory factor analysis along with determination of face validity, construct validity, criterion validity, internal consistency reliability and test retest reliability were performed. For construct validity data from 47

patients who were simultaneously administered two standard BI tools: Body Image Quality of Life Inventory (BIQLI) and Situational Inventory of Body Image Dysphoria (SIBID-S). Criterion validity was assessed against health status. P value = 0.05 were considered significant on two tailed tests. *Results:* The ethnic composition of the study subjects (N=165) was: African Americans (59%), Caucasian (20%), Hispanic (14%) and Asian (7%). 94% were women and 41% were currently married. The mean age of the subjects was 42.5± 13.3 years, with a mean age at disease onset of 32.7± 12.1 years. The mean SELENA-SLEDAI and SDI scores were 4.7 ± 5.4 (median 4, range 0-31) and 1.4±1.7 (median 1, range 0-7). Exploratory factor analysis for BI-SLE v 1.1, confirmed one factor loading that explained 80% of the variance, with an eigenvalue of 4. On confirmatory factor analysis, model fit indices obtained were: goodness of fit index 0.98 and chi-square 7.3. The Spearman's correlation coefficient between BILS and SIBID-SF and BIQLI scores were 0.70 (p=0.001) and -0.41 (p=0.003) respectively. BILS was able to differentiate between patients based on their health status (p=0.001).The internal consistency reliability was 0.94.The test retest reliability was 0.92. *Conclusions:* These data confirm that BILS has acceptable psychometric properties. This brief tool can be used to screen patients' body image in SLE and facilitate early intervention.

PO2D Clinical Outcomes

PO2.D.3

Prolonged serologically active clinically quiescent (SACQ) systemic lupus erythematosus (SLE): predictors of flare

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Objectives: Some patients with SLE are clinically quiescent despite persistent serologic activity, thus presenting a clinical dilemma. While some patients remain SACQ indefinitely or become serologically quiescent (SQCQ), others' SACQ periods are terminated by flare, for which reliable predictors have not been identified. Some suggest fluctuations in anti-dsDNA antibody and complement levels may be instructive. We analyze the two SACQ visits prior to flare for potential predictors thereof. *Methods:* Patients followed in the Lupus Clinic between 1970 and 2008 with visits ≤ 18 months apart were identified. SACQ was defined as a ≥ two year sustained period without clinical activity and with persistent serologic activity (increased anti-dsDNA antibody by Farr assay and/or hypocomplementemia at each visit), during which patients could be taking antimalarials, but not steroids or immunosuppressives. Anti-dsDNA antibody and complement levels at the two visits immediately preceding flare (FLARE group), were compared to those drawn at the third- and second-last visits in patients who remained SACQ or became SQCQ at their last visit (NON-FLARE group). Difference in anti-dsDNA antibody levels was analyzed categorically (normal (≤7), low (8-20), moderate (21-50), or high (>50)) and continuously, and if complement changed to or from normal between visits in the FLARE and NON-FLARE groups. Descriptive statistics were used. Comparisons were made using t-tests and chi-squared tests. *Results:* 56/924 (6.1%) patients were SACQ. Median time between visits, and between last SACQ visit and outcome (e.g. flare) was 0.5 years. 33/56 (58.9%) patients comprised the FLARE group, and 23 (41.1%) the NON-FLARE group. Serologic profile over the visits analyzed did not differ between groups (p=0.83). Of the 25 patients with elevated anti-dsDNA antibody in the FLARE group, nine (36%) had significant categorical change in level: increased in five patients, decreased in four. Of the 18 NON-FLARE patients with elevated anti-dsDNA, two (11.1%) had significant change in levels; both increased. When analyzed as a continuous variable, anti-dsDNA antibody level did not differ between FLARE and NON-FLARE groups. Among FLARE patients with hypocomplementemia, 2/27 (7.4%) changed between normal and abnormal levels in the two visits preceding flare versus 4/21 (19%) in the NON-FLARE group. *Conclusions:* Changes in complement and anti-dsDNA levels do not predict flare in SACQ

patients. Decision to treat these patients must be based on clinical acumen from close observation; alternate predictive biomarkers must be studied. Anti-dsDNA antibodies in SACQ require further characterization.

PO2.D.4

Outcomes in patients with systemic lupus erythematosus (SLE) with and without a prolonged serologically active clinically quiescent (SACQ) period

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Objectives: SACQ SLE patients' discordance presents a clinical dilemma: does active serology alone warrant treatment? We explore outcomes in patients with and without a prolonged SACQ period by comparing the rate of damage accrual, as measured by the SLICC/ACR Damage Index (SDI), and incidences of renal damage and of coronary artery disease (CAD), over 5-10 years. *Methods:* SLE patients followed from 1970-2008 with visits ≤18 months apart were identified. SACQ was defined as a ≥2-year sustained period without clinical activity, with persistent serologic activity (increased anti-dsDNA and/or hypocomplementemia), during which antimalarials were permissible, but not steroids/immunosuppressives. SACQ patients were matched for age, sex, disease duration, decade of clinic entry, and SDI at the beginning of the SACQ period, with SLE controls. Groups were compared on the bases of change in SDI, and incidences of CAD and renal damage. Descriptive statistics were used; comparisons made using paired t- and Mc-Nemar tests. *Results:* 55 SACQ patients and 110 controls were identified. The median SACQ period was 158 weeks. Fewer SACQ patients used antimalarials (60% vs 77.3%) (p=0.004), steroids (18.2% vs 76.4%) or immunosuppressives (5.5% vs 43.6%) (p<0.0001 for both) over the 5 year period. SDI at 3 years from the start of the SACQ period was 0.70 ± 1.27 vs. 1.13 ± 1.54 in controls (p<0.0001), at 5 years was 0.89 ± 1.37 vs 1.36 ± 1.66 (p<0.0001), at 7 years was 0.94 ± 1.28 vs 1.71 ± 1.86 (p=0.0001), and at 10 years was 1.26 ± 1.68 vs 2.26 ± 2.23 (p=0.001); intergroup difference in damage significantly increased over 10 years. SDI difference was mainly independent of corticosteroid effects. Two (3.6%) SACQ patients had CAD prior to study start vs. 7 (6.4%) controls (p=0.32), with 1 (1.8%) new case in SACQ patients vs 8 (7.3%) in controls over 10 years (p=0.06). Baseline serum creatinine did not differ between groups (p=0.90). By definition, SACQ patients had no baseline proteinuria, versus 13 (12.3%) controls (p<0.0001). One (1.8%) SACQ patient and 17 (15.5%) controls had renal damage at 5 years (p=0.0006), and two (3.6%) SACQ patients, vs 26 (23.6%) controls had renal damage at 10 years (p<0.0001). *Conclusions:* SLE patients with a prolonged SACQ period have significantly less damage accrual over 5-10 years compared to matched controls, supporting the practice of active surveillance without treatment (with steroids/immunosuppressives) during the SACQ period.

PO2.D.6

SLEDAI-2K 10 days versus SLEDAI-2K 30 days in a cross-sectional and longitudinal evaluation

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Background: The SLEDAI (Systemic Lupus Erythematosus Activity Index) was developed in 1985 and is based on the presence of 24 features in 9 organ systems over the patient's past 10 days. An updated version SLEDAI-2000 (SLEDAI-2K) was introduced and validated in 2002 again documenting findings in the past 10 days. *Objective:* To evaluate SLEDAI-2K 30 days in a cross-sectional study design and over time and to compare with the original SLEDAI-2K 10 days. *Methods:* In the first phase we enrolled 131 consecutive lupus patients seen at a single centre Lupus Clinic over 6 weeks. In the second phase 41 patients were followed at monthly intervals for 12

months. A complete history, physical examination and laboratory tests were performed to allow the determination of SLEDAI-2K. The SLEDAI-2K score was completed twice, once for a 10-day window and the second for a 30-day. *Results:* Among the 131 patients, 97 had a SLEDAI-2K of 0 and 34 patients had varying levels of disease activity (12 had SLEDAI-2K of 2; 11 had 4; 2 had 6; 3 had 8; 1 had 10; 2 had 12; 1 had 14 and 2 had 16). In all patients but one there was agreement between the SLEDAI-2K 10 and 30 days. Among the 41 patients followed at monthly intervals for 12 months, 419 patient-visits were recorded. 268 patient-visits had varying levels of disease activity (3 patients-visits had SLEDAI-2K of 1; 133 had 2, 2 had 3, 68 had 4, 9 had 5, 35 had 6, 11 had 8, 4 had 10, 2 had 12 and 1 had 15). In all but one patient-visit there was an agreement between the SLEDAI-2K 10 and 30 days. This patient experienced skin rash as a minor lupus flare, however in the last 10 days prior to the visit his rash completely faded. *Summary:* This study confirmed in both phases that it is unusual to have a manifestation of active lupus present at -11 to -30 days prior to a visit and have complete resolution in the 10 days prior to the visit. SLEDAI-2K 30 days scores were concordant with SLEDAI-2K 10 days scores, both in patients in remission and in patients with a spectrum of disease activity levels in both phases of the study. *Conclusion:* SLEDAI-2K 30 days was validated against SLEDAI-2K 10 days and may now be used in clinical studies and clinical trials to describe disease activity over the previous 30 days.

PO2.D.7

SLEDAI-2K Responder Index-50 (SRI-50)

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Purpose: A number of outcome measures to assess disease activity in SLE patients have been developed. SLEDAI-2K (Systemic Lupus Erythematosus Activity Index-2K) is a reliable valid, simple, one-page activity index recording features of active lupus as present or not present. Thus its utility in clinical trials is limited as it cannot reflect partial improvement in a disease manifestation. The objective of this study is to develop a SLEDAI-2K responder measure which could document a minimum 50% improvement in disease manifestations among lupus patients. *Method:* Derivation of SRI-50 (SLEDAI-2K Responder Index-50) from SLEDAI-2K. A new definition for each of the original descriptors of SLEDAI-2K was created to reflect a minimum improvement of 50%. The definitions of descriptors of SRI-50 were constructed based on a literature review for each specific organ system. The new assigned scores for the descriptors of SRI-50 were derived by dividing the score of SLEDAI-2K by 2. Assessing the content validity of the draft instruments; SRI-50 form was assessed by expert rheumatologists reviewing the instruments and providing critical feedback. Testing of SRI-50; One hundred patients who had experienced lupus flares or had persistently active disease were assessed initially and then reassessed after treatment was initiated. SLE-DAI-2K was determined on the first visit and again at the second visit along with SRI-50. Results: SRI-50 and the data retrieval form to accurately document the clinical and laboratory findings of each descriptor were developed. Seventy two patients didn't change their SRI-50 because their manifestations resolved or didn't meet the definition of SRI-50 Twenty eight patients with varying levels of disease activity at the first visit (3 had SLEDAI-2K 2, 3 had 4; 6 had 6; 6 had 8; 3 had 10; 2 had 12; 1 had 16, 1 had 18; 2 had 20; 1 had 21) were further studied with SRI-50. SRI-50 was able to demonstrate incomplete (but ≥50%) improvement which would not have been discerned using SLEDAI-2K. Such incomplete improvement was demonstrated in 13 of the 24 SLEDAI-2K descriptors and in 6 of the 9 organ systems that were present in these patients.

SLEDAI-2K visit 1	2	6	10	4	4	8	6	6	2	16	12	8	8	4	6	20	8	8	21	8	20	10	18	10	12	6	2
SLEDAI-2K visit 2	2	6	6	4	4	8	6	6	2	16	8	8	4	4	4	10	8	8	3	8	18	10	18	10	12	4	2
SRI-50 visit 2	1	5	4	3	2	6	5	4	4	1	8	4	7	3	2	2	6	4	4	1.5	4	10	9	10	6	6	2	1

Conclusion: SLEDAI-2K Responder Index-50 is a promising instrument that can describe partial improvement in disease activity between visits in lupus patients.

PO2.D.8

Health-related family functioning in SLE – patient interviews support six domains

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Purpose: The objective of this research was to identify domains of family social functioning that could be adversely impacted by SLE. *Methods:* Based on a literature review, six potential domains for health-related family functioning were identified: 1) fatigue-related activity impairment, 2) ability to plan activities in advance, 3) mental health/well-being, 4) isolation vs. social support, 5) love and intimacy, and 6) social role functioning. A semi-structured interview was developed based on these domains. Twenty patients with SLE were interviewed by telephone. Data were transcribed and descriptive statistics calculated. Domains were extracted using qualitative analysis evaluating relative support for the importance of each proposed domain. *Results:* Participants were 18 females and 2 males (mean age = 38 years). Most reported moderate to marked work disability, high-moderate social life disability and moderate disruption in family and home life. The meaning of "family" varied with 95% of patients listing immediate family members as their family, but 70% included extended family. Our domains were well supported by patient reports. 1) Fatigue: When asked about the most bothersome symptom of SLE, fatigue was most frequently volunteered (65%; n=13) and reported as problematic by 90%. 2) Activities: 55% reported that flares were unpredictable, but in contrast to what we anticipated, most patients learned how to cope with flares. Instead of having concerns about planning ahead, "active" activities (especially outdoor activities) were reportedly greatly affected by SLE. 3) Mental health: 88% reported that poor mental health impaired their ability to do the things that they liked to do with their families. 4) Isolation: Withdrawal tended to occur during disease flares (e.g., retreat to bed or hospital). Such times were also often complicated by poor mood and extreme fatigue which contributed to the desire to be alone. 5) Of the 19 sexually active participants, 84% reported that SLE had a negative impact on their ability to have a gratifying sex life. Close to half reported that this distanced them from significant others. 6) Role Functioning: 80% described ways in which family roles were impacted, while 10% volunteered that SLE had significantly impacted the family financially. *Conclusions:* We found good support for six domains related to family functioning: F = Fatigue Fatigue-related family activity impairment; A = Activities Ability to engage in active activities; M = Mental Health Mental health as it relates to the family; I = Isolation Feelings of isolation from family; L = Love Love/intimacy; Y= You Family social role functioning.

PO2.D.9

Development of the SLE-FAMILY for the assessment of health-related family functioning

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Purpose: A self-report instrument was developed with the goal of assessing the impact of SLE on family functioning by evaluating six key domains: Fatigue (fatigue-related family activity impairment), Activities (general family

activity impairment), Mental Health (emotional impact on family), Isolation (feelings of isolation from family members), Love (loss of intimacy) and You (fulfilling family roles). The major objective of this study was to develop the SLE-FAMILY self-report questionnaire to assess health-related family functioning. *Methods:* 25 patients with SLE responded to 16 candidate items created by the authors based on a literature review, interviews with 20 SLE patients and clinical judgment. In addition to the candidate-items questionnaire, patients completed the SF-36, Sheehan Disability Scale (SDS), Fatigue Severity Scale (FSS), Multidimensional Scale of Perceived Social Support (MSPSS), Satisfaction with Life Scale (SWLS), Positive and Negative Affect Scale (PANAS) and the Systemic Lupus Activity Questionnaire (SLAQ). Next, data were analyzed by grouping items by domain with Cronbach's alpha calculated for each domain. Items for which the domain's alpha increased when they were dropped from the domain were eliminated. Correlations for the remaining items and reference scales, both general and those specific to each domain, were used as a basis to select one item from each domain. *Results:* Nine of the 16 items were considered "strong" in that they contributed significantly to domain alpha and were highly correlated with the total SLE-FAMILY score based on all 16 items and the SDS Family subscale. The remainder of the items was chosen for their superior correlations with domain-related reference scales. The Fatigue item chosen correlated highly with the SLAQ fatigue item and FSS. The Activities item selected was highly correlated with SF-36 Social Functioning and MSPSS social support. The best Mental Health item had the highest correlations with SF-36 Mental Health and MCS. The strongest Isolation item had the best correlation with SDS Family and SDS Social. The best Love/loss of intimacy item was strongly correlated SF-36 Social Functioning and SF-36 Role Emotional. The You/social role item was highly correlated Social Role Functioning and SDS Family. The 6 chosen items combined as a single questionnaire have an excellent alpha (0.88) and were highly correlated with SDS Family (r=0.89) and SF-36 Social Functioning (r=0.88). *Conclusions:* The SLE-FAMILY is a promising new instrument for the more robust measurement of family functioning, which is often central to overall quality of life. Studies to validate the SLE-FAMILY are underway.

PO2.D.10

Bimodal pattern of mortality in SLE in African-Americans versus Caucasians

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Purpose: A bimodal pattern of mortality in SLE was identified by Urowitz and Gladman. Early deaths tended to be from active lupus or infection, whereas later deaths were from cardiovascular disease. Whether this bimodal pattern applies to the United States, and to both Caucasians and African-Americans (Af-Amer), has not been studied. *Methods:* 1375 patients were followed longitudinally for a total of 9763 person years. The patients were 93% female, 39% African-American and 56% Caucasian with median age at start of follow-up equal to 35 years. We compared the rates of death at different time periods with respect to SLE duration.

Results:

Group	Year since Dx	Deaths Rate/ 1000P-yr	Number of deaths due to specific causes			
			SLE	Cancer	Infection	Vascular
Everyone	< 2	11.3	1	0	6	0
	2 - 5	8.5	2	1	5	3
	5 - 10	10.4	2	5	8	2
	10+	15.4	9	6	6	13
Caucasians	< 2	5.5	0	0	1	0
	2 - 5	5.8	1	1	2	0
	5 - 10	8.5	0	4	3	1
	10+	12.0	3	0	1	6
African-American	< 2	18.5	1	0	4	0
	2 - 5	11.7	1	0	2	3
	5 - 10	13.7	2	1	5	1
	10+	20.0	6	6	5	7

Comparison of SLE < 10 years to SLE>= 10 years, shows a statistically significant difference in the distribution of causes of death (p=.0148), attributable to more infections in the former, and more vascular deaths in the latter. *Conclusion:* Very early (<2 years SLE duration) deaths are due predominantly to infection, which remains the largest cause of death until after 10 years of SLE, when vascular deaths take over. However, the early peak in infection deaths is almost entirely explained by African-American SLE. In this cohort, active lupus deaths peak after 10 years duration of SLE. The bimodal pattern of mortality persists, in terms of early deaths from infection and late deaths from vascular disease, but deaths due to SLE have shifted to long duration SLE.

PO2.D.11

Mortality rates in SLE: effect of gender, race/ ethnicity, age, and disease duration

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Purpose: Male lupus, in spite of being a small proportion of SLE, carries a worse prognosis. African-American SLE differs from Caucasian SLE in multiple aspects including autoantibodies, disease manifestations (such as more lupus nephritis) and more organ damage. In a prospective cohort, we asked how race/ethnicity, gender and age affect mortality rates. *Methods:* 1375 patients were followed longitudinally for a total of 9763 person years. The patients were 93% female, 39% African-American and 56% Caucasian, with median age at start of follow-up equal to 35 years. *Results:* For age < 60, the highest mortality rate was 12.3/1,000 person-years for age 40-49. Caucasians had a lower mortality rate (9/1,000 person-years) vs African-Americans (16.5/1,000 person-years), p = 0.0008. Men had a higher mortality rate: 22.5/1,000 person-years vs 11.4/1,000 person-years in women (p=0.02). Duration of SLE had no effect on all causes of mortality, SLE-related and vascular deaths, or cancer mortality rates. In contrast, there was a non-significant reduction in infection mortality rates for more than 5 years duration of SLE (2/1,000 person-years vs 6.2/1,000 person-years for duration less than 2 years, p= 0.061).

	SLE-Related Deaths/ 1000 P-yrs	Infection Deaths/ 1000 P-yrs	Vascular Deaths/ 1000 P-yrs	Cancer Deaths/ 1000 P-yrs
African-American	2.4	3.8	2.6	1.7
Caucasian	0.8	1.3	1.3	1.0
p-value	0.082	0.029	0.25	0.51

Conclusion: African-American SLE has higher death rates for all causes, reaching statistical significance for infection deaths. In both races, infection-related deaths were most frequent, followed by vascular deaths and then SLE-related deaths. Although infection-related death has been emphasized in the third world, it is the most frequent cause of death in this U.S. SLE cohort. The current emphasis on reduction in atherosclerosis deaths needs to be broadened to include infection-related deaths, as well.

PO2.D.12

Change in SF-36 summary and sub-scale scores in SLE patients who have clinical improvement and deterioration in neuropsychiatric (NP) events

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Objective: Neuropsychiatric (NP) events contribute to morbidity, including health-related quality of life (HRQoL), in systemic lupus erythematosus (SLE). We examined the change in HRQoL in association with the clinical outcome of NP events in a prospective study of SLE patients. **Methods:** An international multi-center inception cohort study prospectively evaluated SLE patients for NP events. Assessments were performed upon enrollment and annually. NP events were classified according to the American College of Rheumatology (ACR) case definitions for 19 NP syndromes and decision rules were used to attribute NP events to SLE and non-SLE causes. The outcome of NP events was determined on a physician-completed 7-point Likert scale indicating change in NP status. Data on HRQoL was collected with patient-completed SF-36 questionnaires which provide 2 summary scores and 8 subscale scores pertinent to HRQoL. Global SLE disease activity was measured with the SLE disease activity index (SLEDAI) and cumulative organ damage with the SLICC/ACR damage index. Statistical analysis used multiple linear regression models with patient specific random effects. **Results:** 274 patients (92% female; 68% Caucasian, 16% Black, 8% Asian, 5% Hispanic) in a cohort of 1400 had $\geq$ 1 NP event with a documented clinical outcome and 2 completed SF-36 questionnaires. 17/19 ACR defined NP events over 587 visits were recorded. The mean (SD) interval between assessments was 12.3 (1.94) months. The overall difference in the change in mental component summary (MCS) scores in 3 patient groups reached statistical significance (p<0.0001) following adjustments for gender, race, research center and initial score; in patients whose NP status consistently improved (N=295) the mean (SD) MCS score increased by 3.66 (0.89) more than in patients without a consistent change (N=262) over the same interval. In contrast, for patients whose NP status consistently deteriorated (N=30), the MCS score decreased by 4.00 (1.96). For the SF-36 physical component summary (PCS) scores the corresponding changes associated with improvement and deterioration in NP status were +1.73 (0.71) and -0.61 (1.58) (p<0.05) respectively. Changes in the 4 subscales of mental and physical health were in the same direction as the summary scores (p<0.05; with one exception (physical health)). Sensitivity analyses that modified the definition of clinical improvement in NP status confirmed the previous findings. Adjustment for multiple covariates (age, education, medications, global SLE disease activity, cumulative organ damage, attribution of NP events and disease duration) did not substantially alter the results. **Conclusion:** Changes in SF-36 summary and subscale scores, in particular those related to mental health, are strongly associated with the clinical outcome of NP events in SLE patients. This emphasizes the importance of detecting and treating NP events, regardless of cause, in order to improve HRQoL of SLE patients.

PO2.D.13

Long-term outcomes – mycophenolate mofetil treatment for lupus nephritis with addition of tacrolimus for resistant cases

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Objectives: Although mycophenolate mofetil (MMF) is increasingly used to manage lupus nephritis (LN), little is known about its long-term benefits and the role of anti-calcineurin agents for MMF-resistant nephritis. The aim is to report our long-term experience with MMF therapy as continuous induction-maintenance treatment in a cohort of 70 patients with LN followed during 5 years. We also report the efficacy of adding tacrolimus as a rescue therapy for resistant cases. **Methods:** Seventy patients with LN were included (84% with type III/IV GMN and 40% with relapsing disease) and followed up to a mean of 60±23 months. Patients received 3 pulses methylprednisolone and MMF (2g/d) for induction and tapering doses of MMF for maintenance therapy. Tacrolimus (0.075 mg/kg/day into divided doses) was added for resistant cases. Primary end-point was the achivement of complete remission (presence of an inactive sediment, proteinuria <0.3 g/day and estable renal function) at 6, 24 and 60 months. Secondary end-points included partial remission, treatment failure, relapse and side effects. **Results:** Forty-eight patients (69%) already met criteria for an early response at 12 weeks. See Table 1 for treatment outcomes. Long-term complete response was higher in patients with newly diagnosed glomerulonephritis than those with relapsed nephritis (64% vs. 32%, p=0.014). Renal function was permanently impaired at last follow-up in fourteen patients (20%), six of them had their creatinine level doubled (9%) and two of them reached ESRD. Time to treatment failure was associated with persistent hypoalbuminemia and higher proteinuria (HR=0.87; 95%CI 0.81-0.95, p=0.001, and HR=1.29; 95%CI 1.03-1.62, p=0,030, respectively) and fewer early responses (HR 0.28, 95%CI 0.10-0.77, p=0.014). Twenty-six (37%) patients suffered a renal relapse. Time to flare was associated to persistent anti-dsDNA titres and younger age at renal flare (HR=1.001; 95%CI 1.001-1.003, p=0.005, and HR=0.36; 95%CI 0.14-0.90, p=0.29, respectively). Tacrolimus was added to MMF in seventeen (24%) patients. After a mean follow-up period of 23±3 months, twelve (70%) patients achieved response (6 CR and 6 PR). Proteinuria levels were significantly reduced by 3 months (p=0.0018). GI side effects (23%) were the most common reported. Three patients developed herpes Zoster and one developed pulmonary tuberculosis in the tacrolimus-combined therapy. **Conclusions:** MMF is an effective treatment for induction-maintenance of remission for LN. Combination therapy with tacrolimus is an effective and safe alternative for MMF-resistant patients.

PO2.D.14

The causes of death in Korean patients with systemic lupus erythematosus over 11 years

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Objectives: To investigate the causes of death and to analyze the prognostic factors in Korean patients with systemic lupus erythematosus (SLE). **Methods:** We evaluated retrospectively 1010 SLE patients who were seen in Seoul St. Mary's Hospital from 1997 to 2007. All clinical characteristics and serologic parameters were investigated. The cause of death was defined on the basis of clinical records. Changing patterns of survival and the causes of death were analyzed. Survival and the predictive factors were calculated by Kaplan-Meier method and the Log-rank test. **Results:** The 5-year survival rate increased from 93.7% in the mid-1990s to 97.8%. Over the 11-year period of the study, 59 deaths occurred. Among 44 patients who died in our hospital, the most common cause of death was infection (37.3%) and SLE-related death was the next frequent cause (22.0%). In comparison to earlier data, the portion of SLE related death in the causes of death has fallen and the proportion of infection has risen. SLE related death was the most frequent cause in early death, while infection was the most common cause in both early death and late death. Damage related with SLE and cyclophosphamide treatment were associated with the death (p<0.001 each). The late-onset of SLE and renal involvement were predictive factors of poor outcome (p=0.03 and p<0.001). **Conclusion:** The most common cause of death in Korean SLE patients was infection. To decrease infection by the more judicious use and choice of immunosuppressive agents may be important for improvement of survival in SLE patients.

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PO2.D.15

Patients of hispanic background develop lupus nephritis (LN) early in the disease course: data from a multiethnic US cohort

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Background and *Purpose:* Lupus patients of Hispanic background develop LN with increased frequency though it is not known whether this manifestation occurs earlier in the disease course compared to other ethnic groups. *Methods:* SLE patients (ACR criteria), age ≥16 years, disease duration ≤5years, African-American, Hispanic or Caucasian ethnicity, from a longitudinal cohort were studied. LN was defined per the ACR criteria. Time to LN was examined using multivariable Cox proportional hazards regression adjusting for pertinent baseline clinically and sociodemographic variables. *Results:* Four hundred and forty nine patients were included. Hispanic (hazard ratio [HR] 2.3 95% confidence interval [CI] 1.0-5.2, p=0.0430) and African American (HR 2.6 CI 1.3-5.3, p=0.0097) ethnicities, photosensitivity (HR 2.1 CI 1.1-4.0, p=0.0251), serositis (HR 2.0 CI 1.1-3.7, p=0.0250) and immunological criterion (HR 5.5 CI 2.1-14.6, p=0.0005) were associated with a shorter time-to the development of LN; while age (HR 0.9 CI 0.9-1.0, p=0.0329) was associated with a longer time to its development. Other variables are depicted in Table 1. *Conclusions:* Patients of Hispanic ethnicity not only develop LN more frequently but also earlier in the course of the disease (as compared to Caucasians). Thus, these patients along with those of African ancestry should be carefully monitored from the outset so that they can be aggressively treated when LN supervenes to prevent the occurrence of renal damage and its devastating consequences.

Table 1. Multivariable Cox model time to renal disorder

Variable	HR	95% CI	P- value
Age	0.97	0.94-0.99	0.0329
Gender	1.04	0.41-2.69	0.9275
Ethnicity			
Hispanic	2.32	1.03-5.25	0.0430
African American	2.57	1.26-5.26	0.0097
Caucasian	--	Reference Group	--
Malar Rash	1.00	0.53-1.91	0.9897
Discoid Rash	0.98	0.46-2.09	0.9613
Photosensitivity	2.10	1.09-4.01	0.0251
Ulcers	1.54	0.87-2.73	0.1376
Arthritis	1.28	0.56-2.91	0.5615
Serositis	2.00	1.09-3.67	0.0250
Neurological involvement	0.73	0.31-1.72	0.4648
Hematologic	1.90	0.88-4.11	0.1034
Immunologic	5.56	2.12-14.60	0.0005
Antinuclear antibody	0.38	0.13-1.13	0.0815
Hydroxychlroquine	1.05	0.55-2.00	0.8943

PO2.D.16

Fibromyalgia is a major predictor of fatigue in SLE patients

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Objectives: To evaluate the relation between fatigue and disease activity, damage, fibromyalgia and depression in patients with systemic lupus erythematosus (SLE). *Material And Methods:* Consecutive patients fulfilling the 1997 ACR Classification Criteria for SLE and followed in the Coimbra Lupus Cohort were included. Study evaluation was done at time of a scheduled Rheumatology outpatient visit. Patients filled the validated Portuguese version of MOS SF-36 quality of life questionnaire. Outcome measure for fatigue was the SF-36 vitality domain. Disease activity was assessed with SLEDAI 2K and cumulative damage with SLICC-DI. Criteria for Fibromyalgia (FM) was a clinical diagnosis by the attending Rheumatologist and for Depression a clinical diagnosis and treatment with anti-depressive drugs. Statistical analysis was performed using the software SPSS 17.0. Continuous data are presented as mean and standard deviation, categorical variables as proportions. Comparison of scores between Depressed and non-depressed patients, as well as between FM and non-FM groups, was made using Man-Whitney U test. The SF-36 variables "energy", "jade" and "tiredness" were transformed into dichotomous variables and its association with the diagnosis with FM and depression was evaluated using Chi2 test. Spearman Coefficient evaluated correlation between Vitality scores and SLEDAI, SLICC, SLE disease duration and age. p<0.05 was considered statistically significant. *Results:* 121 SLE patients were included (90.1% female, mean age= 40.55±14.20 years) with a mean disease duration of 8.02±6.6 years. The majority of patients presented low disease activity (mean SLEDAI= 4.16±4.24; 71.6% with SLEDAI lower or equal to 4) and no cumulative damage (54.8% SLICC=0). 19.8% of the patients had clinical depression and 13.3% FM. SLE patients presented a low mean Vitality score (48.52±23.9%) with 71.07 % never or few times feeling full of energy. Depressed patients presented lower Vitality scores (37.71 vs 51.65%, p=0.025). Vitality was significantly lower in patients with FM (27.75 vs 52.30%, p<0.001). FM patients present a higher risk of feeling tired (OR=6; 95%CI: 1.9-18.7) and feeling jade (OR=9.05; 95%CI 2.4-33.9) most of the time and feel full of energy few time or never (OR=7.3; 95%CI 0,9-57,47). No association was found between fatigue and depression, SLE disease activity, cumulative damage, disease duration or age. *Conclusion:* Fatigue level is high in this SLE cohort and FM is its major predictor. No association was found between Fatigue level and SLE outcome measures. FM prevalence in SLE (13.3%) is higher than estimated in the female Portuguese general population (5.1%).

PO2.D.17

Accumulation of atherosclerotic risk factors and lupus disease risk factors in the first 5 years of SLE: results from the SLICC inception cohort

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Background/Purpose: Patients with systemic lupus erythematosus (SLE) develop premature atherosclerosis (AS). This study examines the accumulation of AS and lupus related risk factors over 5 years in a multicenter, international inception cohort. *Methods:* An inception cohort of SLE patients from 26 centres in 8 countries has been assembled according to a standardized protocol between 2000 and December 2009 to study risk factors for atherosclerosis. In addition to traditional AS risk factors, SLE factors included in these analyses were: disease activity measured by the SLE disease activity index (SLEDAI-2K), accumulated damage measured by the SLICC/ACR damage index (SDI), quality of life and function measured by the SF-36 physical component summary (PCS) and mental component summary (MCS) scores, as well as corticosteroid therapy, antimalarial and immunosuppressive use. *Results:* Of the inception cohort of 1512 SLE patients, 390 (87.2%F, 53.3% Caucasian, age at diagnosis 34.8y) have now been followed for 5 years and constitute the population for this study. Accumulation of potential risk factors for AS are shown in the table.

Table 1. Accumulation of Atherosclerotic and Lupus-related Factors in 390 patients with 5 years of follow-up.

Variable	Enrolment	At 3 years	At 5 years
SLEDAI-2K*	5.74 ± 5.41	3.49 ± 3.79	3.33 ± 4.08
SDI*	$0.37 \pm 0.78\dagger$	0.75 ± 1.13	0.97 ± 1.34
SF-36 PCS*	38.2 ± 11.2	41.7 ± 11.2	42.8 ± 12.0
SF-36 MCS*	45.0 ± 12.0	46.4 ± 12.2	47.7 ± 11.8
Corticosteroid therapy	259 (66.4%)	270 (77.4%)	304 (78.0%)
Antimalarials	230 (59.1%)	263 (75.4%)	319 (81.8%)
Immunosuppressive drugs	145 (37.2%)	209 (59.9%)	249 (63.9%)
Diabetes	12 (3.1%)	18 (5.2%)	22 (5.6%)
Menopause	52 (15.3%)	70 (22.9%)	93 (27.4%)
Smoking	58 (14.9%)	51 (14.7%)	56 (14.5%)
Obesity	112 (29.4%)	117 (35.5%)	129 (36.3%)
Low physical activity	116 (34.0%)	169 (54.2%)	182 (55.7%)
Hypertension or specific treatment	137 (35.1%)	194 (55.6%)	235 (60.3%)
Hypercholesterolemia or specific treatment	131 (33.6%)	214 (61.3%)	260 (66.7%)

*variables are not cumulative

† N = 178 as SDI performed only in those with > 6 months disease duration

Accrual of CAD related risk factors continued through the first 5 years, the majority of the increase occurred by year 3. This may be due to increased use of antihypertensives (from 80% at enrolment to 90% at year 3 and 92% at year 5) and lipid lowering agents (24%, 52% and 53% at enrolment, 3 and 5 years respectively). *Conclusion:* Patients with SLE demonstrate improvement in disease activity but accumulate organ damage over the first 5 years of their disease. Accumulation of CAD risk factors occurs throughout but more prominently over the first 3 years.

PO2.D.18

Greater mortality in late-onset systemic lupus erythematosus in a Latin American inception cohort

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Objectives: The objective of this study was to evaluate the clinical differences and mortality in late-onset systemic lupus erythematosus (SLE) in a prospective inception cohort of Latin-American patients. *Methods:* SLE patients from 34 centers in Argentina, Brazil, Chile, Colombia, Cuba, Guatemala, Mexico, Perú and Venezuela with a recent SLE diagnosis (≤2 years) had been recruited and followed-up longitudinally. Patients who developed SLE after the age of 50 years were considered late-onset SLE. Demographic characteristics, and cumulative clinical manifestations, laboratory data, SLE disease activity index (SLEDAI), Systemic Lupus International Collaborating Clinics damage index (SLICC) and mortality were compared between patients with disease onset before and after 50 years of age. *Results:* Of 1192 GLADEL patients older than 18 years included in this study 82 (6,9 %) were late-onset SLE. Cumulative clinical features are shown in table 1. Mortality incidence rate ratio was 2.3 (95% CI: 1 - 4.6) in late-onset SLE. After adjusting for gender, education, socioeconomic level, ethnicity, diagnosis delay, activity and damage indexes, corticosteroids and cyclophosphamide use, onset of SLE after 50 years of age remained as a risk factor for mortality in a Cox regression model: Hazard ratio: 3.96 (95% CI: 1.6-9.6; p=0.002) *Conclusion:* In this cohort of Latin American patients with late-onset SLE presented less frequently skin and musculoskeletal manifestations and more frequently pleuro-pulmonary involvement and higher mortality rate than those with younger onset SLE.

Characteristics and cumulative clinical features	Early onset (n=1110)	Late Onset (n=82)	p value
Gender, Female, % Gender, Female, %	90.4	85.4	0.145
Age at diagnosis, years*	31.2	60.6	0.0001
Delay in diagnosis, months*	16.1	21.7	0.0894
Ethnic group, %			
Caucasian	44.1	40.2	
Mestizo	42.6	48.8	0.586
African-Latin American	12.8	8.5	
Residence rural, %	8.2	7.3	0.780
Hypertension, %	38	45.1	0.202
Clinical features, %			
Photosensitivity	61.1	42.7	0.001
Malar rash	64.6	37.8	0.0001
Arthritis	92.7	84.1	0.006
Raynaud	33.1	21.9	0.038
Keratoconjunctivitis sicca	2.3	4.9	0.157
Pleuritis	24.9	37.8	0.010
Lung involvement	7.8	13.4	0.076
Renal involvement	59.7	50	0.084
Neurologic involvement	35.3	36.5	0.816
Anti Ro antibodies	53.1	50	0.316
Maxim SLICC *	0.77	0.84	0.6618
Mean SLEDAI *	4.3	4.5	0.7404
Medications, %			
Cyclophosphamide	36.6	23.1	0.014
Antimalarials	81.2	87.9	0.694
Glucocorticoids	94.3	87.8	0.018
Death during follow-up, %	5	10.1	0.022

* Mean values for each group

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PO2.D.19

Evaluating cardiac risk in systemic lupus erythematosus versus other inflammatory arthritis patients

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Objective: Systemic Lupus Erythematosus (SLE) and other inflammatory arthritides (OIA) are independent risk factors for cardiovascular disease (CVD). Cardiovascular risk stratification scoring systems are a starting point in evaluating CVD risk. The main study objective was to determine how effective rheumatologists are at CVD risk stratification in SLE patients and compare this to OIA patients. **Methods:** A retrospective chart review of 504 patients attending the practices of nine rheumatologists at the University of Alberta Hospital was performed with pre-specified case report forms reviewing disease indices and medications, cardiac risk factors and Framingham 2008 and Reynolds risk scores. **Results:** In this group of 504 patients, 64 (12.7%) had SLE (M:F =4:60), 440 (87.3%) had an OIA (M:F =117:323). Of the SLE patients, 33 (51.6%) met four or more ACR criteria, 31 (48.4%) had less than four ACR criteria. Of the OIA patients, 156 (35.5%) were CCP positive and 257 (58.4%) were RF positive. Complete Framingham risk scores were calculable for 1 (1.6%) SLE patient and 3 (0.68%) OIA patients. The Reynold's risk score was not calculable for any patients. The number (%) of SLE vs. OIA patients where common cardiac risk variables were not recorded included: (1) positive family history of MI 62 (96.9%) SLE vs. 440 (100%) OIA patients, (2) diabetes 62 (96.9%) SLE vs. 421 (95.7%) OIA patients, (3) lipids status 48 (75%) SLE vs. 322 (73.2%) OIA patients and (4) smoking status 35 (54.7%) SLE vs. 275 (62.5%) OIA patients. The number (%) of SLE vs. OIA patients where cardiovascular medications (whether positive or negative) were recorded included: ASA 62 (96.9%) SLE vs. 311 (70.7%) OIA patients, anti-hypertensives 62 (96.9%) SLE vs. 317 (72.0%) OIA patients, and lipid lowering medications 61 (95.3%) SLE vs. 310 (70.5%) OIA patients. Systolic blood pressure was documented in 60 (93.8%) SLE and 247 (56.1%) OIA patients. **Conclusions:** Cardiovascular risk assessment in both SLE and OIA is sub-optimally performed by rheumatologists. Cardiovascular medication history and blood pressure documentation in SLE patients, however, is better than that of the OIA's. The multi-system nature of SLE including renal disease potentially leads to closer monitoring of CVD risk factors such as diabetes and hypertension. Increased documentation of CVD risk factors and possible use of existing risk scores is the first step in establishing effective CVD risk reduction in these higher risk rheumatic disease groups.

PO2.D.20

Observational study of outcome for all biopsied SLE patients with glomerulonephritis in one unit between 1986 and 2004, detecting the predictive value of both the new ISN classification and the response to therapy within 6 months

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Objective: To study possible predictors for renal response or reduced glomerular filtration rate (GFR) in lupus nephritis and to compare the WHO with the new ISN lupus nephritis classification. **Method:** All 52 biopsy verified cases of lupus nephritis in the local catchment area between the years 1986 and 2004 were identified and GFR, serum-creatinine, proteinuria, hematuria, SLEDAI-2K, renal variables and complement were retrieved at biopsy, after 6, 12 months and at latest visit. Information about given therapy was also extracted both from medical records and our local prospective SLE registry. Two cases were lost to follow up and in one case the biopsy was unsatisfactory, why 49 biopsied cases remained. All renal biopsies were reevaluated with the new ISN classification. Outcome was defined by the ACR renal response criteria. **Results:** Mean follow up time was 9 years (2-24 y), complete renal response was achieved in 11 cases, partial renal response in 32 cases, end stage renal disease in 5 cases and nephritic syndrome in one case. Of all biopsied patient

15 have died at a median age of 68 years. The final GFR correlated with the age at biopsy (p<0.01), with the ISN classification (p<0.05) and especially the presence of interstitial manifestations (p<0.01); also with the decrease of proteinuria (p<0.01) and serum-creatinine (p<0.05) after six months of treatment. The outcome also correlated with the nefrological components of SLEDAI 2K after 6 months of therapy (p<0.01, positive predictive value 90%). The WHO classification did not correlate with outcome. The local practice was to give azathioprin as induction therapy during the first part of this observational study and was then changed to individually designed pulse cyclophosphamid as induction therapy during the second half. There was a trend towards worse outcome in the azathioprin treated patients. **Conclusions:** A good outcome is correlated with the response to treatment after 6 months and the ISN classification, especially the addition of interstitial manifestations, might add useful information for prediction. Since this is not a randomized study data do not allow conclusions about effectiveness of different therapies.

PO2.D.21

The clinicopathological characteristics and outcome of Chinese patients with late-onset lupus nephritis: a study from a single center

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Objective: This study is to assess the clinicopathological features and outcome of patients with late-onset lupus nephritis in a large cohort of Chinese patients. **Methods:** Renal histopathological data of 319 patients with lupus nephritis were reviewed. The detailed data of patients with late-onset (onset at age>/=50years) lupus nephritis were retrospectively analyzed. **Results:** 30 cases were classified as late-onset lupus nephritis. 22 were female and 8 were male with an average age at 56.0±5.0 (50 to 70) years at presentation. Among the 30 patients, 1 cases were classified as class II, 7 as class III, 12 as class IV (1 cases of class IV-segmental and 11 cases of class IV-global), 9 as class V, 1 as class VI, respectively, by ISN/RPS system. All of the patients received oral prednisone therapy. The majority of patients completed treatment with oral cyclophosphamide (2/30) or monthly intravenous cyclophosphamide (600-800mg/month) (18/30). The other patients received mycophenolate mofetil (3/30), leflunomide (4/30) and azathioprine (2/30). One patients received prednisone alone. Most of patients achieved clinical remission, 18 with complete remission and 9 with partial remission. Three patients presented with treatment failure. During follow-up of 70.5±24.3 months, no patient died. Regarding long-term renal outcome, 2 patients reached endpoint including 1 with doubling of serum creatinine and 1 with end stage renal disease. There were significantly higher male proportion (36% vs. 15%, P=0.038) and interstitial inflammation score (1.50±0.820 vs. 1.18±0.774, P=0.033) in renal histopathologic evaluation in late-onset group compared with early-onset group. There was no significant difference in other indices between the 2 groups. The long-term renal survival rate was similar (93.3% vs. 86.2%, P=0.269). **Conclusions:** There are some differences in clinical and pathological manifestations between late-onset and early-onset lupus nephritis which needs further investigation.

PO2.D.22

ACR criteria at inception and accumulation over 5 years in SLE

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Background/*Purpose:* In long term observational studies and in therapeutic trials, patients with SLE are often recruited if they have 4 or more SLE criteria. We report the mean number of criteria in each of the first 5 years of disease, the frequency of occurrence of each criterion at enrolment and over 5 years and the relative increase in each of the criterion. *Methods:* An international research network comprising 26 centres from 8 countries has followed an inception cohort of SLE patients yearly according to a standardized protocol between 2000 and December 2009. These analyses included 390 patients who had been followed for at least 5 years. The ACR criteria was ascertained at each visit and assessed by ethnicity. Descriptive statistics were used. *Results:* Of the 390 patients followed for at least 5 years, 87.2% were female, 53.3% were Caucasian, 13.1% were Black/African descendant, 13.8% were Asian, 16.9% Hispanic and 2.8% other. 42.6% were married and 62.9% had at least some college education. Their age at enrolment was 35.2 years and SLEDAI-2K at enrolment was 5.7. The duration from diagnosis to enrolment was 5.4 months.

Table 1. Accumulation of ACR criteria in 390 patients with at least 5 years of follow-up.

	At Enrolment (%)	At 5 Years (%)
Malar rash	40.0 ± 48.8	50.8 ± 50.1
Discoid rash	9.2 ± 29.0	16.7 ± 37.3
Oral ulcers	41.8 ± 49.4	55.6 ± 49.7
Serositis	24.4 ± 43.0	32.1 ± 46.7
Arthritis	73.1 ± 44.4	84.6 ± 36.1
Photosensitivity	39.0 ± 48.8	47.9 ± 50.0
Renal disease	31.3 ± 46.4	44.9 ± 49.8
Neurologic disorder	6.7 ± 25.0	11.3 ± 30.7
Hematologic disorder	64.6 ± 47.9	80.5 ± 39.7
Immunologic disorder	79.2 ± 40.6	86.7 ± 34.0
Antinuclear antibody	93.8 ± 24.1	96.7 ± 18.0

The mean number of criteria per patient increases over 5 years from 5.02 to 6.08. Hematologic disorder, renal disease and oral ulcers increased the most over this time. The second most frequent increase occurred in malar rash, arthritis and photosensitivity. At each annual assessment in non-Caucasian patients, renal disease and hematologic disorder were more frequent and photosensivity was less frequent. *Conclusions:* ACR criteria continue to accrue after diagnosis. In the first 5 years after diagnosis, 13.6% of patients develop new renal disease. Renal disease and hematologic criteria were more frequent in non-Caucasian patients at each annual assessment.

PO2.D.23

Clinical-serologic discordance in a multicentre, international cohort of patients with SLE

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Background/Purpose: Anti-DNA antibodies and serum complement levels are considered important biomarkers for disease activity in SLE. Despite this many patients present serologically active but clinically quiescent (SACQ) while others present as clinically active but serologically quiescent (CASQ) disease. These states create therapeutic dilemmas for clinicians. We aim to determine the frequency of SACQ and CASQ in a large international inception cohort of patients with SLE. *Methods:* An international research network comprising 26 centres from 8 countries has followed an inception (within 15 months of diagnosis) cohort of SLE patients yearly according to a standardized protocol between 2000 and April 2009. Of these, 232 patients followed for a minimum of 5 years constitute the study population. Anti-DNA antibody levels and serum complement values are assessed at each visit. Clinical disease activity was assessed using SLEDAI-2K minus anti-DNA antibody and complements. SACQ is defined as presence of elevated anti-DNA ± decreased complement variables of SLEDAI-2K only and CASQ as SLEDAI-2K > 0 in the absence of anti-DNA or complement variables. Descriptive statistics were used. *Results:* Of the 232 patients followed for at least 5 years, 86.2% were female, 61.2% were Caucasian, 13.4% were Black/African descendant, 17.2% were Asian, 6.5% Hispanic and 1.7% Other. 44.4% were married and 59.7% had at least College education. Their age at diagnosis was 36.5 years and SLEDAI-2K at enrolment was 5.63. The duration from diagnosis to enrolment was 5.2 months. At some point in their 5 visits 121(52.2%) were SACQ and of the 232, 47(20.3%) were SACQ on no medication other than antimalarials. In 17(7.4%) patients this state was sustained for at least 2 consecutive years. At some point in their 5 visits 137(59.1%) were CASQ and in 63(27.2%) this state was sustained for at least 2 consecutive years. *Conclusions:* Clinical-laboratory discordance regarding anti-DNA antibodies and complement levels presenting as SACQ or CASQ represent a significant subgroup of SLE patients who require close clinical follow-up. New biomarkers to better reflect disease activity in SLE are required.

PO2.D.24

Damage due to glucocorticoids in an SLE inception cohort

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Background/Purpose: As survival has increased in patients with SLE, organ damage has been shown to accumulate over time as a result of both the disease and its treatment especially corticosteroids. We determined the contribution of corticosteroids to the pattern of damage accumulation in a multicentre, inception cohort of SLE patients followed for 5 years. **Methods:** An international consortium comprising 26 centres from 8 countries has established an inception cohort followed yearly according to a standardized protocol between 2000 and December 2009. Of these, 390 patients have been followed for a minimum of 5 years and constitute the study population. Accrued damage was measured using the SLICC/ACR Damage Index (SDI). Organ damage was categorized into the following groups: definitely related to steroids (cataracts, osteonecrosis, osteoporosis); possibly related (cardiovascular, peripheral vascular, neuropsychiatric, diabetes); independent of steroids (renal, pulmonary, gastrointestinal, skin, gonadal failure and malignancy). Descriptive statistics were used. **Results:** Of the 390 patients followed for at least 5 years, 87.2% were female, 53.3% were Caucasian, 13.1% were Black/African descendant, 13.8% were Asian, 16.9% Hispanic and 2.8% Other. 42.6% were married and 62.9% had at least some College education. Their age at enrolment was 35.2 yrs and SLEDAI-2K at enrolment was 5.7. The duration from diagnosis to enrolment was 5.4 months. By 5 years 47.0% (181/390) of patients had some damage. Glucocorticoids were taken by 65.3% of the patients at enrolment, 75.6% at year 1 and persistently in 78% of the patients thereafter. The mean SDI increased progressively from 0.10 to 0.97 over 5 years.

Table 1. Contribution of corticosteroids to damage accumulation in patients with damage present over 5 years.

Year	# Patients with damage Present	SDI (Mean ± STD)	SDI Definitely glucocorticoid (Mean ± STD)	SDI Possibly glucocorticoid (Mean ± STD)	SDI Independent of glucocorticoids (Mean ± STD)
0	23* (6.4%)	1.57 ± 0.90	0.17 ± 0.39	0.65 ± 0.57	0.74 ± 1.01
1	105 (31.1%)	1.62 ± 0.94	0.28 ± 0.58	0.70 ± 0.84	0.64 ± 0.74
2	129 (37.1%)	1.72 ± 1.05	0.33 ± 0.62	0.66 ± 0.82	0.73 ± 0.81
3	142 (41.2%)	1.82 ± 1.07	0.37 ± 0.67	0.70 ± 0.88	0.76 ± 0.84
4	156 (45.0%)	1.97 ± 1.17	0.48 ± 0.78	0.69 ± 0.83	0.81 ± 0.94
5	181 (47.0%)	2.07 ± 1.24	0.54 ± 0.83	0.66 ± 0.84	0.87 ± 1.00

* SDI performed only in those with > 6 months disease duration

Damage definitely due to glucocorticoids increased over 5 years whereas damage independent of glucocorticoids remained stable over 5 years. **Conclusions:** With improving survival, a significant portion of accumulated organ damage experienced by patients with SLE is related to glucocorticoids. In this study 26.1% (0.54/2.07) of accrued damage in the first 5 years of disease is definitely related to glucocorticoids therapy.

PO2.D.25

Prevalence and persistence of metabolic syndrome in a multicentre, international inception cohort of patients with SLE

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Background/Purpose: Metabolic Syndrome (MetS) may contribute to atherosclerosis risk in SLE. The purpose of this study was to determine the prevalence and persistence of MetS over 2 years in a multicentre, international SLE inception cohort. **Methods:** The SLICC Atherosclerosis Registry (SLICC-RAS) is an international inception cohort registry which has enrolled 1512 recently diagnosed patients with SLE from 30 centres in 11 countries. All patients are assessed according to a standard protocol including clinical and laboratory variables, SLEDAI-2K and SDI at enrollment and annual follow-up visits. The International Diabetes Federation definition for MetS was used and included the following criteria: increased waist circumference (≥ 94 cm

for men, ≥ 80 cm for women) or Body Mass Index (BMI) of greater than 30 kg/m^2 plus any two of raised triglycerides or specific treatment for this lipid abnormality and/or reduced high density lipoprotein (HDL) cholesterol or specific treatment for this lipid abnormality and/or raised blood pressure and/or increased fasting plasma glucose or previously diagnosed type II diabetes. *Results:* Of the inception cohort of 1512 SLE patients (87.2%F, 53.3% Caucasian, mean age at diagnosis 34.8y), 1360 patients had sufficient data to determine their MetS status. Of these 1360 patients, 13.5% had MetS at baseline.

Table 1. Presence of MetS criteria in inception cohort of 1360 patients.

	Present	Normal
Metabolic Syndrome	183 (13.5%)	1177
Increased waist circumference	585 (42.6%)	643
Body Mass Index (BMI) > 30 kg/m^2	224 (16.8%)	1106
Either increased waist circumference or BMI > 30 kg/m^2	621 (45.7%)	739
Raised triglycerides or specific treatment	358 (29.8%)	843
Reduced HDL cholesterol or specific treatment	217 (33.2%)	437
Raised Blood Pressure	629 (46.4%)	728
Increased fasting glucose or diagnosed type II diabetes	200 (16.3%)	1027

In total, 596 patients had two years of follow-up data of whom 14.1% (84/596) had MetS at baseline and 23.7% (141/596) had MetS at some point over the two years. Persistence across all 3 time points occurred in 16.7% (14/84) of these patients. *Conclusion:* MetS is common in newly diagnosed SLE patients and up to one quarter have MetS at one or more time points over the first 2 years of follow-up. Better understanding of the factors that influence variability in MetS expression over time may help inform cardiovascular preventative strategies in these patients.

PO2.D.26

Dyslipidemia in patients with systemic lupus erythematosus

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Studies on dyslipidemia among US subjects with Systemic Lupus Erythematosus (SLE) are negligible. *Aim:* 1.) To estimate the prevalence of dyslipidemia and 2.) To study the association between demographics, disease variables and dyslipidemia among US subjects with SLE. *Methods:* Study data from 70 SLE subjects receiving longitudinal outpatient care at Rush University Medical center pertaining to their demographic, clinical and laboratory information were reviewed for serum cholesterol, low density lipoproteins (LDL), Triglycerides (Tg) and high density lipoproteins (HDL). 45 subjects had received fasting lipid profile testing in the past 5 years. Lipid levels were categorized as abnormal using the current National Cholesterol Education program (NCEP) guidelines. A lipid score was obtained indicating the number of abnormal lipid parameters present per subject. Predictor variables studied included: Demographic (age, ethnicity, education); Clinical (body mass index, age at disease diagnosis, duration of disease, SELENA-SLEDAI, SDI, cardio-vascular and renal disease and use of hydroxychloroquine or prednisone). Descriptive statistics were obtained. ANOVA and Fischer exact test were utilized to these variables categorized by their lipid scores. A p value of ≤ 0.05 was considered significant. *Results:* The mean age and disease duration of the 45 subjects was 46.7± 13 and 12.4 ±9.1 years respectively. 96% were females. The ethnic composition was: African American 64%, Caucasian 20%, Hispanic 14% and others 2%. The mean SELENA-SLEDAI and SDI scores were 10.5 ± 8.8 and 2.8 ± 2.2 respectively. 64% of the subjects had abnormal lipid profile. The mean LDL (114 ± 33.6 vs 73.1 ± 20.9, p 0.001), Triglyceride (143.2 ± 82.5 vs 88.6 ± 31.7, p 0.02) and Cholesterol (205.0 ± 47.0 vs 153.7 ± 19.6, p 0.001) levels were significantly higher among subjects with abnormal lipid profiles. Patients with proteinuria ≥ 3.5 gm/24 hours had higher lipid scores than subjects without (p=0.011). Subjects currently using hydroxychloroquine trended towards lower lipid scores as compared to those not on hydroxychloroquine (p=0.07 on 2-sided test and 0.03 on 1-sided test). There was no association between dyslipidemia and disease activity or damage measures. *Conclusions:*

Only 63% of SLE subjects had received a fasting lipid profile test in the past 5 years, 64% of which had lipid levels above those recommended by the NCEP. Proteinuria ≥ 3.5 gm/ 24 hours was found to be directly associated with dyslipidemia while the use of hydroxychloroquine was possibly indirectly associated with dyslipidemia.

PO2.D.27

Positive health behaviors and health outcomes in systemic lupus erythematosus

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Risky health behaviors can adversely affect treatment compliance and clinical outcomes. However the effects of positive health behaviors (PHB) on clinical outcomes especially in a chronic disease model such as systemic lupus erythematsosus (SLE) are not known. *Aims:* 1. Determine the association of positive health behaviors with current health status in SLE. 2. Determine the demographic and clinical correlates of positive health behaviors in SLE. *Methods:* Positive health behaviors were defined by level of active participation in self health promotion. The 4 self health promotion activities assessed were current use of tobacco and alcohol; and use of Papanicolaou and mammogram screenings (in age appropriate settings) during the previous 2 years. A score of "0" denotes "best" and "4" the "worst" PHB. We obtained demographic, clinical, health outcomes and PHB data from 67 women with SLE, receiving longitudinal outpatient care at Rush University Medical Center, utilizing survey techniques. Variables studied included: Demographic (age, ethnicity, education, and marital status, insurance); Clinical (age at disease diagnosis, duration of disease, SELENA-SLEDAI, SDI, disease manifestations, treatment); Health Outcomes (SF-36, EQ5D). ANOVA and Fischer exact test were utilized to compare continuous and discrete variables categorized by PHB score. A p value ≤ 0.05 was considered significant. *Results:* Of 78 patients approached, 67 consented, and complete data were available for 42 subjects. The ethnic distribution was: 60% African American, 29% Caucasian and 11% Hispanic. The mean (SD) age and disease duration were 44.1± 13.4 years and 10.8 ± 8.1 years respectively. The mean (SD) SELENA-SLEDAI and SDI were 10.5 (8.8) and 2.6 (2.1) respectively.41% and 0% of the subjects had the best (0) and the worst (4) PHB scores. Subjects with best (0) PHB had worst health status (EQ5D Index 0.59 ± 0.21 vs 0.74 ± 0.18 vs 0.76 ± 0.17, p=0.04), more bodily pain (39.1 ±22.0 vs 55.9 ± 24.8 vs 61.3 ±21.4, p 0.05), older at disease onset (38 ± 16.4 vs 35.3 ± 10.3 vs 23 ± 5.8 years, p 0.05), shorter disease duration (7.9±6.5 vs 10.4 ±7.1 vs 20.4± 8.9 years, p 0.008) and had a higher prevalence of skin rashes (82% vs 50% vs 29%, p 0.03) as compare to those with PHB scores of "1" and "2" respectively. *Conclusions:* Patients with shorter disease duration, physically evident disease manifestations and worse health status have better positive health behaviors. This may be a surrogate for heightened health awareness due to recent diagnosis, concern or anxiety for future health; better comprehension of disease effects due to visually perceptible symptoms and worsened health status. SLE patients with disease features not visually perceptible may need greater education from their physicians to improve their overall health outcomes.

PO2.D.28

Frequency and characteristics of lupus patients achieving steroid-free remission

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Objective: To determine the frequency of steroid free remission and to define disease characteristics of lupus patients achieving steroid-free remission. *Methods:* We retrospectively reviewed clinical charts of 289 patients (male

20, female 269, age 45.8±15.3 y.o., disease duration 15.0±12.2 years) who met 1997 ACR SLE Classification Criteria followed in our university hospital. Steroid free remission was defined as a 3-month consecutive period of no disease activity and without corticosteroid treatment. Corticosteroid use, SLEDAI-2K and clinical characteristics were examined. **Results:** Frequency of current corticosteroid users of all the SLE patients was 79.2%, and that of life time users was 96.9%. Forty six patients (15.9%) achieved steroid free remission. Five patients (1.7%) experienced disease flare after cessation of corticosteroid, 9 (3.1%) patients had never received corticosteroid. Patients in steroid free remission were older and renal involvement was less frequent than the other patients. Other factors including gender, disease duration, number of positive items in the SLE classification criteria, disease activity (SLEDAI-2K) at the initiation of steroid therapy, initial dose of steroid and use of immunosuppressant did not reach significant difference. **Conclusion:** Although the frequency of steroid free remission was low, older age and absence of nephritis were associated with discontinuation of corticosteroids. Steroid free remission might be a realistic goal in some patients with lupus.

PO2.D.29

Association between disease damage and health related quality of life in a Peruvian population with systemic lupus erythematosus

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Aim: To determine the association between disease damage and health related quality of life (HQRoL) in a Peruvian population with Systemic Lupus Erythematosus (SLE). **Methods:** A cross-sectional study was performed. Patients with SLE who were consecutively seen in our Rheumatology Department since January to September 2009 were included. An interview, chart review, physical exam and laboratory tests were done. HRQoL was evaluated using SF-36. Disease damage was measured using the SLICC/ ACR damage index (SDI). Disease activity was measured using SLEDAI. Socioeconomical level was evaluated using Graffar's Score. Association between HRQoL and SDI was evaluated using Spearman's correlation. After that, a linear regression model was performed in order to evaluate the association between the subcategories of SF-36 and SDI, adjusted to age, sex, disease duration, socioeconomical level and disease activity. **Results:** 128 patients were evaluated, 95.3% were female, with an average age of 42.04 (SD: 13.88) years. Socioeconomical level was high in 2.3%, middle high in 34.4%, middle in 30.5%, middle low in 30.5% and low in 2.3% patients. Disease duration was 7.23 (SD: 6.67) years, disease activity was 6.78 (SD: 4.83). SDI was 1.16 (SD: 1.40). In the univariate analysis, SDI was associated with lower physical function (R: -0.394, p<0.001), lower emotional role (R: -0.186, p: 0.049) and lower mental health. Of the 2 main subcategories, SDI was associated with lower physical health (R:-0.237, p: 0.012), and also, SDI was associated with a lower general SF-36 (R: -0.232, p:0.014). After adjustment for age, sex, disease duration, socioeconomical level and disease activity, SDI only remained associated with physical function (β: -0.302, p:0.001). **Conclusion:** Higher levels of disease damage are associated with a poor HRQoL. Disease damage has a stronger impact on physical rather than mental health.

PO2.D.30

Low bone mineral density is independently associated with a higher level of disease damage in mestizo Peruvian patients with systemic lupus erythematosus

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Aim: To determine if low bone mineral density (LBMD) is independently associated with a higher level of disease damage in patients with Systemic Lupus Erythematosus (SLE). **Methods:** In a cross-sectional single center study, we evaluated 115 consecutive SLE patients, who were seen at the Rheumatology department of our hospital. SLE was defined using the ACR criteria, LBMD was defined as a T-score≤-2.5 in postmenopausal or elderly patients or a Z-score ≤-2.0 in young patients according to ISCD Official Positions. A chart review, clinical evaluation and laboratory exams were performed. Also, an interview, in order to evaluate calcium intake and osteoporosis risk factors, was carried out. We defined damage using SLICC /ACR damage index (SDI); the point of severe osteoporosis was exclude for the analysis. Disease activity was measured using SLEDAI. For univariate analysis we used Mann-Withney U test or chi square test, after that, we performed (a logistic regression model adjusted to disease activity, accumulated dose of prednisone, age, gender, disease duration, previous fracture, tobacco use, calcium intake and body mass index. **Results:** 115 SLE patients with a mean age of 42.45 (SD: 14.21) years, 95.7% female were included. Almost all of them were mestizo, only one was African Latin-American and one was amerindian. LBMD was found in 28 (24.3%) patients. There were no statistically significant differences between patients with and without LBMD in age (43.90 vs 41.98 years, p: 0.56), disease duration (8.62 vs 7.27; p: 0.30), accumulated dose of prednisone (25.63 vs 16.70 g; p: 0.18), SLEDAI (7.32 vs 6.70; p: 0.31), use of tobacco (25.0% vs 26.4%; p: 0.88), previous fracture (21.4% vs 12.6%, p: 0.26), calcium intake (960.23 vs 846.26 mg/d; p: 0.20) and body mass index (25.42 vs 27.02; p: 0.09). The SDI was higher in patients with LBMD (1.75 vs 0.90; p: 0.006); unadjusted OR was 1.58 (95% CI: 1.14-2.17; p: 0.006). After adjustment for disease activity, accumulated dose of prednisone, age, gender, disease duration, tobacco, calcium intake, body mass index and previous fracture the SDI remained associated with the presence of LBMD (OR: 2.20 (95% CI: 1.14-4.27), p:0.019). **Conclusions:** LBMD is associated with a higher level of disease damage as measured by SDI in our SLE patients, independently of disease activity, accumulated dose of prednisone, age, gender, disease duration, calcium intake, tobacco use, body mass index and previous fracture.

PO2.D.31

Quality of life in cutaneous lupus erythematosus

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Objective: Cutaneous lupus erythematosus (CLE) is a chronic, potentially disfiguring, autoimmune disease. The purpose of this study was to obtain an overview of quality of life (QOL) in CLE, to compare QOL in CLE to other diseases, and to determine which independent variables are associated with poor QOL. **Methods:** All patients with CLE or SLE were invited to participate in the study. Subjects were asked to complete the Skindex-29 and to provide demographic and basic medical history information. Disease severity was assessed with the Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI). Mean Skindex-29 scores in CLE were compared to norms for other diseases. Mean scores within the CLE population were compared between different genders, ethnicities, and disease subtypes. Pearson's correlation coefficients were calculated for Skindex-29 scores and disease severity, age, and age at diagnosis. **Results:** 157 subjects were included in the analysis. Overall, patients with lupus were most affected in the emotions domain (m=48, SD=28) relative to symptoms (m=40, SD=23) and functioning (m=28, SD=25). The subscales were highly intercorrelated, such that a high score in one tended to be associated with high scores in the others (Pearson's r range 0.65-0.78, all p<0.0001). Compared to 8 other diseases, QOL in lupus was sig-

nificantly impaired across all three domains; with respect to symptoms, only vulvodynia (m=50, SD=17) and eczema (m=48, SD=23) had higher scores than CLE (all p<0.0001). In the emotions domain, lupus was comparable to dermatomyositis (mean=45, SD=27) and vulvodynia (m=50, SD=20), and the other diseases were all less affected (all p<0.0009). For functioning, only patients with vulvodynia (m=44, SD=22) were more impaired than patients with lupus (p<0.0001). Female gender was associated with poor QOL in all three domains (all p<0.006), however there was no significant difference in QOL amongst different ethnicities or disease subtypes. There was a correlation between increased disease severity, as measured by the CLASI, and worse QOL across all three subscales (Pearson's r range 0.27-0.38, all p<0.0006). There was a small correlation between younger age and worse symptoms (Pearson's r -0.16, p=0.04) and emotions scores (Pearson's r -0.22, p=0.005). There was also a small correlation between younger age at diagnosis and worse symptoms scores (Pearson's r -0.19, p=0.04). *Conclusions:* CLE has a profoundly negative impact on QOL, particularly in women and individuals with severe disease. Poor QOL has been linked to psychiatric comorbidity (Sampogna et al, Psychosom Med 2004), therefore it is important to acknowledge and address this issue with patients.

PO2.D.32

Incidence of and predictors of hospitalization of patients with systemic lupus erythematosus

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Objective: Hospitalization has an impact on patient morbidity and plays a role in health care burden. Annual rates of hospitalization for lupus in some cohorts are as high as 30 to 40%. There have not been many recent studies looking at hospitalization of patients with SLE in Canada. The aim of this study was to look at the incidence of hospitalization of patients with systemic lupus erythematosus (SLE), the causes of hospitalization, and to determine any predictors of hospitalization. *Method:* Data was collected from the 1000 Canadian Faces of Lupus database, a prospective multi-centre cohort study. Data on hospitalization was taken from the annual visit follow-up form sections on lupus flares and hospitalization due to flares. Baseline participant data used include demographics, medication history, disease activity and damage, and co-morbidities. *Results:* Of 1802 enrolled patients, data on hospitalization was available on 724 participants. Of these 724 participants included in our analysis, there were 68 reported hospitalizations related to SLE (hospitalization rate of 9.4%). The most common causes of SLE-related hospitalization were hematologic (18.1%), serositis (16.9%), musculoskeletal (13.3%), and renal (12.0%). Some participants were hospitalized for more than one reason. The occurrence of hospitalization was significantly associated with being of Aboriginal descent (p=0.004), having a lower household income (p=0.009), being work disabled (p=0.001), having greater disease activity as indicated by the SLAM-2 score (SLE activity measure) (p=0.004), and having a greater number of ACR criteria (p=0.006). There was also a significant relationship between hospitalization and presence of anti-Sm (anti-Smith) antibody (p=0.018) and use of azathioprine (p=0.039). There was no significant relationship between hospitalization and gender, age, number of co-morbidities (as indicated by the Charlson Co-morbidity Score), or number of medications used. *Conclusions:* The rate of hospitalization of patients with SLE was lower than expected, however this may indicate that the care of lupus is improving, that the cohort is biased towards healthy patients, or that flares are more likely to be handled as an outpatient. Some hospitalizations may have been under ascertained or may not have been considered secondary to a lupus flare. The causes of hospitalization were varied and different than what other studies have shown. Several predictors of hospitalization are related to ethnicity, socioeconomic factors and disease activity, which is consistent with results of other studies.

PO2.D.33

Pulmonary fibrosis; beneficial effects of treatment with rituximab

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Objective: To establish if B cell depletion may be beneficial in Pulmonary Fibrosis based on observations in the treatment of systemic lupus and clues from the literature that B cells may have a role in the development of fibrosis in scleroderma. *Method:* Administration of rheumatological protocol doses of Rituximab to four patients with Pulmonary Fibrosis as an addition to the standard of care (1). *Results:* The first patient with Lupus, Polymiositis and rapidly progressive Pulmonary Fibrosis unresponsive to pulsed steroids and cyclophosphamide and about to be placed on a respirator, showed improvement after receiving rituximab and is alive and working 6 years later with pulmonary function tests values close to normal and O2 saturation of 98%. A second patient with psoriatic arthritis and pulmonary fibrosis awaiting a pulmonary transplant improved her functional capacity after two doses of the protocol. A third patient with scleroderma and pulmonary fibrosis who failed abatacept (experimental protocol at a major academic center) improved significantly with the standard of care and showed additional improvement when rituximab was added and now plays tennis six years into his ailment with only mild reduction of the pulmonary diffusing capacity, and normal O2 saturation of 98%. A fourth patient with idiopathic pulmonary fibrosis felt that the progression of his symptoms were halted by the standard of care at six months into his treatment but his functional capacity improved only when rituximab was added. *Conclusion:* Rituximab appears useful in pulmonary fibrosis of diverse etiology when added to the standard of care as seen in these four cases. Furthermore, when used as first and sole agent in two other cases, (rheumatoid arthritis in one and bleomycin induced the other) significant improvements were recorded (2). It would appear that these observations on a limited number of patients should be furthered investigated and triggered clinical trials.

References:

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(2): Rumore, P; unpublished observations

Disclosure: M. A. Cima, None.

PO2.D.34

Prospective disease activity evaluation in a SLE cohort from a Mediterranean country

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Objectives: To determine the annual flare rate and mean disease activity in a cohort of SLE patients. To evaluate concordance between different lupus disease activity indexes. *Material And Methods:* Consecutive patients fulfilling the 1997 ACR Classification Criteria for SLE and within the Coimbra Lupus Cohort were included. All patients were prospectively evaluated for one-year. One rheumatologist (LI) assessed disease activity using SLEDAI-2K, SLAM-R and Physician Global Assessment (PGA) (VAS 0-100) at each visit. The Adjusted Mean SLEDAI (AMS) was calculated using the formula defined by Ibanez et al. Disease flare was defined as an increase in SLEDAI-2K of at least 3 points between consecutive visits. The flare rate per patient-year was calculated. Remission was defined as SLEDAI-2K =0, low disease activity as SLEDAI =1-5 and moderate-to-high disease activity as SLEDAI>5. Concordance between the different indexes was tested with Pearson correlation. p<0.05 was considered statistically significant. *Results:* We included 135 SLE patients. Mean time between visits was 3 months. At baseline, 64% of the patients were in remission or with low disease activity. The mean AMS was 4 (0-15.8) and 27% of patients presented persistent moderate-to-high disease activity. The flare rate in this cohort was 0.28 flares/patient-year of follow-up. In this study, SLEDAI-2K was strongly correlated with PGA (r =0.75, p<0.001) and moderately with SLAM (r=0.51, p<0.001). SLAM presented

a moderate correlation with PGA (r=0.56, p<0.001). *Conclusion:* The SLE disease activity in this cohort was usually low during prospective follow-up, both from the perspective of flare rate and AMS. The three indexes used to assess disease activity showed a good correlation. AMS can be used to describe disease activity over a period of follow-up time. The flare incidence rate is similar to that found in other cohorts.

PO2.D.35

Long-term follow-up of 98 patients with primary antiphospholipid syndrome (PAPS): if they are develop systemic lupus erythematosus (SLE)?

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Aim: Retrospectively to analyze clinical and serological date at onset of APS and during their medical observation to monitor if they involve SLE or other autoimmune disease. *Patients and Methods:* The medical records of 98 (25M; 73F) inpatients with primary APS hospitalized in the Institute of Rheumatology of RAMS since September of 1987 to December of 2009 were retrospectively analyzed. Mean age was 35,6±9,9 years and disease duration - 11,9±8,5 years. None of them had any clinical signs of autoimmune disease before the enrollment in the studies. The diagnosis of APS was based on the Sapporo criteria and laboratory evidence. *Results:* The patients were followed up for about 19.8 (ranged from 3 to 20) years. Recurrent thrombotic events occurred in 81 (83%) patients and the most common ones were deep vein thrombosis (63/98), stroke (36/98) and pulmonary embolism (25). A total of 43 of the 73 female patients had 1 or more pregnancies and 18 (42%) had one or more live births, the other 25 (58%) had fetal losses. Development of SLE in patients, who only had clinical signs of APS at the beginning of the disease, was registered in 13/98 (13%) cases. Nine out of 98 patients died during the follow-up. Two of 9 cases of death had the transformation of the primary APS into the secondary one associated with SLE 2 and 10 years later after the onset. 12 follow-ups disclosed haematological abnormalities at the onset of the disease: 6 of them had thrombocytopenia and hemolytic anemia; 5 of them only had thrombocytopenia and 1 had hemolytic anemia. The 11 out of these 12 evolved the definite SLE. 35/98 (35%) of patients revealed immunological disorder at the beginning of the follow-up (except for the rise of aPL): 25/98 with VDRL; 7 patients with hypocomplementemia, the elevation of anti-ds-DNA, positive ANA; 19 with the elevation of anti-ds-DNA and ANA+ and only 8 patients with ANA+. Univariate analysis revealed the statistical significance of the following signs of risk of further development of SLE (p<0.05), which included autoimmune disease heredity, Raynaud's phenomenon, migraines, CNS involvement, hemolytic anemia, low levels of C3 and C4, positive Coomb's test. After the logistical regression analysis only positive Coomb's test (OR 76,4 CI 95% 1,6- 271 p=0,017) had the statistical significance. *Conclusion:* our research confirms the possibility of transformation of primary APS into SLE even after a long-term observation. 35% of patients with APS onset had immunologic disorder except for positive aPL so only the occurrence of the positive Coomb's test was the reason for the patients with primary APS to develop SLE.

PO2.D.37

Marital status and health outcomes in lupus

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Objective: The effect of marital status on health outcomes (patient and physician reported) in systemic lupus erythematosus (SLE) is not known. We aimed at studying the association between marital status and disease features (disease activity, damage) and health related quality of life (HRQOL) of SLE patients. *Methods:* Demographic, disease activity and HRQOL data were available for 326 SLE patients. HRQOL was assessed using the SF36. Disease activity was determined using SELENA-SLEDAI. Clinical and serologic disease characteristics were obtained through medical chart reviews. Summary statistics were obtained for the study cohort. Mann Whitney and chi square analysis were used to compare demographics, disease and HRQOL features among currently married and unmarried study groups. Linear regression analysis was performed for HRQOL with marital status and age. A p value of ≤ 0.05 was considered significant. *Results:* 156/326 reported being currently married. Ethnic composition: 38% African American, 31% Caucasian, 26% Hispanic and rest were others. Married subjects were older in age (Mean ± SD) 44.9 ± 12 vs. 40.2 ± 13.6 years (p= 0.02). The groups were no different by ethnicity, disease activity or damage. Married subjects had better scores (Median, IQR) on physical functioning [65, 45 vs. 50, 45 (p=0.004)], general health [45, 25 vs. 35, 27 (p=0.002)] and physical component summary score (p=0.02) were observed. On regression analysis for physical functioning and general health; marital status remained a significant predictor after adjusting for age. *Conclusions:* Married SLE subjects reported better physical functioning and general health than unmarried subjects, despite no significant differences in disease activity and damage. These benefits were not due to better psycho-social health frequently reported among married subjects. These data may suggest that worse HRQOL may contribute to being currently single; alternatively, the state of being married may itself positively affect physical functioning. Prospective studies evaluating the effects of SLE on marital health are indicated.

PO2.D.38

Socio-economic status predicts disability in patients with systemic lupus erythematosus

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Purpose: Both minority ethnicity and socioeconomic status (SES) have been linked to disability in patients with SLE. However these factors may not be independent. We evaluated the effects of SES and ethnicity on self reported work disability status in patients with SLE. *Methods:* SLE patients were enrolled from 2 academic hospitals. Demographic information, including age, gender, ethnicity, educational level and marital status, were collected. All patients fulfilled ACR classification criteria for SLE. Education level was divided into 4 categories: less than high school, high school, any college, and graduate studies. Information about work disability was obtained by self-report. Disease assessments included duration of disease, disease activity (SELENA-SLEDAI) and damage (SLICC-ACR). Univariate and multiple logistic regression were used to determine the impact of level of education on disability, using disability as a dependent variable. The independent baseline variables used were: age at diagnosis, disease duration, gender, ethnicity, marital status and SES. Level of education was used as a surrogate for SES. *Results:* 210 SLE patients (92.8% females) were enrolled. The ethnic composition of the study group was: 121/210 (57.62%) African American, 46/210 (21.90%) Caucasian, 30/210 (14.29%) Hispanic and 13/210 (6.19%) Asian. The mean (SD) age was 42.09 (13.29) years and disease duration was 9.57 (9.29) years. 58/188 (30.85%) subjects were on work disability. The mean (median) SLEDAI and SDI score were 6.3 (5.9) and 1.95 (1), respectively. On univariate analysis, SES predicted work disability (OR, 0.62, 95%CI 0.43-0.89, p=0.008). Ethnicity did not reach significance to predict work disability on univariate analysis (OR 0.74, 95% CI 0.54 - 1.01, p=0.051). However on multiple logistic regression, higher education was significantly associated with lower odds of work disability in SLE patients after controlling for other independent predictors: ethnicity, gender, age at diagnosis, duration of disease, marital status. The odds ratio for disability significantly decreased by 0.60 (95% C.I 0.39 - 0.91, p= 0.017) with each increased level of education from less than high school to graduate level. Ethnicity was not predictive of work disability in the multivariate analysis. *Conclusion:* SES but not ethnicity predicts work disability in

patients with SLE. The association of ethnicity with work disability appears to be through its association with SES. Interventions aimed at improving health resources, especially for lower SES category SLE patients may lead to decreased work disability.

PO2.D.39

Charlson co-morbidity index is associated with mortality in systemic lupus erythematosus

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Objectives: To investigate whether co-morbidity as assessed by the Charlson co-morbidity index (CCI) is associated with mortality in the long term follow-up of systemic lupus erythematosus (SLE) patients. *Patients and Methods:* Data from 499 SLE patients attending the Lupus Clinic at the McGill University Health Center, Montreal, Canada and 170 SLE patients from the Department of Rheumatology at Lund University Hospital, Sweden were collected from available databases. These patients constitute long term follow-up cohorts. In the Montreal cohort, data on the CCI was obtained prospectively for patients still active in follow-up in 2006 and 2007 (56%) and was verified through chart review. For the other Montreal patients (44%) and for all patients in the Lund cohort, data were retrieved through retrospective chart review. Data on demographics, disease activity over time, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI), smoking, education and anti-phospholipid syndrome (APS) were also retrieved. Variables were then entered into a Cox proportional hazards survival model. The Montreal and Lund cohorts were analyzed separately. *Results:* At the time of data analysis, 81 of 499 patients in the Montreal cohort, and 46 of 170 patients in the Lund cohort were deceased. The median follow-up time was 13 years in both cohorts. Mortality risk in the Montreal cohort was associated with the CCI (HR 1.57 per unit increase in the CCI, 95% CI 1.18-2.09) and age (1.04 per year increase in age, 95% CI 1.00-1.09). The CCI and age at diagnosis were also associated with mortality in the Lund cohort (CCI: HR 1.35, 95% CI 1.13-1.60; age: HR 1.09, 95% CI 1.05-1.12). Furthermore, the SDI was associated with mortality in the Lund cohort (HR 1.40 per unit increase in the SDI, 95% CI 1.19-1.64), while a wide confidence interval for the estimate in the Montreal cohort prevents a definitive conclusion (HR 1.20, 95% CI 0.97-1.48). We did not find a strong association between mortality and sex, race/ethnicity, disease activity, or APS in either cohort. *Conclusions:* The presence of substantial co-morbidity in SLE is widely recognized. In this study, co-morbidity as measured by the CCI was associated with decreased survival independent of age, lupus disease activity and SDI. Including the CCI in studies of prognostic factors in SLE may be of importance and the CCI may also be of value in the prognostic assessment of individual patients

PO2.D.40

Body Image directly affects health outcomes in systemic lupus erythematosus.

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Body Image (BI) and its longitudinal effects on patient reported health outcomes are not been previously addressed in Systemic Lupus Erythematosus (SLE). We have reported an association between BI, disease damage and HRQOL in SLE; however the direction of this association is unclear. *Goal:* To study the direction of association, direct effects of body image (BI) on patient reported health related quality of Life (HRQOL) among SLE patients.

Methods: 63 SLE subjects receiving care at Rush University Medical Center underwent longitudinal assessments of BI (BI-T1 and BI-T2) and HRQOL (HRQOL-T1, HRQOL-T2) at two time points (T1 and T2). BI was evaluated using the 5 item Body Image Lupus Scale (BILS) developed and validated by the authors at the Rush University. HRQOL was determined using the EQ5D and SF6D preference based indices. Disease damage and disease activity were determined using SLICC and SELENA-SLEDAI respectively. Paired t test was utilized to compare the BI and HRQOL variables. Path analysis was performed using structural equation modeling on AMOS software. The dependent variables were HRQOL-T1 and HRQOL-T2. The independent variables were BI-T1, BI-T2, SLEDAI-T1, SLEDAI-T2, and SLICC-T1. A path model specified was based on our a priori results of univariate linear regression analysis for HRQOL using BI as the independent predictor variable. Model identification, estimation, testing and modifications were performed. Model fit, regression estimates for direct and indirect effects for the final model were evaluated. Higher BI score=worse BI. Higher EQ5D and SF6D scores=better health status. *Results:* The mean age of the participants was 45.3± 12.6 years. The two visits were 8.3±4.3 months apart. The mean SLEDAI –T1 and SLEDAI-T2 scores were 6.4 ± 5.9 and 4.2 ± 4.2 respectively (p=0.01). The mean BI-T1 and BI-T2 scores were 38.4 ±31.7 and 26.6±27.6 respectively (p=0.001). The mean EQ5D-T1 and EQ5D-T2 scores were 0.73± 0.18 and 0.73± 0.21 respectively (p=0.97). The mean SF6D-T1 and SF6D-T2 scores were 0.64± 0.15 and 0.66± 0.15 respectively (p=0.07). At time 1 (Figure 1), BI-T1 independently contributed to HRQOL-T1 (be= -1.0, p=0.001). At time 2, BI-T2 contributed to HRQOL-T2 (be= -0.46, p=0.009). Specifically, BI-T1 predicted HRQOL-T2 (be= -0.61, p=0.009). HRQOL-T1 was a poor predictor of BI-T2. The goodness of fit of this model was 0.98.Root mean square error of approximation and Chi square value were <0.05 and 3.4 respectively. *Conclusions:* Not only is BI associated with HRQOL, but it is also predictive of HRQOL over time. HRQOL does not predict BI longitudinally. Improvement in BI may result in improvement of HRQOL in SLE.

PO2.D.41

Use of body image lupus screening (BILS) tool in systemic lupus erythematosus: what can we learn?

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Objective: To compare Body Image (BI) among Systemic Lupus Erythematosus (SLE) patients with healthy controls (HC), and determine its modifiable correlates. *Methods:* Body Image Lupus Screening (BILS) tool has been developed and validated for assessment of BI among SLE patients. It is a brief five-item tool which can be self- administered. BILS was administered to 233 consecutive SLE patients and 70 HC at a University Center along with SF-36. Women constituted 94% of each group. Demographics, body mass index (BMI), disease activity (SELENA-SLEDAI), disease damage (SLICC-ACR), health status (SF6D), medical chart review for organ involvement, serologies, co-morbid illness, and medications were assessed. A higher BILS score indicates better BI. A t test with unequal variance was utilized to compare age and BILS scores between SLE and HC subjects. Correlation with modifiable factors (history of depression, fibromyalgia, SLEDAI, SDI, active cutaneous manifestations (rash, hair loss), BMI, and prednisone use was examined. Significant correlates were entered into a stepwise linear multiple regression model for BI. *Results:* The mean ages of the SLE and HC groups were 42.4±13.2 and 41.5±12.4 years, respectively. The mean BILS score among SLE and HC were 72.3±26.8 and 78.5±21.3 respectively (p=0.04). Among SLE patients BILS score correlated with depression (r = -0.40, p=0.001), use of prednisone (r = -0.16, p=0.01), health status SF6D (r = 0.46, p=0.001), BMI (r = -0.19, p=0.004), active skin rash (r= -0.24, p=0.001), and active hair loss (r= -0.31, p=0.001). In a stepwise regression model for prediction of BI among SLE patients, health status (B 0.29, 95% CI 29,78, p=0.001), depression diagnosis (B -15, 95% CI -22,-8, p=0.001), BMI (B -0.45, 95% CI -0.8, -0.08, p=0.02), active hair loss (B -3.2, 95% CI -6, -0.4, p=0.02), and current use of prednisone (B -6.5, 95%CI -13,-0.3, p=0.04) explained 34% of variance in BI. Patients were receptive to using the BILS tool in the clinical

setting. *Conclusions:* BI in SLE patients is poor as compared to healthy age and gender matched subjects. Modifiable variables that impact BI in SLE, including BMI, health status, active hair loss, depression, and prednisone use, may be amenable to intervention with favorable effects on BI.

PO2.D.42

Health related quality of life using the LupusQoL-US tool© among systemic lupus erythematosus patients

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Aim: To describe Health Related Quality of Life (HRQOL) among US patients with Systemic Lupus Erythematosus (SLE) using the modified LupusQoL© (LupusQoL-US©). *Methods:* LupusQoL-US© is the modified LupusQoL© version, which has been validated for use among US patients with SLE. 177 consecutive SLE patients meeting the ACR criteria were self administered the LupusQoL-US© tool. At the visit, demographic information and assessment of disease activity (SELENA-SLEDAI) and damage (SLICC-ACR) were obtained. Medical records were reviewed to ascertain organ involvement and serologic characteristics. Data was analyzed using SPSS software for descriptive statistics. Eight LupusQoL domain summary scores were obtained. Scores range from 0-100. Higher score denotes better HRQOL. *Results:* 93% of the participants were women. 59% of the subjects were African American, 23% Caucasian, 11% Hispanic and 7% Asian. The mean age was 42.5±12.9 years. The mean SLEDAI and SDI scores were 5.8±5.5 and 2.0±2 respectively. The mean eight domain (UK) scores were as follows: Physical Health (45.0±20.2), Pain (43.2±23.3), Planning (49.9±23.9), Intimate Relationship (55.2±27.2), Burden to Others (45.4±22.8), Emotional Health (52.9±19.6), Body Image (55.3±21.6) and Fatigue (35.9±23.8). When compared with the referent LupusQoL scores from UK patients, our patients had a worse HRQOL. *Conclusions:* Summary scores for HRQOL using LupusQoL-US© among US SLE patients have been presented here. These can serve as benchmarks for HRQOL comparisons among SLE patients from various centers within the US. Longitudinal studies are indicated to assess responsiveness to change of the LupusQoL-US©.

PO2.D.43

The lupus clinic at the center for autoimmune diseases research (CREA)

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Background and Objective: The Center for Autoimmune Diseases Research (CREA) evaluates and monitors patients with autoimmune diseases (ADs)–mainly systemic lupus erythematosus (SLE)–through a specialized clinic which includes their families. The main goal of CREA is to do research and develop knowledge on the etiology and treatment of ADs. The objective of this study was to establish a cohort of SLE patients by evaluating the clinical, genetic, serologic, and risk factors related to morbidity and mortality which will be a base for scientific research and genetic prediction. *Methods:* In 2007 CREA was founded in Bogotá (Colombia, South America) and has been working on immunogenetics in partnership with several national and international clinics and specialized SLE centers that have set up a legal and ethical framework. The clinical case report form (CRF) is systematized and based on internationally validated formats. Patients and their relatives are followed longitudinally. A comprehensive family medical history is completed to determine the presence of familial autoimmunity through a systematized pedigree form. Saliva, blood and tissue samples are taken and stored at the cellular biology and molecular laboratory. Genetic and immunological analyses are done by using molecular techniques as well as a specifically designed family-based clinical studies data

system. This includes clinical and pedigree management, complete genotype management and sample inventory tracking. *Results:* The results are descriptive in a preliminary analysis. 148 SLE patients with trio families have been included. Seven specialized national centers have participated. A total of 419 people were included of which 254 were first degree relatives and 17, second degree relatives. Family trio distribution was as follows: 43.2% (64) were parents-offspring trio; 5.4% (8), sib trio and 37.2% (55), combined trio. In addition, 14.2% (21) were patient-healthy control individuals. At least one relative was affected with autoimmunity in 39.2% (58) of the cases and 30.4% (45) reported SLE within their families. 55 relatives with AID were evaluated at CREA and 176 were self reported through their pedigree description. SLE patients with at least one more AID came to 40.5% (60). One patient died during follow up. More than 170 individuals still to be included. *Conclusion:* This cohort shows the results of studying and including SLE families and will facilitate future research in etiology, genetic prediction and treatment of the disease.

PO2.D.44

Does Sjögren syndrome or autoimmune thyroid disease influence lupus nephritis?

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Background and Objective: Polyautoimmunity (i.e. autoimmune diseases (ADs) co-occurring within patients) is frequent in systemic lupus erythematosus (SLE) with Sjögren syndrome (SS) and autoimmune thyroid disease (AITD) being the most often associated with it. Lupus Nephritis (LN) is one of the most serious complications of SLE (SLE) and is a major predictor of poor prognosis. The objective of this study was to investigate whether SS or AITD associated with SLE influences the appearance of LN. *Methods:* This was a multicenter cross-sectional study. Patients with LN with and without polyautoimmunity (SS or AITD) were compared. Data were obtained by using a pre-tested questionnaire that sought information about clinical, laboratory and histopathologic characteristics. Bivariate logistic analysis and multivariate logistic regression models were applied to analyze data. *Results:* Out of a total of 889 patients with SLE, 455 (51.2%) had LN. Histological kidney biopsies were obtained for 292 (64.1%) patients. Of these 186 (63.7%) had OMS Class III and IV. Of the SLE patients, 147 (16.5%), 59 (6.6%) and 26 (3%) had AITD, SS and the two diseases together respectively. LN patients had earlier SLE onset (27.2±11.7 vs. 31.8±12.5 years old; p <0.0001) and were younger (35.4±13.5 vs 39.5±14.1 years old; p <0.0001) than non LN patients. Lung Involvement [AOR: 1.5, 95% CI: 1.05–2.07, p=0.02), arterial hypertension (AOR: 3.02, 95% CI: 2.09–4.38; p<0.0001) and dyslipidemia (OR: 1.67, 95% CI: 1.1–2.57; p=0.018) were found to be risk factors for LN. SS or AITD had no influence on the appearance of LN. The renal expression in SLE patients with polyautoimmunity was similar to those without it. *Conclusion:* Polyautoimmunity in patients with SLE does not influence the development of LN nor its clinical expression.

PO2.D.45

Identification of predictive markers for the development of flares after immunoablation and autologous hematopoietic stem cell transplantation in refractory SLE

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Background: Clinical trials have indicated that immunoablation followed by autologous stem cell transplantation (ASCT) has the potential to induce long-term clinical remissions in SLE. However, relapses of SLE may occur in a subset of these patients after ASCT. We therefore longitudinally analyzed the immune reconstitution of these patients to identify predictive cellular or serologic markers for long-term remission or development of lupus flares. **Methods:** Since 1998, eight patients with SLE underwent CD34-ASCT after immunoablation with cyclophosphamide and rabbit-ATG as part of a monocentric phase I/II clinical trial. Autoantibody titers were evaluated with ANA-Immunofluorescence and ELISA, and peripheral T- and B lymphocyte subsets immunophenotyped using multicolor flow cytometry, including assessment of TCR Vβ-repertoire. **Findings:** Clinical remission (SLEDAI ≤ 3) could be achieved in all patients accompanied with disappearance of anti-dsDNA antibodies and protective antibodies in serum. Two patients died due to transplant related infections. From the remaining six patients, three patients are in long-term clinical remission for up to eleven years after ASCT, while three patients suffered a relapse of SLE at 18, 36 and 80 months after ASCT, respectively. Complete eradication of antinuclear antibodies (ANA) was achieved in two out of three patients with long-term remission. In contrast, from relapsed patients only one patient showed disappearance of ANA. Flow cytometric analyses revealed no significant differences between long-term responders and relapsed patients at baseline and in terms of T- and B-cell reconstitution. However, flow cytometric analyses revealed an expansion of memory B cells (IgD- CD27+ and IgD- CD27- DN) and circulating CD27high plasmablasts, increased coexpression of Siglec-1 on monocytes as a surrogate marker for type-I interferon signature and a predominance of CD45RO+ memory T cells preceding the clinical flares. **Conclusion:** Patients with ANA-persistence seem to be at higher risk to develop lupus-flares after immunoablation and ASCT may be because of an incomplete eradication of the autoimmune immunologic memory. Moreover, lupus flares may be predicted individually by flow cytometry with plasmablast expansion, predominance of memory B- and T-cells and increased coexpression of Siglec-1 on monocytes.

PO2.D.47

Outcome measurement of type iv systemic lupus nephritis by classification and regration tree (CART)

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The prognosis of Systemic Lupus Erythematosus depends partly on renal involvement. Lupus nephritis has evolved from a frequently terminal process such as type IV lupus nephritis according to WHO classification to one in which a fairly normal quality of life and good outcome are possible. The aim of this study was to try to predict the short-term outcome of type IV lupus nephritis by looking at the disease variables before the treatment. A way to achieve this goal is to look at patients with bad and good outcome to determine if some sign or symptom, or a combination of them, will differentiate the two groups. For this purpose 73 variables were analyzed by the computer assisted Classification and Regression Tree method in 163 patients with SLE nephritis. The final tree had 12 terminal nodes. The short outcome of renal involvement was related to the systolic blood pressure, the C3 and C4 complement levels, the level of proteinuria, the creatinine clearance, ESR, and monocyte infiltration and tubular atrophy on renal biopsy. The prognosis is bad if the systolic blood pressure is more than 140 mm/hg plus monocyte infiltration on renal sample and daily urine protein excretion which is above 3 gr. At the looking to

the left side of the tree where the blood pressure is normal. If the C4 and C3 complement is low and the tubular atrophy is detected in renal specimen, the prognosis is bad, too. The prognosis is bad, if the C4 complement is low and the daily urine protein excretion is more than 3 g, the creatinine clearance is low plus erythrocyte sedimentation rate more than 50 mm/1thhour. At the end, the short- term prognosis is bad if the combination of tubular atrophy of renal sample and low level of C3 complement is exist.

PO2.D.48

Analysis of poor prognostic factors associated with mortality of patients with systemic lupus erythematosus in China

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Objective: To investigate the mortality of hospitalized systemic lupus erythematosus (SLE) patients in China and determine the influential factors associated with the poor prognosis. **Methods:** The medical records of 1956 SLE in-patients during Jan 1, 1999 and Dec 31, 2009 from 15 hospitals were reviewed. All patients were followed up thorough Jan, 2010. Potential factors might associate with mortality were analyzed by comparing patients who are still alive with those who deceased. **Results:** Among the 1,956 patients, 166 died during follow-up. The overall mortality rate was 8.49%. The mean age at death was 38.9±14.1 years old. 80.8% of these patients died <50 years old. The mean time from the disease onset to death was 6.7±7.1 years. 57.2%, 22.0% and 11.3% of the patients died <5, 5-10 and 10-15 years after onset of SLE, respectively. 52 patients died in hospital. The mean time from hospitalization to death was 18.6±24.0 days (1-127 days, N=52). 63.1% (29/52) of these patients died within two weeks after hospitalization. Major causes of death included: infection (30.1%; lung infection, tuberculosis, sepsis, biliary infection, peritonitis), kidney failure (18.1%), encephalopathy (18.1%), interstitial lung diseases (6.0%) and cardiovascular diseases (5.4%). Additional death causes included thrombocytopenia (n=2), pancreatitis (n=1), suicide (n=1), and hemophagocytic syndrome (n=1). The mean age of onset of SLE between alive and deceased patients was comparable (31.3±12.9 vs. 31.3±11.2 years old). However, the time from onset of SLE to confirming diagnosis was significantly longer in deceased patients (22.3±61.9 vs. 13.5±33.8 months). As compared with the alive patients at admission, deceased patients showed higher SLEDAI score (14.12±6.96 vs. 11.54±5.57) and more organ injuries: central nervous system (16.27% vs. 5.31%, OR=3.06), cardiac and respiratory system (40.96% vs. 23.63%, OR=1.73), eye (3.01% vs. 0.61%, OR=4.93) and kidney (71.08% vs. 57.88%, OR=1.23), thrombocytopenia (50.6% vs.32.8%, OR=1.54), anemia (80.1% vs. 61.2%, OR=1.31), abnormal serum albumin (74.7% vs. 53.3%, OR=1.40) and elevated serum creatinine (14.5% vs. 4.0%, OR=3.63). **Conclusion:** Infection, kidney failure and encephalopathy were the main causes of death. The peak of mortality occurred at the age of 25-30 years. Among those who died in hospital, more than 50% died within two weeks after hospitalization. Delayed diagnosis of SLE associated with mortality. Central nervous system, cardiac and respiratory system, eye or kidney involvements were more frequently observed in deceased patients. Elevated serum creatinine, but not urine protein is associated with the poor prognosis.

PO2.D.49

Outcome of children born to mothers with SLE

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Aims: Immunosuppressive agents are commonly used in SLE during pregnancy, to ensure the optimum outcome for both mother and child. However there is little literature regarding long term outcomes of these children. This

pilot study aims to assess whether the mother's auto antibodies and or medications taken during pregnancy can be used to predict adverse outcomes. ***Methods:*** Women regularly attending specialist lupus clinics, whose pregnancy data was available, were identified and consented to take part in this study. A standard questionnaire developed for a multi-centre study was used to collect the data. ***Results:*** To date 95 women have filled out questionnaires for 132 children, 47 mothers were from Birmingham clinics and the remainder were from St Thomas's Hospital in London. In total 94 children had no adverse outcomes reported and the outcome of the remaining 38 children are shown in:

Table 1. Children with adverse outcomes – other hospital admissions

	# of children	% all children	AZA+/- Pred	Pred +/- HCQ	HCQ alone	No DMARDs	Ro/La positive	Ro/La negative	APS positive	APS negative
									Number of Children Affected	
Any adverse outcome	48		39	61	11	21	38	54	44	50
% of total children		36	30	46	8	16	29	41	33	38
Congenital heart block	2	1.5	1	0	0	1	1	1	0	2
Neonatal lupus	2	1.5	2	0	0	0	2	0	1	1
Other congenital abnormalities	6	4.5	3	3	0	0	3	3	5	1
Dyslexia/ learning difficulties	2	2.6	1	1	0	0	0	0	1	1
Infection with hospital admission	19	14	11	5	0	3	4	14	7	12
Other hospital admission	17	13	4	9	3	3	11	6	8	9
Mean birth wt (Kg)			2.68	2.77	3.00	2.94	2.78	2.90	2.80	2.90
Mean pregnancy duration (weeks)			35.5	35.6	39	37.5	36.4	38	37	36.7

There were 6 children with more than one event.
AZA Azathioprine
Pred Prednisolone
HCQ Hydroxychloroquine
APS Antiphospholipid Antibodies (anticardiolipin IgG /IgM &/or lupus anticoagulant)

Conclusions: This pilot study provides preliminary data regarding the outcomes of children born to mothers with SLE. Most children (94/132, 71%) had none of the above complications. Surprisingly a child was born with CHB, despite no recorded maternal anti-Ro or anti-La autoantibodies. This mother was not on DMARD therapy during pregnancy. A total of 34 children (26%) required hospital admission, mostly for infections (19, 14% of all children). In 14/19 cases. the mothers were either on steroids alone or in combination with 1 or more DMARDs during pregnancy. More work is required to determine whether or not these infections can be associated with duration of gestation, birth weight or the medications given to the mother. Congenital abnormalities reported were accessory digits, renal abnormalities and a child with undescended testis. The data collected demonstrates little difference in the outcome of children born to mothers with or without anti-cardiolipin antibodies. A larger multicentre study is currently underway and further multivariate analysis will be undertaken on risk factors for adverse outcomes in children born to mothers with lupus.

PO2.D.50

Outcome of systemic lupus erythematosus: data from a cross-sectional study in Shanghai Renji Hospital lupus cohort
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Due to a deeper understanding of SLE and its management, the outcome for patients has been improved during recent decades. In an attempt to clarify the long-term outcome of Chinese patients with SLE, a cross-sectional study was made in Renji Hospital Lupus Cohort in Shanghai. Objectives of the project were: i) to determine the demographic data and quality of life in the Chinese SLE group; ii) to evaluate the morbidity of the SLE patients; iii) to establish the mortality rate and the main causes of death in the Chinese population. In the study, totally 1169 patients with SLE are involved. Among these, 8.3% (97/1169) patients were died. Of a total of 1072 survived patients with SLE, 575 (53.64%) have been followed up for more than 10 years (139 for more than 20 years). In the cohort, 5.6% (60/1072) patients are male. 71.6% of the patients finished high school, college or graduate student course education and the unemployment rate is 4.85%. 759 patients (83%) are married with only 1.16% divorce rate. Among those married female patients, 555 (73%) had successful child birth. In the cohort, 607 patients (56.78%) have organ involved and the average score of SLICC is 0.4±0.9. Of these, 195 (18.21%) with lupus nephritis; 26 (2.4%) with IBN of femoral head (24 of 26 patients appeared IBN before first visiting to our department); 70 (6.54%) with ILD; 32 (2.99%) with PAH ; 15 (1.4%) with diffuse central nervous system disorders and 12 (1.12%) with CAD. In the investigation, 831 patients (77.6%) were stratified by SLEDAI scores to stable disease group (<3). The mortality rate of the cohort is 8.3%. The first three causes of death are ordered as follow: cardio-cerebral vascular events, severe infection and renal failure. Compared with age- and gender-matched controls, SLE patients have lower SF-36 scores in some domains besides role limitations because of emotional problems. The SF-36 scores from different domains in SLE patients are correlated with family income, gender, marriage or birth status, education, personal income and organ damage. Therefore, over the past decades, there has been significant improvement in the outcome of SLE in Shanghai. The clinical pattern of lupus has changed from an acute fatal disease to a treatable and controllable inflammatory autoimmune disease. These improved outcomes may be attributed to a fine balance emphasized on the risk/benefit ratio of therapy in our routine practice. We also emphasized the importance of early diagnosis, early treatment and long term follow-up study. To prevent the therapeutic complication, combined therapy was advocated. We first recommend PMC (low-dose prednisone with MTX and Chloroquine) combined therapy for treating mild to moderate lupus without major organ involved.

PO2E Clinical Trials

PO2.E.10

Ability of nonfasting and fasting triglycerides to predict coronary artery disease in lupus patients
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Introduction: Hypertriglyceridemia is a metabolic disorder associated with atherosclerosis. We have previously shown that there is no clinically significant difference in individual lupus patients in the levels of fasting triglycerides (FTG) and nonfasting triglycerides (NTG). ***Objective:*** We aimed to determine whether nonfasting triglycerides predict coronary artery disease (CAD) in lupus patients. ***Methods:*** Patients are followed at regular intervals (at 2-6 months) according to a standard protocol which includes: complete history and physical exam, SLE Activity Index (SLEDAI-2K), and Systemic Lupus International Collaborative Clinics Damage Index (SDI). Fasting lipid profile was measured once yearly and nonfasting was determined at all other visits.

We included the entire patient cohort and all the values of TG available in our data. Time-dependent covariate survival analysis was conducted to determine the predictive ability of TG for CAD – whether fasting and nonfasting. Variables considered were: sex, age at diagnosis, age, SDI, SLEDAI-2K, smoker, glucocorticoid, anti-malarial, immunosuppressive drugs, cholesterol. Variables retained for multivariate analysis were selected through the variable reduction strategy (Harell) – selecting variables which alter the parameter estimate of TG by ±10% when include in the model. Using this variable selection, age, SDI, and immunosuppressive drugs were selected to be included in the models. *Results:* We identified 1289 patients since the date of the first TG available. Eighty eight percent of the patients were female. Five hundred forty one patients had elevated cholesterol level and the length of follow-up from the 1st TG to CAD was 8.82±8.19 (table 1). One hundred eight patients (8.1%) developed CAD. We identified 89 events of CAD in 1137 patients in the nonfasting model and 35 events of CAD in 707 patients in the fasting model(table 2). Both nonfasting and fasting model showed ability of the variables to predict CAD; triglycerides, age and SDI while immunosuppressive drug use predicted CAD only in the nonfasting model. *Conclusion:* Both fasting and nonfasting TG predicted CAD in lupus patients. Nonfasting TG levels can be used in clinic to detect CAD event in lupus patients.

Table 1. Patients' demographics

Number of patients	1289
Number of CAD events	108 (8.1%)
Mean number of visits with TG available prior to CAD / last visit	18.8 ± 19.8
Sex F	1143 (88.7%)
Age at diagnosis (years)	30.7 ± 13.7
Age * (years)	34.9 ± 13.5
Disease Duration * (years)	4.2 ± 5.8
SDI *	0.33 ± 0.82
SLEDAI-2K *	8.81 ± 7.21
Smoker *	249 (19.7%)
Steroids *	834 (64.9%)
Antimalarial *	516 (40.2%)
Immunosuppressive *	296 (23.0%)
Elevated Cholesterol *	541 (42.1%)
Length of FU from 1st TG to CAD / last visit (years)	8.82 ± 8.19

* as of date of 1st TG available

Table 2. Hazard ratio for CAD in both group of analysis

	Non-fasting Model			Fasting Model		
	HR	95% CI	p	HR	95% CI	p
TG	1.80	1.10, 2.93	0.02	2.76	1.07, 7.10	0.04
Age	1.06	1.04, 1.08	<0.0001	1.06	1.03, 1.09	<0.0001
SDI	1.11	1.00, 1.23	0.06	1.18	1.00, 1.37	0.04
Immunosuppressive	2.20	1.41, 3.44	0.0005	1.27	0.61, 2.65	0.52

PO2.E.11

Atorvastatin therapy reduces a interferon regulated chemokine CXCL9 plasma levels in SLE patients
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IFN-regulated chemokines seem to be important in SLE pathogenesis, as suggested by various studies in the literature. Serum and urinary CCL2 levels were associated with active lupus nephritis. CXCL11, CXCL10 and CCL3 were also found at higher levels in serum of patients with active renal disease and the chemokines CXCL9, CXCL10 and CXCL11 were found at higher levels in SLE skin lesions. These data suggested monitoring blood chemokine levels in lupus patients may be useful in assessing disease activity, as well new therapies aimed to block chemokine may be effective in SLE. *Objective:* to evaluate the efficacy of atorvastatin to reduce the plasma levels of IFN-regulated chemokines (CCL2, CCL3 and CXCL9) in SLE patients. *Methods:* Eighty-eight SLE women were divided in 2 groups: 64 received atorvas-

tatin 20 mg/day for 8 weeks (intervention group) and 24 without intervention were followed for the same period (control group). Chemokines plasma levels was measured by ELISA sandwich technique using commercial kits (DuoSet, R & D Systems MN, USA) at baseline and after 8 weeks for all participants. *Results:* 64,8% of patients were white, the mean age was 32±8 years and mean disease duration was 8.9±4.8 years. The mean SLEDAI and SLICC scores were 4.16 and 1.06, respectively. The plasma levels of CCL2 were higher in patients with dyslipidemia, CHD risk factors and with current serositis, vasculitis and nephritis. Patients with current serositis and nephritis presented a higher plasma level of CXCL9. In an univariate analysis, we find a positive correlation between the plasma levels of CCL2 (r=0.04; p<0.001), CXCL9 (r=0.30; p=0.01) and CCL3 (r=0.27; p=0.01) and SLEDAI score. In the intervention group we observed a significant decrease in the plasma levels of CXCL9 when comparing baseline and at the end of 8 weeks (p=0.04). However no differences were observed regarding CCL2 neither CCL3 plasma levels along the study. No significant difference was observed in the plasma levels of these chemokines in the control group. A multiple linear regression model considering SLEDAI score as a dependent variable and including the following variables: triglycerides, total cholesterol, body mass index, current prednisone dosage (mg/day) and the plasma levels of CCL2, CXCL9 and CCL3 were constructed for 88 SLE patients. In this analysis, the current prednisone dosage (p<0.001), plasma levels of CCL2 (p= 0.001) and plasma level of CXCL9 (p=0.006) were independently associated with the SLEDAI score. *Conclusion:* atorvastatin use was associated with a significant decrease in the plasma levels of CXCL9 in SLE patients. As the plasma levels of CXCL9 correlated with the SLEDAI score, we question whether reducing levels of this chemokine could help to control SLE activity.

PO2.E.12

Five-year experience with belimumab, a BLyS-specific inhibitor, in patients with systemic lupus erythematosus (SLE)
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Objective: Provide 5-year safety and efficacy data in SLE patients treated with belimumab. *Methods:* 449 SLE patients with SELENA-SLEDAI (SS) ≥4 enrolled in a phase 2, 52-week, double-blind study of belimumab (1, 4, or 10 mg/kg, q28d) vs placebo plus background SLE therapy. At week 56, placebo patients received belimumab 10 mg/kg; belimumab patients remained on their current dose or received 10 mg/kg. At week 80, 296 patients received belimumab 10 mg/kg in a continuation trial. The 5-year dataset was divided into ten 6-month intervals for reporting adverse events (AEs) and flare rates. Analyses of disease activity included the SS SLE Flare Index (SFI) and SLE Responder Index (SRI): improvement in SS (≥4-point decrease), no new BILAG A or 2 B flares, and no Physician's Global Assessment (PGA) worsening (<0.3-point increase) vs baseline. SRI assessments are limited to the seropositive (ANA titer ≥1:80 and/or anti-dsDNA ≥30 IU/mL) patient subgroup (n=321). *Results:* By 5 years, overall belimumab exposure was 1,394 patient-years (pty). The incidence rates (per 100 pty) of AEs generally were stable or decreased over 5 years (Table 1). In seropositive patients, SRI rate was 46% at week 52 (vs placebo 29%; p<0.05), which increased to 55% by week 76 and was maintained through week 272 (59%); the frequency of new BILAG A or 2 B flares decreased from 30% at 6 months to 25% at 1 year (vs placebo 33% and 26%, respectively) and declined to 10% at 5 years; the frequency of flares by SFI decreased from 72% (severe 13.2%) at 6 months to 64% (severe 8.7%) at 1 year (vs placebo 76% [severe 9.4%] and 74% [severe 11.3%], respectively) and declined to 21% (severe 1.6%) at 5 years.

Table 1: AE Incidence (rate per 100 pty)[a]

Interval	1	2	3	4	5	6	7	8	9	10
Years	0-0.5	0.5-1	1-1.5	1.5-2	2-2.5	2.5-3	3-3.5	3.5-4	4-4.5	4.5-5
No. patients [pty]	424 [206]	398 [183]	353 [166]	314 [147]	284 [136]	261 [128]	252 [122]	240 [116]	227 [105]	188 [85]
Overall AEs	400 (194)	337 (184)	304 (184)	271 (184)	244 (179)	226 (177)	209 (171)	204 (176)	163 (156)	131 (155)
Serious AEs	43 (20.8)	33 (18.0)	28 (16.9)	25 (17.0)	24 (17.6)	26 (20.4)	15 (12.2)	19 (16.4)	16 (15.3)	12 (14.2)
Overall infections	246 (119)	197 (108)	170 (103)	165 (112)	145 (107)	130 (102)	108 (88)	121 (104)	81 (78)	67 (79)
Serious infections	14 (6.8)	10 (5.5)	6 (3.6)	8 (5.4)	4 (2.9)	5 (3.9)	2 (1.6)	6 (5.2)	1 (1.0)	3 (3.5)
Malignancies	0 (0)	1 (0.5)	3 (1.8)	2 (1.4)	0 (0)	1 (0.8)	1 (0.9)	3 (2.6)	1 (1.0)	2 (2.4)

[a] Interval 1 includes the placebo patients who initiated belimumab treatment at week 56

Conclusions: Belimumab added to standard of care therapy was generally well tolerated over 5 years. Seropositive patients treated with belimumab showed sustained improvement in disease activity and a decline in BILAG and SFI flares over 5 years. (NCT00071487/NCT00583362)

PO2.E.13

Concentration-controlled therapy for mycophenolate mofetil in active lupus nephritis

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Background: Mycophenolic acid (MPA) is an effective treatment for active lupus nephritis despite its variable efficacy seen in different ethnic groups. This study evaluated the benefits of controlling concentrations of MPA levels 1 hour after administrating the drug (C1) in active lupus nephritis (LN). **Methods:** This was a prospective, open-label study that enrolled SLE patients with biopsy-proven proliferative lupus nephritis (class III or IV of ISN/RPS category). Major inclusion criteria required all patients to have proteinuria over 1 g/d and active urine sediments. Pure membranous (class V) LN was excluded. The dosage of mycophenolate mofetil (MMF) was initiated at 1-1.5 g per day and was adjusted to achieve targeted MPA C1 levels (> 13 μg/mL). Prednisolone (0.5 MKD) was given and rapidly tapered to 5 mg per day. Early response (12th week) was determined by the following criteria: a 50% reduction of urine protein and less than 2 g per 24 h. All responders had to have an improved or stable renal function. **Results:** Median (interquartile range: IQR) age of patients (n=9) was 32 (20-40). Median (IQR) proteinuria and erythrocyturia were 2.18 (1.25-3.87) g/ 24 h and 20 (6-65) cells/ high power field, respectively. Median (IQR) activity and chronicity indices of renal histology were 8.5 (7-11.5) and 1.5 (0.25-3.75), respectively. Six out of nine patients (responders) responded to treatment within 12 weeks. All responders (n=6) consistently achieved targeted MPA C1 (>13 μg/mL) and MPA-AUC (>47 h*μg/mL) whereas all non-responders (n=3) failed to achieve targeted MPA C1 or MPA AUC. There were no differences in MMF doses between the responders and non-responders. Even though two patients had herpes zoster and upper respiratory tract infection, however these were not related to MPA levels. None of the patients experienced declining renal function, end-stage renal disease or death during the study period. **Conclusions:** Therapeutic drug monitoring of MPA may be required to manage active lupus nephritis.

Key words: therapeutic drug monitoring, mycophenolate mofetil, mycophenolic acid, lupus nephritis

PO2.E.14

Leflunomide: the safety profile in the treatment of chinese SLE patients

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Purpose: To study the safety profile of leflunomide (LEF) in the treatment of systemic lupus erythematosus (SLE) in Chinese population. **Methods:** Leflunomide was approved in China in 2000. A perspective cohort study was performed to investigate adverse events (AE) in LEF therapies. A specifically designed questionnaire form (QF) was completed by practice physician at the time of each clinic visit by each patient, which included information of : patient's general information; drug combination (glucocorticoid, NSAIDs, DMARDs, Traditional Chinese Medicine); LEF Dose; AE; AE management and AE consequence. Data were entered into an Access data base and AEs rates were analyzed using Chi-square analysis. By Oct 31, 2009, 1405 physicians from 495 hospitals across China have participated in the study. **Results:** Among 59,531 enrolled patients, 5,159 suffered SLE who took at least one dose of LEF, 87.90%(4,535 cases) of them were female. 1,554 cases were treated with LEF monotherapy and 3,605 cases were treated with LEF-based combination therapies. Average dosing time of LEF was 10.38 ± 10.10 months (range 1 - 47 months), mean age was 32.69 ± 13.96 (3-79) years old, mean course of disease was 23.88 ± 40.06 (1-480) months. Average dose of LEF was 18.16 ± 8.28 mg/d (range 5 - 90 mg). The general AE rate was 2.81%. The most common AE was elevation of the level of ALT or AST (0.74%), followed by gastrointestinal(GI) events (0.58%), including diarrhea, nausea, vomit, dyspepsia, and gastr-burning sensation; skin rash (0.48%), alopecie (0.29%), hypertension (0.17%) and hematocytopenia (0.14%). The AE rate was 2.83% in LEF monotherapy group and 2.80% in combination group, P>0.05. In monotherapy group, the most common AE was elevation of the level of ALT or AST (1.03%), followed by GI events (0.51%) and skin rash (0.32%). In combination group, the most common AE was GI events (0.67%), followed by skin rash (0.58%), and elevation of the level of ALT or AST (0.50%).

	LEF monotherapy	combination therapy	Overall
Case	1554	3605	5159
Average dose (mg/d)	18.11 ± 7.57	18.18 ± 8.58	18.16 ± 8.28
All AE (%)	2.83	2.80	2.81
Hepatic (%)*	1.03	0.50	0.74
GI (%)	0.51	0.67	0.58
Skin (%)	0.32	0.58	0.48

*P<0.05

Conclusions: The overall AE rate in Chinese SLE patients is 2.81%, mainly in hepatic, GI and skin events. The profile of risk is slightly higher than those observed in RA patients in the same cohort. There was no significant difference in safety profile between LEF monotherapy and LEF-based combination therapies.

PO2.E.15

Belimumab, a BLyS-specific inhibitor, significantly improved physical functioning, fatigue, and other health-related quality of life (HRQoL) measures in patients with seropositive systemic lupus erythematosus (SLE): BLISS-52 study

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Objective: To assess the effect of belimumab, a BLyS-specific inhibitor, on physical functioning, fatigue, and other HRQoL measures in patients with active seropositive (ANA ≥1:80 and/or anti-dsDNA ≥30 IU/mL) SLE. ***Methods:*** A phase 3, multicenter, randomized, double-blind, placebo-controlled 52-wk study (BLISS-52) was conducted in 865 seropositive SLE patients treated with belimumab. Inclusion criteria required that patients have baseline SELENA-SLEDAI (SS) ≥6. Patients were randomly assigned to receive either belimumab (1 or 10 mg/kg) plus standard of care (SOC) SLE therapy or placebo plus SOC, with all patients on a dosing schedule as follows: days 0, 14, 28, then every 28d for 48 wks. SF-36 Physical and Mental Component Summaries (PCS and MCS) and domain scores and the FACIT-Fatigue questionnaire were assessed at baseline and at wks 4, 8, 12, 24, 36, and 52. ***Results:*** At week 52, mean improvements (MI) from baseline in SF-36 PCS, Physical Functioning, and Bodily Pain domains were significantly greater with belimumab (1 and 10 mg/kg) vs placebo (Table 1).

Assessment		Placebo n=287	Belimumab (1 mg/kg) n=288	Belimumab (10 mg/kg) n=290
SF-36				
	n	286	283	284
Baseline Physical Component Summary (PCS)	mean ± SE	41.3 ± 0.5	41.4 ± 0.6	41.7 ± 0.5
Mean Improvement (MI) in PCS at wk 52	mean ± SE	3.0 ± 0.5	4.2 ± 0.5*	4.2 ± 0.5*
	n	287	287	290
Baseline Physical Functioning (PF)	mean ± SE	62.9 ± 1.5	62.5 ± 1.5	61.8 ± 1.4
MI in PF at wk 52	mean ± SE	4.8 ± 1.2	8.9 ± 1.2†	9.1 1.2†
	n	286	287	290
Baseline Role Physical (RP)	mean ± SE	54.6 ± 1.6	56.6 ± 1.6	57.2 ± 1.4
MI in RP at wk 52	mean ± SE	9.9 ± 1.5	10.0 ± 1.6	9.3 ± 1.4
	n	287	287	290
Baseline Bodily Pain (BP)	mean ± SE	52.6 ± 1.4	54.0 ± 1.5	52.9 ± 1.4
MI in BP at wk 52	mean ± SE	8.1 ± 1.5	11.4 ± 1.6*	12.6 ± 1.4*
	n	287	284	286
Baseline General Health (GH)	mean ± SE	44.8 ± 1.1	44.0 ± 1.1	45.2 ± 1.1
MI in GH at wk 52	mean ± SE	6.7 ± 1.0	9.8 ± 1.1*	9.1 ± 1.2
	n	286	283	284
Baseline Mental Component Summary (MCS)	mean ± SE	40.9 ± 0.6	41.1 ± 0.6	40.0 ± 0.6
MI in MCS at wk 52	mean ± SE	2.7 ± 0.6	3.7 ± 0.6	3.8 ± 0.6
FACIT-Fatigue				
	n	281	283	278
Baseline FACIT-Fatigue	mean ± SE	33.5 ± 0.6	33.6 ± 0.6	33.3 ± 0.6
MI in FACIT-Fatigue at wk 52	mean ± SE	2.1 ± 0.6	3.9 ± 0.6†	4.8 ± 0.6‡

*p<0.050, †p<0.010, ‡p<0.001, otherwise the p value was not significant.
SE=standard error.

The 1 mg/kg belimumab treatment group showed significantly greater improvement in the General Health domain compared with placebo at wk 52. The 10-mg/kg belimumab treatment group demonstrated greater mean improvement in FACIT-Fatigue vs placebo by wk 8 (3.9 ± 0.5 [SE] points vs 1.8 ± 0.5 placebo; p=0.0015), and further improved, with significant differences observed at wk 36 (4.4 ± 0.6 vs 2.7 ± 0.6; p=0.0241) and wk 52 (4.8 ± 0.6 vs 2.1 ± 0.6; p=0.0003). ***Conclusions:*** In BLISS-52, belimumab improved multiple dimensions of HRQoL in seropositive SLE patients, with significant improvements observed vs placebo for physical functioning, bodily pain, general health, and fatigue at wk 52. Improvement in fatigue with belimumab was noted as early as wk 8. (NCT00424476)

PO2.E.17

Factors determining response in patients with active lupus nephritis treated with glucocorticoids and mycophenolate mofetil (MMF)

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Purpose: To evaluate the clinical variables associated with response after induction treatment of active lupus nephritis with combined prednisolone and MMF. ***Method:*** Data were extracted from an open randomized controlled trial of the efficacy of MMF vs tacrolimus for induction treatment of lupus nephritis. All patients who were recruited into the MMF arm were treated with a standard protocol of prednisolone (0.6mg/kg/day for 6 weeks and then slowly tapered) and MMF (2-3g/day in 2 divided doses). Clinical response and remission was assessed at 6 months and factors associated with the achievement of a good clinical response were studied by logistic regression analysis. ***Results:*** Data from 53 patients who had completed the induction phase of the controlled trial were analyzed. The mean age was 34.2±11.3 years and the mean SLE duration was 56.4±64 months. 33(62%) patients had first time nephritis. The histological classes of lupus nephritis (RPS/ISN) were IVg (36%), IVs (8%), III (19%) and V/V+III/IV (38%). The activity and chronicity scores were 7.6±3.8 and 2.8±1.6, respectively. 21(40%) patients were hypertensive and 23(43%) were nephrotic at presentation of nephritis. The mean daily MMF dosage administered was 2.23±0.42g (2g in 74% and ≥2.5g in 26%). At the end of 6 months, 31(58%) patients achieved good clinical response (defined as urine P/Cr <1.0, improvement in lupus serology and urinary sediments and no deterioration of creatinine clearance [CrCl]) by ≥10%) and 5(9%) patients achieved ACR renal remission (CrCl ≥90ml/min, urine P/Cr <0.2 and inactive urine sediments). Regression analysis revealed that the presence of membranous component in the renal histology (RR 0.29[0.09-0.93]; p=0.04) and increasing anti-dsDNA titer at presentation (RR 1.01[1.001-1.012]; p=0.03) were unfavorable factors for a good clinical response. More serious proteinuria at onset was also less likely to respond well to the treatment regimen. 25(47%) patients reported ≥1 adverse events. The distribution of these adverse events was: diarrhea / nausea (13%), major infections requiring hospitalization (13%), minor infections including herpes zoster (62%), transient hyperglycemia (5%) and others (8%). ***Conclusions:*** Combined prednisolone and MMF is a reasonably safe and effective regimen for the initial treatment of active lupus nephritis. Patients with histological evidence of membranous nephropathy, lower anti-dsDNA titer and more serious proteinuria at onset are less likely to achieve a good clinical response. Alternative initial regimens may be considered.

PO2.E.18

Low-dose combination of mycophenolate mofetil and tacrolimus for the treatment of refractory lupus nephritis

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Objectives: To evaluate the efficacy and toxicity of low-dose combination of mycophenolate mofetil (MMF) and tacrolimus (Tac) for refractory lupus nephritis. ***Design:*** an open-label prospective single-arm study. ***Patients and methods:*** Adult patients with refractory lupus nephritis were recruited. Inclusion criteria were: (1) Active lupus nephritis documented by renal biopsy within 24 months; (2) Failure to respond to treatment with at least 2 immunosuppressive regimens which consist of high-dose corticosteroid with one other immunosuppressive drug (eg. CYC, AZA, MMF, cyclosporin A [CSA], Tac) and the use of angiotensin converting enzyme (ACE) inhibitors ± angiotensin receptor blockers (ARB). Each regimen should be used for >= 6 months, unless not tolerated; (3) Serum creatinine (Scr) >=200umol/L. Exclusion criteria were: (1) Previous intolerance to MMF or Tac; (2) Informed consent could not be obtained. Treatment failure to previous regimens was defined as any one of: (1) Failure of proteinuria to improve to <3g/day or urine protein-to-creatinine (uP/Cr) ratio to <3.0; or <50% of pretreatment values; (2) Deteriorating Scr by >20% or loss in creatinine clear-

ance (CrCl) by >30% caused by active lupus nephritis; (3) Persistent active urinary sediments (>=5/HPF). While corticosteroid (prednisolone <=10mg/day), ACEI/ARB were continued, other immunosuppressive agents were discontinued and replaced by MMF (1g/day) and Tac (4mg/day). Patients were followed every 2 months and the primary end-point was clinical efficacy at 12 months. *Results:* Up to January 2010, 11 patients were recruited. The mean age was 35.7±10 years and the mean SLE duration was 106±47 months. The distribution of the histological classes of lupus nephritis was: ISN/RPS class IVG or III (36%), pure V (27%), V+III (36%). Previous treatment regimens received by these patients were: high-dose prednisolone (N=11), CYC (pulse/oral) (N=4), AZA (N=11), MMF (N=8), CSA (N=2) and Tac (N=7). All patients had been receiving the maximally tolerated doses of ACEI±ARB. The mean Scr, CrCl, uP/Cr, 24-hour proteinuria and serum albumin was 80.5±29umol/L, 85.3±32ml/min (8 patients [73%] had CrCl of <90ml/min), 2.85±1.4, 2.67±1.0g and 31.3±4.8g/L, respectively. Seven(64%) patients had active urinary sediments and 9(82%) patients had active lupus serology. Five patients had completed 12 months' follow-up. Significant improvement in proteinuria and urinary sediments was observed in 4 patients and improvement started to occur at month 4. At month 12, a proteinuria of <1g/day and an improvement in CrCl was achieved in these patients. One patient was refractory to this regimen and withdrawn. The following adverse events were reported: minor infections (N=3), diarrhea (N=1) and leg cramps (N=1). *Conclusions:* Low-dose combination of MMF and Tac is a viable option to be considered in patients with refractory lupus nephritis. Expansion of the study sample and a proper comparative trial with the novel biological agents is warranted.

PO2.E.19

Lupus nephritis patients treated with rituximab-results from long-term follow-up

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Objective: The purpose of this study was to investigate the long term clinical, histological and serological effects of B-cell depleting therapy with a combination of rituximab (RTX), intravenous (i.v.) cyclophosphamide (CYC) and steroids in patients with lupus nephritis (LN) refractory to conventional therapy. Furthermore our aim was to analyze if serological or cellular parameters could be predictive of renal response. *Patients and Methods:* Twenty-five patients with therapy resistant Systemic Lupus Erythematosus (SLE) and LN were treated with RTX, CYC and steroids, followed for a mean time of 35 months (9-95), were included. The patients were evaluated clinically every third month during the first year after treatment and every sixth month thereafter until renal relapse or re-treatment for relapse in non-renal systems. Renal disease activity was evaluated with British Isles Lupus Assessment Group (BILAG) index and renal response was determined according to the Lupus Nephritis European consensus statement. Renal biopsies were performed for histological evaluation at baseline and after approximately 6 months. *Results:* Clinical improvements with at least a partial response (PR) were observed in 22/25 after a median time of 12 months. Sixteen patients achieved a complete renal response (CR) after a median time of 24 months. Six patients experienced a renal relapse, 4/6 within the first 18 months and the remaining not until 5 years after treatment. One patient was regarded a non-responder with persistent BILAG A. Albuminuria decreased and serum albumin increased significantly (p=0.0002 and 0.007 respectively) from baseline to 36 months. Low baseline values of IgM and CD19+ cells were indicative of a faster clinical response (r=0.53, p=0.04 and r=0.50, p=0.06 respectively). The flare rate was low, most of the flares occurring within the first 12 months. *Conclusion:* A majority of the patients achieved a partial renal response within the first year and a substantial proportion continued to a complete response between one and two years of follow-up. A trend towards lower absolute numbers of CD19+ cells and low levels of IgM at baseline was associated with faster responses, suggesting that the clinical benefit may be linked to effective suppression of autoreactive plasmablasts. Although formal evidence of RTX efficacy in LN is lacking, our data may

provide guidance to clinicians considering therapeutic options in patients with refractory SLE renal disease.

PO2.E.20

Study design and baseline patient characteristics of BELONG, the randomized double-blind, placebo-controlled phase III trial of ocrelizumab, a humanized anti-CD20 antibody, in lupus nephritis

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Objectives: Ocrelizumab (OCR), a humanized monoclonal antibody that selectively targets CD20 positive B cells, is in development for the treatment of autoimmune diseases. The contribution of B cells to lupus nephritis (LN) pathogenesis and open-label studies with the anti-CD20 antibody, rituximab, prompted the creation of the BELONG protocol. The objective is to determine the benefits and risks of OCR compared to placebo in patients (pts) with active class III/IV LN also receiving corticosteroids plus mycophenolate mofetil (MMF) or Eurolupus (EL) cyclophosphamide (CYC) regimen. *Methods:* BELONG enrolled pts with active proliferative LN defined by renal histology and urinary protein/creatinine ratio ≥1. Pts were randomized equally to Placebo, OCR 400 mg, or OCR 1000 mg IV on days 1 and 15; single infusions were then administered every 16 weeks to maintain CD20 B cell depletion. Open-label was an option after 1 year for pts not achieving a partial response. At investigator discretion all pts received either MMF (upto 3 g/d) or EL (IV CYC 500 mg q2 weeks x 6 followed by azathioprine 2 mg/kg, upto 200 mg/d). IV methylprednisolone was permitted (upto 3 g total) until day 15, whilst 100 mg was given before all OCR/placebo infusions. A protocol-defined steroid taper was mandated. Stratification was by race (black vs other) and background therapy (MMF vs EL). The primary endpoint at 48 wks consisted of: complete or partial renal response defined by normal or stable serum creatinine, proteinuria reduction, urinary sediment improvement. Additional endpoints included: time to response, flare rates, extra-renal activity changes, steroid dose, anti-dsDNA antibody, C3/C4. *Results:* 381 pts were randomized in 28 countries (Latin America: 42%, Europe/S. Africa: 25%, Asia: 23%, US/Canada: 10%). Table 1 shows pretreatment characteristics.

Table 1. Pre-treatment characteristics

Disease Characteristics	Mean (SD)	Median (Min-Max)
24 h urine protein:creatinine ratio All Pts	3.9 (2.9)	2.8 (0.1-16.9)
24 h urine protein:creatinine ratio MMF Pts	3.6 (2.8)	2.7 (0.4-16.9)
24 h urine protein:creatinine ratio Eurolupus Pts	4.2 (3.1)	3.6 (0.1-16.2)
Serum albumin (g/dL)	3.1 (0.7)	3.2 (1.1-4.4)
Serum creatinine (mg/dL)	1.0 (0.5)	0.8 (0.2-3.9)
eGFR (CG) (ml/min)	101 (42.8)	95 (29-286)
C3 (mg/dL)	68 (36.1)	61 (8-502)
C4 (mg/dL)	15 (7.6)	12 (10-63)
	Geometric Mean	**Median (Min-Max)**
Anti-dsDNA antibody (IU/mL) (based on existing data)	145.4	149.5 (2-9755)
Additional Characteristics		
ISN/RPS or WHO biopsy class	Class IV	~80%
ISN/RPS or WHO biopsy class	Class III	~20%
ISN/RPS or WHO biopsy class	Concomitant Class V	~15%
Mean (SD) interval from biopsy to study entry (days)	44 (44)	
Mean (SD) Age (years)	31.3 (9.7)	
Female	87%	
Immunosuppression regimen (% Pts)	MMF	63%
Immunosuppression regimen (% Pts)	Eurolupus	37%
Median LN disease duration (years)	MMF Pts	1.2
Median LN disease duration (years)	Eurolupus Pts	0.4
Race and Ethnicity		
Ethnicity:	Hispanic	43%
Race:	White	48%
Race:	Asian	27%
Race:	Black	5%
Race:	Native Americans/mixed race	21%

Conclusions: BELONG was designed to evaluate the benefit and safety of ocrelizumab added to steroids plus MMF or EL regimen. Most patients had class IV disease and the majority received MMF. The study was stopped early due to an imbalance in serious infection. Results will be reported separately.

PO2.E.21

Does carotid arterial stiffness predict aortic arterial stiffness in patients with systemic lupus erythematosus?

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Objectives: The premature development of carotid and aortic atherosclerosis is common in patients with systemic lupus erythematosus (SLE). No data exist about the temporal relationship of peripheral to central arterial stiffness in patients with SLE. Therefore, we sought to determine the temporal relationship of carotid versus aortic stiffness in these patients. *Methods:* 21 patients with SLE (21 women, age 36 ± 12) and 11 age and gender matched healthy controls (9 women, age 32 ± 13) underwent carotid ultrasonography and transesophageal echocardiography (TEE) for assessment of vessel luminal diameters and intima media thickness using M-mode imaging. Measurements of the right and left carotid arteries as well as proximal, mid, and distal aorta were performed using electronic calipers. Also, simultaneous blood pressure measurements were obtained. Right and left carotid and an averaged aortic stiffness were determined using the **Pressure-Strain Elastic Modulus (PSEM) as = [k(sBP − dBP)/(sD-dD/dD)]/100** where k = 133.3 is a conversion factor from mmHg to Pascal units, sBP = systolic blood pressure, dBP = diastolic blood pressure, sD = systolic diameter, and dD = diastolic diameter. Experienced

observers unaware of the subjects' clinical data interpreted both carotid and TEE studies. *Results:* Right and left carotid PSEM was similar in patients with SLE as compared to controls (7.2 ± 6.15 versus 5.3 ± 2.86 and 7.7 ± 5.26 versus 5.4 ± 2.21, p = 0.24 and 0.09, respectively). In contrast, aortic PSEM was significantly higher in patients with SLE as compared to controls (8.36 ± 4.67 versus 4.87 ± 1.6, respectively, p = 0.005). *Conclusions:* Our data suggest that young patients with SLE develop aortic stiffness prematurely and prior to the development of carotid stiffness. Thus, carotid stiffness in patients with SLE may be a marker of generalized premature arterial stiffness and early stage atherosclerosis.

PO2.E.22

Effect of rituximab (RTX) on anti-dsDNA and C3 levels and relationship to response: results from the LUNAR trial

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Objectives: Previous studies have suggested that improvements in anti-dsDNA titers and complement C3 levels are associated with improvement in lupus nephritis (LN). Changes in these serologic markers and correlations to renal response were assessed in the LUNAR trial. *Methods:* Pts with a diagnosis of active class III/IV LN and urine protein to creatinine ratio > 1 were randomized 1:1 to receive RTX (1000mg) or placebo (PBO) on days 1, 15, 168, and 182 in addition to mycophenolate mofetil and steroids. Changes in anti-dsDNA titers and complement C3 levels were assessed at Week 52 and correlated to renal responses. *Results:* 144 pts (72 to each arm) comprised the intent-to-treat population. Mean daily MMF dose was 2.4+0.63g in PBO and 2.7+0.41g in RTX. Overall, there were no significant differences in renal response rates between the PBO and RTX groups (p=0.55). Baseline median anti-dsDNA and mean C3 were 168.5 IU/mL and 74.1 mg/dL in the PBO group, respectively, and 122.5 IU/mL and 73.6 mg/dL in the RTX group. At Wk 52, there was a greater decrease in anti-dsDNA (p=.007) and greater increase in C3 (p=.025) in the RTX group compared to PBO. Changes by response status are presented in the table. Spearman's correlation coefficient between improvement in C3 and renal response was 0.55 (p<0.001) in PBO and 0.03 (p=0.8) in RTX. Correlation of change in anti-dsDNA to response was not statistically signfficant in either PBO or RTX. *Conclusion:* RTX statistically significantly lowered anti-dsDNA titers and increased C3 levels compared to PBO. However, both RTX responders and RTX non-responders had similar levels of improvement in anti-dsDNA titers and C3 levels, suggesting that changes in these serologic markers at one year do not correlate with renal response in RTX-treated pts.

	All Patients				Placebo				Rituximab			
	All (n=144)	Resp (n=74)	NR (n=70)	Difference (95% CI)	All (n=72)	Resp (n=33)	NR (n=39)	Difference (95% CI)	All (n=72)	Resp (n=41)	NR (n=31)	Difference (95% CI)
Mean % reduction in log (a-dsDNA)	16.6	20.5	12.4	8.2 (1.9, 14.5)	11.9	17.8	6.8	11.0 (0.9, 21.1)	21.3	22.8	19.3	3.4 (-3.9, 10.7)
% Pts with a-dsDNA <30	37.5	41.9	32.9	9.0 (-7.0, 25.0)	33.3	39.4	28.2	11.2 (-11.4, 33.8)	41.7	43.9	38.7	5.2 (-18.5, 28.9)
Mean increase in C3 (mg/dL)	31.7	42.3	20.5	21.8 (12.1, 31.4)	25.9	44	10.6	33.5 (20.3, 46.7)	37.5	40.9	33.1	7.8 (-5.8, 21.4)
% Pts with C3≥90	72.2	81.1	62.9	18.2 (3.7, 32.8)	63.9	81.8	48.7	33.1 (12.0, 54.2)	80.6	80.5	80.6	0.16 (-19.2, 18.9)

PO2F Immunology – Dendritic Cells

PO2.F.1

1α,25-Dihydroxyvitamin D3 suppresses differentiation, maturation and activation of dendritic cells from patients with systemic lupus erythematosus

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Background: Dendritic cells (DC), professional antigen presenting cells, are believed to play a crucial role in the pathogenesis of systemic lupus erythematosus (SLE). 1α,25-Dihydroxyvitamin D3 (VitD3), in addition to its effect on bone metabolism, has been increasingly recognized to have immunomodulatory action. **Objective:** To examine the effect of VitD3 on the differentiation, maturation and activation of DCs in SLE patients. **Method:** CD14+ monocyte derived-DCs from SLE patients and age- and sex- matched controls were derived from growth medium cultured with IL-4 and GM-CSF. Mature DCs were induced by addition of lipopolysaccharide (LPS) and tumour necrosis factor-α (TNF-α) in the presence or absence of VitD3 ($1×10$-10 M) and/or dexamethasone ($1×10$-6 M). The expression of CD1a and co-stimulatory molecules was examined by flow cytometry. After stimulation of DCs with CD40L transfected cell line for 24h, the production of pro-inflammatory cytokines including IL-12 and IL-6, were measured by ELISA kits. **Results:** 50 SLE patients and 32 normal controls were studied. VitD3 was found to suppress differentiation of monocytes into DCs with lower level of expression of CD1a for both controls ($p<0.05$) and SLE patients ($p<0.01$) compared to untreated condition. With or without dexamethasone, VitD3 inhibited upregulation of maturation markers, including CD86, CD40 and CD83 ($p<0.05$), but not CD80 and HLA-DR (Table 1&2). In terms of pro-inflammatory cytokine production, stimulated and unstimulated DCs produced less IL-12p70 and IL-6 in SLE patients as well as controls under the effect of VitD3 with or without dexamethasone compared to pre-treatment DCs. **Conclusion:** VitD3 is found to inhibit differentiation, maturation and activation of DCs in vitro in both SLE patients and controls and may be considered as immunomodulatory agent in the treatment of SLE.

Table 1

Normal	CD1a(%)	CD86(%)	CD40(%)	CD83(%)	IL-12p70 (pg/ml)	IL-6 (pg/ml)
Medium	82.10± 15.75	87.29± 8.14	74.17± 23.70	51.86± 14.67	36.59± 39.08	2653.00± 1647.38
VitD3	6.35± 7.95*	83.24± 9.85	61.59± 19.12*	20.97± 13.47***	8.01± 6.30*	380.67± 62.69
VitD3+Dex		53.47± 35.57**	30.36± 25.06*	3.30± 2.43**	0.82± 0.18	728.20± 189.72*
Medium+ CD40L		91.87± 6.90	80.17± 17.64	69.19± 12.36	34.73± 38.48	2944.00± 1733.30
VitD3+ CD40L		87.79± 13.70#	64.25± 19.90	33.81± 18.04###	2.95± 3.75	829.80± 387.75#
VitD3+Dex+ CD40L		53.99± 34.81##	33.55± 25.54##	3.60± 3.88##	1.12± 0.93	170.80± 66.20#

Table 2

SLE	CD1a(%)	CD86(%)	CD40(%)	CD83(%)	IL-12p70 (pg/ml)	IL-6 (pg/ml)
Medium	82.69± 21.06	77.27± 14.52	61.83± 20.94	29.67± 14.45	10.14± 11.76	1853.22± 646.06
VitD3	2.85± 0.86**	75.05± 13.53	49.80± 21.64**	12.73± 11.10***	2.93± 2.11*	173.50± 145.38
VitD3+Dex		48.84± 23.64**	28.65± 27.54**	2.51± 1.96**	1.76± 1.01*	1212.78± 407.50
Medium+ CD40L		82.01± 16.59	67.86± 15.46	41.56± 11.28	11.76± 23.18	1624.33± 615.12
VitD3+ CD40L		76.72± 17.11##	49.43± 22.60##	13.16± 9.99###	2.49± 3.22	1198.43± 434.17
VitD3+Dex+ CD40L		47.14± 24.34##	23.02± 25.19##	4.18± 4.66##	1.01± 0.35#	797.78± 601.96##

Vs Medium, *p<0.05, **p<0.01, ***p<0.001
Vs Medium+CD40L, #p<0.05, ##p<0.01, ###p<0.001

PO2.F.2

Serum from active SLE patients induces a distinct pattern of monocyte chemokine receptor expression

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Objective: Monocytes from some Systemic Lupus Erythematous (SLE) patients function as antigen-presenting cells in vitro, as they induce the proliferation of allogeneic CD4+ T cells. Furthermore, serum from SLE patients induces normal monocytes to differentiate into dendritic cells (DCs) in an interferon-alpha (IFNα)-dependent fashion. To understand the molecular events underlying the effects of SLE serum on monocytes, we adopted a systems biology approach and analyzed the serum-induced transcriptome and protein expression changes in vitro after 6 and 24 hr culture respectively. **Methods:** Negatively and positively selected monocytes from healthy donors were cultured in the presence of either 20% autologous serum, 20% autologous serum with 1,000 IU of IFNα-2b, or 20% serum from SLE patients with a wide range of disease activities. Microarray analysis was performed after 6 hr using HG-U133A & HG-U133B Affymetrix GeneChip® arrays. Surface protein expression was analyzed by flow-cytometry after 24-hour culture. Monocyte trans-migration to chemokine receptor ligands (CCL) was performed using an in vitro transwell migration assay. **Results:** Among the many changes induced by SLE serum at the RNA and protein levels, we observed a distinct program of chemokine receptor (CCR) expression that included the early upregulation of CCR7. This CCR drives naïve and central memory T cells as well as DCs to the lymph nodes and it represents a maturation marker for DCs, but is not normally found on cells with a monocyte phenotype. CCR7 upregulation was mainly induced by sera from active patients and it correlated with monocyte migration towards its ligand CCL19. This SLE serum effect requires concomitant CD14 signaling but it seems to be independent of IFNα, as it could not be blocked by addition of anti-IFNα together with anti-type I IFN receptor antibodies, nor could it be fully reproduced by adding recombinant IFNα to autologous serum. **Conclusions:** SLE serum-induced expression of surface CCR7 could drive lupus monocytes endowed with DC function to the T cell-rich areas of lymph nodes. This might contribute to disease flares, especially in the context of bacterial and viral infections where a concomitant engagement of CD14 takes place.

PO2.F.3

Functional characterization of peripheral blood dendritic cells and monocytes in systemic lupus erythematosus

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Objectives: To evaluate abnormalities of dendritic cell (DC) numbers and function related to SLE and disease activity. *Methods:* We recruited patients with SLE fulfilling the 1997 ACR classification criteria and age- and gender-matched healthy controls. The SLE disease activity at time of study evaluation was scored with SLEDAI 2K. Patients were divided into two groups, one with active (SLEDAI $\geq$5) and the other with inactive SLE (SLEDAI <5). Peripheral blood was collected from each subject and divided into stimulated (LPS and IFN-gamma) and control samples. Immunofluorescent staining and flow cytometry data acquisition and analysis was done for quantification of monocytes and DC myeloid (mDC), plasmacytoid (pDC) and CD14-/lowCD16+ subpopulations and their production of pro-inflammatory cytokines (TNF-alpha, IL-10, IL-6 and IL-12). Statistical analyses of differences between groups of active and inactive SLE patients and healthy controls were done using the non-parametric Mann-Whitney test. *Results:* The study included 47 subjects, 15 with active and 19 with inactive SLE and 13 healthy controls. We found an overall decrease of absolute number and relative frequency of total DC in SLE patients with active disease when compared to those with inactive disease and healthy individuals, although this decrease did not seem to have an effect on the distribution of DC subsets. The number of monocytes in SLE patients were similar to those found in healthy individuals, whereas without prior stimulation a higher frequency of monocytes producing cytokines and of the amount of each cytokine per cell was found, particularly in those patients with active disease. After stimulation, we observed a higher frequency of IL-12-producing monocytes in active SLE patients. On the other hand, we found in DC a higher frequency of cytokine-producing CD14-/lowCD16+ DC as well as in the amount of cytokines produced per cell, particularly in active disease. *Conclusions:* These findings suggest an increased production of inflammatory cytokines by antigen-presenting cells in active SLE, mostly with abnormalities in CD14-/lowCD16+ DC subset.

PO2G Interferon

PO2.G.6

Platelet interferon-regulated proteins are associated with platelet activation and cardiovascular disease in SLE

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Objective: Patients with SLE have a marked increased risk to develop cardiovascular disease not fully explained by traditional or known disease specific risk factors. We therefore undertook a study to investigate platelet mRNA and protein expression as well as corresponding platelet functions in SLE patients and matched healthy controls. *Methods:* Platelets were isolated and mRNA expression quantified by real-time PCR and protein expression by flow cytometry. *Results:* We found that platelets from SLE patients have a type I IFN mRNA signature as well as an increased expression of type I IFN-regulated

proteins such as PRKRA, IFITM1 and CD69 (p<0.0001). The megakaryocytic cell-line MEG-01 increased the expression of CD69 and IFITM1 upon IFNα stimulation, compatible with IFNα interacting with megakaryocytes in vivo. Importantly, patients with a history of cardiovascular disease had increased expression of type I IFN-regulated proteins as well as more activated platelets as compared with patients without a cardiovascular disease. *Conclusions:* We suggest that interferogenic immune complexes stimulate production of IFNα which up-regulates the megakaryocytic type I IFN-regulated genes and proteins and affects the platelet activation. Altogether, our findings show that SLE patients have more activated platelets and increased expression of type I IFN-regulated proteins as compared with healthy controls which may contribute to the development of cardiovascular disease in SLE.

PO2.G.7

Atorvastatin therapy reduces a interferon regulated chemokine CXCL9 plasma levels in SLE patients

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IFN-regulated chemokines seem to be important in SLE pathogenesis. High CCL2, CXCL11, CXCL10 and CCL3 levels were associated with active lupus nephritis and high CXCL9, CXCL10 and CXCL11 with active skin lesions, suggesting monitoring blood chemokine levels may be useful in assessing SLE activity. As well new therapies aimed to block chemokines may be effective in SLE. *Objective:* to evaluate the efficacy of atorvastatin to reduce the levels of IFN-regulated chemokines (CCL2, CCL3 and CXCL9) in SLE patients. Methods: Eighty-eight SLE women were divided in 2 groups: 64 received atorvastatin 20 mg/day for 8 weeks (intervention group) and 24 without intervention were followed for the same period (control group). Chemokines plasma levels was measured by ELISA sandwich technique using commercial kits (DuoSet, R & D Systems MN, USA) at baseline and after 8 weeks for all participants. *Results:* 64,8% of patients were white, the mean age was 32±8 years and mean disease duration was 8.9±4.8 years. The mean SLEDAI and SLICC scores were 4.16 and 1.06, respectively. The plasma levels of CCL2 were higher in patients with dyslipidemia, CHD risk factors and with current serositis, vasculitis and nephritis. Patients with current serositis and nephritis presented a higher plasma level of CXCL9. In a univariate analysis, we find a positive correlation between the plasma levels of CCL2 (r=0.04; p<0.001), CXCL9 (r=0.30; p=0.01) and CCL3 (r=0.27; p=0.01) and SLEDAI score. In the intervention group we observed a significant decrease in the plasma levels of CXCL9 when comparing baseline and at the end of 8 weeks (p=0.04). However no differences were observed regarding CCL2 neither CCL3 plasma levels along the study. No significant difference was observed in the plasma levels of these chemokines in the control group. A multiple linear regression model considering SLEDAI score as a dependent variable and including the following variables: triglycerides, total cholesterol, body mass index, current prednisone dosage (mg/day) and the plasma levels of CCL2, CXCL9 and CCL3 were constructed for 88 SLE patients. In this analysis, the current prednisone dosage (p<0.001), plasma levels of CCL2 (p= 0.001) and plasma level of CXCL9 (p=0.006) were independently associated with the SLEDAI score. *Conclusion:* atorvastatin was associated with a significant decrease in the plasma levels of CXCL9 in SLE patients. As the plasma levels of CXCL9 correlated with the SLEDAI score, we suggest that this may be the mechanism related to the atorvastatin capacity to help control SLE activity.

PO2.G.8

Interferon-α-induced expression of myxovirus resistance 1 (MX1) requires the sequential activation of PI3K, PKC δ and JNK

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A multitude of interferon (IFN)-inducible genes (IFIGs), including Myxovirus Resistance 1 (MX1), are coordinately expressed in peripheral blood mononuclear cells (PBMCs) of patients with systemic lupus erythematosus (SLE), emphasising the globle activating of signal pathway mediated by IFN-I in SLE. In this study, we investigated the mechanisms of expression regulation of MX1 by IFN-α. We found that IFN-α failed in inducing MX1 in STAT1-negative U3A cells. Ectopic expression of STAT1, but not mutant STAT1-S727A, almost completely restored IFN-α2a-induced MX1 expression. IFN-α induced the expression of MX1 and STAT1 in THP-1 cells, and this process was significantly antagonized by the specific inhibitors of PI3K, PKCδ and JNK or their dominant negative mutants respectively. The inhibition of JNK activity by its specific inhibitor or its dominant negative mutant suppressed both MX1 expression and serine phosphorylation of STAT1 but not the activation of PKCδ, while inhibition of PKCδ suppressed activation of MX1, STAT1, and JNK. Our results suggest that the induction of Mx1 transcription by IFN-α depends upon sequential activation of PI3K , PKCδ, JNK and STAT1, and that the influence of PI3K, PKCδ or JNK on IFN-α-mediated induction of MX1 is dependent upon the phosphorylation of STAT1 at Ser-727. The results in our experiment provide an in vitro model of the signaling mechanisms of IFIGs regulated by IFN-α, that is putatively thought to occur in vivo as the one of pathogenesis of SLE.

PO2.G.9

The role of genetic variation near interferon-κ in systemic lupus erythematosus

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Objective: Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by increased type I interferons (IFN) and multi-organ inflammation which frequently targets the skin. IFN-kappa is a member of the type I IFN family which is expressed in skin, and a pooled genome-wide scan implicated the IFNK locus in SLE susceptibility. **Methods:** We studied

18 single nucleotide polymorphisms (SNPs) in the IFNK region in approximately 4000 SLE cases and as many controls, including 242 European (EU) male cases and 931 controls, 2268 female EU cases and 2036 controls, 629 female African-American (AA) cases and 531 controls, 54 male AA cases and 252 controls, and 843 female Asian cases and 777 Asian controls. Analyses were conducted to detect associations with SLE susceptibility, skin phenotypes, and serum type I IFN, and each sex was examined individually. All associations were corrected for potential population stratification. **Results:** rs12553951C was associated with SLE in EU males (OR=1.93, p=2.5x10-4), but not females. Associations with skin phenotypes in EU and AA females were found, and interestingly allele frequencies of these skin-associated SNPs showed strong sex-skewing as they related to skin phenotypes. Specific IFNK SNPs were associated with increased serum type I IFN in EU and AA. **Conclusions:** Our data suggest a sex-dependent association between IFNK SNPs and SLE and skin phenotypes. The serum IFN association suggests that IFNK variants could influence type I IFN producing plasmacytoid dendritic cells in affected skin.

PO2.G.12

Endogenous retroelement LINE-1 is a candidate trigger of the innate immune response in systemic lupus erythematosus

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Objectives: Endogenous triggers of type I interferon (IFN) might include DNA or RNA encoded by genomic retroelements. Previous studies from our lab demonstrated that human plasmacytoid dendritic cells (pDC) transfected with a plasmid containing a full length LINE-1 (L1) element induced robust IFNa2 expression and documented significant elevation of L1 mRNA in renal biopsies from patients with class IV lupus nephritis. To extend these findings, we investigated the effect of L1 mRNA on induction of type I IFN by pDC and studied the relationship of L1 and IFN-inducible gene (IFIG) transcripts in longitudinal PBMC samples from SLE patients and healthy donors (HD). **Methods:** Human pDCs (CD4+, CD123+ and BDCA-2+) were isolated from PBMC by negative selection with magnetic beads (Mylteni). Full-length element L1.4 from the X chromosome of a SLE patient was cloned into the pDONR221 vector. L1, U1, hY3 and unrelated control RNAs were obtained by in vitro transcription of T7 RNAP (NEB) from corresponding plasmids followed by purification with Trizol (Invitrogen). pDCs were transfected with either plasmids or RNA using DOTAP (Roche). Relative expression of IFNa2, IFIGs (IFIT1, IFI44, PKR, Mx1), L1 and housekeeping gene HGPRT was determined by RT-PCR and normalized to mock transfection. Statistical analysis was performed using GraphPad Prism software. Methylation analysis of L1 promoter was done by EpigenDx (Worcester, MA). L1 and IFIG mRNA expression was quantified in longitudinal PBMC samples from 54 SLE patients and 23 HD. **Results:** Transfection of human pDC with RNA from classic lupus antigens U1 and hY3 or with L1 mRNA fragments revealed that U1 and L1, but not hY3 or control RNA, induced similar high level expression of IFNa2 mRNA. This effect was blocked by bafilomycin A, suggesting involvement of the TLR7 signaling pathway. Elevated expression of L1 mRNA was found in 29 out of 54 (53%) SLE patients followed longitudinally, and this expression showed a trend toward correlation with IFIG expression (p=0.013, r²=0.196). Methylation analysis of the L1 promoter across the genome revealed an average demethylation rate of 5% in SLE patients compared to HD (p=0.0021). **Conclusions:** Correlation of L1 overexpression with IFIG expression in SLE PBMC, together with demonstration of the induction of IFN by L1 mRNA, points to the plausible involvement of this endogenous retroelement in innate immune activation in SLE. Demethylation of the L1 promoter is one of the possible mechanisms facilitating L1 expression *in vivo*.

PO2.G.13

Abnormalities in lymphoid subsets and architecture associated with the development of autoimmunity in the interferon-accelerated NZB/NZW lupus model

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Objectives: In the IFNα accelerated NZBxNZW F1 (BWF1) mouse model of lupus, we observed the loss of the follicular dendritic cell (FDC) network, which correlated with the disrupted B/T cell compartmentalization, abnormal positioning of the GL7+ B cells and reduction in homeostatic chemokines. We wanted to explore two questions: 1) are these changes a result of the adenoviral infection, and 2) would we see similar changes in a non-autoimmune prone strain of mouse. *Methods:* 9 week old female BWF1 mice and Balb/C mice were intravenously injected with 3 x 109 particles of adenovirus-IFNα or adenovirus-GFP. Mice were euthanized at 3, 5 and 7 weeks post injection, and spleen was collected for flow cytometry, histology, IHC and RNA analysis. Serum and urine were also collected for clinical chemistry analysis. Five age-matched mice from each strain were included as naïve controls. *Results:* Only BWF1 mice that received adenovirus-IFNα developed classic symptoms of lupus disease, as defined by elevated proteinuria, anti-nuclear antibodies and immune complex deposition in the kidney. At 3 weeks post adv-IFNα, FDC markers CD35, FDC-M2 and MFG-E8 (FDC-M1) staining were reduced, and B and T cell zone staining of the splenic white pulp showed disrupted architecture. Memory CD4+ T cell (CD44+CD62L-) and activated CD3+ T cell (CD69+) populations were significantly elevated as early as 3 weeks post adv-IFNα, which correlated with the loss of naïve CD3+ T cells. *Conclusions:* The collapse of the FDC network and disruption of splenic architecture is not due to adenoviral infection in BWF1 or high IFNα levels in a non-autoimmune strain. Since the loss of FDC occurred during the period when activated T cells are increasing, the FDC loss could be due to excessive T cell activation. Moreover, as the decay of CXCL13 production lagged behind CCL19 and CCL21, the FDC effects could be secondary to T-cell zone collapse. Balb/C mice lacked significant T cell activation and migration into the FDC areas, which is consistent with the absence of lupus disease symptoms. Aberrant or mispositioned FDC networks could exacerbate the pathogenesis (disease progression) of SLE based on two models: 1) An FDC-less germinal center may impair selection of autoreactive B cells, and 2) lack of FDC derived MFG-E8 may affect proper clearance of apoptotic cells, resulting in more nuclear chromatin debris and production of autoantibodies.

PO2.G.14

Paradoxical increase in both IFN-α and Th17 cells in systemic lupus erythematosus

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Objectives: Type I interferons (IFN) are strongly implicated in the pathogenesis of SLE. They contribute to disease in a number of ways including stimulation of auto-reactive naïve T cells and promoting their differentiation to the Th1 subset, enhancing isotype switching and antibody production, and activation of antigen presenting cells. Recently, several groups have reported increased numbers of Th17 cells in the blood and in the kidneys of SLE patients with nephritis, suggesting that Th17 cells also promote inflammation. Detection of increased numbers of Th17 cells is surprising because most studies indicate that IFN-α suppresses Th17 proliferation and differentiation. Since the impact of type I IFNs on Th17 differentiation during the disease course in SLE is unknown, we asked whether IFN-α and IL-17 are positively or negatively correlated in SLE patients and whether SLE patients were resistant to the suppressive effects of type I IFN on IL-17 production. *Methods:* To examine these questions, we collected blood samples from lupus patients and controls (n=8-15). In order to measure IFN activity in our subjects, we used real-time PCR to quantify mRNA expression of the three interferon response genes (IRGs) (MX1, PKR, and CXCL10) relative to the housekeeping gene,

18S. We quantified Th17 cells in two ways: 1) by quantifying relative mRNA expression of IL-17A and RORc (a transcription factor specific for Th17 cells) and 2) by determining the percent of positive cells by intracellular cytokine staining for IL-17 using flow cytometry. Expression was quantified at baseline and after stimulation with PMA/Ionomycin (4-6 hours). *Results:* We identified up-regulation of IRGs in over half of the lupus patients but in none of the controls. At baseline, similar relative levels of IL-17 and RORc mRNA was seen in both patients and controls, regardless of the IFN score. Without stimulation, no IL-17 producing cells were detected by flow cytometry in either group. Lupus patients were found to have an increased percentage of IL-17 positive T cells after stimulation with PMA/Ionomycin by flow cytometry. Preliminary analysis suggests that patients with high IFN scores also had an increase in the Th17 phenotype. Higher IFN scores and increased numbers of IL-17 producing cells were seen in patients with more active disease as measured by SELENA-SLEDAI score. *Conclusions:* These results suggest that SLE patients with active disease can express high levels of both type 1 IFN as well as Th17 T cells. Whether the failure of IFN to suppress IL-17 production reflects a unique cytokine profile, intrinsic defect in inhibitory signaling or unique antigen drive, remains to be determined.

PO2.G.15

Enriching for sialylated IgG increases the inhibition of interferon-alpha production by human plasmacytoid dendritic cells in response to TLR7 and TLR9 activation

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Objectives: IgG in the form of intravenous immunoglobulin (IVIg) has been used as an anti-inflammatory agent in multiple autoimmune diseases. Recent studies reported that it is the small fraction of sialylated IgG in human IVIg preparations that attenuates arthritis or immune thrombocytopenia. However, these studies were performed across species (human IgG injected into mouse models of disease). Our goal was to study the importance of the sialylated subset of IVIg in the inhibition of the inflammatory responses of human cells stimulated with TLR agonists. *Methods:* Human IVIg preparations were enriched (SA+, 7- to 10-fold) or depleted (SA-, 2- to 5-fold) of the sialylated subset by lectin affinity chromatography. PBMC were isolated from whole blood of healthy individuals, cultured overnight in the presence of TLR7 (Loxoribine, CL097) or TLR9 (CpG) agonists with or without SA+ or SA- IgG at two doses (0.5 and 5 mg/mL). In certain experiments, cells were depleted or isolated from PBMC using magnetic beads. Supernatants were collected 20 hours post-treatment and analyzed by ELISA for IFN-α, TNF-α, IL-8, and IL-6. *Results:* In response to the TLR agonists tested, SA+ IgG inhibited IFN-α production significantly more than SA- IgG (p<0.05), and the inhibition by SA+ IgG increased with higher doses (p<0.05). Surprisingly, levels of IL-6, IL-8, and TNF-α in response to TLR agonists were not inhibited by SA+ or SA- IgG. Inhibition of IFN-α by SA+ IgG was decreased in the absence of CD14+ monocytes, but not CD19+ B cells or CD56+ NK/NKT cells. Supernatants from isolated monocyte cultures treated with SA+ IgG could transfer inhibition to fresh cultures of TLR agonist-stimulated PBMC or isolated pDC. *Conclusions:* SA+ IgG is more potent at inhibiting IFN-α production in response to TLR agonist stimulation compared to SA- IgG. A soluble factor produced by CD14+ monocytes mediates this inhibition by acting directly on pDC, and identification of this factor and the specific receptor for SA+ IgG is currently under investigation. The greater potency of SA+ IgG has therapeutic implications specifically for SLE because these results suggest that a lower dose of IVIG enriched for the active, sialylated subset may be efficacious. Also, identification of the receptor(s) and signaling pathways involved in the SA+ IgG anti-inflammatory effects could reveal targets for immune modulation of type 1 IFN in SLE.

PO2H Management – Biologic Therapies

PO2.H.1

Refractory childhood-onset systemic lupus erythematosus treated with Rituximab
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Objective: Systemic lupus erythematosus (SLE) is an multisystem auto-immune disease, induced by activation of autoreactive T cells and over-production of autoantibodies by B cells. B cell dysfunction has been thought to be critical in the pathogenesis of (SLE). Thus, the concept of B cell depletion as treatment for SLE seems rational. To describe the safety and efficacy of rituximab in the treatment of refractory childhood-onset (SLE). Children with SLE tend to have more severe hematologic and renal involvement compared with adults. **Methods:** In this study we have 5 patients who had been previously diagnosed with SLE based on the American College of Rheumatology criteria. Rituximab was administered at different doses(375 mg/m2). We present five cases Iranain retrospective study of refractory childhood-onset SLE treated with rituximab. **Results:** three girls and one boy with refractory SLE, including 3 girls with class IV and one boy with severe autoimmune cytopenia. The mean age at onset of rituximab treatment was 10.5years. All of Patients received 4 to 8 intravenous infusions of rituximab (3750/mg/m2/infusion), with corticosteroids. Remission was achieved in 2 of 3 patients with lupus nephritis and in the 1, patients with autoimmune cytopenia. Steroid therapy was tapered in 2 patients who responded to treatment, and low-dose prednisone treatment was maintained in 3 patients. The mean follow-up period was 8 months (range, 6-12months), and remission lasted in all who patients who responded to treatment, except 1 patient who was successfully retreated with a second course of rituximab. Anti-double-stranded DNA antibody levels decreased in 4 of 5 patients. Severe adverse events developed in 3patients. Effective depletion of peripheral blood B cells was observed in 2 of 5 patients who were examined, and this paralleled the remission. **Conclusion:** Our study showed that rituximab is useful as a new treatment for such cases. However, recurrence after rituximab treatment was noted in our patients. Rituximab may be an effective co-therapy; however, further investigations are required because severe adverse events occurred in the patients in this study.

PO2.H.2

Epratuzumab preferentially affects trafficking of naïve CD27- B cells from SLE patients
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Objective: In SLE, disturbances of B-cells in the peripheral blood, the production of auto-antibodies as well as experiences in clinical trials with B-cell directed therapy suggest a key role of B-cells in its pathogenesis. Epratuzumab, a humanized monoclonal antibody targeting CD22, has been studied in patients with systemic lupus erythematosus (SLE), leading to reductions of peripheral B-cells. The mechanism of action of this monoclonal still remains to be fully delineated. The current study addressed the influence of epratuzumab on migration and adhesion molecule expression. **Methods:** We analyzed the distinct binding of PE-labeled epratuzumab on peripheral B-cell subsets in terms of its impact on migration towards gradients of chemokines (CXCL12) and the expression of adhesion molecules (CD62L and β7 integrin). **Results:** While no binding of the antibody on T-cells and low binding on monocytes was ob-

served, there was enhanced binding to CD22 on naïve (CD19+ CD27-) and to a lesser extent on memory B-cells (CD19+ CD27+) from SLE patients. Consistent with this, epratuzumab pre-incubation led to enhanced migration towards CXCL12 of naïve B-cells only (13% ±6.5 to 23% ±9.3). The migration of monocytes from SLE patients towards CXCL12 was also moderately enhanced under the influence of epratuzumab (27% ±17 to 33% ±13), but did not reach significance (P=0.078). In terms of adhesion molecule expression, a significant down-modulation of CD62L occurred after incubation with epratuzumab (P=0.0078) on B-cells, whereas no significant effect was observed on T-cells and on monocytes. Interestingly, the expression of this molecule was found to be significantly decreased on naïve B-cells from SLE patients only (P=0.0039).Without epratuzumab treatment, 70% of the naïve B-cells expressed β7 integrin. After epratuzumab exposure, this expression was reduced to 60% on naïve B-cells (P=0.0039) which was again restricted to naïve B-cells. **Conclusion:** Epratuzumab leads to distinct changes in migration and adhesion molecule expression on B-cells from SLE patients, suggesting that impaired trafficking by affecting migration and adhesion molecule expression mainly restricted to naïve B-cells contribute to its mechanism of action in SLE.

PO2.H.3

Somatic B cell immunization with tolerogenic consensus DNA construct ameliorates lupus disease in NZBxNXW F1 mice : towards a therapeutic vaccine for SLE
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Objectives: (NZB x NZW)F1 (NZB/NZW) lupus-prone mice have high serum level of autoantibodies which can form immunocomplexes and can deposit in the kidney causing glomerulonephritis. Autoantibody production is driven by autoreactive Th cells among which there are Th cells specific for antigenic sequences within the Vh regions of autoantibodies to DNA. B cells can process and present such epitopes and activate T cells. A consensus peptide, pCONS, derived from anti-DNA Ig antigenic sequences has been shown to have tolerogenic properties in lupus-prone mice. This study aims at providing preclinical data on a somatic B cell immunization with an Ig-pCONS DNA construct. **Methods:** A chimeric gene coding for the Fc portion of a human IgG and for pCons (Ig-pCONS) has been inserted in a plasmid under a CMV promoter. NZB/NZW lupus mice received syngenic B cell spontaneously transfected with Ig-pCONS plasmid or control plasmid. Lupus disease was monitored by checking autoantibody production, renal disease development, and T cells phenotype, number, and function. **Results:** Treatment of mice with Ig-pCONS plasmid induced CD8+CD28- T cells that suppressed the antigen-specific stimulation of CD4+ T cells promoting immunological tolerance, retarding the development of nephritis, reducing autoantibody levels and improving survival. **Conclusions:**This novel B cell somatic Ig-pCONS immunization protocol is a physiologically tolerogenic vaccination approach that could be transferred to human in a near future.

PO2.H.4

Biologics use in SLE in 4 centers – data from the international registry for biologics in SLE (IRBIS)
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Objective: To characterize the use of biologics in SLE. **Background:** No biologic agents are approved for use in SLE, but off-label use is possible in various health-care settings. The degree to which such use is in fact taking place is not known, nor are specifics on the types of patients who are treated in this manner and the short- or long-term results. In order to obtain information regarding biologics use in SLE, members of the SLICC group recently initiated the International Registry for Biologics in SLE (IRBIS). **Methods:** SLICC/IRBIS investigators were asked to provide retrospective data on all patients treated with a biologic for SLE at their center. Standardized case report forms (CRFs) were used to collect demographic, disease-specific, and treatment data at the time of biologic initiation and, if available, at yearly follow-up. A retrospective data collection is currently in progress, and data from the first four reporting centers were analyzed. **Results:** 55 patients received a biologic, in all cases rituximab (RTX). Age (mean±SD) was 38.9±12.2, 91% were female, and 91% Caucasian. Disease duration when the biologic was initiated was 10.0 ± 7.6 years. SLEDAI at biologic start was 12.0 ± 6.8, SLICC-damage index 0.98 $\pm$ 1.49, and glucocorticoid (GC) dosage (prednisone equivalent) 18.7 ± 14.5 mg. Two different dosing regimes for RTX were used: 375 mg/m2 x 4 (58%) and 1000 mg x2 (42%). Concomitant cyclophosphamide (CYX) was used in 77% of patients. The major organ manifestations leading to biologic treatment were lupus nephritis (LN) in 33 patients (60%), skin disease in 8 (14%), hematological in 8 (14%), musculoskeletal in 5 (9%), CNS in 4 (7%), and others in 6 (11%) (some patients having more than one). Both disease-control and GC-sparing were given as reasons for choosing a biologics. At 1-year follow-up (n=49) additional immunosuppressives (ISs) had been started in 7 patients. Both SLEDAI and GC dose had decreased (SLEDAI to 5.0±4.1; GC to 7.8±6.5 mg, p<0.0001 for both comparisons, and excluding patients on new ISs). SLEDAI at baseline was higher in LN patients than in non-LN but similar at follow-up. The two RTX regimes achieved similar changes in SLEDAI, and regardless of concurrent CYX use. **Conclusions:** Biologics use in this multi-center international cohort was limited to rituximab, employed mainly in LN. At 1-year follow-up, lupus activity and concomitant GC dosage had decreased, and this could not be attributed to the start of other IS treatment. The complete retrospective dataset from all participating centers, and the initiation of the prospective IRBIS registry, will provide much-needed data on the use of and results achieved with biologics in SLE.

PO2.H.5

Infliximab-induced lupus erythematosus

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Objectives: TNF alpha (TNFα) inhibitors have greatly improved our ability to effectively control rheumatoid arthritis and other inflammatory disorders. The side effects of the use of TNFα agents include the development of autoantibodies and a lupus-like syndrome. To our knowledge, there are only isolated reports of switching patient from one TNFα inhibitor to another after the development of drug related lupus (DRL). We report a case of a patient who developed drug-related lupus while receiving infliximab therapy which resolved spontaneously upon discontinuation of the agent and did not recur with subsequent institution of certolizumab. **Methods:** A case was investigated retrospectively and literature was reviewed. **Results:** A 47-year-old man presented with a 2-week history of cutaneous lesions located on his face, polyarthritis, asthenia, and myalgias. The patient had a 7-year history of Cronh´s disease and sacroileitis. The patient was initially treated with azathioprine and steroids with inadequate response. Azathioprine was discontinued, and he received metrotexate during several months without remission of the sacroiliac pain. He was started on infliximab infusions 5 mg/kg every 8 weeks with subsequent remission of his complaints. Seven months after initiation of infliximab, he developed gradual onset of joint pain and swelling in his shoulders, wrists, metacarpophalangeal, proximal interphalangeal-hands, knees, ankles, and spine, in addition to a facial rash, and fatigue. High titres of anticardiolipin

(IgM and IgG) and antibeta-2 glycoprotein I (IgM and IgG) antibodies, were observed. Antinuclear antibodies, and anti-double-stranded DNA antibodies tests were positive. Antibodies to histones were positive. Other serologic tests for autoimmune disorders were negative. A diagnosis of DRL secondary to infliximab was made. Infliximab was discontinued, and prednisone 30 mg/day was added, with rapid resolution of his symptoms. Progressive disappearance of antiphospholipid, antihistones, and antinuclear antibodies, was observed. Subsequently he had a reactivation of Crohn disease, and certolizumab was instituted. The patient's Crohn disease went into remission again, and he had not developed any recurrence of his lupus-like symptoms. **Conclusions:** Physicians should considerer the possibility of DRL in patients under treatment with infliximab who develop arthritis and cutaneous lesions. Our patient tolerated the switch to certolizumab without recurrence of DRL suggesting that the development of DRL secondary to one TNFα inhibitor should not prohibit switching patients from one TNFα inhibitor to another.

PO2.H.6

Lower dose rituximab therapy for refractory thrombocytopenia in patients with systemic lupus erythematosus

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Objectives: To evaluate the safety and efficacy of lower dose rituximab therapy for refractory thrombocytopenia in patients with systemic lupus erythematosus. **Methods:** Eight adult patients with systemic lupus erythematosus were enrolled in this study. The mean platelet count at baseline was 9.5×10^9/L(range $4\text{-}14 \times 10^9$/L). All patients had been resistant to high dose corticosteroids in combination with immunosuppressants(cyclophosphamide or ciclosporine A), or methylprednisolone pulse therapy. Patients were scheduled to receive intravenous rituximab at the dose of 100 mg once weekly for 4 weeks. Previous dose of corticosteroids were continued, while immunosuppressants in all patients were withdrawn. Patients were followed for 12-24 weeks. **Results:** Complete responses (platelet count > 100×10^9/L) were observed in 4/8 (50%) and 3/8 (37.5%) patients at weeks 12 and 24, respectively. Partial responses (platelet count $50\text{-}100 \times 10^9$/L) were observed in 3/8 (37.5%), 0/8(0%) and 1/8(12.5%) patients at weeks 4, 12 and 24, respectively. Overall response rates were both 50% at weeks 12 and 24. Peripheral B cells were depleted (defined as < 5 cells/μl) in all subjects after the rituximab regimen. Infusion reaction was observed in 1 patient, but was of modest intensity and did not require discontinuation of treatment. **Conclusions:** Lower dose rituximab therapy is safe and effective for refractory thrombocytopenia in patients with systemic lupus erythematosus.

PO2.H.7

Rituximab induces a rapid and sustained remission in patients with severe systemic lupus erythematosus refractory to standard immunosuppression

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Objectives: To evaluate the efficacy of rituximab in a cohort of north western Colombian patients with severe SLE; refractory to standard immunosuppression. To determine the relapse time and indications of new rituximab infusions. To describe the complications and adverse effects of rituximab in these patients. **Methods:** An open, prospective, and descriptive cohort study was conducted in patients older than 16 years, with active (SLEDAI score above 8 points), severe (SLEDAI score above 18 points or with life-threating clinical manifestations) and refractory SLE (non-achievement of ACR lupus

nephritis remission criteria or non-reduction of SLEDAI score over 5 points after three months with prednisone 1mg/kg/day). The patients received RTX 1 g IV every two weeks (two doses) and were evaluated quarterly with clinical and laboratory variables before and after rituximab infusion. Data was analyzed statistically with univariate analysis and Friedman´s test. **Results:** The cohort included 31 patients; 67.74% with nephritis and 25.8% with neuropsychiatric SLE. Mean follow-up period of time: 8.5 months (3-18). SLEDAI decreased from 13.25 to 2.48 (80% variation; p < 0.05); creatinine clearance increased from 62.29 mL/min to 99.71 mL/min (59.5% variation; p < 0.001); prednisolone dose was reduced from 60 mg (40-70) to 10 mg (5-15) (68% variation; p=0.001) and 24-hour proteinuria decreased from 2440 mg to 150 mg (94% variation; p < 0.001). Five patients required new RTX infusion witin a period of time of 16.33 months (9-33) after the first dose, due to proteinuria increase (60%) and neurological relapse (20%). Eighteen infections occurred; the urinary tract involvement was the most frequent (11 patients). Two non-severe infusional reactions occurred. One patient died due to acute lupus pneumonitis six months after rituximab infusion. **Conclusions:** A rapid and sustained response was observed with rituximab in patients with severe and refractory SLE, especially in 24-hour proteinuria reduction, as well as renal function. Global control of lupus activity and steroid sparing were also achieved.

PO2.H.8

Good safety profile of rituximab therapy in systemic lupus erythematosus: an observational study over a 5-year period

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The antibody against CD20 Rituximab (RTX) is a novel therapeutic option for patients affected by systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) refractory to conventional immunosuppressive treatment. No studies compared the prevalence of adverse events (AEs) related to RTX in patients affected with SLE respect to RA. We report herein the results of an ongoing, longitudinal, observational study specifically designed to evaluate the safety profile of RTX in patients affected by SLE and to compare these results with those obtained from a cohort of patients affected with RA. **Methods:** We prospectively over 5 years collected data on safety and efficacy of RTX treatment in 21 SLE patients. RTX was administered intravenous with two different protocols. Thirty-one patients affected by RA treated with RTX according to standard protocol were also enrolled. In SLE patients the clinical activity was assessed by SLEDAI and ECLAM scores; in RA patients by DAS28 index. All early and/or late AEs eventually occurred were registered. **Results:** Similar percentages of infections, mainly upper respiratory and urinary tract, were registered, occurring in 2 patients with SLE versus 4 with RA. SLE patients didn't show any infusion related AE, while 7 RA patients suffered from such AE (P=0.04). Premedication, infusion rate, different RTX protocols, concomitant immunosuppressive treatment, disease duration were not related with such higher prevalence of infusion related reactions in RA group. However, when considering the week prior to the infusions, the SLE group received higher weekly prednisone dosage respect to RA (110.1±104.8 mg versus 87.8±65.3 mg, P=n.s.) and, surprisingly enough, when considering separately the 7 RA patients suffering from infusion related reactions, such patients received a much lower mean weekly prednisone dose (54.5±15.6 mg) both when compared with the SLE patients, but also with the 24 remaining RA patients (91.6±70.3 mg). **Conclusions:** The results of this study confirm that repeated cycles of RTX in combination with different immunosuppressant drugs could be a safe therapeutic option in SLE and RA patients. It might be suggested that a higher weekly dosage of corticosteroids could prevent the occurrence of infusion related AEs.

PO2.H.9

Variable responses to retreatment with rituximab and ocrelizumab in SLE: a study with highly sensitive flow cytometry

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All open label case report that an initial cycle of rituximab is effective in resistant SLE. However, time to relapse is unpredictable and there are few reports on response to retreatment. We have previously shown that highly sensitive flow cytometry (HSFC) is predictive of response and relapse in SLE. **Objective:** To report time to relapse and response to retreatment in SLE in relation to B cell subset depletion and repopulation, as measured by highly sensitive flow cytometry. **Patients and Methods:** 41 patients with active SLE despite conventional immunosuppressants were treated with a first cycle of 2 x 1000mg rituximab with oral and intravenous corticosteroids (C1). Highly sensitive flow cytometry (HSFC) was performed as previously described (1). 500 000 events were evaluated by 6 parameter flow cytometry over 2 tests with extensive sequential gating. Total B cell count below 0.0001 x 109/L after both infusions of rituximab defined complete depletion. All patients had BILAG A or 2xB at baseline. Response was defined as improvement to a maximum of 1 BILAG B. Relapse was defined by A/B flare and retreated with rituximab (C2). **Results:** C1: 17/41 patients had complete depletion. 35/41 responded. All non-responders had incomplete depletion (p = 0.030). C1 relapse: Of 28 responders with ≥ 18mo follow up, 14 relapsed within 18mo (median 10mo, "early relapse"). The others had much longer responses (after follow up 20-67mo, 5/14 had relapsed at median 33mo, "sustained response"). These outcomes were predicted by repopulation of memory (p=0.02) and preplasma cells at 6 months (p<0.001). Retreatment of early relapse: 16/17 responded to retreatment. All patients had B cell depletion by conventional flow cytometry. By HSFC, rate of complete depletion rose from 36% to 50%. Estimated median relapse-free survival increased from 42 to 61 weeks (p=0.04). Retreatment of sustained responders: After retreatment 2/5 failed to respond, failed to achieve >50% depletion and had prolonged infusion reactions. However, after retreatment with ocrelizumab both had complete depletion and clinical response. Retreatment of non-responders: 2 C1-non-responders were retreated. Neither responded to C2, and both failed to achieve depletion on retreatment. However one has received ocrelizumab and had complete depletion and response. **Conclusions:** 50% of responders have early relapse, which is predicted by repopulation at 6 months - all these patients responded to retreatment. Patients with longer lasting responses had slower B cell repopulation; however some failed to deplete or respond after retreatment. These patient depleted and responded after ocrelizumab.

PO2.H.10

Rituximab as first option in early severe lupus microangiopathic hemolytic anemia?

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Objectives: Describe a successful case of use of Rituximab as savage therapy for treating Systemic Lupus Erythematosus (SLE) presenting as Microangiopathic Hemolytic Anemia (MAHA). **Methods:** Revision of the patient's medical records and revision of published information concerning the use of Rituximab in auto-imune diseases. **Results:** 17 years old female presenting with new onset of mild thrombocytopenia, severe immuno-hemolytic anemia,with microangyopathy, with hemolysis markers (elevated bilirrubin, serum lactic dehydrogenase and schizocytes), and SLE criteria (polyarthritis, positive ANA and anti-DNA antibodies, lymphopenia, thrombocytopenia and hemolytic anemia). As standard approach, she initiated glucocorticoids (reaching 2mg/kg/day) and daily plasmapheresis, achieving transient clinical improvement (platelets > 150000/uL; hemoglobin > 9 g/dL). At day 7, tapering plasmapheresis to every other day was associated with hematological relapse,

as described in more than 30% of patients, requiring other approach. Considering reports with evidence of prompt and successful response of MAHA to anti-CD20 antibody, in cases worsening on plasma exchange, the patient initiated Rituximab - 2 doses, 15 days apart, and suspending plasmapheresis.72 hours after the first administration, thrombocytopenia remitted (> 150000/uL), without other imunossupression besides prednisolone 1mg/Kg/day, tapered along 3 months to less than 20mg/day. Recovery was complete by the fourth week. 15 months after Rituximab, there were no exacerbations, maintaining only hydroxichloroquine, without glucocorticoid. *Conclusion:* Treatment of MAHA in SLE is not well established, though several options are available. Our patient displayed a fast and sustained – out of steroids – improvement after administration of Rituximab. Further studies are needed in order to consider Rituximab in the initial therapeutic approach, specially in early severe disease, enhancing the probability of long and sustained remission, without major iatrogeny.

PO2.H.11

Is dsDNA vaccination to activate CD4+CD25+ suppressor/regulator T cells in systemic lupus erythematosus a feasible treatment.?

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Basis and Rationale
CD4+ CD25+ FOXPro3 Suppressor/Regulator T cells (T Regs), are depressed in function and or in numbers in SLE1, Rheumatoid Arthritis,2 recurrent pregnancy loss,3 Multiple Sclerosis4, Crohns Disease and Colitis,5 Psoriasis,6 Aging7, Alzheimers Disease,8 Transplantation rejections,9 and also in chronic inflammatory infections where chronic immune stimulation is the norm.- Parvovirus B 19, Hepatitis B and C, Malaria, Leprosy, TB , and HIV AIDs10. When this happens, immune dysregulation, Hyperimmunity and Autoimmunity follows. A need for upregulating T Regs is therefore an important pursuit not just for SLE but for all autoimmune and chronic inflammatory disorders in which deficiency or dysfunction of T Regs has been found. We will show that Mammalian dsDNA is non antigenic for human T cells (normal or Lupus PBMs) when measured by T cell blastogenesis in vitro. It in fact suppresses T celll blastogenesis to memory antigens -(SK/SD): T and B cell mitogens-(PHA, CON A, and PWM); as well as Alloantigens in a dose dependent manner , which is abolished by prior DNAase treatment. The method of this suppression we found to be induction of suppressor/regulator T cells.11 The exact mechanism of this suppression is still unknown. Cell contact, IL 10 and TGFβ have been cited.12 Mammalian DNA differs from bacterial DNA, which is antigenic in vitro,13 and in vivo, in animals and man14, by the presence of CpG motifs in the latter15, which are absent in the former, and methylation of these when present in man.16 This antigenicity has been shown to be dependent, on TOLL receptor 9 activation, endosomal processing and antigen presentation to the TCR and BCR receptors resulting in immune responses.17 Mammalian dsDNA and inhibitory oligonucleotides (ODNs) have also been shown to block stimulatory ODNs at this endosomal site.18 This is the first evidence however that mammalian dsDNA is not only non antigenic, but tolerogenic in vitro, by activating suppressor/regulator T cells in Lupus and normals from PBMs.

PO2.H.12

Efficacy and safety of rituximab in clinical practice

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Objectives: To report the efficacy and safety of rituximab in 15 SLE patients with predominantly non-renal lupus disease activity that had failed multiple immunosuppressive regimes. *Methods:* Disease activity was assessed using the BILAG index, anti-dsDNA antibodies, C3 and C4 levels. BILAG haematological scores were excluded due to uncertainty of attribution to SLE. Complete response (CR) and partial response (PR) were defined as BILAG D/E only and C/D/E in 7 systems, respectively. Rituximab was administered usually as 2 doses of 1000mg with IV cyclophophamide and methylprednisolone 15 days apart and oral prednisolone < 0.5mg/kg/day. All patients showed significant B cell depletion after each course. Serious adverse events were determined retrospectively. *Results:* Mean age ± SD of 15 SLE patients was 37.9 ± 7.2 yrs. Mean disease duration was 8.5 ± 3.3 yrs; 46% Afro-Caribbean, 27% South Asian, 20% Caucasian & 7% other. Within 1 month, 10 patients exhibited CR or PR. By 6 months, 3 patients were unresponsive and 12 had PR or CR and 50% of these had PR/CR for >1year (n=6). The mean time to CR was 3.4 ± 2.3 months in the 8/12 responders (67%). Eleven patients had 2nd course with CR in 64% by six months and 27% lasting >1yr. Time to significant A flare (mean ± SD) after 1st course 7.0 ± 3.9 months was similar to that after 2nd course 7.2 ± 4.3 months. Time to mild B flare was less: 1st course 4.3 ± 4.4 months, 2nd course 4.4 ± 2.3 months. Reduction in daily steroids was significant at 6 and 12 months; 1st course (n=15): mean reduction: 7.6 mg/day by 6 months, 9.2 mg/day by 12 months, 2nd course (n=11): 13.0 mg/day by 6 months, 14.8 mg/day by 12 months. Four patients had a 3rd course, but only 2 patients required 6 courses for neurological disease showing benefit each time. Very few patients had low C3 or C4 levels initially. The 4 patients with raised anti-dsDNA antibodies showed marked reductions & 3 normalized with mean time ± SD of 3 ± 2.5 months after 1st course and all improved with subsequent courses. Admissions for infection were rare affecting only 2 patients after multiple courses. A nonresponder who smoked developed lung cancer >12 months post-rituximab (single course) while on IV cyclophosphamide pulses. *Conclusion:* Rituximab is safe and efficacious treatment for patients with refractory SLE but risk of infection may increase with repeated courses.

PO2.H.13

Effects of rituximab treatment on normal immunological parameters; B cell counts, immunoglobulin levels and functional antibody levels in 9 SLE patients

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Objectives: A retrospective analysis to report the effects of Rituximab treatment on immunological parameters; B cell counts, immunoglobulin subsets and functional antibody levels in 9 SLE patients. In addition, we assessed infections requiring hospital admission following Rituximab treatment. *Methods:* We describe 9 patients treated with Rituximab for whom monitoring of normal immune function comprised B cell counts, immunoglobulin levels (IgG, A M), functional antibodies (Pneumococcal, Haemophilus influenzae b (HiB) and Tetanus). Laboratory data was recorded pre and post treatment with rituximab, usually 1000mg on 2 occasions, 15 days apart, to ascertain the influence of the B cell depleting drug on immunological responses. Effects of rituximab on the disease and autoantibodies have been reported separately. The patients were not routinely immunised prior to receiving rituximab. *Results:* Mean age ± SD of 9 SLE patients was 39.2 ±7.4 years. Mean disease duration was 8.9 ± 3.2 years. Ethnicity of these patients comprised 45% Afro-Caribbean; 33% South Asian; 11% Caucasian; 11% other/mixed. Number of rituximab treatment courses for the patients in the study group were; 1 (n=1), 2 (n=5), 3 (n=1), 6 (n=2). Significant depletion in B cell numbers was seen after every course in these 9 patients. Reduction in immunoglobulins IgG and IgM and functional antibodies was seen in 2 patients who had received 6 courses of rituximab: reduction occurred in pneumococcal antibodies (below the normal level) but preserved antibodies to Tetanus and HiB. Documented infections requiring inpatient treatment (2 episodes each for both patients) comprised lower respiratory tract infection and persistent diarrhoea for the first patient and urinary tract infections and infected leg ulcers for the second patient (who was paraplegic due to transverse myelitis, catheterised and had developed pressure sores with persistent MRSA infection). One patient who had received 2 courses of rituximab had low IgA, sub-optimal levels

of pneumococcal and tetanus antibodies at baseline with low IgM levels and lower functional antibody levels (below normal/protective range) following the first course. However, she continues to do well (12 months after second course) without infections requiring in-patient therapy. *Conclusions:* B Cell depletion was seen in patients regardless of number of courses of Rituximab. Reduced IgM levels were seen in patients with re-treatment (2-6 courses). Pneumococcal antibodies were reduced below the normal levels in both patients who had received 6 courses whilst antibodies to Tetanus and HiB remain at protective levels. Immunisation should be considered in patients prior to receiving rituximab.

PO2I   Management – Non-Pharmacologic

PO2.I.1

Self-management in Thai adolescents with systemic lupus erythematosus
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Self-management (SM) is the key element to improve quality of life in chronic illness children. Systemic Lupus Erythematosus (SLE), a kind of chronic conditions, affects on adolescent health more than other age groups. Minimal knowledge about SM behaviors in adolescents with SLE existed, especially in Thai context. Aim of this study was to explore the patterns of SM behaviors in adolescents with SLE. Eighteen adolescents with SLE, age 12-19 years old, and their parents were interviewed by using in-depth interview. Content analysis was used to analyze saturated qualitative data. Results of this study found that adolescents with SLE had five significant patterns for established SM behaviors for caring their health. First was "Learning from self-experience", adolescents who had the experience of crisis period or exacerbation time must be learned about reality of life and try to have safety life styles. Second was "Self-acceptance". Accepting on uncertainty of illness, changing images, and unpredictable of prognosis, were the concepts that they learned to accept the nature of SLE disease. Adolescents could have the good future plan when they accepted the nature of chronic illness. Then was "Self-empowerment". Empowerment was the key concept to promote adolescents with SLE to develop their good health behaviors. They could develop self-esteem by self-empowerment. "Self-control" was the fourth elements of SM of adolescents with SLE. Adolescents managed the illness by controlling both physical activities, and emotional expression. The last element was "Self-monitoring". This was the strategy that they used to aware how disease flaring. The five elements could be contributed the strategies to promote SM behaviors in adolescents with SLE. The finally results expect that SM behaviors will be improved quality of life in these patients.

Key words : self-management, adolescent, SLE

PO2.I.2

A case of active SLE treated with vitamin D
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Objective: Our case emphasizes the need to consider the role of vitamin D in the treatment of SLE. *Clinical presentation:* Mrs S is a 33-year-old lady who is known to have SLE for eight years, manifested as a butterfly rash, polyarthritis, pleural effusion, tonic-clonic seizures, leucopenia, neutropenia, lymphopenia, positive ANA, positive anti-ds-DNA antibodies and positive anti-Ro and anti-La antibodies. Her disease was controlled on azathioprine and an average daily dose of 7.5 mg prednisolone. Upon reducing the dose of prednisolone one year ago the disease flared with mainly cytopenia, joint pain and fatigue. Because of persistent generalized bony ache 25-hydroxy vitamin D

was measured which showed a level below 10 nmol/L (ideal > 125) and parathyroid hormone was 24.1 pmol/L (normal 1.0-7.5). Serum cacium, alkaline phosphatase, liver and renal functions were normal. She was already receiving 600 mg calcium and 200 IU of vitamin D daily. Treatment with vitamin D was initiated which included three intramuscular injections of 600,000 IU each, given one month apart. *Results:* After starting treatment with vitamin D the patient improved gradually. Fatigue, joint pain and bony ache disappeared, leucocyte count raised from 1.6 to 4.6 X109/L after completing vitamin D treatment, absolute neutrophil count from 0.8 to 3.2, absolute lymphocyte count from 0.4 to 1.1 and hemoglobin from 107 to 12 g/L. She continued to be seizure free. A repeat MRI brain and EEG remained normal. Prednisolone was tapered down and successfully discontinued. Azathioprine was also discontinued. She remained disease free and off treatment except for calcium and vitamin D for six months. *Conclusion:* Vitamin D deficiency should be screened and treated in all patients with SLE. Our case suggests a crucial role for vitamin D in the pathogenesis and treatment of SLE which should be further studied.

PO2.I.3

A randomised controlled study of effectiveness of patient information leaflets on systemic lupus erythematosus (SLE) in improving knowledge on SLE
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Introduction: Providing information about the disease and treatments is important particularly for patients with a complex chronic disease. Providing Patient information leaflets(PILs) is only rarely done in Sri Lanka due to llimited resources. Therefore we developed PILs in Sinhalese and Tamil for SLE patients in the University Lupus Clinic of National Hospital of Sri Lanka.
Objectives: To evaluate the effectiveness of PILs in improving the knowledge on SLE compared to a control group who did not receive leaflets.
Methodology: A patient knowledge questionnaire(PKQ) was developed and validated. Patients who fulfill ACR criteria for diagnosis of SLE were randomised to intervention and control groups by block randmisation. The PKQ was administered to both groups at baseline and after 4-6weeks of giving the leaflets to intervention group. The mean knowledge score(MKS) at baseline and after intervention compared using unpaired t test in SPSS and Graphpad Instat statistical packages. The control group was given the leaflets after administering the PKQ for the 2nd time and steps were taken to shield the control group from leaflets until then. *Results:* Out of 100 participants (48 in Intervention), 89 (41 in intervention) completed the 2nd session. The MKS in the total population at baseline was 38.9 out of 63 (62 %). Although the total MKS was not significantly different between the two groups at baseline, total MKS significantly improved in intervention compared to control group following intervention (Table 1). The baseline knowledge was poor on contraception, pregnancy and side effects of drugs. Significant improvement in knowledge occurred on these aspects and on signs and symptoms, common myths, cyclophosphamide and drug storage (Table 2). Statistically significant improvement occurred in compliance as reported by patients, in the intervention group (SND=3.35, P< 0.001). *Conclusion:* Patient information leaflets provide significant improvement in overall knowledge and on important aspects of SLE.

Table 1

	Control group (%)	Intervention group (%)	P value	Significance
Baseline mean score	39.3 (62%)	41.0 (65%)	0.31	Not Significant
After intervention mean score	45.9 (73%)	53.6 (85%)	0.0001	Extremely significant

Table 2

Aspect of disease (maximum score)	Control group		Intervention group		P value	Significance
	Mean	SD	Mean	SD		
Total score (63)	45.9 (73%)	10.22	53.6	7.14	0.0001	Extremely Significant
Aetiology	3.4	1.23	3.8	1.35	0.1775	Not Significant
Signs and symptoms	4	0.94	4.6	0.54	0.0006	Extremely Significant
Common myths	4.1	1.2	4.6	0.74	0.0166	Significant
Exacerbating factors	4.3	2.06	4.6	0.81	0.4638	Not Significant
Pregnancy and contraception	2.0	1.41	3.0	1.52	0.0021	Very Significant
Complications	3.7	1.08	4.6	0.59	<0.0001	Extremely Significant
Life style modification	7.3	1.32	7.7	0.69	0.0857	Not Significant
Awareness on names of drugs used	4.8	0.66	4.9	0.53	0.442	Not Significant
Reason for use of drugs	4.4	1.16	4.7	0.78	0.11	Not Significant
Cyclophosphamide	1.8	1.75	2.9	1.93	0.0065	Very Significant
Side effects of drugs	3.0	1.43	4.2	0.69	0.0001	Extremly Significant
Storage of drugs	4.4	0.96	4.8	0.63	0.037	Significant

PO2.I.4

Living well with lupus: sacrificing disease management strategies to live a meaningful life?

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Objectives: Research shows that some people live well with CDs despite the economic hardship, pain, suffering, and disability they create. This raises questions about what characterizes these experiences and how they develop in the context of extensive physical and psychological challenges. The objective of this qualitative study was to understand the experience of living well with lupus, MS or scleroderma: specifically, to understand some of the physical, psychological and social processes by which it develops and how these dimensions inter-relate over time. *Methods:* 31 participants (20 years or older who had been diagnosed with lupus, MS or scleroderma for at least 5 years, self-identified as living well, and spoke English) were recruited through advertisements. Interviews were conducted, transcribed and analyzed in an iterative process using grounded theory and interpretive methods. *Results:* Living well was having meaningful experiences amidst the daily struggles of CD. Participants made decisions about how to live with these challenges in relation to their goals and values rather than in relation to just managing, coping or adapting to illness-related difficulties. They used a number of attitudinal and behavioural strategies to attain meaningful experiences and changed their understanding of what was important when then encountered situations and losses they could not overcome. Participants' priorities were not managing disease per se but rather living a fulfilling and meaningful life in the context of CD. Accordingly, they sometimes sacrificed the disease management strategies for maintaining physical well-being because they hindered their ability to experience something of value. *Conclusions:* This study provides a framework for understanding how individuals with lupus and other CDs live well. The value comes from advancing an understanding of truly living well rather than one of coping or managing disease. Findings suggest that it would be beneficial for health care providers to look at self-care decisions in relation to an individual's values and the disease-related challenges they face rather than just medical goals. Being able to provide effective care for those with CDs requires an understanding of approaches that can enable individuals to meet the needs of their disease and bodies within the larger framework of what is important in their lives. Since CD individuals interact with health care professionals on an ongoing basis over many years, this would likely promote better compliance of medically-related treatments and greater acceptance of the times when individuals choose not to follow recommended treatments in order to best meet life goals.

PO2J Management – Traditional Therapies

PO2.J.3

How to make friends with lupus

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Systemic Lupus Erythematosus (SLE), also known as "the Great Imitator", is a chronic disease where the cause and cure are still unknown. In facing a chronic disease, the best approach is not to make the disease into an enemy, but to make it a friend. That way, people living with Lupus, (I will call them "Lupies"), can maintain their energy for "fighting" the disease, stay wise and rational, and retain the ability to find blessings behind the adversity. The key words here are: Befriend Lupus. Ways that we can live with Lupus and make it into a friend can be categorized into two dimensions: those in the physical/ medical dimension and those in the mental/psychological/spiritual dimension. The ultimate aim of a human existence is to be happy here in the world and in the hereafter. Happiness can be defined as freedom from rejection, anger, depression in the form of sadness, unease, anxiety fear and other negative feelings. For those living with Lupus, the ups and downs of their physical condition affect the psyche. For some, this can really affect their psychological condition. For instance, someone with a previously upbeat mental/psychological/ spiritual condition can decline as their physical health deteriorates (accompanying a prolonged physical disease), resulting in a prolonged sadness. This condition should be avoided. Those with low spiritual and psychological conditions feel emptiness and cannot experience happiness even when their physical health is good or there is no physical pain. In this condition, emptiness and unhappiness can still show. Conversely, some whose mental/ psychological/spiritual condition is very good are not robbed of their happiness but their physical condition deteriorates. Although "the body is sick, the soul remains healthy". Those who have good psychological and spiritual conditions are serene and remain positive even when their physical condition is low. Therefore, for Lupies whose health is always in yoyo, it is important to aim to keep in a good mental/psychological/spiritual condition. It is clear that Lupies need to synergize their efforts in maintaining these two dimensions - the physical and the mental/psychological/spiritual - in a balance to achieve happiness and quality of life. A matrix of combination between high/ low physical condition and high/ low mental/psychological/spiritual condition was develop. Ten (10) tips how to make friend with Lupus are discussed that cover both dimensions.

* The writer has been living with lupus since 1999 and now lives with only 5 percent of her vision. She has dedicated herself to supporting friends living with Lupus through Care for Lupus and Care for Low Vision support group under the umbrella of the Syamsi Dhuha Foundation, of which she is the chairwoman. She lives in Bandung, Indonesia.

PO2.J.4

Intentional and unintentional treatment non-adherence in patients with systemic lupus erythematosus

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Objective: Patients may be defined as non-adherent if they do not take their medications as prescribed by their physicians. Non-adherence can be divided into intentional non-adherence, e.g., taking less than instructed, or unintentional non-adherence, i.e., forgetting to take the medications. Underlying

causes for non-adherence may vary between and within patient groups. This study investigated the extent to which patients with systemic lupus erythematosus (SLE) show intentional and unintentional non-adherence, and the associations with psychological and medical parameters. *Methods:* The study included 106 patients who were on at least one immunosuppressive agent to control their SLE. Level of self-reported adherence was assessed with the Medication Adherence Self-Report Inventory (MASRI). The distinction between intentional and unintentional non-adherence was made using the Medication Adherence Response Scale (MARS). Questionnaires were completed to assess the association between adherence measures and psychological characteristics: the Cognitive Symptoms Inventory (CSI), the Beliefs about Medicines Questionnaire (BMQ), the Brief Illness Perception Questionnaire (B-IPQ), and the Emotional Health domain of the LupusQol. The SLE Disease Activity Index (SLEDAI) was used to measure disease activity at the time of assessment. *Results:* The mean self-reported adherence rate for the total patient group was 86.7%. At least occasional intentional non-adherence was reported by 46.2% of patients and 58.5% of patients were at least occasionally unintentionally non-adherent. Patients who reported low adherence rates were more likely to experience problems with cognitive functioning, to show concerns about potential negative effects of their SLE medication, and to be affected emotionally by their SLE. The distinction between intentional and unintentional non-adherence showed that both were associated with stronger concerns about possible side effects, but only unintentional non-adherence was associated with problems with cognitive functioning. Compared with patients who were never intentionally non-adherent, intentional non-adherers had lower emotional health and had stronger beliefs that physicians overuse medicines in general. Disease activity showed no relationship with measures of (non-)adherence. *Conclusion:* Although SLE patients report high adherence levels on average, they commonly report intentional and unintentional non-adherence. Adherence measures were associated with both cognitions and emotions. Non-adherence may be reduced by targeting emotional and cognitive functioning and by fine-tuning doctor-patient communication to address patients' individual concerns about their medications.

PO2.J.5

Systematic review and meta-analysis of randomized trials of mycophenolic acid/ mycophenolate mofetil for induction treatment of lupus nephritis

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Objective: To systematically review the efficacy and safety of Mycophenolic Acid (MPA) and Mycophenolate Mofetil (MMF) compared to Cyclophosphamide (CYC) for the induction treatment of lupus nephritis (LN). *Methods:* MEDLINE, EMBASE, the Cochrane Center Register of Controlled Trials and abstracts presented in major international conferences were searched for randomized controlled trials without language restriction. The primary outcome was renal remission (complete, partial and overall); secondary outcomes were adverse events and long-term follow up data. *Results:* Four trials of total 618 patients were identified. We observed no significant difference for renal remission (partial RR 0.94, 95% CI 0.80 to 1.12; complete RR 0.67, 95% CI 0.35 to 1.28 and overall RR 0.89, 95% CI 0.71 to 1.10) comparing MMF with CYC. There was a significant reduction in alopecia and amenorrhea when MMF was compared to CYC. There was no significant difference for infections, leucopenia, gastrointestinal symptoms and herpes zoster. The analysis of the extended follow-up data showed a significantly lower number of subjects with ESRD but not death when MMF was compared to CYC. *Conclusion:* There was no significant difference for the induction treatment of LN when comparing MMF to CYC. Patients treated with MMF showed reduced risk for alopecia, amenorrhea. MMF is an alternative to CYC for the treatment of LN.

Outcomes	Number of studies	Number of participants	Relative risk
Partial remission	4	310/308	RR 0.94, 95% CI 0.80 to 1.12
Complete remission	4	310/308	RR 0.67, 95% CI 0.35 to 1.28
Overall remission	4	310/308	RR 0.89, 95% CI 0.71 to 1.10
Leucopenia	3	243/235	RR 1.29, 95% O.35 to 4.70 p=0.07 I2=81%
Infections	4	310/308	RR 1.56, 95%0.66 to 3.69
Gastrointestinal	4	310/308	RR 0.74, 95% 0.54 to 1.02
Herpes zoster	4	310/308	RR 0.88, 95% 0.43 to 1.80
Amenorrhea	4	310/308	RR 6.64, 95% 2.00 to 22.07
Alopecia	3	285/289	RR 5.77, 95% 1.56 to 21.38
ESRD	4	310/308	RR 2.06, 95% 1.07 to 3.98
Death	4	310/308	RR 1.15, 95% 0.47 to 2.82

PO2.J.6

Withdrawal of immunosoppressive therapy in lupus nephritis: an achievable goal?

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Introduction: The treatment of diffuse proliferative glomerulonephritis (DPGN) is largely represented by an induction phase based on the administration of cyclophosphamide (CYC) followed by a maintenance phase during which different drugs such as Azathioprine, Mycophenolate or Cyclosporin have proved to be effective. Little data exist on the optimal duration of the maintenance therapy and on the withdrawal of immunosuppressive drugs. *Objectives:* The aim of the present study was to evaluate the long term evolution of the disease in SLE patients with LN in whom immunosuppressive therapy was withdrawn at a certain point of the disease course. *Methods:* Thirty SLE patients with DPGN and treated with pulse steroids and a mean of 7 monthly pulses of CYC not followed by additional immunosuppressive therapy, were included in this analysis. Complete response was defined as a normal serum creatinine, proteinuria < 500 mg/24hours and normal urinary sediment at the end of therapy; a partial response was defined as reduction of proteinuria with values > 500 mg/24 hours with normal urinary sediment, and stable serum creatinine values. Renal flares (RF) were defined as an increase of at least two of the following parameters after a remission was achieved: dysmorphic erythrocytes, cellular casts, proteinuria, serum creatinine. A poor renal outcome was defined as the doubling of serum creatinine. *Results:* Three patients did not respond to therapy and developed ESRD and were excluded from the analysis; 17 patients had a complete and 10 a partial response to therapy. Fourteen patients (52%) presented at least one RF after a mean period of 30.3 months after the withdrawal of immunosuppressive therapy. Thirteen patients did not present RF after a mean follow up of 141 months after the suspension of CYC. The occurrence of RF was associated with a partial response to therapy and a younger age at kidney biopsy (p=0.036); in addition patients who relapsed tended to have higher AI. A poor renal outcome was correlated with an earlier occurrence of the first renal flare (within 12 months after withdrawal) and with the cumulative number of renal flares (p= 0.03). *Conclusions:* These data suggest that in selected patients with low activity on renal biopsy and with a complete response to CYC therapy, IS therapy can be stopped with a good control of LN and suggest the possibility of tailoring immunosuppressive therapy on the basis of prognostic factor at baseline.

PO2.J.7

Evidence for antimalarial beneficial effect in cardiac arrhythmic events in systemic lupus erythematosus (SLE)

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Objective: There are no prevalence studies of arrhythmias and conduction disturbances in a large SLE population determining the influence of disease factors and long-term antimalarial use. *Methods:* Three-hundred seventeen consecutive SLE patients (ACR criteria) age>18 y were evaluated by resting-ECG and 24-hour Holter monitoring (n=142, randomly selected) for arrhythmia, conduction disturbances, heart rate variability, and repolarization parameters. Data were obtained in an ongoing electronic database protocol which consists of an extensive clinical/ laboratorial/treatment evaluation. *Results:* The majority was female (91%) with medians of age and disease duration of 40.25 years and 11.36 years, respectively. Renal involvement was observed in 26.2% and hypertension in 18.9%. Antimalarial therapy (minimum of 6 months) was observed in 69% with a mean duration of 8.47 ± 6.74 years. Resting-ECG abnormalities were detected in 66 patients (20.8%): long QT in 14.2%; RightBBB in 1.9%; LeftBBB in 0.6%; 1st degree AV-block in 1.6%; sinus bradycardia in 1.3%; sinus tachycardia in 1.3%, and supraventricular tachycardia in 0.3%. Prolonged PR interval was associated with less chloroquine use (p=0.01), shorter chloroquine treatment duration (1.00 ± 2.45 vs. 6.10 ± 6.88 years, p=0.018) and older age (54.17 ± 7.33 vs. 42.26 ± 13.25 years, p=0.029). Holter monitoring events were observed in 121 patients (85.2%): HR<50bpm in 31.6%; pauses>2.0s in 2.8%; atrial tachyarrhythmia in 18.3%; atrial isolated ectopies in 63.4%, ventricular ectopies in 45.7%, and ventricular tachycardia in 2.8%. Tachyarrhythmias were associated with shorter chloroquine treatment duration (7.05 ± 7.99 vs. 3.63 ± 5.02 years, p=0.043) with a trend to less use of chloroquine (p=0.054), and older age (40.19 ± 11.54 vs. 52.50 ± 12.02 years, p<0.001). Clinical and laboratorial variables such as renal and cardiac insufficiency, hypertension, and anti-La/SS-B were not associated with conduction abnormalities (p>0.05), except for anti-Ro/SS-A with an association with supraventricular arrhythmia (p=0.042). Logistic regression model revealed that predictors for supraventricular tachyarrhythmia (AT/AF) were age (p<0.001; OR=1.100; IC95%=1.050-1.154) and shorter antimalarial use (p=0.035; OR=0.921; IC95%=0.853-0.994). *Conclusions:* Antimalarials seem to have a protective role in the unexpected high rate of cardiac arrhythmias and conduction disturbances observed in SLE. Further studies are necessary to determine if this anti-arrythmogenic effect is due to the disease control or a direct effect of the drug.

PO2.J.8

Outcome of mycophenolate mophetil (MMF) treatment in patients with proliferative lupus nephritis (LN)

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Introduction: LN is a major cause of morbidity and mortality in SLE. MMF is an immunomodulatory agent that has been shown to be effective in LN. *Patients and Methods:* We retrospectively analysed 63 SLE patients with proliferative LN (class III and IV) that have received MMF for 6 months or longer. Demographic and clinical-laboratory data were obtained from patient records. The clinical status (complete response (CR), partial response (PR), and nonresponse (NR)) as suggested by Boumpas and Balow was determined at the 6th and 12th months and at the last doses of induction, maintenance and re-induction therapies. *Results:* Of all the biopsies, 22% showed WHO Class III and 78% Class IV LN. MMF was used for early maintenance in 33 patients with CR, for non-CR/flares in 27 and for remission induction in 3.

The mean time of MMF treatment was 28 months and the mean dose was 2g. In all MMF groups, comparisons were made in CR (n=37) and no CR (n=26) groups. Mean proteinuria at the onset of MMF treatment and the number of patients presenting with active urinary sediment in no CR group was significantly higher (0.2 ± 0.9 vs 1.6 ± 1.8; p=0,002; 74% vs 94%, p=0.002) and class IV histopathology in renal biopsies of no CR group was significantly prevalent (92 % vs 59 %, p=0.002). Activity and chronicity indices did not show any statistically significant relationship with remission status. There was a total of 14 patients who experienced a flare and the number of patients who were on MMF for early maintenance was significantly higher in non-flare group compared to flare (+) group (68% vs 23%, p= 0.004, OR=0.14). 6 patients developed chronic renal failure (CRF) (4 on hemodialysis and 3 transplanted). Nephrotic range proteinuria, hypertension and hemodialysis at presentation were significantly higher in CRF group compared to non-CRF group (83% vs 28, p=0.013, OR=4.3; 100% vs 33, p=0.033, OR=0.3; 50% vs 4, p=0.005, OR=0.07 respectively). The biopsies of all patients with CRF were proliferative nephritis with high activity and low chronicity scores. *Conclusion:* None of our patients that reached CR and given MMF for early maintenance had a flare. MMF may be the drug of choice for early maintenance after CR considering the favorable safety profile. Class IV histopathology was frequent in patients without CR . Nephrotic range proteinuria, hypertension and hemodialysis at presentation were the risk factors associated with the development of chronic renal failure.

PO2K Management – Treatment in Development

PO2.K.3

A single arm, open label study to evaluate the safety, tolerability and short term efficacy of extracorporeal specific immunoadsorption on the LUPUSORBTM column for the treatment of SLE patients

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Purpose: We have previously shown that Systemic Lupus Erythematosus (SLE) patients have autoantibodies that bind VRT 101, a 21-mer peptide located at the globular part of the laminin alpha chain. Anti-VRT 101 level correlated with disease activity, and these antibodies could be removed in vitro on VRT 101-Sepharose beads (the LUPUSORBTM column). Our purpose was to evaluate the safety, tolerability and short term efficacy of extracorporeal specific immunoadsorption on the LUPUSORBTM column for the treatment of SLE patients. *Methods:* SLE patients were treated with a single LupusorbTM immunoadsorption session using a routine plasmapheresis procedure. Patients were followed up for 8 weeks after the apheresis. Primary endpoints were safety, tolerability and short term efficacy on SLE serological markers. Secondary endpoints included SLE activity and the kinetics of anti-VRT101 serum levels. *Results:* Ten SLE patients were enrolled (9 females; mean age 36.2), with a mean duration of disease of 7.2 years, and mean SLE-DAI of 10.4 at baseline. Patients were on stable treatment in the three months before the procedure and during the 8 weeks of follow up. Toxicity: Three non-severe adverse events were related to the apheresis treatment including myalgia, dyspnea, and leg cramps. *Efficacy:* Mean serum anti VRT 101 level decreased significantly from 0.8 at baseline to 0.48 at week 3 (P value < 0.009) and gradually increased up to 0.59 at week 8. Anti-DNA levels decreased from 2.1 to 1.22 (µg/ml) 8 weeks after the treatment. Mean CRP decreased from 6 (mg/dl) at baseline to 4.6 (mg/dl) at week 3 and returned to 5.3 (mg/dl) at weeks 4-8. Mean of C4 and C3 increased from 18 / 88 mg/dL at baseline to 19.5 / 93.2 mg/dL respectively after three weeks and decreased to 17.4 / 89.8 mg/dL respectively at week 8. Mean SLEDAI decreased from 10.4 at baseline to 8.4 at week 3 and to 8.2 after 8 weeks. Mean of 24 hours urinary protein excretion decreased from 350 mg to 240 mg at week 3 and increased to 480 mg after 8 weeks. These changes did not reach statistical significance. *Conclusions:* Treatment with anti-VRT 101 extracorporeal plasmapheresis for SLE is feasible and safe. Anti VRT 101 levels decreased significantly 3 weeks

after the apheresis and increased gradually from week 4, with no rebound phenomenon. A decrease in SLEDAI, CRP and proteinuria and an increase in complement levels was seen on week 3. A decrease in anti DNA level was observed on week 8. Based on these results we are now planning a pivotal study to determine the efficacy of repeated apheresis sessions of the LupusorbTM column in SLE patients.

PO2.K.4

Sustained stabilization of disease activity in lupus nephritis treated with prolonged immunoadsorption (IAS)

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Objective: Pathogenic autoantibodies and immune complexes are a hallmark of SLE. They can effectively be removed by extracorporeal procedures such as IAS. After up to one year of IAS, we had previously observed a reduction of proteinuria, disease activity and pre-treatment autoantibody levels in highly active SLE with renal involvement and contraindications or refractoriness to cyclophosphamide (CYC). Antibody removal, however, does not block the formation of new autoantibodies; thus, a large proportion of these patients underwent prolonged IAS (>1 yr for up to 10yrs) and are the focus of this report. We evaluated patients under prolonged IAS for sustainability or further improvement of the primary response to IAS (proteinuria, disease activity, anti-dsDNA-Abs) and for the number of flares, infections, adverse events and tumors. **Patients and Methods:** IAS therapy was started in highly active SLE patients with lupus nephritis (proteinuria 7.1±4.8 g/day, SIS 15±6, SLEDAI 20±8, anti-dsDNA 394±712 IU/ml) if i.v. CYC was contraindicated or not effective enough to control disease activity. 13 patients responding to initial IAS therapy were included into the prolonged program, showing moderate disease activity at the start of the extension period (proteinuria 2.0±2.4, SIS 4±2, SLEDAI 3±2, anti-dsDNA 47±36 IU/ml). We defined the end of observation (EoO) upon either completion of 10 yrs of IAS therapy or by 1 JAN 2009. During IAS, oral immunosuppression and ACE/ATII-inhibitors were kept constant, steroids were tapered as clinically feasible. IAS was performed with high affinity columns and the effective removal of serum Ig was monitored. Severe infections were defined as requiring i.v. therapy or hospitalization, flares according to the SELENA protocol. **Results:** Under prolonged IAS (mean observation period of 6.7±3.5 years), proteinuria further decreased from 2.0±2.4 g/d to 0.9±1.7g/d (p<0.05) at the EoO while the Creatinine clearence increased to normal ranges in all patients. Disease activity and anti-dsDNA levels could be stabilized at low levels (SIS3±3 , SLEDAI 3±4, anti-dsDNA 26±24 IU/ml at EoO). Complete remission (proteinuria <0.5g/d, SIS/SLEDAI ≤4, pre-treatment dsDNA <25 IU/ml) was achieved in 9 (69%) patients. One patient flared and was discontinued. Ten (77%) patients are still under IAS therapy at the EoO. In 2 patients, IAS was stopped because of a sustained response. Severe infections (0.1±0.3 per patient year) and severe flares (0.1±0.2 per patient year) were uncommon. Tumors, anaphylactic or orthostatic adverse events were not observed. **Conclusion:** Prolonged IAS leads to stabilization of disease activity in moderately active SLE patients and can induce sustained remission in previously refractory SLE while showing an acceptable safety profile.

PO2.K.5

The induction of apoptotic cells by ultraviolet A1 irradiation on spontaneous lupus erythematosus-like skin lesions in MRL/lpr mice

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In skin lesions of systemic lupus erythematosus (SLE), the histamine-N-methyltransferase (HMT) activity is much lower than that of controls, and the decreased activity plays a particular role in the development of immune-complex-mediated skin lesions (Furukawa, Dermatologica 1989). We confirmed that the expression of histamine 2 receptor (H2R) was seen on infiltrated mast cells in spontaneous lupus erythematosus (LE)-like skin lesions of MRL/lpr mice (Yoshimasu, Bentham Open J Dermatol 2008). The skin lesions of lupus erythematosus (LE) is known to be exacerbated by ultraviolet B light (UVBL). It is still obscure the effects of ultraviolet-A1 (UVA1) on the skin lesions of LE. **Objectives:** We investigated the effects of UVA1 irradiation on the skin lesions of MRL/lpr mice, using a disease-prevention model. **Methods:** All these mice were females and 4-6weeks old at the start of an experiment, and 58 mice were randomly divided into 3 groups including non-treatment (22 mice), 5 J/cm2 of UVA1 (18 mice) and 10 J/cm2 of UVA1 (18 mice). UVA1 was irradiated on shaved dorsal skin of these mice at 5 times / week, and the irradiation was continued 4months. We examined clinical changes, immunohisitochemical staining of the skin and anti-nuclear antibody in sera. **Results:** LE-like skin lesions were developed at 27.3% (6/22) in non-treatment group of MRL/lpr mice, whereas both UVA1 irradiation groups of 5 J/cm2 and 10 J/cm2 did not develop skin lesions at 0/18 and 0/18 respectively. UVA1 irradiation significantly inhibited the development of LE-like skin lesions, without obvious changes of the disease including renal disease, serum anti-nuclear antibody levels, and mortality. The number of mast cells in MRL/lpr skin lesions was significantly increased than those of UVA1-irradiated (5 J/cm2 or 10 J/cm2) non lesional skin by toluidine blue staining (p<0.05). Although apoptotic cells were remarkably seen in the dermis of UVA1-irradiated non-lesional skin, those cells were hardly detectable in the dermis of the non-irradiated non-lesions skin by tunnel staining. Further analysis showed that some of those apoptotic cells were mast cells. **Conclusion:** UVA1 irradiation inhibited the development of MRL/lpr skin lesions and induced the apoptosis of pathogenic mast cells.

PO2.K.6

Body image intervention improves health outcomes in systemic lupus erythematosus

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Systemic Lupus Erythematosus (SLE), a multi-systemic autoimmune disease of young women. It can be potentially disfiguring, involve multiple organs and adversely affect their physical and emotional health. Our previous work shows Body Image (BI) is poor among women with SLE and is predictive of poor health outcomes. **Aims:** To determine if BI intervention leads to an improvement in (1) BI and (2) Health outcomes, among women with SLE. **Methods:** Body Image Intervention comprising of education, cognitive behavioral therapy and cosmetic training was offered to 13 SLE patients once a week for 10 weeks. Each session lasted 1hr and 45 minutes. 9 SLE patients were followed without any intervention. Body Image in Lupus Screen (BILS) and Body Area Satisfaction Scale (BASS) were used to measure BI. Center for Epidemiological Studies Depression (CES-D), Self Esteem (SE), Positive and Negative anxiety Scale (PANAS), State Trait Anxiety Scale, LupusPRO based Lupus Symptom Scale (LSS) and Non HRQOL scores were used for health outcomes. Data was obtained baseline, post intervention, followed by

8 and 14 weeks post intervention. Descriptive statistics and longitudinal data analysis were performed. P value of ≤ 0.05 was considered significant on two tailed test. *Results:* The mean age of the intervention and control group were 42.7±10.9 and 39.7±7.8 yrs respectively. Body Mass Index, disease duration and disease activity were not different between the study groups or time periods. BILS and BASS (Table 1) improved over time in the intervention group. Also noted were improvements in depression (CES-D), Lupus Symptom Scale (LSS) and N-HRQOL in the intervention group, while worsening in self esteem (SE), positive PANAS and Lupus Symptom Scale (LSS) were noted over time in the control group (Table 1).On longitudinal analysis, change in BI lead to changes in SE, CES-D and State Anxiety over the study duration. *Conclusions:* Body Image is modifiable in SLE. The proposed intervention was successful; improvements in BI persisted at 14 weeks after the intervention and lead to significant improvements in other health outcomes. Further research is ongoing.

PO2.K.7

Octreotide improves systemic lupus erythematosus:
an *in vitro* study on its therapeutic mechanism

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Background and Objectives: Our previous study has shown that increased serum level of growth hormone (GH), together with high expression of its receptor on peripheral blood mononuclear cells (PBMCs), correlates with SLE activity. On this basis, we conducted a pilot study treating SLE with Octreotide (OCT), an analog of somatostatin which could suppress the release of GH. Interestingly, in this randomized, prednisone-controlled clinical trial, we observed that patients in OCT group obtained a favorable improvement in SLEDAI, dsDNA titer, complement levels and ESR. This finding prompted us to further study the therapeutic mechanism of OCT in treating SLE. The objective of this study is to investigate effects of OCT on the *in vitro* proliferative capacity and cytokine expression of PBMCs from patients with SLE. *Methods:* PBMCs from 13 active and 13 inactive SLE patients as well as 11 healthy controls were cultured with various concentrations of OCT, pretreated with or without GH. The proliferative capacity of PBMCs was detected by MTT assay. Cytokine levels in PBMCs were quantified by ELISA. *Results:* OCT inhibited PBMCs proliferation in a dose-dependent manner: from 0.1, 1 to 10ng/mL with no, slightly and markedly inhibitory effects respectively. The secretion levels of IL-6, IL-10 and IFN-γ in PBMCs treated with OCT were significantly decreased than those in untreated groups. Such decreases were more obvious in groups treated with both GH and OCT rather than those with GH only. *Conclusion:* OCT extensively inhibited PBMC proliferation and its secretion of IL-6, IL-10 and IFN-γ stimulated by GH. This may indicate the therapeutic mechanism of OCT in patients with SLE.

PO2.K.8

Mechanisms for the induction and treatment of lupus
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Silica and EBV virus are the dominant risk factors cited for the causation of Lupus. The IL1/TIR/ Toll R superfamily, and their cytokine products are critical in the balance achieved between the effector Th 1 & Th 17 T cells, and the suppressor regulator T cells (T regs). Thus the pro-inflammatory IL-6, in the presence of TGFβ, generates Th-17 cells, while IL-6, abrogates T Regs. (Pasare & Medzhitov). Loss of T Reg function is permissive to autoantibody formation, epitope scpreading, antibody avidity enhancement, and immune complex formation, the hallmark of SLE. IL-18 is elevated in SLE and found in the blood, kidneys, and urine of SLE patients. It increases IFNγ, IL 2, IL 12, potentiating Th 1 and TH-17 responses. With less affects on Th 2 responses.

IL-18 together with IL-1 are the products of the NALP 3 inflammasome. The known activators of this are silica, aluminum, titanium, UVL, amyloid β protein, and the crystals of uric acid, and calcium pyrophosphate dehydrate. These are notably non protein non peptide triggers of inflammation and are ubiquitously present in our environment, vaccines, implanted materials, cosmetics, and as ceramics in dental veneers, and in all tooth pastes. And in all medications as inert FDA approved ingredients. While genes are fundamentally important, the environmental challenges are ever more complex and need increased emphasis, as evidenced by the recent citings, of lipsticks, pesticides and herbicides as SLE risk factors. We propose a 2 point strategy once these triggers are eliminated: (1) Use of anti interleukins which will down regulate IL-6, IL-1, IL-17& IL-18 followed by (2) dsDNA vaccination with methylated dsDNA to upregulate T Regs (Patent Pending). Hydroxy chloroquine has now been shown to reduce the levels of IL-18. Tocilizumbab an anti IL-6 R monoclonal is now available for treatment of RA. IL-18R, and IL-18 binding protein, IL-1 RA, as Kineret or Rilonacept also. Monoclonal anti IL-17 & IL-18 antibodies are in development.

PO2L   Management – Microparticles

PO2.L.1

Distinct features of circulating microparticles
in systemic lupus erythematosus
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Objectives: Microparticles (MPs) are small membraneous vesicles released by apoptotic or activated cells. Circulating MPs may exert regulatory functions in inflammation, immunity, and cancer. Hypothetically, the occurrence of systemic autoimmunity in systemic lupus erythematosus (SLE) has been linked to defective clearance of apoptotic cells and cell fragments. Thus we here investigate the number, origin, and characteristics of circulating MPs in a group of well-characterized SLE patients. *Methods:* Seventy-one SLE patients and 29 matched healthy controls were included. MPs were isolated from citrate blood by differential centrifugation and analyzed by flow cytometry. Gating was accomplished using size standards. The numbers of unlabelled MPs and MPs binding Annexin V (Anx V MPs), anti-CD42a (PMPs, platelet-derived MPs), anti-CD146 (EMPs, endothelial cell-derived MPs), anti-CD45 (LMPs, leukocyte-derived MPs) and anti-human IgG were obtained. *Results:* The MP preparations were estimated by sizing and transmission electron microscopy to be consistent with heterogeneous populations of membranous bodies measuring less than 1 μm in diameter. The total number of MP counts and proportions of EMPs and LMPs did not differ between patients and controls. However, highly significant (p<0.0001) reductions of the fractions of Annexin V-binding MPs and PMP were observed in SLE patients. This was paralled by a pronounced increase in the SLE-MPs of small, granular MPs negative for the tested surface markers. Thus, there were significant correlations between Annexin V-negativity and small sized,highly granular MPs in SLE patients. Also, a significant increase (p=0.02) of MPs negative for other markers but positive for IgG was found in the SLE patients. *Conclusions:* SLE-patients have a qualitatively and quantitatively different circulating MP profile than healthy controls. This SLE-MP profile is characterized by highly significantly reduced fractions of Anx V-binding MPs and platelet-derived MPs. Instead, highly increased populations of small granular MPs negative for the surface markers of this study were observed. SLE patients also had significantly higher numbers of IgG positive MPs of unknown origin. While reliable sizing of such small particles is difficult by flow cytometry the reduced Annexin V binding and the size range in which they are found suggest these particles may be of exosomal origin. Ongoing studies focus on identifying the nature of these small particles, the specificity of the associated IgG and clinical correlates.

PO2M Overlap Syndromes

PO2.M.1

True undifferentiated connective tissue diseases, proposal for diagnostic criteria

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In previous studies we have proposed to define as Undifferentiated Connective Tissue Diseases (UCTD) conditions characterized by (i) Signs and symptoms suggestive of connective tissue diseases, not fulfilling criteria for defined CTD and (ii) Positive ANA in 2 different determinations. This definition identifies clinically different conditions. In fact, it may refer to systemic autoimmune diseases which cannot be classified according with the existing classification criteria but clinically diagnosed conditions (incomplete connective tissue diseases), to overlap syndromes and finally systemic autoimmune diseases which cannot be classified nor diagnosed on the basis of clinical manifestations. Aim of the present analysis is to describe a cohort of UCTD patients which cannot be classified nor diagnosed as defined CTD and to attempt to develop a set of preliminary diagnostic criteria for these conditions. One hundred patients with a diagnosis of UCTD with a disease duration of more than 60 months (mean follow up 134.9 months, min 60- max 372 months) and an undefined clinical picture were identified and were analyzed. The observed clinical manifestations resulted: arthralgias 85%, Raynaud's phenomenon 52%, arthritis 44%, leukopenia 41%, sicca syndrome 36%, photosensitivity 27%, fatigue 23%, fever 28%, serositis 14%, cutaneous rash 11%, thrombocytopenia 10%, lymphadenopathy 9%, malar rash 9%, livedo reticularis 7%, haemolytic anaemia 2%. Antinuclear antibodies were positive in all patients; additionally the following specificities were identified: anti-Ro 31%, anti- RNP 19%, anti- dsDNA 12%, aCLA 15%. Over the follow up these patients remained oligosymptomatic, did not develop major organ involvement and maintained an unchanged autoantibody profile. This analysis clearly shows the existence of patients with systemic autoimmune diseases which cannot be classified according with the existing classification criteria nor can be diagnosed as defined CTD. It is our opinion that this subgroup represents the true UCTD. We therefore propose the following changes in the previously published criteria: UCTD are conditions characterized by: (i) Signs and symptoms suggestive of connective tissue diseases, not fulfilling criteria for defined CTD and not clinically diagnosed as defined CTD, (ii) positive ANA in 2 different determinations, (iii) stability of the undifferentiated clinical picture for 3 years. Patients with a clinical picture suggestive of defined CTD "spectrum" (e.g. scleroderma, anti-phospholipid antibodies spectrum) should not be included among UCTD. Further analysis is underway to better define the clinical and serological exclusion criteria based on a systematic literature review.

PO2.M.2

Renal amyloid deposition in lupus nephritis

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Objectives: Amyloidosis in patients with lupus nephritis is a very rare finding. Only 26 cases have been described in the literature so far. We encountered a 52-year old female patient with renal AA-amyloidosis after a 21 year history of SLE. In a period of nine months two biopsies were taken. The first biopsy showed class IV lupus nephritis, consistent with clinical parameters at that time. The second biopsy, however, showed overt amyloidosis of AA-amyloid, mainly in glomeruli, in the absence of active lupus nephritis. In retrospect, minute amounts of amyloid were demonstrated with an AA-amyloid staining in the first biopsy. This case led us to investigate if AA-amyloid (as stained for by immunohistochemistry) is present in renal biopsies of patients with lupus nephritis, in cases without blunt light microscopic changes. *Methods:* Renal biopsies of 27 patients with lupus nephritis were stained for the presence of AA-amyloid. The time between SLE diagnosis and biopsy ranged from zero to sixteen years, with a mean of four years. Patients had a mean age of 31, ranging from 17 to 50, at time of biopsy. *Results:* AA-amyloid was not present in glomeruli or vessels in the renal biopsies of these patients. However, in all patients except one, the AA-amyloid staining showed positivity in a granular staining pattern in tubes, both at intra-epithelial sites and colocalizing with the brush border of proximal tubes. It is likely that this positivity reflects the presence of the precursor protein of AA-amyloid, but further investigations are necessary to establish whether in some cases, AA-amyloid is in fact present in the tubes. A preliminary control study of 5 cases with over proteinuria in the absence of SLE and clinical signs of amyloid, did not show the staining patterns observed in the patients with SLE. *Conclusions:* AA-amyloid in lupus nephritis is a rare finding. We describe a case with massive amyloidosis in the renal biopsy, 9 months after an initial biopsy was only demonstrated positive for AA-amyloid in retrospect. In this series, we investigated the presence of amyloid in 26 patients with lupus nephritis. Glomeruli and vessels were negative, however, there the AA-amyloid staining showed granular positivity in almost all patients in the tubes. We are currently investigating what this pattern signifies. AA-amyloidosis should be considered in patients with SLE, particularly in those with nephrotic syndrome who do not improve after immunosuppressive therapy.

PO2N Paediatric Lupus

PO2.N.7

Prescribing practices in juvenile-onset lupus: the 1000 Canadian Faces of Lupus study

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Purpose: Systemic lupus erythematosus (SLE) in children is a severe disease requiring aggressive treatment. Factors associated with prescription of immunosuppressive (IS) drugs have not been identified. This study describes current medication prescribing practices in 4 pediatric rheumatology centres in Canada (Vancouver, Montreal, Toronto, Halifax) participating in a longitudinal outcome study of lupus. *Methods:* Between 2005-2008, children and adolescents with study baseline visits within one year of SLE diagnosis were selected for analysis. Data included demographics, disease activity, physician global assessment by VAS (MDVAS) and medications prescribed within the previous 4 wks. Logistic regression was performed to assess the relationship between organ system disease (renal, neurologic, serositis, hematologic) and specific medication use; medications included were prednisone, hydroxychloroquine, azathioprine (aza), cyclophosphamide (cyclo), methotrexate (mtx), and mycophenolate mofetil(mmf). *Results:* One hundred thirteen patients were included in this analysis (median age 17 yr, range 7-21 yr, IQR 16-19; F 83%: M 17%; median SLEDAI 2, IQR 0-5; median SLAM 3, IQR 5.75). The patient group is multi-ethnic, with 46% Caucasian, 32.7% Asian, 7% African, 8% Latin/Hispanic, 2.6 % Arabic, and 1% Native American. Aza was most commonly prescribed (44%), followed by cyclo (17.8%), mmf (14.6%), and mtx (8.7%). Neither gender nor ethnicity were associated with specific IS use. Six percent of patients were not prescribed prednisone, and 10% were not prescribed hydroxychloroquine. There was significant variation in use of prednisone (p=0.004)and plaquenil (p=0.031) across the 4 centres. Renal or neurologic disease were strongly associated with use of either aza or cyclo (p<0.01), whereas serositis was not associated with any IS use. Renal disease was associated with mmf (p<0.01). A high MDVAS was also associated with use of aza or cyclo (p<0.01). SLEDAI or SLAM scores were not associated with IS use. *Conclusions:* Prescribing practices for children and adolescents

with SLE vary across Canada, with the most significant differences in use of prednisone and hydroxychloroquine. The most frequent IS used are aza and cyclo. Use of these two drugs was highly associated with presence of renal or neurologic disease, and a high MSVAS; in constrast a high disease activity score was not associated with IS use. Current treatment paradigms for juvenile onset SLE differ across Canada, but impact on outcomes will not be known until long term followup is analyzed.

PO2.N.8

Mannose binding lectin expression genotype in pediatric-onset systemic lupus erythematosus: Associations with susceptibility to nephritis and protection against infections

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Objective: To study associations between extended mannose binding lectin (MBL) expression genotypes and clinical manifestations and infections in systemic lupus erythematosus (SLE) children. ***Methods:*** This was a prospective study of a cohort of pediatric-onset SLE patients. MBL gene polymorphisms were genotyped by polymerase chain reaction. Serum MBL concentrations were measured by ELISA. ***Results:*** The frequencies of MBL variant alleles and extended MBL expression genotypes did not differ between 128 pediatric-onset SLE patients and 137 controls. Comparing patients with a high MBL expression genotype to a group with low and medium MBL expression genotypes showed more lupus nephritis (65.5% versus 43.2%, P=0.016) and fewer serious bacterial infections (SBI) (26.2% versus 45.5%, P=0.027). By logistic regression, SLE patients with high MBL expression genotypes had an odds ratio of 2.409 (95% CI = 1.132-5.129, P =0.023) for lupus nephritis and a protective effect against SBI (OR = 0.352; 95% CI = 0.148-0.834; P=0.018). SLE patients had significantly higher MBL levels compared with controls in different MBL genotype groups. During a one-year SLE course follow-up, patients with persistent active SLE had constantly high MBL levels, significantly higher than controls. Patients with the highest MBL levels at SLE onset experienced flare-ups of SLE and MBL level changes reflected the clinical course of SLE flare-ups. ***Conclusion:*** Our findings suggest that a high MBL expression genotype is a risk factor for lupus nephritis, while it has a protective effect against infection. Serum MBL level changes correlate with SLE clinical course and may be useful for disease monitoring.

PO2.N.9

Relative gene expression analysis of cytokines IFN-γ and TNF-α genes and their association with autoantibody profile and disease activity in pediatric lupus

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Objectives: To analyze the relative gene expression of cytokines IFN-γ and TNF-α by Real time RT PCR and its association with autoantibody profile, clinical manifestations and disease activity in a pediatric SLE in North Indian cohort. ***Methods:*** 40 children with SLE and 20 healthy children were recruited. A real-time RT-PCR assay was used to quantify target gene transcripts. The mRNA levels were measured by a Light Cycler machine (Roche Diagnostics, Germany) and expressed as threshold cycle (CT). Quantitative real-time RT-PCR curves were analyzed by Light Cycler 3.5 software (Roche Diagnostics). For relative quantification of TNF-α and IFN- γ genes expressions were normalized by expression of beta actin. The amount of target was calculated by 2−ΔΔCT method. Line Immuno blot assay was carried out for multiplex determination of auto antibodies to 14 different antigens-nRNP, Sm,SS-A(SS-A native and Ro-52 ,SS-B Scl70,PM-Scl, Jo-1,CENP-B,PCNA, dsDNA,nucleosomes,histones,ribosomalP-protein and AMA-M2.Quantitative determination of ds DNA antibodies Anti-cardiolipin antibodies (IgG and IgM

isotype) in serum of SLE patients was carried out through ELISA. Disease activity was evaluated by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Relative gene expression was correlated with autoantibody profile and clinical manifestations using Fisher T test and Chi square test. ***Results:*** We observed an over-expression of TNF-α in 90% (36/40) and IFN-γ in 80% (32/40) patients as compared to controls. However, no statistically significant correlation was seen with SLEDAI scoring (p=0.075).A statistically significant association of TNF-α gene expression seen with anti Sm antibody (p=0.004), renal involvement (p=0.012) and hematological involvement (p=0.003). The IFN-γ gene expression was found to be significantly associated with anti-SSB (p=0.036), anti-ds DNA (p=0.038), anti-histones (0.017) auto antibodies, renal involvement (p=0.043), ds DNA titers (p=0.037) and with positive renal biopsy (WHO histopathological grading) (p=0.022). However relative gene expression for both TNF-α and IFN-γ genes were also found to be statistically significantly associated in patients testing positive for SSA (p=0.020 and p=0.020), Ro52 (p=0.013 and p=0.013), ds DNA (p=0.001 and p=0.000), nucleosomes (p=0.033 and 0.001),histones(p=0.003 and p=0.000) and Ribosomal P auto-antibodies(p=0.011 and p=0.011). ***Conclusion:*** A highly sensitive method, real-time RT-PCR analysis showed that both genes (TNF-α and IFN-γ) were significantly up-regulated in SLE patients and no correlation with SLEDAI observed. Association of TNF-α and IFN-γ gene expression with autoantibodies and clinical manifestations suggests that these two cytokines have crucial role in the SLE pathogenesis and highlights TNF-α and IFN-γ cytokines as potential targets for immunotherapy.

PO2.N.10

Th1 and Th2 cytokine profile in pediatric lupus and their association with disease activity in a north India cohort

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Objectives: To Study the analysis of Th1 and Th2 cytokine profile in pediatric SLE and their association with disease activity. ***Methods:*** 40 children with SLE and 20 healthy children were recruited. Peripheral blood mononuclear lymphocytes (PBMCs) were cultured from whole blood and stimulated with concavalin-A, for 24 hr at 37°C and their supernatants were collected. Cytokines (IL-6 and TNF-α) profile were analyzed by Instant ELISA kit (BenderMed GmBH) method.TH1 and TH2 cytokines(11plex cytokines:- IL-2,IL-4,IL-6,IL-8,IL-10,IL-12 ,IFN-γ,TNF-α,TNF-β,IL-1 β and IL-12p70) profile by Flow cytometry (using human Th1 and Th2 11plex cytokines analysis kit,(Bender Med systems GmBH, Austria).Disease activity was determined by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI).The student's unpaired t-test was used to compare the difference in means of cytokine concentrations. The Spearman's rank correlation test was used to ascertain the correlation among cytokine concentrations, the ratio of Th1 and Th2 cytokines, and SLEDAI. ***Results:*** Increase productions of Th2 type cytokines (IL-4, IL-6, IL-8 and IL-10) were observed in the SLE patients. No significant association of Th1 and Th2 cytokines with SLEDAI was observed. Concentration of cytokine IL-6 in patients was found to be significantly higher as compared to controls (p=0.001), whereas concentration of cytokine TNF α did not show any significant difference (p=0.246) when compared with controls. Ratio of mean concentrations of Th1/Th2 cytokines (IL-6) was calculated and correlated with SLEDAI.TNF-α/IL-6 ratio was found to be significantly correlated with SLEDAI (p=0.041).However, this ratio was not statistically significant in IL-12p70/IL-6(p=0.067), IFN-γ/IL-6(p=0.292) and TNF-β/IL-6(p=0.193). ***Conclusion:*** In our study we observed an increase level of Th2 type (IL-4, IL-6, IL-8 and IL-10) cytokines. However, this was independent of disease activity. None of the cytokine parameter i.e. Th1, Th2 and Th1/Th2 was associated with SLEDAI except TNF-α/IL-6 (p=0.041).This suggests that an imbalance cytokine profile and disruption of cytokine homeostasis might play a role in SLE pathogenesis. Cytokine IL-6 level was significantly increased in patients as compared to healthy controls and targeting IL-6 cytokine may provide valuable option for treating SLE in our cohort.

PO2.N.11

Juvenile systemic lupus erythematosus in the Sultanate of Oman: clinical and immunological comparison between familial and non-familial cases

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Objective: There is a wide variation in the natural history of systemic lupus erythematosus (SLE) among different ethnic and geographic groups. Consanguineous marriage is quite prevalent in the Middle East including Oman with up to 45% of marriages being consanguineous (1). Given the high rate of consanguineous marriage in Oman, the objective of this study was to determine demographic, clinical and serological characteristics between familial and non-familial cases of juvenile SLE in Oman. **Method:** Hospital medical records were retrospectively reviewed for 44 of consecutive children with juvenile SLE seen at Sultan Qaboos University Hospital (SQUH), one of the two pediatric rheumatology referral centers in the country. Personal interviews were conducted to fill in missing family history data. All the children included in the study were Omani, diagnosed before13 years of age, and fulfilled the 1982 revised American College of Rheumatology (ACR) criteria. Patients are considered to have familial SLE if at least two individuals in the pedigree met four ACR criteria. Analyses were performed using descriptive statistics. To avoid spurious results due to multiple comparison tests, the Bonferroni correction procedure was applied. **Results:** A total of 44 children were included in the study, 16 with familial SLE compared to 28 with non-familial SLE who were matched for age since diagnosis and disease duration. There were largely no significant differences in the clinical and serological manifestations between familial childhood SLE cases and their matched cases in Oman. (Table1).

Clinical features	Familial n=16 (%)	Non Familial n=28 (%)	P value
Fever	10 (63)	10 (36)	0.086
Weight loss	5 (31)	14 (50)	0.227
Alopecia	4 (25)	13 (46)	0.208
mucocutaneous manifestations	13 (81)	17 (65	0.316
Arthritis/arthralgia	11 (69)	20 (71)	0.851
Renal disease	9 (56)	18 (64)	0.0598
Pulmonary involvement	2 (13)	9 (32)	0.278
Cardiac manifestations	1 (6)	4 (14)	0.638
Neuropsychiatric manifestations	1 (6)	6 (21)	0.393
Lymphadenopathy	6 (35)	10 (36)	0.977
Hepatomegaly	2 (13)	7 (25)	0.450
Splenomegaly	1 (6)	4 (14)	0.638
Hematological involvement	9 (56)	21 (75)	0.199
Leucopenia	2 (13)	5 (18)	1.000
Lymphopenia	1 (6)	4 (14)	0.638
Hemolytic anemia	9 (56)	16 (57)	0.954
Thrombocytopenia	1 (6)	6 (21)	0.689
ANA	16 (100)	28 (100)	1.000
anti-dsDNA	15 (94)	24 (86)	0.638
anti-SSA	1 (6)	5 (18)	0.392
anti-SSB	2 (13)	4 (14)	1.000
anti-RNP	4 (25)	1 (4)	0.051
anti-Sm	4 (25)	4 (14)	0.434
ACA	4 (25)	6 (21)	0.100

Conclusion: Even though the rate of familial SLE is higher in the sultanate of Oman (36%) compared to the Western world (10-12%) (2), it appears that familial and non-familial SLE cases are in fact similar disease entities in both the West and the Middle Eastern countries (3-7). Hence, so as to improve statistical power, the two disease entities can be grouped together in the future analysis of genetic studies and the development of therapeutic strategies.

PO2.N.12

Mycophenolate mofetil treatment in paediatric onset systemic lupus erythematosus

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Introduction: We present our safety and efficacy data on the use of mycophenolate mofetil (MMF) treatment in children with systemic lupus erythematosus (SLE) and lupus nephritis (LN). **Patients and Methods:** Thirty-one children and adolescents (28 (90%) female), aged 5-22 (median 16) years with SLE were treated with MMF at Great Ormond Street Hospital for Children NHS Trust and University College Hospital, London, UK. All patients fulfilled at least four of the 1982 revised American College of Rheumatology classification criteria of SLE. 71% (22 of 31) patients had biopsy-proven LN. Treatment outcome was monitored through assessment of the British Isles Lupus Assessment Group (BILAG) index and blood and urine parameters. Group 1 patients were commenced on MMF induction and/or maintenance therapy (n=15) and Group 2 children who were converted from azathioprine (AZA) due to inadequate disease control (n=16). **Results:** 77% of all (10 (67%) group 1 and 14 (88%) group 2) patients experienced an improvement in BILAG score from a median of 9 (range 2-23) to 3 (1-8) and from 9 (4-21) to 2 (0-8) respectively at 12 months after initiation of MMF treatment (p < 0.05 in both groups). Group 1 children with hypocomplementaemia increased their C3 and C4 levels significantly (C3 from 0.53 to 1.15 and C4 from 0.08 to 0.17g/l, p<0.05), whereas group 2 patients' C3, but not C4 showed increased levels (0.56 to 0.12g/l, p<0.05). Renal function and albuminuria improved in children with active lupus nephritis (p<0.01). Significant improvements were seen in both groups in haemoglobin, erythrocyte sedimentation rate and lymphocyte counts. Prednisolone dose was weaned in Group 1 patients from 25 (range 5-60) mg, p<0.05 to 7 (0-10) and in Group 2 from 13 (0-60) mg to 5 (0-20) mg), p<0.05. Side-effects of nausea, diarrhoea, leucopenia and viral infection were seen in seven (22.5%) but none were judged to be severe enough to discontinue treatment. **Conclusions:** MMF treatment in our cohort of children with SLE and LN seemed to be safe, well-tolerated and effective. Randomized controlled trials are urgently needed to further define the role of MMF in childhood SLE.

PO2.N.13

Jaw osteonecrosis in juvenile systemic lupus erythematosus patients

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Objective: The aim of this study was to assess temporomandibular joint (TMJ) involvement in juvenile systemic lupus erythematosus (JSLE) and controls. Patients and Methods: Twenty six children and adolescents with JSLE (ACR criteria) and 28 healthy controls were evaluated by radiographic panoramic examination of TMJ. Multislice computer tomography (MCT) of TMJ was performed on those with flattening and/or destruction of the mandibular condyles. Demographic data, oral health indices, clinical manifestations, laboratory tests, SLEDAI, disease damage and therapies were also carried out. **Results:** Flattening and/or destruction of the mandibular condyles alterations compatible with jaw osteonecrosis and confirmed by MCT of TMJ were observed in 2/26 (8%) of JSLE patients vs. 0% of control group (p=0.22). Mild clinical dysfunction of TMJ was observed in 67% of JSLE patients. The age of JSLE onset, disease duration and current age were comparable in JSLE patients with and without jaw osteonecrosis (9.3 vs. 10.8 years, p=0.77; 3.3 vs. 2 years, p=0.63; 12.6 vs. 13.5 years, p=0.74; respectively). Likewise, no differences were evidenced in both groups regarding gender, socio-economic

class, oral health indices, clinical manifestations, laboratory exams, disease activity, disease damage and therapies (p>0.05). These two JSLE patients with jaw osteonecrosis had a chronic active course, were teated with corticosteroids for a long period of time and had mild TMJ dysfunction. None of them had antiphospholipid antibodies and previously treated with bisphosphonate. **Conclusions:** Jaw osteonecrosis with mild clinical dysfunction of TMJ were observed in JSLE patients, thus indicating the importance of oral heath evaluation during clinical follow up.

PO2.N.14

Cardiovascular responses during graded exercise in juvenile systemic lupus erythematosus patients

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Background: Juvenile systemic lupus erythematosus (JSLE) is an inflammatory autoimmune disorder that affects all organs and system including the cardiovascular. In addition, alterations in left ventricular function or structure have been identified in JSLE patients. There is however, scarce data regarding cardiovascular responses during graded exercise in this population. **Purpose:** To identify cardiovascular responses (CR) during graded exercise in JSLE patients and controls. **Methods:** Nine consecutive adolescent JSLE (ACR criteria) patients (7 females) without cardiopulmonary involvement were selected and compared to 7 healthy controls (5 females). All patients and controls performed a progressive treadmill cardiopulmonary test until exhaustion to determine the maximal aerobic capacity (Peak) and ventilatory anaerobic threshold (VAT). Cardiovascular response was evaluated by oxygen pulse response during graded exercise. The cardiovascular responses during graded exercise test were analyzed between two groups at rest, VAT and peak of exercise using two-way analysis of variance (ANOVA) with repeated measures to test possible differences between groups. When significance was found, Scheffés' post-hoc comparison was performed. P values of < 0.05 were considered statistically significant. **Results:** Age (13.0 ± 0.9 vs. 12.0 ± 0.8 years, p=0.42) and body mass index (22.1 ± 1.6 vs. 20.2 ± 1.1 Kg/m2, p=0.36) were alike between JSLE and controls. Of note, JSLE patients had significant lower peak workload (4.6±0.2 vs. 5.3±0.3 mph, p=0.05) and relative aerobic fitness [VO2peak] (28.0±1.4 vs. 36.9±4.0 mL.kg-1.min-1, p=0.01). In addition, O2 pulse was significantly altered during graded exercise in JSLE patients compared to controls (VAT=6.5±0.5 vs. 8.3±0.6 ml/bpm, p=0.01) and (Peak= 7.4±0.5 vs. 9.5±0.9 ml/bpm, p=0.001). However, we not observed differences between groups for heart rate at (VAT=142.6 ±4.2 vs. 132.3±3.0 bpm, p=0.65) and (Peak= 180.8±6.1 vs. 187.3±4.2 bpm, p=0.92) in JSLE patients and controls, respectively. **Conclusion:** The novel finding of a decreased cardiovascular response in JSLE patients probably associated to abnormalities in inotropic response and also oxygen extraction in the peripheral muscles may be a relevant contributing factor to aggravate the known decreased aerobic capacity observed in these children. The reduced maximal aerobic capacity in adolescents with JSLE might be a reflection of the underlying limited cardiac reserve.

PO2.N.15

Anti-C1q, anti-nucleosome and anti-dsDNA antibodies in 62 juvenile systemic lupus erythematosus patients

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Objectives: To evaluate the presence of anti-C1q, anti-nucleosome and anti-doble strand-DNA (dsDNA) antibodies in juvenile systemic lupus erythematosus (JSLE) and controls; to establish a comparison between the sensitivity, specificity, positive and negative predictive values of the three auto-antibodies and to assess the association of these antibodies with disease activity and lupus nephritis. **Methods:** Sixty-two JSLE (ACR criteria) and 34 age and gender matched healthy controls were analyzed for the presence of anti-C1 (Inova Diagnostics, San Diego, USA), anti-nucleosome (Inova Diagnostics, San Diego, USA) and anti-dsDNA antibodies (The Binding Site, Birmingham, UK) by ELISA. Statistical analysis was carried out according to Mann-Whitney and Fisher's exact tests. **Results:** The mean current age was similar in JSLE patients and controls (14.6 ± 3.86 vs. 13.6 ± 2.93 years, p = 0.14). The female gender was similar in both groups (83% vs. 79%, p = 0.58). The age at JSLE onset ranged from 3 to 16 years. A higher frequency of anti-C1q, anti-nucleosome and anti-dsDNA antibodies was observed in JSLE compared to controls (20% vs. 0%, p=0.0037; 48% vs. 0%, p<0.0001 and 69% vs. 3%, p<0.0001, respectively). The medians of anti-C1q, anti-nucleosome and anti-dsDNA were also significantly higher in JSLE patients versus controls [9.6Units (5.5-127) vs. 7.5Units (5-20), p=0.0006; 18Units (1.9-212) vs. 3.2Units (1.7-17), p<0.0001 and 67IU/ml (6-741), p<0.0001, respectively]. The sensitivities for anti-C1q, anti-nucleosome and anti-dsDNA were 21% (CI 11-33%), 49% (CI 36-62%) and 70% (CI 57-81%); while specificities were 100% (CI 88-100%), 100% (CI 88-100%) and 97% (CIn83-99%), respectively. Positive predictive values were 100% (CI 75-100%), 100% (CI 88-100%) and 97% (CI 87-99%) and negative predictive value was 39% (CI 28-50%), 50% (CI 37-62%) and 62% (CI 47-76%) for anti-C1q, anti-nucleosome and anti-dsDNA, respectively. Comparing JSLE patients with and without nephritis (50 vs. 12 JSLE patients), no differences were observed in the frequencies of anti-C1q (26% vs. 8%, p=0.26), anti-nucleosome (48% vs. 50%, p=1.00) and anti-dsDNA antibodies (72% vs. 58%, p=0.30). Additionally, JSLE patients with (SLEDAI≥4) and without (SLEDAI<4) disease activity did not differ for the frequencies of the three auto-antibodies (p>0.05). Interestingly, a positive correlation was found between anti-dsDNA levels and both anti-C1q (r=0.51, CI 0.29-0.68, p<0.0001) and anti-nucleosome (r=0.87, CI 0.79-0.92, p<0.0001) levels. **Conclusion:** Although anti-C1q and anti-nucleosome auto-antibodies presented a lower sensitivity compared to anti-dsDNA, the exceedingly high specitivity of both antibodies could help in diagnosis of JSLE patients, mainly in those cases with negative anti-dsDNA.

PO2.N.16

Blindness in juvenile systemic lupus erythematosus

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Introduction: Juvenile systemic lupus erythematosus (JSLE) may affect multiple organs and systems, including ocular involvement. Ocular vasculitis leading to permanent amaurosis is rare, particularly in the pediatric population. From 1983 to 2009, 5,367 patients were followed in the Pediatric Rheumatology Unit of our University Hospital and JSLE was diagnosed in 263 patients (ACR criteria). Of note, 2 (0.76%) of them had blindness due to retinal vasculitis and were described. **Case Report:** Case 1 – A 15 year-old female had JSLE diagnosis, presenting with malar rash, serositis, antinuclear and anti-dsDNA antibodies, and proteinuria. Intravenous methylprednisolone pulse

was started followed by oral prednisone with improvement of pericarditis. However, after two weeks, she had cutaneous vasculitis associated to pain and redness of both eyes and sudden decrease of visual acuity. Her laboratory exams revealed hemoglobin 8g/L, hematocrit 23%, white blood cell (WBC) count 16,400/mm³ (72%neutrophils, 24%lymphocytes and 4%monocytes), platelets 142,000/mm³, urinalysis (5,000 leukocytes and 64,000 erythrocytes), low levels of C4, erythrocyte sedimentation rate (ESR) 48mm/1st hour, urea 68mg/dL, creatinine 0.7mg/dL and renal biopsy with focal and segmental lupus nephritis. Ophthalmologic exam showed severe iridocyclitis and retinal vasculitis with hemorrhage. Prompt treatment with topic prednisone, atropine and phenylephrine was associated to intravenous metilprednisolone pulse, prednisone (2 mg/kg/day), hydroxychloroquine and monthly intravenous cyclophosphamide (1 g/m²/month). However, despite treatment, she rapidly evolved to an irreversible blindness in the first month of follow-up. Case 2 – A 19 year-old female with JSLE diagnosis was hospitalized with macroscopic hematuria and edema of lower limbs. At that moment, her laboratorial exams showed hemoglobin 14g/dL, hematocrit 40%, WBC 24,000/mm³ (67%neutrophils, 5%lymphocytes, 5%monocytes), platelets 260,000/mm³, ESR 30mm/1st hour, CRP 4.21mg/dL, antidsDNA 419IU, anticardiolipin IgM 15MPL and IgG 30GPL, positive lupus anticoagulant and anti-P antibody, low levels of C3 and C4, urinalysis (leukocytes 100,000/mL, erythrocytes 12,000/mL), proteinuria 2.16g/day, urea 60mg/dL and creatinine 0,8mg/dL. During hospitalization, she had peripheral polyneuropathy of the four limbs and received intravenous methylprednisolone pulse therapy, intravenous cyclophosphamide (750mg/m²/month) and azathioprine. Three weeks after admission, she had an infection by varicella-zoster virus with ocular involvement and presented acute blindness secondary to bilateral necrotizing retinal vasculitis. After intravenous acyclovir and intravenous immunoglobulin (2g/kg/month) and two applications of intra-vitreous gancyclovir, she had a poor vision recovery with irreversible blindness. **Discussion:** Retinal vasculitis associated to JSLE is a rare manifestation. However, this may be a severe manifestation with permanent blindness associated with disease activity and viral infection.

PO2.N.17

Improvement in pediatric automated neuropsychological assessment metrics (PedANAM) over time in childhood-onset SLE

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Purpose: The Pediatric Automated Neuropsychological Assessment Metrics (PedANAM) is a computerized test battery that assesses neurocognitive (NC) function in children $\geq$ 10 yrs. Preliminary results in cSLE suggest improvement in concentration and learning over the first 9 months of testing. The objective was to examine for longitudinal change in PedANAM performance. **Methods:** The PedANAM was administered 3 times at baseline (to eliminate practice effects) and every 3 months. NC function was assessed by PedANAM performance parameters: accuracy, speed and throughput (a measure of accuracy and speed) for each subtest. **Results:** 35 cSLE subjects enrolled in a longitudinal cohort study of NC function had a minimum of 21 months of repeated testing. Subjects (M:F = 11:24) had a median age of 16.0y (range 12.1 - 21.7y) and a median disease duration of 3.1y (range 0.4 - 12.4y) at entry. The population was 69% Hispanic, 11% Black, 11% Asian, and 9% White. By paired t-test analysis, 4 subtests demonstrated significant improvement in ≥1 performance parameter.

SUBTEST	ACCURACY	SPEED	THROUGHPUT
Code Substitution Learning (CDS)	1.2 (p=NS)	11.3 (p<.001)	11.6 (p<.001)
Code Substitution Delayed (CDD)	9.9 (p<.01)	10.1 (p=NS)	10.9 (p<.001)
Matching to Sample (MSP)	0.6 (p=NS)	3.9 (p=NS)	2.6 (p=NS)
Mathematical Processing (MTH)	1.3 (p=NS)	22.4 (p<.05)	11.3 (p<.001)
Procedural Reaction Time (PRO)	-3.7 (p=NS)	6.6 (p=NS)	.02 (p=NS)
Spatial Processign (SPD)	9.8 (p<.001)	25 (p<.05)	25 (p<.05)
Simple Reaction Time (t1) (SRT1)	.06 (p=NS)	6.3 (p=NS)	6.4 (p=NS)
Simple Reaction Time (t2) (SRT2)	1.9 (p=NS)	13.3 (p=NS)	11.0 (p=NS)
Sternberg Memory Search (STN)	1.3 (p=NS)	5.8 (p=NS)	3.0 (p=NS)

CDS assesses concentration and learning, CDD is a memory recall task, MTH assesses working memory, and SPD assesses spatial analysis. Five subtests (MSP, PRO, STN, SRT1 and SRT2) demonstrated no significant change over the study period. To consider all time points for each subject random effects modeling of the difference in scores over time was used; results were similar; however, significant improvement in subjects' mean reaction time on 5 subtests (CDS, CDD, MSP, MTH, SPD) was observed. Subsequent analysis of 21 vs. 9 month data demonstrated significant change on only the MTH subtest (data not shown). Multiple regression analysis did not identify contributory factors including disease duration, SLEDAI, ethnicity or SLICC damage score. **Conclusion:** Significant improvement in performance from baseline on subtests assessing concentration, learning and working memory were detected over a minimum of 21 months of repeat testing, without significant change on subtests assessing short-term memory, reaction time and attention. Overall improvement was similar to 9 months of follow-up, suggesting an early plateau in procedural learning. Further data analysis will utilize reliable change indices to assess for subtle change in NC function of individual subjects.

PO2.N.18

Type 1 diabetes mellitus and juvenile systemic lupus erythematosus

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Introduction: Juvenile systemic lupus erythematosus (JSLE) may affect the endocrine system and association between this disease and type 1 diabetes mellitus (DM1) was rarely reported, particularly in paediatric population. From 1983 to 2009, 5367 patients were followed in the Pediatric Rheumatology Unit of our University Hospital and 263 had JSLE (ACR criteria). Of note, 3 (1.1%) of them had DM1 diagnosis and were described. **Case Reports:** Case 1– A 18 year-old female had JSLE diagnosis at the age of 11, with malar rash, arthritis, serositis, antinuclear, anti-dsDNA and IgG-anticardiolipin antibodies. Patient's family history was remarkable for consanguineous parents and sister with Graves' disease. Past medical history revealed asthma and chronic diarrhea since 6 and 9 years old, respectively. Six months prior to admission, she had acute pancreatitis, developing DM1. Three years later, insulin pump therapy was started with a better clinical response. Additionally, selective IgA deficiency and autoimmune thyroiditis diagnoses were found during follow-up. JSLE clinical manifestations have been well controlled with prednisone and chloroquine diphosphate. Case 2– A 17 year-old female was admitted to our Hospital with a two-year history of recurrent arthritis, photosensitivity, arterial hypertension, proteinuria and leukopenia. Past medical history revealed DM1 and Graves' disease diagnosed at 1.5 and 10 years old, respectively. Renal biopsy showed a segmental and focal glomerulonephritis and laboratory exams demonstrated positive antinuclear and anti-dsDNA. JSLE was diagnosed and prednisone, azatiophrine and chloroquine diphosphate were started, with clinical improvement. At the age of 16, she started

insulin pump therapy, with a marked improvement in blood glucose levels. Of note, a random screening found a selective IgA deficiency. Case 3– An 11 year-old girl was admitted with an eight-month history of fever, arthritis, myalgia, lymphadenopathy, weight loss and seizures. She was hospitalized during the first 3 months for diagnostic investigation. At that period, she had an acute necrohemorragic pancreatitis and developed DM1. Laboratory exams showed anemia, lymphopenia and thrombocytopenia, positive antinuclear antibody, anti-dsDNA, anti-Sm, anti-RNP and lupus anticoagulant. JSLE diagnosis was made and patient was started on intravenous metylprednisolone pulses and immunoglobulin, followed by prednisone and hydroxichloroquine. One year later, she developed nocardiosis and sulfamethoxazole-trimethoprim was initiated. Since then, patient's clinical manifestations have been well controlled by immunosuppressive and insulin therapy. *Discussion:* The prevalence of DM1 and JSLE was rare. Two of the patients described also had thyroiditis and selective IgA deficiency associated, suggesting a common pathophysiology underlying these complex clinical presentations.

PO2.N.19

Safety and immunogenicity of the quadrivalent HPV vaccine Gardasil in female systemic lupus erythematosus patients aged 9 to 26 years and its effects on the autoantibody profile

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Background: Women with SLE have higher rates of persistent HPV infections and precancerous lesions than healthy women. HPV vaccine is safe and effective in healthy females aged 9-26 years. There are no data on the safety and immunogenicity of HPV vaccine in females with SLE. *Objectives:* To evaluate safety and immunogenicity of Recombinant Quadrivalent Human Papillomavirus Vaccine in female SLE patients aged 9-26 years. *Methods:* Prospective, open-label, pre-post intervention, historical control study. All patients met ACR Criteria for SLE. Exclusion criteria were: disease exacerbation within past 30 days resulting in ≥6 points increase in SLEDAI, increase in corticosteroid dose, initiation of new immunosuppressive medication or hospitalization; rituximab in the past 6 months; current cyclophosphamide treatment; previous HPV vaccination; pregnancy. HPV vaccine was administered at months 0, 2, and 6. Patients were monitored by physical examination, SLEDAI scores and laboratory studies at months 0, 2, 4, 6 and 7. Antiphospholipid, anti-ENA and HPV antibody titers were measured at months 0 and 7. Each patient's SLEDAI scores and laboratory profile in the year prior to vaccine administration were used as controls for that patient. Primary outcome measures were change in SLEDAI and mean geometric HPV antibody titers. The secondary outcome measure was induction of autoantibodies. *Results:* 26 patients, ages 12 to 26 years, were enrolled. To date, 24 and 19 patients have received two and three doses of HPV vaccine, respectively. None had acute allergic, local or vasovagal reactions after receiving HPV vaccine. Six patients had mild/moderate lupus flares, typically with symptoms similar to flares experienced before vaccine administration. Of 12 patients with history of lupus nephritis, two with Class IV nephritis experienced worsening renal function during/after the study; both progressed to renal failure. Seven patients dropped out of the study: 2 pregnancies, 1 non-compliant, two moved out-of-state, 2 opted out secondary to increased arthralgias. In 17 patients who completed the study, there was a significant reduction in the mean SLEDAI scores from a mean of 6.36 pre-vaccination to 4.77 post-vaccination. (p=0.024, 95% CI: -0.24 TO -2.94) *Conclusions:* Our preliminary results show that HPV vaccine does not result in an increase in mean SLEDAI scores and is well tolerated in young women with SLE. It is not clear if the progression of lupus nephritis in two of 12 patients with lupus nephritis is related to HPV vaccine. Data on HPV vaccine's immunogenicity and its effects on auto-antibody profile are being evaluated.

PO2.N.20

Rapidity of pediatric rheumatology consultations for children with systemic lupus erythematosus

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Objective: To determine the time interval between the onset of symptoms and pediatric rheumatology consultation in a referral center in south-east Brazil. To determine patient characteristics and physician characteristics associated with the referral associated. *Patients and Methods:* Data were collected from 81 patients followed in the pediatric rheumatologist Unit. Patients were eligible if they had 4 or more criteria for SLE, were less than 16 years at time of diagnosis. We assessed the effects of various demographic and clinical factors on referral time using univariate logistic regression analyses. We also performed multivariate models which included age, sex, referral unit, initial diagnosis and positive ANA. *Results:* We identified 81 SLE patients (67 female and 14 males) with mean age at disease onset of 115 months (SD=44; range 18-209). Mean referral time was 17.3 months (SD=3vc4 months; range: 0-192 months), however, 46 (57%) patients had a referral time longer than 3 months for the first pediatric consultation. Fifty eight (71.8%) patients were referred as SLE, 7 (8.6%) patients as juvenile arthritis, 2 (2.5%) as reactive arthritis and 14 (17.3%) without diagnosis. Twenty one patients (26%) were referred from hematology units or local hospitals, 60 (74.1%) from primary units or private clinics. In univariate analysis shorter referral time was associated with older age (r=-0.6; p=0.01), diagnosis of SLE (r=0.76; p=0.009), referral from hematology or local hospitals (p=0.02) and positive ANA (r=0.68; p=0.02) . In multivariate analysis shorter referral time was associated with older age (OR=-3.1; 95%CI=0.01-0.3) and positive ANA (OR=1.7; 95%CI=1.1-5.2). *Discussion:* 57% of jSLE had a referral time longer than 3 months. Older age and positive ANA was associated with shorter referral time. Continuous education is necessary to reduce the referral time for children with SLE.

PO2.N.21

Urinary biomarkers may differentiate between children with ISN/RPS class IV versus class V of lupus nephritis (LN)

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Purpose: Biopsy-proven LN occurs in up to 75% of all children with SLE. ISN/RPS classes of LN differ in major histological features, clinical manifestations and prognosis. Our aim was to identify urinary biomarkers that can differentiate between ISN/RPS classes IV and V in children with LN. *Methods:* In this ongoing study, urine samples from children with LN ISN/RPS class IV (n=6) and pure class V (n=7) collected within 60 days of a kidney biopsy and those of controls with focal segmental glomerulosclerosis (n=4) were tested. Two proteomic methods were employed that reliably measure large and mid-molecular weight proteins, i.e. 2-dimensional gel electrophoresis (2-DGE) and surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS), respectively. Candidate biomarker proteins were selected if levels differed significantly when comparing class IV vs. V, class IV vs. controls, or class V vs. controls. The identity of relevant protein spots seen on 2-DGE was obtained by MALDI-TOF-MS/MS. *Results:* Using 2-DGE and MALDI-TOF-MS/MS, we found human serum albumin fragments (25kDa) and α1-B glycoprotein (60kDa) significantly over-expressed in class IV vs. class V LN. For SELDI-TOF-MS/MS four different chromatographic surfaces were tested; spectra were analyzed

with ProteinChip Data Manager 3.07. The resulting urinary signature of mid-molecular weight proteins that differed between groups of samples based on robust and reproducible peaks is shown in the Table, with areas under the receiver operating characteristic curves > 0.7 (all p < 0.05). ***Conclusion:*** We have identified two proteins and a urinary biomarker signature that appear to be significantly different between LN ISN/RPS class IV and class V. Further studies in larger patient groups, including adults with SLE, are needed to confirm our findings and to assess the diagnostic accuracy of these candidate biomarkers.

SELDI ProteinChip type of chromatographic surface #	Class IV vs. class V *	Controls vs. class IV **	Controls vs. class V **
Cation exchange	7807	3273	
Normal phase	3266, 3278	3936, 4270, 4478, 7787, 23119	23119
Hydrophobic binding	3816, 3876, 4247, 5835, 9075, 9452, 16673	3876, 6796, 16134, 25835, 28101	4475, 4631, 7634, 11830, 11958, 13080, 47905
Metal affinity	4349, 4639, 4702, 8846	15096, 15298, 66411, 138089, 148232	7035, 15096, 15298

Values are MS peaks at m/z of Da; * Peaks (Da) with fold change >2;
** Peaks (Da) with fold change > 10

PO2.N.22

Race/ethnicity predicts disease severity in pediatric lupus: results from a single-center, multiethnic inception cohort

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Objectives: We observed a high rate of morbidity in patients of ethnic/ racial background with pediatric lupus referred to our pediatric rheumatology center in Northern California. The purpose of this study was to determine whether Caucasian ancestry protects against lupus severity. ***Methods:*** 112 patients with pediatric lupus (age <18 at diagnosis) were asked to participate and all consented to the study. 98 patients had sufficient medical records and completed a questionnaire on racial/ ethnic background and socioeconomics. Race/ ethnicity was assessed using the ancestry of four grandparents. Having any Caucasian ancestry was used as the primary predictor of interest. The primary outcome variable was time to development of severe lupus nephritis (ISN/ RPS class III, IV, V). The secondary outcome variable was time to initiation of aggressive immunosuppression for any affected organ system. Aggressive immunosuppression is defined as cyclophosphamide (CYC), mycophenolate mofetil, azathioprine, cyclosporine A, and rituximab. Predictor and outcome variables determined a priori were analyzed by multivariate Cox regression. ***Results:*** In our pediatric lupus cohort, 35% identified themselves as Hispanic, 35% as Asian American/ Pacific Islander, 2% as African American, and 12% were of mixed descent. Based on 4 grandparent inquiry, 10% had exclusive Caucasian ancestry and 30% had at least one grandparent of Caucasian ancestry. 82% of the cohort received aggressive immunosuppression, which was used to treat neuropsychiatric lupus (21%), hematologic lupus (62%), pulmonary involvement (19%), and severe nephritis (58%). In the total cohort, 8% patients required short-term dialysis, but only 3% had end-stage renal failure. 55% of the cohort received CYC for at least 6 months. Patients of color were more likely to have lower maternal education status (p=0.01), lower insurance status (p=0.001), lower family income (p=0.001), lower proportion of English spoken at home (p=0.03), and lower income (p=0.0002) by t-test and Chi squared testing. Having at least one Caucasian grandparent decreased the development of severe lupus nephritis (HR 0.5, p= 0.02) and remained significant after controlling for age, gender, and maternal education. There was an association between having at least one Caucasian grandparent and later time to aggressive immunosuppression initiation (HR 0.6, p= 0.08) after controlling for the same covariates listed above. ***Conclusion:*** Caucasian ancestry appears to be protective against severity of lupus, especially lupus nephritis.

PO2.N.23

Fungal infections in pediatric SLE — prevalence, presentation, and outcome

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Objective: To report the prevalence, presentation and outcomes of invasive fungal infections requiring systemic antifungal therapy in a pediatric systemic lupus erythematosis (pSLE) cohort. ***Methods:*** We queried STRIDE (Stanford Translational Research Integrated Database Environment), an electronic clinical database at Stanford University to identify pSLE patients diagnosed between Jan 2000 through Jan 2010. Patients were included if treated primarily at Lucile Packard Children's Hospital at Stanford. To find all patients who received systemic antifungal therapy, we queried pharmacy orders, medication lists, and all dictations for both inpatient and outpatient encounters. ***Results:*** 135 pSLE patients were followed at Stanford pediatric rheumatology (mean follow-up time 5.5 years). 67 patients (50%) received cyclophosphamide (CYC) and high dose corticosteroids as part of treatment induction. 39 patients (29%) were treated with aggressive immunosuppression (azathioprine, mycophenolate mofetil, cyclosporine, and rituximab) but did not receive CYC induction. Six patients developed invasive fungal infection during immunosuppressive treatment requiring systemic antifungal therapy and hospitalization: all had negative fungal blood cultures. Prevalence of fungal infection was 7.5% among patients receiving CYC induction and 2.6% among patients receiving aggressive immune suppression but no CYC induction. Contributing factors include renal failure (4), nephrosis (6), neutropenia (4), lymphopenia (6). At the time of fungal infection, all patients had decreasing platelets despite improving markers of pSLE activity (complements and dsDNA antibody). All patients had resolution of fungal infection with systemic antifungal therapy, except one patient who died from herniation as a result of brain hemorrhage from aspergillosis brain abscess (Clinical characteristics of patients).

	Patient 1	Patient 2	Patient 3
Age	16F	17M	15M
Time from SLE diagnosis to fungal infection	5 months	31 months; SLE flare 1 month prior to fungal presentation	2 months
Steroids (mg/kg/day)	0.42	1.61	1.10
Immuno-suppressives on, 2 months before fungus	CYC, MP, PP, Ritux	MMF, MP, PP, Ritux	CYC, MP
Cumulative cytoxan dose (mg/mÂ²)	2186	NA	962
Fungal presentation	Fever, cutaneous lesion; pulmonary nodules (CT)	Hemoptysis; pulmonary nodules & tree in bud-opacities (CT)	Right facial weakness, dysarthria, ataxia; cavitary lesions (CXR)
Organism	Mucor (skin); fungal elements (nasal turbinate)	Aspergillus Fumigatus (pleural fluid); Candida Albicans (sputum)	Aspergillus Fumigatus (brain tissue)
IgG nadir within 2 weeks prior to fungus	413	563	162
ANC/ALC nadir within 2 weeks prior to fungus	280/220	7530/100	370/50
Platelets nadir within 2 weeks prior to fungus	76	142	37
Normal complements/ positive anti-dsDNA	Yes/No	Yes/No	Yes/No
Renal insufficiency requiring hemodialysis/ nephrosis	Yes/Yes	Yes/Yes	Yes/Yes

	Patient 4	Patient 5	Patient 6
Age	12F	16F	16M
Time from SLE diagnosis to fungal infection	3 months	2 months	2 months
Steroids (mg/kg/day)	2.20	2.30	1.37
Immuno-suppressives on, 2 months before fungus	CYC, MP	CYC, MP	CYC, MP
Cumulative cytoxan dose (mg/mÂ²)	1145	1010	2000
Fungal presentation	Erythe-matous papules involving hands	Left inguinal swelling and redness; soft tissue nodules and hyperemia on CT	Pneumo-thorax, pleural thickening & effusions, pneuma-toceles (CT)
Organism	Aspergillus (skin)	Rhizopus (groin abscess)	Aspergillus Fumigatus (pleural fluid); Candida Albicans (sputum)
IgG nadir within 2 weeks prior to fungus	229	327	170
ANC/ALC nadir within 2 weeks prior to fungus	740/380	7320/240	350/220
Platelets nadir within 2 weeks prior to fungus	72	92	66
Normal complements/ positive anti-dsDNA	Yes/No	No/No	Yes/No
Renal insufficiency requiring hemodialysis/ nephrosis	No/Yes	Yes/Yes	No/yes

Immunosuppressives: PP= plasmapheresis; Ritux- Rituximab; MP=mehtylprednisolone pulse; CYC=Cyclophosphamide; MMF=Mycophenylate Mofetil CsA=Cyclosporin; NA=non-applicable

Conclusion: Invasive fungal infections among patients with pSLE likely result from aggressive immunosuppression and immune dysfunction secondary to lupus and renal failure. While significant morbidity and mortality can arise from fungal infections, in 5/6 of our patients, lupus activity appeared to decrease quickly during the infection, even with aggressive withdrawal of immune suppression. We speculate that fungal infection may have an independent effect on lupus activity.

PO2.N.24

Increased immunoglobulin E in juvenile systemic lupus erythematosus

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Background: Systemic Lupus Erythematosus (SLE) is characterized by a Th2 immune response and a polyclonal activation of B lymphocytes. Immunoglobulin E (IgE) plays a central role in the pathogenesis of atopic diseases and in host immunity against parasitic infections. IgE synthesis is tightly controlled by regulatory T cells. Few studies have reported increased IgE in lupus patients, although no one was carried out in Juvenile SLE (JSLE). **Objective:** To asses the IgE concentration in JSLE patients and to evaluate a possible association with clinical features, disease activity and damage. **Methods:** Sixty-nine JSLE patients had the serum IgE concentration determined by nephelometry (Dade Behring/Siemens, Deerfield, USA). The value of 100IU of IgE/ml was used as the cut-off point. All patients were negative for intestinal parasitc infections. Fifteen patients reported atopic disease and 9 of 69 had severe sepsis. Nineteen lupus patients present an associated primary immune deficiency (PID) – 5 with C2 deficiency (D), 2 with C4D, 2 with complete C1qD, 4 with IgG2D, 3 with IgAD and 3 with IgMD. Statistical analysis included the Mann Whitney's test and the Sperman's rank. **Results:** Increased IgE concentrations above 100IU/mL were observed in 31 of 69 (45%) JSLE patients. The mean IgE concentration was 442.0±163.4 IU/ml (range 3.5-9936.0). Fifty-four JSLE patients without atopic manifestations had a mean IgE concentration of 271.6±699.5 IU/ml and only 9 of 31 (29%) with high IgE reported atopic disease. There were no differences in IgE concentration considering the presence of PID, atopic manifestations, severe sepsis, nephritis, disease damage (SLICC/ACR-DI>1) and disease activity (SLEDAI2K>4) (p>0.05). Interestingly, IgE concentration inversely correlated with C4 levels (r=-0.25, p=0.03) and with SLICC/ACR-DI score (r=-0.34, p=0.0045). No correlation was evidenced between IgE concentration and C3 levels, anti-dsDNA and SLEDAI2K score. Of note, 4 patients had IgE values >1000 IU/ml, one of these with a C1qD and atopic manifestations. **Conclusions:** We demonstrated herein for the first time that JSLE patients have increased IgE concentrations that are not related to the presence of atopic or parasitic disease. A possible association between IgE concentration, disease activity and damage is suggested, although other primary immune abnormalities, such as the regulatory T cells, could also be influencing in the increased IgE concentration observed in JSLE patients.

PO2.N.25

Characteristics of childhood systemic lupus erythematosus in Cape Town, South Africa

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Introduction: Systemic Lupus Erythematosis(SLE) is a multi-system autoimmune disease characterized by the formation of antinuclear antibodies. There have been very few reports of pediatric lupus from Africa. Faller et al reported on a cohort of 36 patients from the Gauteng region in South Africa and suggested that this disease is increasingly recognized in black South African children as the sociopolitical situation has changed. SLE is thought to be more common in the western cape than in other parts of the country and that the disease is more aggressive in this population. The patient profile, disease characteristics, morbidity and complications have not been described in the Western Cape, which has a predominantly mixed race population. **Aim:** To document disease characteristics, disease activity, morbidity and treatment practices in our cohort of patients with childhood onset SLE from Cape Town, South Africa. **Methods:** A retrospective folder review of all patients with Pediatric SLE seen at the Red Cross Children"s Hospital and Groote Schuur Hospital was undertaken and clinical and demographic data collected. **Results:** 26 patients were included. 22 Patients were of mixed race, 3 indigenous African and 2 caucasian. Mean age at presentation was 9.1 (2-15) years. Male/female ratio was 1:13. Average follow up was 5.9 years. The most common clinical/laboratory features at presentation are represented in table 1.

Table 1

Manifestation	(%)
Renal Disease	62
Arthritis, Malar Rash	57
Fever	35
Lymphadenopathy, photosensitivity	27
Pericarditis, Thrombocyopenia	23
Leukopenia, oral ulcers, anaemia	19
Reynaud's Phenomenon, Hypertension	15
Seizures	7
ANA	100
Anti dsDNA	57
Anti SM	30

Table 2

Lupus Nephritis Class	Number(%)
I	0
II	2/18 (11)
III	3/18 (16)
IV	7/18 (39)
V	2/18 (11)
VI	2/18 (11)

Renal disease was present in 18/26. Renal biopsies were done in 17/18 patients. There were no patients with Class I nephritis, 2(11%) with Class II, 3(16%) with class III, 7(39%) class IV, 2(11%) class V and 2(11%) class VI. End stage renal failure requiring dialysis/transplant occurred in 33%. One patient died before transplant and three have had kidney transplants. **Conclusion:** Patients from the Western Cape region appear to have similar features at presentation to those described in studies from other countries, though there appears to be a higher incidence of Class IV nephritis, and a lower incidence of CNS manifestations. The majority of patients (84%) are of mixed race. This may be partially explained by the racial demographics of the Western Cape. The incidence of positive anti-dsDNA is lower than in other studies.[1]

1) Hiraki L et al, Clinical and laboratory manifestations of systemic lupus erythematosis (SLE) in pediatric patients. Arthritis Rheum 46:S315,2002

PO2.N.26

Pancreatitis in juvenile systemic lupus erythematosus

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Objective: Acute pancreatitis (AP) is a rare and severe manifestation of systemic lupus erythematosus (SLE), particularly in juvenile SLE (JSLE) patients. However, to our knowledge, few cases of AP in JSLE have been reported on literature. The objective of this study was to evaluate AP in a population of JSLE patients of a Brazilian Pediatric Rheumatology tertiary service. **Patients and Methods:** From 1983 to 2009, 5367 patients were followed in the Pediatric Rheumatology Unit of our University Hospital and 263 had JSLE (ACR criteria). Of note, 11 (4.2%) of them had AP and were reported. **Results:** All of patients were hospitalized in intensive care unit. At the time of AP, the median age of JSLE patients was 11.5 years (8.8-17.9) and female:male ratio was 10F:1M. AP diagnosis was established previously to JSLE diagnosis in 3/11, simultaneously in 4/11 and after in 4/11. AP clinical features and laboratorial abnormalities observed in our patients were: abdominal pain in 9/11, vomiting in 8/11; increased of amylase in 11/11, lipase in 8/10, triglycerides in 9/11, AST and ALT in 9/11; and decreased albumin in 11/11. Abdominal ultra-sonography collaborated with AP diagnosis in 7/10 (pancreatic edema in 3, necrohemorrhagic pancreatitis in 2 and peripancreatic lymphadenopathy in 2). Of note, SLEDAI-2K score greater than 20 was evidenced in 9/10 patients (median 35, range 12-53). Interestingly, macrophage activating syndrome (MAS) was concomitantly observed in 3 patients. These patients were treated with fasting in 10/11, antibiotics in 10/11, pulse therapy with methylprednisolone in 10/11 and intravenous cyclophosphamide use in 3/11. Despite treatment, 4 patients died, two of them with MAS. AP recovered after a median of 12 days (range 7-30d). Diabetes mellitus was observed in 2 patients, concomitantly at necrohemorrhagic pancreatitis diagnosis in one and one month after AP in the other. **Conclusion:** AP was a rare and life-threatening manifestation in pediatric lupus. The main possible cause of AP in our population was disease activity, suggesting that pancreas could be an involved organ of SLE. The exceptional association between AP and MAS in our cases possibly increased the mortality of these patients.

PO2.N.27

Multiple organ dysfunction syndrome in juvenile systemic lupus erythematosus patients

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Objectives: To analyze multiple organ dysfunction syndrome (MODS) in a population of juvenile systemic lupus erythematosus (JSLE) patients hospitalized in the pediatric intensive care unit (ICU) of a tertiary University Hospital.

Methods: From January 1994 to June 2009, 58 JSLE patients (ACR criteria) were hospitalized in pediatric ICU of our University Hospital and were studied. At admission in ICU, MODS was evaluated according to Goldstein and Wilkinson criteria. Goldstein criteria assessed five organs and systems (cardiovascular, respiratory, renal, hematological and neurological) and Wilkinson criteria evaluated six organs and systems (cardiovascular, respiratory, renal, hematological, neurological and gastrointestinal). Moreover, Pediatric Risk of Mortality (PRISM) was also analyzed. Clinical and laboratorial features necessary to fulfill criteria and PRISM, disease activity (SLEDAI-2K), cumulative damage (SLICC-ACR/DI) and treatment were also collected in the first 72 hours after hospital admission. **Results:** Wilkinson and Goldstein criteria were performed in 48 patients and PRISM in 36. Death was observed in 18/48 (37.5%) patients. JSLE patients fulfilled a median of two affected organs and systems according to both Wilkinson and Goldstein criteria. These patients were divided in two groups according to their **Outcomes:** survivors and death. Of note, the median of Wilkinson and Goldstein criteria were significantly higher in those who died versus survived [3.0 (1-4) vs. 1.0 (0-3), p=0.001; 3.0 (1-5) vs. 1.5 (0-4), p=0.004; respectively]. Compromised cardiovascular system (present in 22 cases) showed the most strongly association with death in both evaluations (p=0.007). The median of PRISM score was significantly higher in those who died versus survived [14.5 (2-33) vs. 4.5 (0-23), p=0.002]. Presence of sepsis (p=0.045), severe sepsis (p=0.002) and septic shock (p=<0.0001) were also considered risk factors for death outcome. In contract, systemic inflammatory response syndrome was similar in both groups (p=0.14). No differences were evidenced in the median of SLEDAI-2K and SLICC-ACR/DI in those who died versus survived [21.0 (2-39) vs. 20.0 (0-42), p=0.733; 1.0 (0-3) vs. 0 (0-3), p=0.801; respectively]. The use of prednisone and intravenous cyclophosphamide were similar in both groups (p>0.05). No Spearman´s correlations were observed between SLEDAI-2K and Wilkinson criteria, Goldstein criteria and PRISM score (p>0.05). **Conclusions:** This study suggests that JSLE patients admitted on ICU with MODS by severe infectious are more susceptible to death, particularly in the presence of compromised cardiovascular system.

PO2.N.28

Toll-like receptor (TLR) expression in juvenile-onset systemic lupus erythematosus (JSLE) and inflammatory controls

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Background: JSLE is a severe multisystem autoimmune disease, characterised by the production of autoantibodies against nuclear material. It is proposed that dysregulated apoptosis may cause autoantigen exposure in JSLE. T and B cells can act as antigen-presenting cells. If they have the ability to detect foreign antigens there is also the possibility they can detect autoantigens. The TLR family are essential components of the innate immune system. TLRs may have a crucial role in the pathogenesis of JSLE. In adult SLE there is up-regulation of TLRs 3,7,8 & 9 in T and B cells which positively correlates with disease activity. TLRs 3, 7-9 can recognise autoantigens typical of SLE. Data concerning TLRs in JSLE is sparse. **Aim:** To determine the expression of TLRs 3,7,8 and 9 in JSLE patients compared to inflammatory controls in T and B cells. **Method:** PBMCs were isolated from JSLE (n=7) and inflammatory controls (n=7;Juvenile Idiopathic Arthritis). T and B cells were obtained using an immunomagnetic cell selection procedure. Total RNA was extracted and reverse transcription performed. Quantitative PCR was used to measure TLR 3,7-9 mRNA and was normalised to 18s (mean±SEM). **Results:** In T cells TLR 3 (34.57±33.55;p=0.003), 8(14.02±4.04;p=0.002), and 9 (13.44±7.07;p=0.002) mRNA expression was higher in JSLE compared to inflammatory controls (TLR3; 4.1±1.114798, TLR8; 4.47±1.04, TLR9; 4.49±1.49). TLR 7 mRNA expression was higher but was not statistically significant. In B cells TLR 7 (3.50±2.34;p=0.038) and 9 (3.94±2.68;p=0.038) mRNA expression was significantly higher in JSLE when compared to inflammatory controls (TLR 7; 0.68± 0.1, TLR 9; 0.80± 0.22). TLR 3 (3.22±2.12;p=0.097) and 8 (3.62±2.32;p=0.073) mRNA expression was also higher in JSLE compared to controls (TLR 3; 0.67± 0.11; TLR8; 0.80± 0.22) but was not statistically significant. **Discussion:** We have demonstrated in-

creased TLR expression in T and B cells in patients with JSLE. TLR stimulation results in inflammatory cytokines production. Increased expression of TLRs is possibly due to increased stimulation by SLE autoantigens acting as TLR ligands. Increased stimulation leads to excessive inflammatory cytokine production, creating a pro-inflammatory environment within the body, characteristic of JSLE . Up regulation of TLRs in B cells suggests that SLE autoantigens may be able to directly stimulate autoantibody production. This is consistent with adult findings, suggesting TLRs may play a role in JSLE. Understanding the true role of TLRs has the potential to provide a significant insight into JSLE and the development of future therapeutic interventions.

PO2.N.29

Paediatric SLE at a tertiary level centre in North India

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Objectives: Systematic study of all diseases is essential to understand the spectrum of the disease presentation, the severity of the disease and the outcome. There is paucity of data from India on details of paediatric SLE. This study aims to add to the existing literature and aims to define: (i) The demographic profile of childhood lupus at our centre; (ii) Describe the clinical features of SLE in children < 18 years of age; (iii) Describe the immunological profile of SLE within six months of disease onset; (iv) To define the mean value of SLEDAI at presentation (within six months of disease onset). *Methods:* This study was carried out from Jan 2008 to June 2009 at the Division of Paediatric Rheumatology, Centre for Child Health, Sir Ganga Ram Hospital, New Delhi. Thirty seven consecutive children less than eighteen years age who presented with Systemic Lupus Erythematosus and fulfilled the ACR 1997 classification criteria through the above mentioned time period were included in the study. These included both new cases and follow up attendees. Data at disease onset was collected on standardized data collection forms. Features present in the first six months after disease onset were taken to calculate the SLEDAI. *Results:* Mean age at diagnosis was12 yrs 3months; F:M::4:1,Family history of autoimmune disorders was present in 18.9% and the mortality in 2.7%. Fever was present in 89% cases on presentation and arthralgia was present in 75.6% children within first six months of disease onset. 64.8% children presented with malar rash and 62.1% had oral ulcers. CNS features were present in 35.1%cases and 48.6% presented with nephritis. In lab markers ANA was positive in 100% cases and dsDNA was positive in 94.5% cases. Hypocomplementemia was present in 83.7% cases. Mean age of onset was 12.3 yrs (Range 5.5 yrs to17.5 yrs). Mean delay to diagnosis: 8.52 months (Range 15 days to 70 months). Age at diagnosis (age / no. / %): 6-10 years / 7/ 20; 11-16 years / 29 / 78.3; >16years / 1 / 2.7. SLEDAI: Mean SLEDAI at our centre was 23.6 (Range 5-49). Mortality in our study is 2.7% that is 1 patient from the study group of 37 died. *Conclusion:* Patients seen at our centre had a significant disease burden with a mean SLEDAI score of >20. This is a small data set from a tertiary level centre and may not be representative of the community disease

PO2O Pregnancy and Fetal Loss

PO2.O.3

The embryotoxicity of sera from patients with autoimmune diseases on post implantation rat embryos in culture persists during remission and is not related to oxidative stress

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Objectives: To evaluate the embryotoxic effect of sera from patients suffering from auto-immune diseases on post implantation rat embryo culture, and determine the association with the clinical presentations, high blood levels of specific antibodies, maternal medications, and oxidative stress parameters. *Methods:* One hundred and eighty, 10.5 day old rat embryos were cultured for 28 hours in their yolk sacs in 33 sera of women with systemic Lupus Erythematosus/Anti phospholipid Syndrome (SLE/APLs) and compared to 84 embryos cultured in rat sera and 88 embryos cultured in control human sera. Embryos were scored, evaluated for the presence of anomalies and for oxidative stress by measuring: superoxide dismutase activity (SOD), catalase like activity (CAT) and for low molecular weight antioxidants (LMWA) measured by cyclic voltametry. Sera were analyzed for the presence and quantification of anti-double stranded DNA, anti-single stranded DNA and the antiphospholipid antibodies: aCL, anti-β2GPI, antiphosphatidylserine, antiphosphatidylcholine and antiphosphatidylethanolamine. Anti-Ro and anti-La were only assessed for the presence of the antibody, but not the titer. Yolk sacs absorptive function was measured by endocytic index. *Results:* The sera proved to be embryotoxic and embryolethal but not teratogenic. Toxic sera, with survival rate under 66% resulted in: smaller yolk sacs and embryos, lower protein level and lower total and neurological score. We found that significantly less embryos cultured in "toxic" SLE/APLs sera had peak 2 of LMWA wave implying a delay in the maturation of the antioxidant defence mechanism. There was a positive correlation between a lower peak 1 of LMWA and recurrent abortions in the embryos cultured in the SLE/APLs sera that were not related to sera toxicity. Embryonic levels of SOD and CAT did not correlate with sera toxicity or patients' clinical history of recurrent fetal loss, thromboembolic events or specific antibodies. The endocytic index did not differ between the control groups and SLE/APLs non toxic sera *Conclusions:* About half of the sera from women with SLE/APLs in remission were embryolethal with no correlation with past clinical history or antibody levels. The possible causes for the high embryotoxicity are currently unknown. Further studies are needed to elucidate the cause/mechanism of the embryotoxicity and the exact role of oxidative stress on the embryos of women suffering from SLE/APLs.

PO2.O.4

Cyclophosphamide therapy in pregnant SLE, does not necessitate termination of pregnancy: case report

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Introduction: Systemic lupus erythematosus (SLE) is a chronic, multi-system autoimmune disease that occurs predominantly in women of childbearing age. The two most commonly used cytotoxic agents in the treatment of SLE are azathioprine and cyclophosphamide. Cyclophosphamide is generally avoided during pregnancy because of its teratogenic risk unless there is no alternative available for life-threatening disease affecting the mother. Fetal loss is a likely outcome of cyclophosphamide administration during pregnancy. *Case Presentation:* We report a young married lady, 22- year old presented with SLE flare. She had CNS manifestations (confusion, abnormal behavior, dystonic movement and recurrent attacks of convulsions). Pancytopenia, vasculitic skin lesion and evidence of nephritis (protinurea more than 4.4 gram/24 hour) supported by renal biopsy cooped with moderate degree of diffuse proliferative GN class IV of systemic lupus erythematosus. So diagnosis of active SLE with cerebritis and nephritis was made. Aggressive therapy started with pulse steroid, followed by oral corticosteroid, antibiotics, anticonvulsant, ACE inhibitors, antimalarial and induction therapy with cyclophosphamide

/IV was started as soon as her blood indices improved. Three months later she got pregnant and although she was informed about the teratogenic effects of cyclophosphamide on the fetus she insisted to continue her pregnancy. Maternal Anti Ro/SSA and anti La/SSB antibodies were requested and Echocardiography also repeated assessing the heart valves and pulmonary blood pressure, all the results were normal. Fetal Echocardiography and fetal electro-cardiogram showed normal P-R interval, no evidence of any degrees of block, myocarditis or hydrops fetalis. The patient was closely monitored for SLE activity, clinically and laboratory. Laboratory investigations include ANA and Anti Ds DNA titer. Serum C3, C4, IgG, IgA, IgM levels. Total serum protein and albumin. Kidney function test and 24/hour urinary protein excretion. She was on regular follow up with the Obstetrician till she gave birth to a healthy full term boy by an uncomplicated CS. *Conclusion:* Termination of pregnancy is not a mandatory procedure for SLE women who got pregnant during cyclophosphomide therapy. As controlling of the disease activity by cyclophosphamide may save their pregnancy and give them chance to get full term healthy baby. But Careful follow up clinically and laboratory to avoid flare up of the disease and complication of cytotoxic drug with regular medical checkup for the well being of the fetus and monitoring the fetal progress by ultrasonography and echocardiography to detect any abnormalities especially heart block is necessary.

PO2.O.5

Pregnancy outcomes in Japanese patients with SLE: retrospective review of 48 cases at a university hospital

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Methods: We performed a retrospective analysis of 38 systemic lupus erythematosus (SLE) patients (48 pregnancies) who were followed at our university hospital from January 2000 to September 2009. *Results:* The mean age of patients was 30.3 years and mean disease duration was 6.0 years. Of the 48 pregnancies, we observed 5 (10.4%) spontaneous abortions, 4 (8.3%) artificial abortions, 2 (4.2%) intrauterine fetal deaths, 11 (22.9%, 1 twin birth) premature births, and 26 (54.2%) live births at full term. After exclusion of artificial abortions, live birth rate was 84.1%. Three patients acquired SLE and five pregnancy-associated flares were documented during pregnancy and postpartum. Neonatal lupus erythematosus occurred in 1 case (skin eruption). *Conclusions:* We conclude that it is necessary to provide SLE mothers with the proper information before the pregnancy. Optimal management of both mothers and infants requires collaborative efforts of both physicians and obstetricians.

PO2.O.7

A comparative study on pregnancy outcomes and contraceptive use in patients with systemic lupus erythematous and those with rheumatoid arthritis or no chronic illness

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Introduction: Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease which affect women primarily, in childbearing age but their fertility rates remain relatively unaffected by the disease. Therefore patients with SLE become pregnant and pregnancy related issues are important in SLE. *Objectives:* To compare the pregnancy out comes and contraceptive practices in SLE with Rheumatoid arthritis (RA) and women with no chronic illness (WNCI) among patients attending a dedicated Lupus clinic in Colombo, Sri Lanka. *Methodology:* Patients with SLE who satisfied ACR criteria for diagnosis and history of pregnancies were identified from University Lupus Clinic,

National Hospital of Sri Lanka(NHSL). Age matched women with history of pregnancy and RA was obtained from Rheumatology clinic, NHSL and WNCI from a surgical clinic. Comparisons between live birth rates (LBR), pregnancy losses, contraceptive use and planned pregnancies in the three groups were done using chi square test. *Results:* Table 1 gives main pregnancy outcomes in the 3 groups. In 78 patients with SLE, 59 pregnancies occurred before and 20 after diagnosis. In 32 patients with RA, 72 pregnancies occurred before and 8 occurred after diagnosis. The mean age at diagnosis was higher (p< 0.01) in RA (35years) than in SLE (25.98years) using student t test. LBR after diagnosis was significantly lower (p<0.01) in SLE (9/20, 45%) compared to RA (6/8, 75%) and WNCI (77/85, 91%). Adverse pregnancy outcomes (fetal loss, pre-maturity, low birth weight) and assisted deliveries were more (p<0.001) in SLE than in WNCI. Unplanned pregnancies were more (p<0.01) in SLE (80%) than in RA (25%) and in WNCI (9.4%). Contraceptive usage was lower in patients with SLE (25.6%) compared to WNCI (56.4%). Disease exacerbations occurred in 20% in SLE during pregnancy and no babies developed neonatal lupus. *Conclusions:* More pregnancies occur in SLE than RA after diagnosis of illness. Unplanned pregnancies and adverse pregnancy outcomes need to be addressed more in SLE than in RA or in WNCI.

	SLE + APS		RA		Control (WNCI) (n= 85)
	Before diagnosis (n = 59)	After diagnosis (n=20)	Before diagnosis (n=72)	After diagnosis (n=8)	
Live Birth at Term	32 (54%)	6 (30%)	60 (83%)	6 (75%)	78 (92%)
Pre term delivery	5 (6%)	3 (15%)	0	0	2 (2.3%)
Normal birth weight	24 (65%)	2 (22%)	53 (88%)	6 (68%)	63 (79%)
Low birth weight	13 (35%)	7 (78%)	7 (12%)	2 (33%)	17 (21%)
Still birth	2 (3%)	4 (20%)	1 (1.4%)	0	0
Spontaneous abortion	20 (34%)	5 (25%)	10 (12%)	1 (13%)	4 (4.8%)
Therapeutic abortion	0	2 (10%)	0	0	0
H mole	0	0	1 (1.4%)	0	0
Ectopic pregnancy	0	0	0	1 (13%)	1 (1.2%)

PO2.O.8

Increased risk of adverse pregnancy outcome in CTDs patients with SSA and/or SSB antibodies and autoimmune thyroid disease

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Objectives: Connective tissue disorders (CTDs) and autoimmune thyroid diseases (AITs) can be frequently associated and both can affect pregnancy outcome. Anti-Ro-SSA/La-SSB antibodies are the most frequently anti-extractable nuclear antigen (ENA) auto-antibodies detected in different CTDs. Beyond neonatal lupus, some papers have advocated them as a marker of other pregnancy complications even if this is not universally supported. A recent paper stated that mothers carrying both these conditions had a 9-fold increased risk over pregnant women with normal thyroid function of having a child with cardiac congenital heart block. In the present study we tried to investigate if mothers with hypothyroidism and anti-Ro-SSA and/or anti-La-SSB antibodies were at major risk for adverse pregnancy outcome. *Methods:* From January 2005 to December 2008 we have prospectively followed 57 pregnancies in 52 women divided into 3 groups. Characteristics of patients and pregnancy outcome are detailed in Table [1]. All women with hypothyroidism had this diagnosis before pregnancy and were adequately supplemented with thyroxine therapy. The women with other ENA specificities, positive antiphospholipid antibodies and/or lupus anticoagulant or any other known congenital thrombofilic condition were excluded. *Results:* Adverse pregnancy outcome was clearly correlated to the presence of hypothyroidism associated with SSA and/or SSB antibodies; indeed, 50% of the pregnancies in this group of patients ended in spontaneous abortions (3 miscarriages and

1 intrauterine foetal death) and 13% in premature membrane rupture at 33rd weeks gestation. Pregnancy outcome in the other two groups of patients was comparable suggesting that the association of the two conditions (e.g. hypothyroidism and presence of SSA and/or SSB antibodies) is a real risk factor for pregnancy complications and, in particular, for recurrent miscarriages (p < 0.0001). *Conclusions:* Even if the number of cases is too small to draw definitive conclusions, we think that our results suggest a careful follow-up of all patients with CTDs and positive SSA and/or SSB antibodies associated with hypothyroidism due to autoimmune thyroid disease.

	CTDs pts with SSA and/or SSA without AIT	CTDs pts without SSA and/or SSB plus AIT	CTDs pts with SSA and/or SSB plus AIT
N° patients	26	20	6
N° pregnancies	27	22	8
Rheumatoid Arthritis	0	4	0
SLE	12	4	3
UCTD	5	12	1
Sjogren syndrome	9	0	2
Median age	33 (23-40)	35 (27-51)	36 (28-44)
N° miscarriages (%)	1 (3%)	1 (4,5%)	4 (50%)
Pre-eclampsia /%)	1 (3%)	1 (4,5%)	0
PROM (%)	0	2 (10%)	1 (12,5%)
Prematurity	0	1 (4,5%)	0
IUGR (%)	1 (3%)	2 (10%)	0
CCHB	0	0	0

PO2.O.9

Occurrence of proteinuria in pregnant patients with lupus nephritis

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Introduction: In pregnant patients with systemic lupus erythematosus (SLE), the differential diagnosis between pre-eclampsia and active lupus nephritis during pregnancy is generally based on the combined analysis of urinary sediment, renal function tests, 24 hrs proteinuria, anti-dsDNA and, when available, anti-C1q antibodies. However some patients may pose diagnostic problems with an impact on therapeutic decision and pregnancy outcomes. *Aim:* To determine the occurrence of pre-eclampsia in a group of patients with lupus nephritis (LN) prospectively followed and to evaluate the misdiagnoses or difficulties in diagnosis encountered. *Methods:* SLE patients with LN were included in this analysis. The following parameters during pregnancy were monitored: renal function, urinary sediment, 24 hour proteinuria, C3, C4, anti-dsDNA antibodies, anti-C1q antibodies, complete blood cell count, blood pressure and body weight. *Results:* Twenty-five pregnancies in patients with LN (3 type II, 4 type III, 18 type IV LN) were selected. The mean age at pregnancy was 32.4 years and the mean disease duration was 9.7 years. In all but one patients LN was inactive at pregnancy and the mean duration of remission was 5.9 years. All patients were receiving therapy: 24 methylprednisolone, 11 immunosuppressive drugs (Azathioprine 8, Cyclosporine 3), 10 heparin, 18 low dose aspirin, 3 IVIg. Two pregnancies ended with a IUFD at 26 and 28 weeks, the remaining had a mean duration of 36.8 weeks; the mean gestational age at delivery was 36.8 weeks, with 9 (39%) preterm deliveries. The mean body weight of offsprings was 2680 g, and 5 babies were small for gestational age. Disease flares were observed in 6/24 patients (excluding the patient with active disease at onset); in 2 cases a renal flare was diagnosed. Two cases of pre-eclampsia were observed occurring at 24 and 26 weeks in the two pregnancies ended with IUFD. An increase in proteinuria was observed in 8 patients not configuring a renal exacerbation nor pre-eclamptic syndrome. In these patients, the raise in proteinuria was observed during the third trimester of pregnancy (mean Delta value 1.2 g/24 hr). Six of these pregnancies ended preterm (mean 36 weeks) with cesarean section decided because of the increase of proteinuria. In all these patients the proteinuria values spontaneously returned to pre-pregnancy levels within 2 months from delivery suggesting

an increase related with pregnancy and excluding a renal flare. In conclusion isolated increases in proteinuria are observed in a high percentage of pregnant patients with LN which cannot be certainly attributed to pre-eclampsia nor to LN. The identification of additional biomarkers or the definition of a diagnostic algorithm might be of help in the correct interpretation of these findings and could help the physician in decision making.

PO2.O.10

Outcome of pregnancies in a group of SLE patients prospectively followed at a single center

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Introduction: Pregnancy in patients with Systemic lupus Erythematosus (SLE) is associated with an increased incidence of adverse outcomes due either to disease flares or to obstetric complications (OC). *Objectives:* To evaluate disease activity and obstetrical outcomes in a cohort of pregnant SLE patients prospectively followed in a single centre. *Methods:* Pregnancy outcomes of SLE patients prospectively followed at the Pregnancy Clinic of our Unit were examined. From the time the pregnancy is detected, patients undergo monthly rheumatological and obstetric evaluations including ultrasound and uterine artery Doppler as appropriate. Obstetric complications included: pre-eclampsia, preterm delivery, PROM, IUGR, IUFD. *Results:* Forty-nine pregnancies were followed; 7 ended with a spontaneous abortion within 10 weeks of gestation and were excluded from the analysis. The remaining 42 were the object of our study. The mean age and the mean disease duration at pregnancy were 32.5 and 8.8 years respectively. In 25 pregnancies (59.5%), a previous history of biopsy- proven lupus nephritis (LN) was present (mean duration of remission before pregnancy 6.2 years). Disease flares were observed in 10 patients (24%), 9 occurred during pregnancy and were characterized by hematological (4 cases), renal (2 cases), articular (2 cases), CNS (1 case) manifestations. In one patient a flare was observed in the puerperium. Overall, obstetric complications were observed in 22 pregnancies (52.3%) with 4 cases of pre-eclampsia. As far as fetal outcome is concerned, two pregnancies ended with a intrauterine fetal death at 26 and 28 weeks; in the remaining successful pregnancies the mean gestational age at delivery was 36.9 weeks, with 13 (32%) preterm deliveries. The mean body weight was 2880 g, 6 babies were small for gestational age. The occurrence of pre- eclampsia was correlated with APL (p<0.05) but not with previous LN nor with disease flare during pregnancy. However, previous LN was significantly associated with a higher incidence of OC overall (p=0.032); when we considered each OC separately, only the association with low body weight (<2500 g) resulted statistically significant (p=0.02). *Conclusions:* These data show that pregnancy in SLE is at high risk as it is associated with the occurrence of complications/flares in 64% of patients. As expected, antiphospholipid antibodies resulted an important risk factors for pre-eclampsia, while previous renal involvement did not. However the presence of LN seems to predispose to obstetrical complication in general and, in particular, to a tendency to have babies with low body weight.

PO2.O.11

Decreased live births in women with SLE

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Objective: Though well-designed studies are lacking, there is a general notion that live births are not decreased in SLE, compared to healthy women.

We calculated live births in women with SLE, and compared this with general population rates. **Methods:** We studied women with SLE from a subset of centers participating in the SLICC Registry for Atherosclerosis inception cohort study. Women diagnosed with SLE before age 50 were included. We determined the number of children borne as of the last follow-up visit, and summed the years from age 15-50, or oldest age attained if the subject was <49. We applied age- and country-specific general population birth rates, and relevant calendar-period rates to these years to determine the expected number of live births. We then calculated the standardized incidence ratio (SIR) of observed to expected live births. We performed a multivariate analysis with the SIR as the dependent variable to explore potential predictors of live births, including marital status, drugs (immunomodulators, prednisone, aspirin, anticoagulants), antiphospholipid antibodies, disease activity and damage. **Results:** 339 women with SLE were studied. Mean age at diagnosis was 35.3 years (standard deviation, SD, 13.3) and mean disease duration at the last visit was 2.7 years (SD 2.0). Most (43%) women were from the US, 27% from Canada, 27% from the UK, and 3% from Sweden. The majority of women (61%) were white, 19% were black, 10% were Asian and 5% were Hispanic. Most (42%) were currently married or living common-law. Overall, the number of live births over the interval (313) was substantially below what would be expected (479) (SIR 0.65; 95%CI 0.58-0.73). In sensitivity analyses, we used race-specific general population birth rates with similar results (SIR 0.65; 95%CI 0.58-0.72). In multivariate analyses, being married or living common-law (SIR 2.07; 95%CI 1.58-2.69) and current use of aspirin (SIR 1.36; 95%CI 1.02-1.80) were associated with increased live births. There were trends for fewer live births in women exposed to cyclophosphamide (SIR 0.82; 95%CI 0.53-1.27) and in those with high disease activity (SLEDAI ≥ 5) (SIR 0.76; 95%CI 0.54-1.07). We did not establish a decrease in live births independently attributable to positive antiphospholipid antibodies (SIR 0.98: 95%CI 0.73-1.32) or disease damage (SLICC score ≥ 2) (SIR 1.02: 95%CI 0.70-1.48). **Conclusion:** Women with SLE have fewer live births compared with the general population. Marital status and current aspirin use were the most important predictors of live births in our sample.

PO2.O.12

Outcomes and disease flares in 36 consecutive lupus pregnancies
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Objective: It is well documented, that pregnant lupus patients are at increased risk of spontaneous foetal loss, preeclampsia and disease exacerbation. In this study we investigated pregnancy outcomes and the pattern of pregnancy related disease flares in a population of predominantly Scandinavian lupus patients. **Method:** At Odense University Hospital, Denmark, a population-based cohort of lupus patients is followed (1). Thirty-six consecutive pregnancies during a 12-year period were studied retrospectively using a research database and medical records. **Results:** Twenty-one patients presented with 36 pregnancies with the following outcome: 20 live births, 10 spontaneous abortions, 4 fetus mortuus and 2 provoked abortions. In 11/21 lupus patients all pregnancies resulted in live births. Ten patients had 1 to 4 miscarriages, however, 5 of these patients also gave birth to live children. Seven of 10 patients with miscarriage had antiphospholipid syndrome (APS). In APS patients, all treated with low molecular weight Heparin (LMWH) and low dose aspirin (ASA), 19% of the pregnancies resulted in live births. Lupus flares were diagnosed in 20/36 pregnancies, pre-partum flares in 14 (arthralgia 50%, rash 40%) and post-partum in 17 patients (arthralgia 40%, rash 55%). Five pregnancies resulted in preeclampsia, all patients with pre-existing nephropathy. HEELP syndrome was diagnosed in two. SSA/SSB autoantibodies were recorded in 17 pregnancies resulting in 7 live children without signs of neonatal lupus or congenital heart block. **Conclusion:** In this ethnically homogenous lupus population the risk of spontaneous foetal loss was significantly elevated (40%) as compared to the general population (estimated risk of miscarriage in Denmark is 20%). APS was a major risk. Nevertheless, APS patients treated with LMWH and ASA were capable of giving live birth in 19% of their pregnancies.

Pregnancy related flares were mostly mild, mainly including joints and skin. However, patients with pre-existing lupus nephropathy seem to be at increased risk of acquiring preeclampsia.

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PO2.O.13

Adjunctive GnRH-a treatment attenuates depletion of ovarian reserve during CYC therapy in pre-menopausal SLE patients
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Objectives: Premature ovarian failure (POF) is a well recognized adverse outcome for women of child bearing age treated with cyclosphosphamide (CYC) for autoimmune disease (including lupus, vasculitis, and scleroderma), as well as malignancies. Anti-mullerian hormone (AMH) is a biomarker of ovarian reserve believed to be produced only by antral follicles possessing growth capacity, and thus may represent the quantity and quality of the ovarian follicle pool. AMH has been demonstrated to have utility for prediction of the timing of natural menopause, and also response to assisted reproductive technologies. We propose that AMH can serve as a surrogate measure of CYC-induced ovarian damage, with the hypothesis that AMH levels will be diminished in patients exposed to CYC versus patients with no exposure, or patients who received adjunctive gonadotropin-releasing hormone agonist (GnRH-a) for ovarian protection during CYC therapy. **Methods:** 50 pre-menopausal patients from the Michigan Lupus Cohort were studied and divided into three treatment groups: CYC alone (n=9), CYC + GnRH-a (n=11), neither (n=30). AMH was measured by a commercial ELISA (Beckman Coulter; Marseille, France) in the CLASS (Central Ligand Assay Satellite Services) lab at the University of Michigan. AMH levels between groups were compared using a non-parametric test (Wilcoxon rank-sum), and multiple linear regression adjusting for age was performed. **Results:** AMH (ng/mL) levels were significantly lower in the CYC vs neither group (mean ± SD: 0.27±0.22 CYC vs 1.11±1.07 neither; p=0.04). However, AMH levels for the CYC+GnRH-a group (0.68±1.04) were non-significantly lower than the neither group (p=0.19), suggesting that adjunctive GnRH-a therapy provided some protection against CYC-induced ovarian damage. When centered on age 30 years, the average AMH levels for the CYC, CYC+GnRH-a, and neither groups were: 0.27, 0.36, and 0.85, respectively. When adjusted for age, AMH levels between all groups were significantly different (p<0.0005). Preliminary examination of trends over time suggests that the slope of AMH decline according to age also differs between groups. Future directions will be to investigate longitudinal trends, cumulative dose effects, and interaction between dose and age. **Conclusions:** These data confirm that after accounting for age, CYC exposure is associated with diminished ovarian reserve, and that co-treatment with GnRH-a provides some, but not full, protection against ovarian damage. These data suggest that AMH is an indicator of CYC-induced ovarian damage, and may have utility as a surrogate measure of ovarian function in clinical trials of candidate agents for ovarian protection such as GnRH-a.

PO2.O.14

Use of effective contraceptive methods among women taking teratogenic medications for systemic lupus erythematosus
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Objectives: Systemic Lupus Erythematosus (SLE) is a disease that primarily affects women of reproductive ages. It is recommended that women with SLE plan their pregnancies for a time when they can minimize their exposure to teratogenic medications and for when their disease is quiescent. Most are candidates for extremely effective contraceptive methods (typical use failure

rate ≤ 3%), including depomedroxyprogesterone acetate, implants, intrauterine contraceptive devices, or permanent sterilization. However, these methods are often underutilized. The primary objective of this study is to assess whether women with SLE who are at risk for pregnancy and using methotrexate (MTX), mycophenolate mofetil (MMF), cyclophosphamide (CYC), or azathioprine (AZA) are also using extremely effective methods of contraception. *Methods:* All women aged 15-44 presenting to the LAC+USC Rheumatology Clinics in January 2010 with a diagnosis of SLE were approached to complete a 30 item researcher-administered survey. *Results:* Of the 15 women meeting inclusion criteria, 14 (93%) completed the survey. Ten women (71%) were sexually active. Of the sexually active women, at the time of the interview, 100% were taking one or more of MTX, MMF, CYC, or AZA, 40% were using extremely effective methods of contraception, and 60% were using either no method of contraception or unreliable methods (typical use failure rate ≥10%). Four women experienced six unintended pregnancies since their SLE diagnosis. Of the four women with unintended pregnancies since their SLE diagnosis, two are currently using extremely effective methods of contraception and two are currently using unreliable methods. *Conclusions:* Many reproductive-aged women with SLE who are taking potentially teratogenic medications are sexually active and not using adequate contraception, putting them at risk of unintended pregnancies. Considering the risks of unplanned pregnancies, further studies should investigate strategies for improving uptake of effective contraception among women with SLE taking teratogenic medications.