Conclusion: PLTs are greatly activated in SSc and this is associated with disease progression. Findings suggest that this activation is greater at less severe patients.

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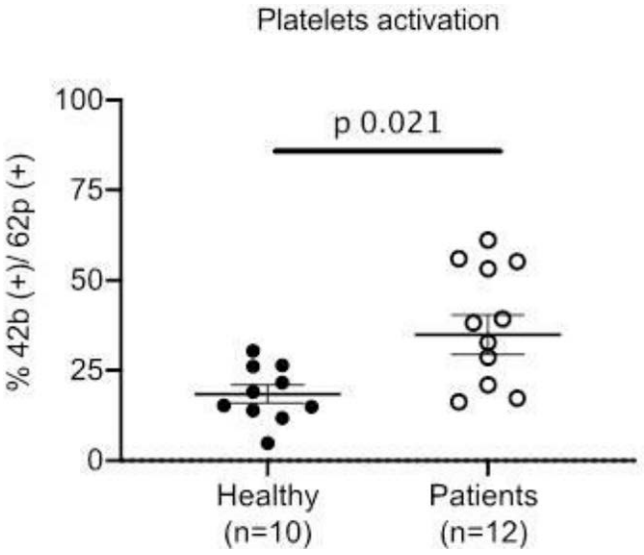

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AB0170 PHENOTYPIC CHARACTERIZATION OF ENDOTHELIAL PROGENITORS CELLS OF SYSTEMIC SCLEROSIS (SSC) PATIENTS: ROLE IN ENDOTHELIAL-TO-WMESENCHIMAL TRANSITION PROCESS

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Background: Endothelial-to-mesenchymal transition (EndoMT), a newly recognized type of cellular transdifferentiation, seems to be involved in Systemic Sclerosis (SSc) pathogenesis. In this process endothelial cells lose their specific markers, and acquire a mesenchymal phenotype, thus expressing cell products such as alpha smooth muscle actin (α-SMA) (1,2).Circulating endothelial progenitors cells (EPCs) derive from bone marrow stem cells and contribute to *de novo* vessels formation. Several studies, although with conflicting results, have shown that EPCs in the peripheral blood of patients with SSc are impaired in their number and function (3).

Objectives: to assess phenotypic characteristics of EPCs fromSSc patients and from patients with Very Early Diagnosis of SSc (VEDOSS) compared with healthy controls (HC). In particular we want to evaluate the expression of α-SMA, as marker of a pro-mesenchymal switch (EndoMT) in:

1. Circulating Early (CD34+KDR+CD 133+) and Late EPCs(CD34+KDR+) in the peripheral blood using flow cytometry
2. Cultured EPCs using Western blot analysis

Methods: we enrolled 11 patients (6 SSc and 5 VEDOSS), classified according to the classification criteria for SSc (4) and for VEDOSS not fulfilling SSc criteria (5), and 5 HC. Phenotypic characterization was performed as previously described by Vasa et al. using a FACS Calibur (BD Immunocytometry Systems). EPCs number was expressed as a percentage of cells within the lymphocyte gate. 5*106 PBMCs were plated on human fibronectin-precoated (10 µg/ml Sigma-Aldrich) 6-well plates and cultured for 7-12 days to obtain EPCs. PBMCs from one HC were also cultured with 20% SSc patient serum. Collected EPCs were lysed and a Western blot analysis for α-SMA detection was performed.

Results: we found a significant higher percentage of α-SMA positive Early EPCs in all patients respect to HC (0,06% ±0,03 vs 0,03% ± 0,01; p=0,0149) particularly in VEDOSS patients (0,07%±0,01 vs 0,03%±0,01 p=0,008). Similarly we found a significant higher expression of α-SMA protein in all patients and VEDOSS patients respect to HC (0,1895±0,16 vs 0,07± 0,06 p= 0,0342; 0,3075 ± 0,14 vs 0,07± 0,06 p=0,0159). After the incubation of HC PBMCs with SSc serum, the α-SMA protein expression seems to be increased respect to its expression in thePBMCs of the same HC cultured without SSc serum (0,33 vs 0,1).

Conclusion: we found higher percentage of Early α-SMA positive EPCs and a higher expression of α-SMA protein in cultured EPCs in patients group (SSc and VEDOSS) than in HC. So we hypothesized a predominant pro-mesenchymal phenotype of this kind of EPCs. This could be considered the expression of the involvement of EPCs in the EndoMT process and it better explain the controversial role of EPCs in SSc pathogenesis. Moreover the modified expression of α-SMA in HC EPCs co-cultured with 20% SSc serum could suggest the presence of a factor inducing the EndoMT process in the disease.

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AB0171 THE IMMUNOMODULATORY AND ANTI-INFLAMMATORY EFFECTS OF BOSENTAN IN SYSTEMIC SCLEROSIS

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Background: Plasma endothelin-1 (ET-1) levels are increased in patients with systemic sclerosis (SSc), playing a central role in the development of fibrosis, vasoconstriction and inflammation[1]. While the beneficial effect of Bosentan, the endothelin receptor antagonists, have been demonstrated on vasoconstriction and fibrosis, its potential anti-inflammatory and immunomodulatory activity needs to be further investigated.

Objectives: To assess whether Bosentan can modulate the gene expression profile of immune cells in sample of patients with limited and diffuse SSc and active digital ulcers.

Methods: We enrolled 34 patients affected by SSc. Twenty-four patients were affected by limited SSc and 12 by diffuse SSc. Blood samples were collected from patients before and after 24 weeks of treatment with Bosentan, in the absence of immunosuppressive therapies. All patients received Bosentan 125 mg twice a day for 24 weeks. Gene expression profiles were assessed by GeneChip® Human Transcriptome Array 2.0 microarray technology. Significantly (p-value<0.05) and differentially (|FC|>1.5) expressed genes pre/post treatment were obtained by paired t-statistics, as implemented in Partek Genomics Suite ver. 6.6. These genes were subjected to functional enrichment analysis by Ingenuity Pathway Analysis. The effect of Bosentan on patients was studied on the "diffuse" and "limited" sub-cohorts, individually, as well as on the whole cohort.

Results: Contrary to the limited cohort where differentially expressed genes resulted to be all non-coding genes which are almost all over-expressed before treatment, the diffuse cohort was characterized by 19 differentially expressed genes that enrich biological functions and pathways related to the immune system and its organic response (in particular T-cells). Comparing the limited to the diffuse cohort, pre- and post- treatment, a distinct genetic fingerprint emerges, that characterizes the response to Bosentan by the latter cohort as increased apoptosis of lymphocytes (z-score=3.28) and a decreased quantity of antigen presenting cells (from z-score=1.06 (pre) to -0.75 (post)).

Conclusion: The presence of an inflammatory microenvironment, as occur in SSc, influence the relative expression of ET-1 receptors on immune cells, which in turn further contribute to the amplification of cellular responses to inflammation. The observed difference response to therapy between the two cohorts of patients was attributed to influence of ET-1 levels on the relative expression of ET-1 receptors on immune cells surface. Interestingly Bosentan, beside the already-known effect on promoting antigen presenting cells apoptosis, seem to exert its immunomodulatory activity also by deregulating functions that mainly involves the T cells and by promoting their apoptosis, which in turn reflect also its anti-inflammatory proprieties.

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AB0172 **PGC-1A REGULATES AUTOPHAGY TO PROMOTE FIBROBLAST ACTIVATION AND TISSUE FIBROSIS**

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Background: Peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) is the best studied member of the family of coactivators. PGC-1α was initially identified through its interaction with PPARγ in brown adipose tissue. Recent evidence further indicates that PGC-1α may also modulate the transcription of autophagy-related genes, which has recently been shown to be required for fibroblast-to-myofibroblast differentiation under fibrotic conditions. However, the role of PGC-1α in the pathogenesis of SSc has not been investigated.
Objectives: The aim of the present study was to evaluate the role of the coactivator PGC-1α on autophagy and to evaluate its role in the pathologic activation of fibroblasts in SSc.
Methods: Expression of PGC-1α was analyzed by RT-PCR, Western blot and immunofluorescence. Modulation of autophagy was analyzed by reporter studies by expression of autophagy related genes. The effects of PGC-1α knockdown on collagen production and myofibroblast differentiation were analyzed in cultured human fibroblasts and in two mouse models with fibroblast-specific knockout of PGC-1α.
Results: PGC-1α overexpression was detected by immunohistochemistry in skin sections of SSc patients and in experimental fibrotic murine skin, particularly in fibroblasts. Knockdown of PGC-1α inhibited the stimulatory effects of TGFβ on fibroblast activation with impaired induction of collagen as compared to control fibroblasts. Fibroblasts specific knockout of PGC-1α ameliorates experimental fibrosis in bleomycin-induced and adTBR-induced murine dermal fibrosis with decreased dermal thickness, hydroxyproline and myofibroblast counts compared to wild-type fibrotic mice. Incubation of dermal fibroblasts with TGFβ activated autophagy in control fibroblasts with increased expression of the autophagy-related genes ATG7 and BECLIN-1, enhanced conversion of LC3 I to LC3 II and decreased ratios of ILC3 I EGFP to LC3 II RFP in LC3 reporter assays. The expression levels of ATG7, BECLIN-1 and ILC3 II of TGFβ-stimulated PGC-1α knockout fibroblasts decreased compare to TGFβ stimulated wild-type fibroblasts. The ratio of ILC3 I EGFP to LC3 II RFP of TGFβ-stimulated PGC-1α knockout fibroblasts in reporter assays were comparable to unstimulated fibroblasts.
Conclusion: PGC-1α is upregulated in SSc and promotes autophagy to foster TGFβ-induced fibroblast activation. Targeting of PGC-1α prevents aberrant autophagy, inhibits fibroblast activation and tissue fibrosis.
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10. Basic and translational science in paediatric rheumatology

AB0173 **ALLELIC POLYMORPHISM OF PROINFLAMMATORY CYTOKINE GENES AS A BASIS FOR THE FORMATION OF PHENOTYPES OF JUVENILE IDIOPATHIC ARTHRITIS**

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Background: The pathological process of juvenile idiopathic arthritis (JIA) largely depends on pro-inflammatory cytokines, the polymorphism of the alleles of some genes of which we have the opportunity to study. No studies have been conducted on the dependence of certain features of the pathological process of JIA on the polymorphism of the IL-6(G-174C) and TNF(G308A) genes.
Objectives: To reveal the dependence of JIA phenotypes and its course on genetic polymorphism of alleles IL-6 and TNF.
Methods: Polymorphism of the IL-6 and TNF genes was studied by PCR-method using allele-specific primers 44 patients 1-17 y.o. (24f, 20m) with JIA. The level of IL-6 and TNFα in the serum was determined using ECLIA and CLIA methods.
Results: There were 73% cases with an unfavorable course of the disease (UCD) of the patients with the CC allele of the IL-6 gene, for most patients average activity was JADAS27 13.5±1.6. oJIA (50%) & uveitis (30%) were the most frequent among subgroups. The level of serum IL6 was 74.1±69.5 pg/ml, TNFα 27.4±17.3 pg/ml (ratio IL6/TNFα=4.3±2.1). Among patients with GC IL6 70% female, 79% with UCD. More often pJIA (36%, including all RF+) and eJIA (35%) were noted with the largest frequency of inclusion of the hip joints (33%), spine (35%), detection of secondary osteoporosis (43%). The metabolic changes were registered on the ECG in 82% cases. The serum IL-6 level was 11.35±2.95 pg/ml, TNF 241.75 pg/ml (IL-6/TNFα=0.047, p<0.05 vs CC allele). Children with GG IL-6 (wild allele) with a more favorable course of the JIA (31%, less than in the CC and GC groups (p<0.05), only 8% had the highest disease activity), the largest number of patients with sJIA (25%) was registered in this group. The detection of HLA B27 was significantly lower (p<0.05) than in other alleles, while 60% cases were ANA+ (more than in the group GC, p<0.05). The highest level of serum IL6 (35.3±18.9 pg/ml) & the highest average number of mutations in folate metabolism genes (4±0.51) were revealed in this group. The wild allele GG prevailed (n=32) among the TNF gene alleles, sex ratio 1:1, UCD in 70%. The number of active joints, ESR, CRP, ANA-positivity (50%), HLA B27+ (53%) were unsignificantly higher than in GA TNF allele, while serum IL6 level (22.8±9.8 pg/ml) & TNFα (12.3±4.1 pg/ml) were lower. In patients with the GA TNF gene allele, an UCD (73%), eJIA (36%) were noted slightly more often. By such parameters as the patient's gender, the presence of uveitis, damage to the hip joints, the type of synovitis, metabolic changes on the ECG, indicators were observed comparable with the wild allele group. IL6 level was 48.3±39.2 pg/ml, TNFα 636.5±420.1 pg/ml, IL6/TNFα=0.07±0.06 (vs 1.9±0.5 in GG group, p<0.05). The genotype of two wild alleles TNF GG with IL6 GG expectedly showed the smallest proportion of the UCD (33%, p <0.05), the most frequency of ANA-positivity (71%), with no uveitis and RF+pJIA in this group. All cases of RF+pJIA had TNF GA and IL6 GC. oJIA prevailed (57%) in the TNF GG&IL6 CC group, there was not a single case of sJIA, and the AJ number was the smallest (2.86±0,5). The largest group was TNF GG & IL6 GC (n=14). 91% of cases had UCD, AJ=6.6±2.4, damage to the hip joints in 40%, ESR 23.7±6.7 mm/h, CRP 14.5±5.4 mg/l, metabolic changes on the ECG in 100%, but ANA+ only at 13%. In general, there was no correlation between the cytokine content in the blood serum during of active disease in the examined children with features of allelic polymorphism of these genes.
Conclusion: Depending on the allele polymorphism of the IL-6 and TNF genes, certain phenotypes of the JIA course may be distinguished. Thus, revealing the polymorphism of these alleles in patients at the onset of the disease, we can predict to some extent its course and take this into account when choosing treatment tactics.
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AB0174 **T REGULATORY CELLS LEVEL IN PERIPHERAL BLOOD OF PATIENTS WITH JUVENILE IDIOPATHIC ARTHRITIS AND ITS RELATION WITH DISEASE ACTIVITY**

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