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Serological Biomarkers in Systemic Lupus Erythematosus

Chan, Madelynn

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SEROLOGICAL BIOMARKERS IN SYSTEMIC LUPUS ERYTHEMATOSUS

Madelynn Tsu-Li Chan

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DECLARATION

I declare that this thesis represents my own work and to the best of my knowledge, does not contain any material previously published or written without due reference. This thesis has not been submitted to any other University in application for another degree or diploma.

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ABSTRACT

Background

Systemic lupus erythematosus (SLE) is a multi-system autoimmune disease characterised by autoantibody production and variable clinical features, ranging from mild to severe disease. Patients with SLE are at increased risk of developing accelerated atherosclerosis. Biomarkers have potential utility in SLE as markers of disease or predictors of future clinical events and mortality.

Objective

The aim of this thesis was to identify serological biomarkers predictive for erosive arthritis (EA), cardiovascular events (CVEs), mortality and subclinical atherosclerosis in SLE.

Methods

In chapters 2 to 4, study subjects were SLE patients from Bath. Anti-cyclic citrullinated peptide antibodies (ACPA) and *HLA-DR* and *-DQ* were studied for markers of EA, and anticardiolipin (aCL) and lipoprotein profiles for markers of CVEs and mortality. In chapters 5 and 6, study subjects were women with SLE from Manchester. B-mode ultrasound scans of subjects' carotid arteries were performed at baseline and follow-up time-points to detect atherosclerotic plaque. Baseline IgG and IgM antiphospholipid (aPL) antibodies and CV risk factors were studied for markers of subclinical atherosclerosis. Clinical data collected for all studies included SLE features and auto-antibody profiles.

Results

ACPA was identified as a marker of a SLE phenotype with EA - "rhupus". Patients with major erosive arthritis were *HLA-DQB1*0302* carriers. Increased aCL GPL levels and total cholesterol : high density lipoprotein-C (TC : HDL-C) ratio were markers for future CVEs, and increased TC : HDL ratio, aCL GPL and lipoprotein(a) concentrations were markers for increased mortality. Lower HDL-C concentrations and anti-annexin A5 (anti-AnxA5) GPL were markers of carotid plaque progression.

Conclusion

This thesis identified new markers for EA, subclinical atherosclerosis and future CVE and mortality risk in SLE. Strategies to incorporate these new CV markers into clinical CV risk assessments may assist in distinguishing the subset of SLE patients most at risk of developing accelerated atherosclerosis.

LIST OF ABBREVIATIONS

%CV	coefficient of variation
2D	two-dimensional
ABCA1	ATP-binding membrane cassette transport protein A 1
aCL	anti-cardiolipin antibody / antibodies
ACPA	anti-cyclic citrullinated peptide antibody
ACR	American College of Rheumatology
ACS	acute coronary syndrome
ACS-ACOD-MEHA	acyl-CoA synthetase - acyl-CoA oxidase - 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline
AECA	anti-endothelial cell antibody
AH	ancestral haplotype
ANA	anti-nuclear antibody
anti-AnxA5	anti-annexin A5
anti-dsDNA	anti-double-stranded deoxyribonucleic acid
anti-LB1	anti-nuclear lamin B1
anti-oxPAPC	anti-oxidised palmitoyl arachidonoyl phosphocholine
anti-PT	anti-prothrombin (anti-PT)
anti-PT/PS	anti-prothrombin/phosphatidylserine
anti-Ro/SSA	anti-Ro/SSA
anti-Sm	anti-Smith
anti-ssDNA	anti-single-stranded deoxyribonucleic acid
anti-U1RNP	anti-U1 ribonucleoprotein
anti-β_2GPI	anti-β_2-glycoprotein I
AnxA5	annexin A5
APC	antigen presenting cell
aPL	antiphospholipid antibody / antibodies
apo(a)	apolipoprotein (a)
apoA-I	apolipoprotein A-I
apoB	apolipoprotein B
apoB100	apolipoprotein B100
apoE	apolipoprotein E
APS	antiphospholipid syndrome

aPTT	activated partial thromboplastin time
AUC	area under the curve
BILAG	British Isles Lupus Assessment Group index
BILAG-2004	British Isles Lupus Assessment Group 2004 index
BIRD	Bath Institute for Rheumatic Diseases
BLK	B-lymphoid tyrosine kinase
BMI	body mass index (kg/m2)
BP	blood pressure
CCA	common carotid artery
CCF	congestive cardiac failure
CD11B	cluster of differentiation molecule 11B / Integrin alpha M (ITGAM) / CR3A
CETP	cholesteryl ester transfer protein
CHB	congenital heart block
CHOD-PAP	cholesterol oxidase - p-amino-antipyrine
CI	confidence interval
CRP	C-reactive protein
CTD	connective tissue disease(s)
CV	cardiovascular
CVD	cardiovascular disease
CVE	cardiovascular event
DC	dendritic cell
DLE	discoid lupus erythematosus
DM	diabetes mellitus
DNA	deoxyribonucleic acid
dRVVT	dilute Russell's viper venom test
dsDNA	double-stranded deoxyribonucleic acid
DVT	deep venous thrombosis
EA	erosive arthritis
EC	endothelial cell
ECLAM	European Consensus Lupus Activity Measurement Index
EDTA	ethylenediamine tetra-acetate
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assay
EMD	endothelium-mediated dilatation

EPC	endothelial progenitor cell
EULAR	European League Against Rheumatism
GPL	IgG antiphospholipid antibody
GPO-PAP	glycerol phosphate oxidase - p-amino-antipyrine
GWAS	genomewide association studies
HCQ	hydroxychloroquine
HDL	high density lipoprotein
HDL-C	high density lipoprotein cholesterol
HL	hepatic lipase
HLA	human leukocyte antigen
HSP	heat-shock protein
IC	immune complex(es)
IDL	intermediate density lipoprotein
IFN	interferon
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IHD	ischaemic heart disease
IL	interleukin
IMT	intima media thickness
IQR	interquartile range
IRF5	interferon regulatory factor 5
ITGAM	integrin alpha M / CR3A / cluster of differentiation molecule 11B (CD11B)
JBS 2	Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice (2005)
JIA	juvenile idiopathic arthritis
KCT	kaolin clotting time
LA	lupus anticoagulant
LCAT	lecithin-cholesterol acyltransferase
LDL	low density lipoprotein
LDL-C	low density lipoprotein cholesterol
LN	lupus nephritis
Lp(a)	Lipoprotein (a)
LpL	Lipoprotein lipase

LPS	lipopolysaccharide
LUMINA	LUpus in MInorities, NAture versus nurture
MCP	metacarpophalangeal
MCP-1	monocyte chemotactic protein-1
MCTD	mixed connective tissue disease
MECP2	methyl CpG binding protein 2
MetS	metabolic syndrome
MHC	Major Histocompatibility Complex
MI	myocardial infarction
MPL	IgM antiphospholipid antibody
MRI	Manchester Royal Infirmary
NA	no arthritis
ND	no data available
ndHDL	nascent discoidal HDL
NEA	nonerosive arthritis
NF-κB	nuclear factor -κB
NHL	non-Hodgkin's lymphoma
NP-SLE	neuro-psychiatric systemic lupus erythematosus
NS	not significant
ONS	Office for National Statistics
OR	odds ratio
oxLDL	oxidised low density lipoprotein
oxPL	oxidised phospholipid
p	probability
PAH	pulmonary arterial hypertension
PAMP	pathogen-associated molecular pattern
PCR-SSP	sequence specific primers
PGN	peptidoglycan
piHDL	pro-inflammatory HDL
PIP	proximal interphalangeal
PL	phospholipid
PON1	paraoxonase 1
PRR	pattern recognition receptor
PS	phosphatidylserine
PsA	psoriatic arthritis

PT	prothrombin
PVD	peripheral vascular disease
RA	rheumatoid arthritis
RF	rheumatoid factor
RNHRD	Royal National Hospital for Rheumatic Diseases
RNP	ribonucleoprotein
ROC	receiver operator characteristic
RR	relative risk
SCLE	subacute cutaneous lupus
SE	shared epitope
SIR	standardised incidence ratio
SLAM	Systemic Lupus Activity Measure
SLAM-R	revised Systemic Lupus Activity Measure
SLE	systemic lupus erythematosus
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index
SLEDAI-2K	Systemic Lupus Erythematosus Disease Activity Index 2000
SLICC	Systemic Lupus International Collaborative Clinics
SLICC DI	Systemic Lupus International Collaborative Clinics Damage Index
SLICC/ACR DI	Systemic Lupus International Collaborative Clinics/American College of Rheumatology Damage Index
SLICC-RAS	SLICC Registry for Atherosclerosis
Sm	Smith antigen
SMR	standardised mortality ratio
SNP	single-nucleotide polymorphism
SS	Sjögren's syndrome
SSc	systemic sclerosis
STAT4	signal transducer and activator of transcription 4
TC	total cholesterol
TFPI	tissue factor pathway inhibitor
TG	triglycerides
Th1	T helper cell type 1
Th2	T helper cell type 2
TIA	transient ischaemic attack
TLR	Toll-like receptor

TNF	tumor necrosis factor
TNFAIP3	tumor necrosis factor alpha-induced protein 3
tPA	tissue-type plasminogen activator
uNGAL	urinary neutrophil gelatinase-associated lipocalin
uPCR	urine protein-creatinine ratio
uTWEAK	urinary TNF-like weak inducer of apoptosis
VLDL	very low density lipoprotein
VLDL-C	very low density lipoprotein cholesterol
vs	versus
vWF	von Willebrand factor
β_2GPI	β_2-glycoprotein I

CHAPTER 1

Literature Review

Introduction

Systemic lupus erythematosus (SLE) is a complex, multi-system, autoimmune disease characterised by autoantibody production, immune complex (IC) formation and complement activation. The clinical course is variable, with unpredictable disease flares. The production of multiple autoantibodies with differing specificities is a hallmark of the disease and intimately linked to mechanisms underlying acute or chronic inflammation which result in target organ damage. Although there may be protean disease manifestations, many patients develop characteristic clinical syndromes belonging to distinct disease subsets with associated autoantibodies. The clinical spectrum of disease ranges from mild disease such as rash or arthritis, to severe, organ or life-threatening disease, such as neuropsychiatric lupus (NP-SLE) or lupus nephritis (LN). A number of clinical features may also be shared with those found in other systemic auto-immune diseases, giving rise to the term "overlap syndrome".

SLE predominantly affects females, with a female-to-male ratio of 8:1[1]. The overall age-adjusted incidence rate of SLE in the UK from 1989 to 1999 was reported as between 3.0 - 4.7 per 100,000 per year. The female incidence rate was 5.3 - 7.9 per 100,000 per year, with male incidence rate of 0.7 - 1.5 per 100,000 per year[1-5]. The highest incidence of SLE occurred in the group of females aged 40 to 54 years[2, 3, 5]. The overall prevalence rate in the U.K. was 7 - 26 per 100,000 over the same 1989 to 1999 period[2, 3]. Compared with Caucasians, SLE occurs more frequently in Afro-Caribbean and South Asian populations. Prevalence rates in the U.K are 112 - 207 per 100,000, 40 - 49 per

100,000 and 20 - 21 per 100,000 for Afro-Caribbeans, South Asians and Caucasians respectively[2-4, 6].

Biomarkers

The Biomarkers Working Definitions Group[7] defined a biological marker (biomarker) as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention". In SLE, biomarkers have great potential utility as markers of specific disease characteristics, measures of disease activity or severity, or as predictors of future clinical events and mortality. Such biomarkers would provide useful information to guide clinicians' therapeutic decisions and monitoring. Due the nature of SLE with its complex pathogenesis, heterogeneous clinical manifestations and unpredictable disease course, multiple biomarkers may be required. However, a major challenge in SLE research is to identify a biomarker with high predictive value, yet is cost-effective and feasible to perform in routine clinical practice.

Review criteria

This chapter reviews the published literature on biological factors associated with arthritis, accelerated atherosclerosis and long-term outcomes of clinical events and mortality in the context of SLE.

The objectives of this chapter were to review:

1. Associations of auto-antibodies with clinical features shared by SLE and other autoimmune diseases
2. Genetic markers associated with SLE and rheumatoid arthritis (RA)
3. Traditional cardiovascular (CV) risk factors and SLE-associated risk factors predictive of mortality in SLE
4. Traditional CV risk factors and SLE-associated risk factors predictive of future cardiovascular events in SLE

5. Traditional CV risk factors and SLE-associated risk factors associated with subclinical atherosclerosis in SLE
6. Inflammatory and autoimmune mechanisms in SLE with potential influence on accelerated atherosclerosis

Inclusion criteria for epidemiological studies of SLE patients were systematic reviews, meta-analyses, SLE cohort studies and case-control studies with the above objectives published in peer-reviewed journals. Case series and case reports were excluded. Publications on SLE disease pathogenesis, atherogenesis and lipoprotein biology were also included in this review.

Full-text publications published in English between 1970 and 2011 were identified from PubMed searches, using the terms (either alone or in combination): "systemic lupus erythematosus", "SLE", "Major Histocompatibility Complex", "HLA", "genetic", "shared epitope", "antibody", "anti-CCP", "rheumatoid arthritis", "erosive arthritis", "Jaccoud's arthritis", "rhupus", "antiphospholipid", "lupus anticoagulant", "anticardiolipin", "annexin A5", "β_2-glycoprotein I", "prothrombin", "mortality", "thrombosis", "subclinical", "atherosclerosis", "endothelial dysfunction", "Toll-like receptor", "Type I interferon", "lipid profile", "lipoprotein lipase", "high density lipoprotein", "apolipoprotein-A1" and "lipoprotein(a)". Further papers were identified by searching the reference lists of the selected articles.

The majority of available evidence identified using the above inclusion criteria comprised cohort studies (both prospective and retrospective), which would be expected for SLE, which is an uncommon disease. The main limitations of cohort studies, in particular retrospective studies, would be selection bias and confounding with respect to multiple predictor variables. These potential effects were taken into account in this chapter's tables by summarising results from studies that used multivariate analyses to adjust for these effects. Highly cited studies were also included in the tables, with clarification where no statistical adjustments were performed. Recall bias may also be a limitation of the

retrospective cohort and case-control studies reviewed in this chapter. However, consistent results were found in several publications, including multi-centre studies with large cohorts of patients with differing ethnicities, which reduces possible bias.

1. Autoantibodies as biomarkers in SLE

1.1. Autoantibodies as diagnostic biomarkers

Anti-nuclear antibodies (ANA) are useful for the diagnosis of SLE, since they are found in over 95% of patients[8, 9]. ANA are usually determined by immunofluorescence in the HEp-2000 assay and comprise one of the American College of Rheumatology (ACR) 1997 revised SLE classification criteria (see Appendix). However, they are not specific for SLE, as they are also found in patients with other autoimmune diseases and in 12% of the healthy population[10]. Apart from a centromere staining pattern, which is associated with limited cutaneous systemic sclerosis (SSc), the pattern and titre of a positive ANA result are not specific for SLE and other antibodies would be more diagnostic for a case of suspected SLE. Anti-double-stranded DNA (anti-dsDNA) and anti-Smith (anti-Sm) are highly specific for SLE and are present in up to 80% and 55% of patients respectively, but in less than 1.1% of the healthy population[9, 11-13].

1.2. Pathogenicity of autoantibodies in SLE

Arbuckle et al. found the presence of autoantibodies in sera of patients with SLE up to 9.4 years before their diagnosis[12]. There was a temporal order of autoantibody appearance, with anti-Ro/SSA, anti-La/SSB, ANA and antiphospholipid antibodies (aPL) appearing first, followed by anti-dsDNA, and finally anti-Sm and anti-U1 ribonucleoprotein (anti-U1RNP). Animal studies, as well as human clinical and tissue-based studies, have provided evidence for the pathogenicity of these autoantibodies[11]. IgG anti-Ro/SSA from sera of mothers whose children had CHB induced complete atrioventricular block in human fetal

heart tissue[14]. Passive transfer of human monoclonal IgG aPL into pregnant mice caused fetal loss[15, 16]. Mechanisms by which aPL cause fetal loss include placental vessel thrombosis, complement activation and disruption of the annexin A5 (AnxA5) anticoagulant shield on syncytiotrophoblasts and umbilical vein endothelial cells[16-19]. Further evidence of autoantibody pathogenicity was provided by studies demonstrating deposition of human monoclonal IgG anti-dsDNA in murine glomeruli with induction of proteinuria[20, 21]. In humans, more severe types of LN were associated with higher levels of IgG anti-dsDNA, but not with IgM anti-dsDNA or IgG anti-single-stranded DNA antibodies (anti-ssDNA)[22]. Higher anti-dsDNA titres were associated with increased disease activity, as defined by exacerbations of SLE clinical manifestations[23]. In contrast, some SLE patients may have elevated IgG anti-dsDNA accompanied by low complement levels for years, without developing disease exacerbations[24]. This suggests that only certain autoantibody subsets are pathogenic in SLE. This factor should be taken into account with respect to studies correlating quantitative measurements of antibodies with clinical outcome measures.

1.3 Autoantibodies as biomarkers of SLE clinical disease subsets

Several studies have demonstrated auto-antibody associations with SLE disease subsets (Table 1.1). The Euro-lupus cohort comprised 1000 European SLE patients studied prospectively from 1991 to 2000[25-27]. Patients with high titres of anti-dsDNA antibodies were more likely to have arthritis, active LN and haemolytic anaemia, and less likely to have a discoid rash. Patients with anti-Ro/SSA antibodies more frequently had subacute cutaneous lupus (SCLE) and patients who were rheumatoid factor (RF) positive had a lower incidence of active LN and thrombosis. Thrombosis and thrombocytopenia occurred more frequently in patients with IgG anticardiolipin antibodies (aCL GPL) or lupus anticoagulant (LA). Arthritis occurred less frequently in aCL GPL positive patients. In other studies, anti-Sm was associated with renal involvement, NP-SLE and lymphopenia[27, 28]. Witte et al. found that RF-positive SLE patients were more likely to have active arthritis and sicca syndrome and less likely to develop

LN[29]. David-Bajar et al. found a distinctive pattern of staining on direct immunofluorescence termed "particulate epidermal IgG deposition" in lesional skin biopsies from seven anti-Ro/SSA positive patients with SCLE. Infusion of anti-Ro/SSA into human skin-grafted mice reproduced this staining pattern, which was absent in patients with discoid lupus erythematosus (DLE)[30].

Several auto-antibodies are more characteristic of other autoimmune diseases, although they may share similar clinical subsets with SLE. Anti-Ro/SSA and anti-La/SSB are markers of Sjögren's syndrome (SS). Babies born to mothers with SLE or SS and anti-Ro/SSA or anti-La/SSB antibodies have an increased risk of developing neonatal lupus, including congenital heart block (CHB)[31, 32]. Alexander et al. found that anti-Ro/SSA positive patients with primary or secondary SS (including SS secondary to SLE) had more frequent extra-glandular manifestations such as vasculitis, purpura, and lymphadenopathy, as well as haematological abnormalities of anaemia, leucopenia, and thrombocytopenia[33]. In the Euro-lupus cohort, anti-Ro/SSA was associated with a higher prevalence of SCLE and the sicca syndrome and a lower prevalence of thrombocytopenia[27], while anti-La/SSB was associated with arthritis, serositis and cutaneous manifestations of malar rash, SCLE, and photosensitivity[27]. Anti-U1RNP is an antibody associated with mixed connective tissue disease (MCTD). In SLE patients, it is associated with Raynaud's phenomenon, arthritis, myositis, pleurisy and pulmonary arterial hypertension (PAH), features characteristic of MCTD[26, 34-36]. Antibodies associated with overlapping autoimmune disease manifestations could therefore be studied as potential SLE biomarkers.

1.4. Antiphospholipid antibodies

Antiphospholipid antibodies (aPL) are a group of autoantibodies directed against complexes of phospholipids (PL) with phospholipid-binding proteins, such as β_2-glycoprotein I (β_2GPI) or prothombin (PT)[37]. Targeted phospholipids include phosphatidylserine (PS) and oxidised low density lipoprotein (oxLDL).

The antiphospholipid syndrome (APS) is a clinical syndrome in which the presence of persistent aPL is associated with arterial or venous thrombosis, thrombocytopenia and pregnancy morbidity, including spontaneous fetal loss[25, 27, 38]. APS may occur alone (primary APS), or in association with SLE and other auto-immune diseases[39, 40], and its prevalence has been reported to be between 23% and 42% in different SLE cohorts[41-43].

ACL, anti-β_2GPI and LA are the 3 types of aPL included in the updated 2006 classification criteria for APS[44] (see Appendix). β_2GPI is the main co-factor for antibody binding to cardiolipin[45]. ACL, anti-β_2GPI and other APL are usually determined by enzyme-linked immunosorbent assays (ELISAs) of serum samples, whereas LA is determined by functional coagulation assays, including the activated partial thromboplastin time (aPTT), dilute Russell's viper venom test (dRVVT) and kaolin clotting time (KCT). LA activity is due to a set of heterogeneous autoantibodies comprising aPL directed against cardiolipin, β_2GPI and other phospholipid-binding proteins such as PT, PS and annexin A5 (AnxA5). ACL and anti-β_2GPI are therefore significantly associated with LA[42, 46, 47]. The overall prevalence of aCL and LA in SLE has been reported as up to 44% and 34% respectively[42].

1.4.1. APL as predictors of future thrombosis

Previous studies have confirmed the utility of aPL (including LA, aCL and anti-β_2GPI) as predictors for future arterial thrombosis, including stroke and myocardial infarction (MI), venous thrombosis and APS[38, 41, 48-57]. In a study of 21 aCL-positive patients attending a London tertiary referral centre, 11 (52%) developed APS during the 10-year follow-up period[51]. Several studies have shown that thrombosis occurs less frequently in non-SLE cohorts with LA than in SLE patients with LA[42]. LA is the strongest aPL predictor for both arterial and venous thrombosis[38, 46, 48, 50, 58, 59]. However, in the Euro-lupus cohort, only 43% of SLE patients positive for LA developed thrombotic episodes[25]. In SLE populations, high-titre aCL are predictors for future thrombosis[42, 52] including stroke[60], however, Petri et al. found that aCL was not a predictor for MI[49, 61].

Danowski et al. showed that anti-β_2GPI GPL increased the risk for arterial and venous thrombosis and anti-β_2GPI MPL increased the risk of arterial thrombosis among patients with SLE and primary APS[62]. In contrast, no associations were found between aPL/LA and thrombosis in the LUpus in MInority populations: NAture versus nurture (LUMINA) cohort[63]. The LUMINA cohort comprises a multi-ethnic (Hispanic, African-American and Caucasian) cohort of 442 American SLE patients followed prospectively. The "2 hit hypothesis" has been proposed to provide an explanation for the observation that thrombotic events occur only occasionally, despite the persistence of aPL[64]. According to the hypothesis, aPL constitute the first hit by inducing a pro-thrombotic state, however, thrombosis only occurs in the presence of a second pro-thrombotic condition (the second hit). Rauch et al. suggested that the second hit may involve activation of the innate immune system, possibly through triggering of Toll-like receptors (TLRs) such as TLR4[65]. Another possible explanation is that not all aPL are pathogenic, due to differences in autoantibody specificities. For example, anti-β_2GPI with LA activity correlate better with thrombosis, due to increased specificity of aPL that bind domain I on β_2GPI. One likely pathogenic mechanism is that anti-β2GPI directed against domain I confer increased resistance to the anticoagulant properties of AnxA5[66]. However, the specificity of LA may vary depending on different laboratory assay methods, which may in turn affect the predictive ability of aPL for APS manifestations. The likelihood of detecting pathogenic aPL may be increased if moderate to high aPL titres are present[67].

Other factors have been found to be protective for thrombosis. The presence of anti-nuclear lamin B1 (anti-LB1) is associated with protection against thrombosis in SLE patients who are LA positive[68]. A recent multi-ethnic study of 1930 SLE patients found that hydroxychloroquine (HCQ) had an independently protective effect for thrombosis[69], a finding that was also reported by another group[70]. These results provide further evidence of the complexity of the pathogenesis of thrombosis in SLE, where multiple interacting factors may modify the final clinical outcome.

1.4.2 APL in atherosclerosis

There is emerging evidence suggesting pro-atherogenic roles for aPL in atherosclerosis. The increased risk of thrombotic events and CVEs in SLE patients with aPL may partly be due to cross-reactivity between aCL and anti-oxLDL antibodies, reflecting a state of pro-atherogenic oxidative stress[71]. Cardiolipin is a component of lipoproteins such as LDL and HDL and hence aCL may play a role in lipoprotein lipid peroxidation[72]. However, although pro-atherogenic actions of aPL have been described, the evidence for aPL as clinical biomarkers of atherosclerosis is less clear. IC have been reported to be risk factors for atherosclerosis in the general population. In a prospective study of 257 healthy, 50-year old men, increased levels of circulating IC and aCL GPL independently predicted future MI[73]. ACL has been associated with MI in several studies[56, 74]. Vaarala et al. prospectively followed middle-aged men prospectively and found that elevated aCL levels were independent predictors for future MI or cardiac death[56]. Bili et al. showed that elevated aCL GPL and low aCL MPL levels were independent risk factors for recurrent cardiac events in post-MI patients[75]. Hamsten et al. found that 21% of post-MI patients aged under 45 years had persistent aCL, a predictor for recurrent CVEs[76]. In this study, high aCL levels were also positively correlated with anti-oxLDL levels. In the Honolulu Heart Program, β_2GPI-dependent aCL GPL was associated with future ischaemic stroke and MI[77]. In contrast, in a cross-sectional study of patients with acute coronary syndromes (ACS), Edwards et al. found no association with aCL GPL or MPL[78]. Other studies have also reported similar negative results[79, 80]. Within the general population, aPL are also strong predictors for initial ischaemic stroke[79, 81, 82].

In SLE patients, the role of aPL in atherosclerosis remains unclear. Gustafsson et al. prospectively followed 182 SLE patients and showed that aPL was an independent predictor for initial CVEs[83]. APL was also an independent predictor for future CVEs in the LUMINA cohort[84]. In the Hopkins Lupus Cohort, LA was associated with stroke and MI, however, other aPL were associated with stroke, but not with MI[61]. Ahmad et al. found that aCL and/or LA was independently

associated with the presence of carotid plaque in women with SLE[85]. In contrast, other studies of SLE patients found no independent associations for aPL with the subclinical atherosclerosis markers of carotid plaque, carotid IMT, or coronary calcification[86-88]. Possible explanations for these inconsistent results include the presence of non-pathogenic aPL which may dilute the overall clinical effect of pathogenic aPL, a non-pro-coagulant state, or low disease activity. Furthermore, it remains to be determined whether specific aPL, such as anti-β_2GPI, are able to exert differential effects in various stages of the atherosclerotic process.

1.4.3. Anti-β_2-glycoprotein I

β_2GPI is a highly-conserved, single-chain glycoprotein with 5 domains which binds to negatively-charged PLs via its fifth domain[89]. β_2GPI also binds other negatively charged structures such as heparin, DNA, oxLDL, lipoprotein(a) [Lp(a)], and apoptotic cell membranes and syncytiotrophoblasts via exposed PS[89]. β_2GPI forms stable complexes with oxLDL, possibly providing an anti-oxidant effect, and inhibits oxLDL uptake by murine macrophages via scavenger receptors[90, 91]. Moreover, β_2GPI binds to cell surfaces of activated ECs, monocytes, and platelets[92]. β_2GPI is thought to play a regulatory function in the coagulation cascade, through inhibition of activation of Factors XI and XII, interference with thrombin generation by the prothrombinase complex[89], and inhibition of platelet adhesion and aggregation by binding to vWF[93].

Multiple studies have demonstrated pro-thrombotic and pro-atherogenic effects for anti-β_2GPI. Kobayashi et al. found that murine macrophage uptake of oxLDL was enhanced in the presence of both β_2GPI and anti-β_2GPI GPL, most likely mediated by Fcγ receptors[94]. In contrast, IgM anti-oxLDL reduced macrophage oxLDL uptake, suggesting opposing effects of aPL GPL and MPL[90]. β_2GPI has been detected in human atherosclerotic plaques, where it is co-located with T cells and macrophages[95]. Kobayashi et al. suggested that antibodies to oxLDL-

β_2GPI complexes promote atherogenesis by enhancing macrophage oxLDL uptake and subsequent foam cell formation[94].

Anti-β_2GPI binds β_2GPI adherent to EC cell membranes via a TLR 4/annexin A2-containing multiprotein complex to activate EC expression of adhesion molecules and chemokine production[92, 96-99]. Anti-β_2GPI has been shown to promote monocyte release of TNF-α and TF, possibly via triggering of TLR2 or TLR4 -mediated NF-κB activation[100]. Furthermore, Lambrianides et al. found that anti-β_2GPI GPL from APS patients with venous thrombosis activated monocyte production of TF via TLR4[101]. These studies highlight the complexity of anti-β_2GPI interactions with innate immune mechanisms in atherogenesis.

Clinical studies have also provided support for the pro-atherogenic effects of anti-β_2GPI in the general population. The presence of anti-β_2GPI GPL or MPL was found to be an independent risk factor for MI in young premenopausal women[102]. IgA anti-β_2GPI was associated with ACS[103]. Greco et al. showed that anti-β_2GPI was the most frequent aPL type in patients with ACS, occurring in 54% of aPL positive patients with IHD[104]. Antibodies to oxLDL-β_2GPI complexes occurred in 48% of aPL positive patients with IHD. Moreover, anti-β_2GPI and/or anti-oxLDL-β_2GPI were associated with increased IHD severity and adverse outcomes, providing support for a pro-atherogenic role for these autoantibodies.

Elevated levels of oxLDL-β_2GPI complexes and anti-oxLDL-β_2GPI GPL have also been found in patients with SLE and APS, and were associated with an increased risk of arterial thrombosis[105, 106]. However, Lopez et al. found no associations of anti-oxLDL-β_2GPI GPL or MPL with carotid plaque or IMT in patients with SLE[106].

1.4.4. Anti-prothrombin

Prothrombin (PT) is a vitamin K-dependent glycoprotein which binds to negatively charged PLs in a Ca^{2+}-dependent manner. PT is activated by the

prothrombinase complex (comprising activated Factors X and V, calcium and PLs), and converted to thrombin. Thrombin has several anticoagulant actions, including converting fibrinogen into fibrin and binding to thrombomodulin on the EC surface to activate protein C. In turn, activated protein C exerts a negative feedback effect on the prothrombinase complex and therefore PT. PT also acts on Factors V, VIII, and XIII and platelets[107] and binds to apoptotic cells, serving as a target for LA[108]. Anti-PT antibodies may be determined by ELISA using PT, or PT bound to PS as the antigen[109]. Anti-PTs targeting PS-PT complexes have been found to correlate best with arterial and venous thrombosis in patients with SLE[110, 111]. Anti-PT has also been associated with atherosclerosis. High anti-PT levels were predictive for MI or cardiac death in middle-aged, dyslipidaemic men[112].

1.4.5. Anti-annexin A5

Annexin A5 (AnxA5) belongs to the annexin family of proteins which bind negatively charged PLs in a Ca^{2+} dependent manner[113]. AnxA5 binds with high affinity to PS, a potent, pro-coagulant PL. PS is usually confined to the inner leaflet of the cell membrane, however, during cell apoptosis, it is translocated to the external cell membrane and is found on the highly immunogenic surface blebs of apoptotic cells[113, 114]. After binding PS on the cell surface, AnxA5 self-assembles into two-dimensional (2D) crystalline arrays[113], forming a shield which inhibits coagulation and apoptosis[115, 116] and promotes repair of disrupted cell membranes[117]. During placental development, villous syncytiotrophoblasts express surface PS, to which AnxA5 binds and forms 2D arrays, preventing coagulation[115]. Plasma from patients with APS demonstrate inhibition of AnxA5 binding to ECs[118] and resistance to AnxA5 anticoagulant activity[119, 120]. APL can also disrupt the organisation of cell surface AnxA5 2D arrays[18]. These mechanisms may explain the association of anti-AnxA5 with arterial and venous thrombosis, and recurrent fetal loss in patients with SLE[121].

AnxA5 may also have a protective role in atherosclerosis. In vitro, AnxA5 binds to negatively charged PLs within oxLDL, which suggests that AnxA5 may directly inhibit the pro-coagulant and pro-inflammatory effects of oxLDL[122]. Endothelial dysfunction is present early in the atherosclerotic process and AnxA5 improves endothelial dysfunction by acting on NO signalling, reducing leucocyte adhesion to activated endothelium, and reducing expression of the pro-inflammatory cytokines MCP-1 and TNF-α[123]. Cederholm et al. demonstrated that AnxA5 was abundant at sites prone to rupture in advanced atherosclerotic plaques, suggesting that AnxA5 may act to stabilise atherosclerotic plaque[124]. They also reported that plasma containing aCL from SLE patients with CVD inhibited AnxA5 binding to endothelium[124]. Hydroxychloroquine inhibits aPL disruption of AnxA5 binding to ECs and hence increases the anticoagulant effects of plasma AnxA5 from patients with APS[125].

1.5. Summary

There are several aspects to consider about autoantibodies as SLE biomarkers.

- Diagnostic biomarkers - ANA is not a specific marker, whereas anti-dsDNA and anti-Sm are.
- Autoantibody pathogenicity - certain autoantibodies cause specific organ damage e.g. anti-dsDNA and lupus nephritis, aPL and fetal loss.
- Biomarkers of disease subsets - some autoantibodies are markers of SLE disease subsets common to other auto-immune diseases e.g. anti-Ro/SSA or anti-La/SSB and sicca symptoms, neonatal lupus or congenital heart block.
- APL - LA, aCL, anti-β_2GPI are associated with arterial and venous thrombosis, however not all aPL are pro-thrombotic and it has been proposed that an additional condition is required for a thrombotic event to occur, possibly involving the immune system.
- APL in atherosclerosis - aPL are associated with CVEs in the general population, however conflicting results have been reported in SLE

populations, including associations of aPL with IHD and subclinical atherosclerosis.

- Anti-β_2GPI has both pro-thrombotic and pro-atherogenic effects. Circulating anti-oxLDL-β_2GPI GPL complexes have been associated with arterial thrombosis in SLE.
- Anti-PT predicts both arterial and venous thrombosis in SLE.
- Anti-AnxA5 - AnxA5 has a protective role against thrombosis and in atherosclerosis, through its ability to form crystalline arrays on endothelial cell surfaces. Anti-AnxA5 exerts a pathogenic effect in atherosclerosis through its inhibition of AnxA5 binding to endothelium.

Table 1.1 below presents the associations of autoantibodies with distinct clinical subsets of SLE disease manifestations.

Table 1.1. Associations of antibodies with SLE clinical features

Authors (year)	Study design	Follow-up period (years)	SLE subject sample size	Antibody	Associated SLE clinical features
Cervera et al (1993)[25]	prospective cohort - cross-sectional (Euro-lupus)	-	1000	anti-dsDNA	LN, haemolytic anaemia, fever; less thrombosis, sicca
				anti-Ro/SSA	SCLE, sicca; less thrombocytopenia
				anti-La/SSB	malar rash, SCLE, photosensitivity, arthritis, serositis, thrombosis; less lymphadenopathy
				anti-U1RNP	Raynaud's phenomenon, myositis, lymphadenopathy
				anti-Sm	oral ulcers, myositis; less sicca
				aCL GPL	thrombosis, spontaneous fetal loss, thrombocytopenia, livedo reticularis
				aCL MPL	thrombosis, thrombocytopenia, haemolytic anaemia
				LA	thrombosis, spontaneous fetal loss, thrombocytopenia, chorea
				RF	discoid rash, sicca; less LN
Cervera et al (1999)[26]	prospective cohort (Euro-lupus)	5	1000	anti-dsDNA	arthritis, active LN, haemolytic anaemia; less discoid rash
				anti-Ro/SSA	SCLE
				anti-La/SSB	SCLE
				anti-U1RNP	Raynaud's phenomenon, myositis
				aCL GPL	thrombosis, fetal loss, thrombocytopenia; less arthritis
				aCL MPL	haemolytic anaemia
				LA	thrombosis, thrombocytopenia
				RF	less active LN, thrombosis
Cervera et al (2009)[27]	prospective cohort (Euro-lupus)	10	1000	anti-dsDNA	LN, haemolytic anaemia, fever; less sicca, thrombosis
				anti-Ro/SSA	SCLE, sicca; less thrombocytopenia
				anti-La/SSB	malar rash, SCLE, photosensitivity, arthritis, serositis, thrombosis
				anti-U1RNP	Raynaud's phenomenon, myositis, lymphadenopathy

Authors (year)	Study design	Follow-up period (years)	SLE subject sample size	Antibody	Associated SLE clinical features
Cervera et al (2009)[27]	prospective cohort (Euro-lupus)	10	1000	anti-Sm	oral ulcers, myositis; less sicca
				aCL GPL	thrombosis, fetal loss, thrombocytopenia
				aCL MPL	thrombosis, fetal loss, thrombocytopenia, haemolytic anaemia
				RF	sicca, less LN
Bastian et al (2002)[126]	prospective multi-ethnic cohort (LUMINA)	up to 7	353	anti-dsDNA, anti-RNP	LN
Hitchon & Peschken (2007)[28]	retrospective cohort	-	330	anti-dsDNA	renal disease
				anti-Ro/SSA	discoid rash, hypocomplementaemia, leucopenia, lymphopenia
				anti-La/SSB	hypocomplementaemia, leucopenia, lymphopenia
				anti-RNP	renal disease, NP-SLE (psychosis, neuropathy), proteinuria, pleuritis, vasculitis, scarring alopecia, deforming arthritis
				anti-Sm	renal disease, proteinuria, NP-SLE (seizures, psychosis), vasculitis, lymphopenia, fever
Hanly et al (2011)[58]	inception cohort (SLICC)	mean 3.6	1047	anti-ribosomal P	psychosis
				LA	intracranial thrombosis
Mittoo et al (2010)[35]	prospective cohort	-	876	anti-U1RNP, anti-Sm	pleurisy
Lian et al (2012)[34]	retrospective case-control	9	41 PAH cases + 106 controls	anti-U1RNP	pulmonary arterial hypertension
Love (1990)[42]	systematic review		29 published reports (total n > 1000)	aCL, LA	thrombosis, neurological disease, thrombocytopenia
Horbach (1996)[48]	retrospective case-control		175 + 23 controls	high titre aCL GPL / MPL, high titre IgG / IgM anti-β2GPI, LA	thrombosis

Authors (year)	Study design	Follow-up period (years)	SLE subject sample size	Antibody	Associated SLE clinical features
Somers et al (2002)[38]	prospective cohort (Hopkins)	up to 14	352	high titre aCL GPL / MPL, LA	venous thrombosis
Danowski et al (2009)[49]	prospective cohort (Hopkins)		105	aCL GPL > 40	venous thrombosis
Danowski et al (2006)[62]	prospective cohort (Hopkins)		413	IgG anti-β2GPI	arterial & venous thrombosis, livedo reticularis
				IgM anti-β2GPI	arterial thrombosis
Bertolaccini (1998)[127]			207	anti-PT	thrombosis
Lakos et al (2000)[128]	retrospective case-control		65, 5 APS/CTD, + 33 SLE controls	IgG anti-β2GPI, IgG anti-PT, IgG anti-AnxA5	venous thrombosis, APS
Kaburaki et al (1997)[121]	retrospective cohort	up to 20	140	anti-AnxA5	arterial or venous thrombosis, fetal loss
Witte et al (2000)[29]	retrospective cohort		352	RF	active arthritis, sicca, Raynaud's phenomenon; less LN, livedo racemosa
Chan et al (2008)[129]	retrospective cohort + controls (Bath)		104 + 130 serum controls	ACPA	erosive arthritis
Qing (2009)[130]	retrospective cohort		267	ACPA	erosive arthritis
Zhao (2009)[131]	retrospective cohort		138	ACPA	erosive arthritis

2. Genetic biomarkers of SLE

Genetic predisposition to the development of SLE is an important factor in the pathogenesis of SLE. Genetic markers of SLE disease susceptibility have been shown to code for proteins involved in innate and adaptive immunity, including autoantibodies.

The extended Major Histocompatibility Complex (MHC) comprises three genomic regions (class I, II and III) found on chromosome 6. In humans, the MHC class I and II regions encode human leukocyte antigen (HLA) molecules, which present peptides to CD8+ and CD4+ T lymphocytes respectively. MHC class I molecules are ubiquitously expressed, whereas MHC class II molecules are primarily expressed by professional antigen presenting cells (APC), such as dendritic cells (DC), macrophages and B cells. In general, MHC class I molecules present intracellularly-derived peptides and MHC class II molecules present exogenous peptides. MHC class I α chains are encoded by three classical HLA genes, *HLA-A, HLA-B* and *HLA-C.* MHC class II α and β chains are encoded by *HLA-DR, HLA-DQ* and *HLA-DP*. All six genes exhibit a high degree of polymorphism. MHC class III genes encode cytokines such as TNF, early complement components, heat shock proteins and other proteins with potential immunomodulatory function[132, 133].

Haplotypes refer to closely-linked clusters of genes that are inherited together. Ancestral haplotypes (AH) contain conserved continuous gene sequences which appear to be derived from a common remote ancestor[134]. The most well-known genetic susceptibility factors for Caucasian SLE patients are the MHC haplotypes *HLA-B8, DR3 (DRB1*03)* and *HLA-B7, DR2 (DRB1*1501)*[135-137]. *HLA-B8, DR3 (DRB1*03)* forms part of the ancestral haplotype *AH8.1*, comprising *HLA-A1, Cw7, B8, TNFAB*a2b3, TNFN*S, C2*C, Bf*S, C4A*Q0, C4B*1, DRB1*0301, DRB3*0101, DQA1*0501, DQB1*0201. AH8.1* is commonly found in Northern European populations and is significantly associated with multiple auto-immune

diseases, including SLE[138]. A microsatellite mapping study of 334 families of predominantly Caucasian SLE patients identified three MHC class II risk haplotypes - *DRB1*1501(DR2) / DQB1*0602, DRB1*0801(DR8) / DQB1*0402, and DRB1*0301 (DR3) / DQB1*0201*[139]. The estimated relative risk (RR) for developing SLE for each of the three haplotypes was between 1.3-fold and 2.3-fold in a gene dose-dependent fashion, with *DRB1*0301 (DR3) / DQB1*0201* conferring a higher risk than the other haplotypes. Compound heterozygotes exhibited the highest risk of 5.2-fold for developing SLE.

Two MHC class III alleles, *C4 "null" (C4A*Q0)* and *TNF-α -308A*, have been proposed as susceptibility alleles[140]. Hereditary deficiencies of early components of the classical complement pathway (C2 and C4) are associated with SLE susceptibility[141] and a significant association of the *TNF-α -308A* allele with SCLE was also reported[142-144]. However, as both alleles are inherited in linkage disequilibrium and form part of the *AH8.1* haplotype[135, 145], it is difficult to be certain of the independent causality of these alleles, thus limiting interpretation of these data.

The MHC class II genes *HLA-DR2* and *HLA-DR3* confer a 2-fold RR for developing SLE in Caucasian populations[146]. However, there are differences in the autoantibody associations with both haplotypes. Several studies have confirmed the association of *HLA-DR3* with the production of both anti-Ro/SSA and anti-La/SSB antibodies[147, 148]. The *HLA-DR2* haplotype is associated with anti-Ro/SSA, anti-Sm and anti-dsDNA, but not with anti-La/SSB[145, 147-151]. Compound heterozygotes for *HLA-DR2 / HLA-DR3* have the highest risk for developing anti-Ro/SSA, with a RR of up to 15-fold[149, 151, 152]. Moreover, there are differences in the clinical phenotypes associated with both haplotypes. Babies born to anti-Ro/SSA positive mothers with the *HLA-A1, B8, DR3* haplotype are at increased risk of developing neonatal lupus, compared with babies born to anti-Ro/SSA positive mothers with the *HLA-DR2* haplotype[153]. The *HLA-A1, B8, DR3* haplotype with anti-Ro/SSA antibodies is also associated with SCLE[154, 155]. Anti-Ro/SSA and anti-La/SSB positive patients with the *HLA-*

DR3 haplotype are more likely to be older at disease onset, with sicca symptoms and less renal involvement[151]. Other *HLA* associations with SLE have also been reported. In the LUMINA cohort, patients with LN were more likely to carry *HLA-DRB1*13* and less likely to carry *HLA-DQB1*0201*[126]. The *HLA-DR4* haplotype was reported to be protective against the development of SLE[150], however it has been associated with aCL and anti-β_2GPI in other SLE studies[152, 156-158]. *HLA-DQB1*0301 (HLA-DQw7)* was found to be associated with the presence of LA in SLE and in primary APS[159].

Candidate gene studies in SLE have yielded non-MHC genes related to type I interferon (IFN) production, including *signal transducer and activator of transcription 4 (STAT4)* and *interferon regulatory factor 5 (IRF5)*[160]. The type I IFN system constitutes a family of cytokines, including IFN-α and IFN-β, that can be produced by all nucleated cells upon recognition of conserved viral and bacterial structures[161]. Protein products of type I IFN-inducible genes have complex regulatory roles in immunological pathways involved with chronic inflammation[161]. Studies of patients with SLE have demonstrated elevated serum levels of type I IFN and type I IFN-inducible genes[162], which were associated with high disease activity, LN, NP-SLE, cutaneous lupus and the presence of autoantibodies such as anti-dsDNA, anti-Ro/SSA, anti-U1RNP and anti-Sm[162, 163].

Recent genome-wide association studies (GWAS) have further identified susceptibility loci for SLE that encode proteins involved in innate and adaptive immune responses and IC clearance[164]. These loci include the *B-lymphoid tyrosine kinase (BLK)* promoter region, *integrin alpha M (ITGAM)* and *tumour necrosis factor alpha-induced protein 3* (*TNFAIP3*)[160, 165]. A recent meta-analysis confirmed the independent associations of genetic variants at the *IRF5*, *STAT4*, *BLK*, *ITGAM* and *TNFAIP3* loci with SLE[166]. IRF5, when activated by triggering of intracellular TLRs, TLR7 or TLR9, induces transcription of type I IFNs and pro-inflammatory cytokines such as TNF-α, interleukin-6 (IL-6) and IL-12. Several functional variants of the *IRF5* gene have been identified, with

three alleles conferring increased risk for SLE and two others conferring protection[160, 167, 168]. Niewold et al. found that high-risk *IRF5* genotypes were associated with higher serum IFN-α activity in Caucasian SLE patients and this effect mainly occurred in patients positive for anti-Ro/SSA and/or anti-La/SSB, or anti-dsDNA autoantibodies. These antibodies were proposed as activators of IRF5 through TLR7 or TLR9 binding, causing differing downstream effects on cytokine production[167, 169]. *STAT4* encodes a nuclear transcription factor that transmits signals induced by several cytokines, including type I IFNs and IL-12/IL-23[170]. Activated STAT4 stimulates transcription of specific genes involved in the T helper-1 (Th1)-type immune response, including *IFN-γ*[171]. The *STAT4* risk gene increases the risk of developing severe manifestations of SLE, including renal disease[172]. *ITGAM* is a major susceptibility gene for SLE which encodes the α-chain of $\alpha_M\beta_2$-integrin (also known as Mac-1, CD11b/CD18, or complement receptor type 3 [CR3]), a cell-surface receptor mediating immune cell adhesion, IC processing and apoptosis regulation[173]. *ITGAM* risk alleles are significantly associated with discoid rash in SLE[174], and impaired $\alpha_M\beta_2$-integrin function may be involved the upregulation of apoptosis genes within evolving discoid lesions[175]. Furthermore, the *ITGAM* SNP rs9888739 is associated with less frequent arthritis in SLE[176]. *STAT4* and *ITGAM* risk alleles are associated with anti-dsDNA production in SLE[176]. *BLK* encodes a Src tyrosine kinase specifically expressed in B-cell lines. BLK influences the proliferation, differentiation and tolerance of B cells[140]. The SLE risk allele is found in the *BLK* promoter region and causes reduced BLK expression, resulting in impaired B-cell signalling[177]. *TNFAIP3* encodes A20, a de-ubiquitinating protein that negatively regulates nuclear factor-κB (NF-κB)-induced pro-inflammatory responses that are stimulated upon triggering of TLRs. A20 has anti-apoptotic and anti-inflammatory effects, which are impaired in SLE[178]. Considered together, these genetic studies provide new insights into the auto-immune and inflammatory pathways involved in the pathogenesis of SLE.

2.1 Summary

- Recently discovered genetic markers have provided new markers of SLE disease susceptibility, in addition to the known MHC Class II genes *HLA-DR2* and *HLA-DR3* and Class III genes *C4A*Q0* and *TNF-α -308A.*
- Recently defined SLE susceptibility genes include non-MHC genes related to type I IFN production *(e.g. STAT4, IRF5),* and innate and adaptive immune responses *(e.g. BLK, ITGAM, TNFAIP3).*
- Some of these susceptibility genes are also associated with SLE clinical features, e.g. *STAT4* and lupus nephritis.

Table 1.2 below summarises the known associations of immunogenetic markers with specific autoantibodies and clinical disease subsets.

Table 1.2. Clinical associations of genetic markers in SLE

Authors (year)	Gene	Marker / SNP	Haplotype	Chromosome	Protein	SLE serological associations	SLE clinical associations	Biological pathways
	MHC Class II							
Hochberg et al (1985)[147]	*HLA-DR3 / HLA-DRB1*03*		*HLA-DR3 / HLA-DRB1*03 / DQB1*0201*	6p	DR3	anti-Ro/SSA & anti-La/SSB (Caucasians)	older age at disease onset	
	*HLA-DR2 / HLA-DRB1*15*		*HLA-DR2 / HLA-DRB1*1501 / DQB1*0602*	6p	DR2	anti-Ro/SSA (Caucasians); anti-dsDNA (African - Americans)	younger age at disease onset	
Watson et al (1984)[153]	*HLA-DR3 / HLA-DRB1*03*		*HLA-A1, B8, DR3, DR52 (MT2), DQ2 (MB2)*	6p	DR3	anti-Ro/SSA	neonatal lupus in offspring	
Smolen et al (1987)[148]	*HLA-DR3 / HLA-DRB1*03, HLA-DR2 / HLA-DRB1*15*		*HLA-DR3 / HLA-DRB1*03 / DQB1*0201*	6p	DR3, DR2	anti-Ro/SSA &/or anti-La/SSB		
	HLA-DR1 or DR4		*HLA-DR1 or HLA-DR4*	6p	DR1, DR4	anti-Sm &/or anti-RNP		
Hamilton et al (1988)[151]	*HLA-DR3 / HLA-DRB1*03*		*HLA-B8, DR3, DR52 (MT2), DQ2 (MB2)*	6p	DR3	anti-Ro/SSA & anti-La/SSB	sicca, older age at disease onset, less LN	

Authors (year)	Gene	Marker / SNP	Haplotype	Chromosome	Protein	SLE serological associations	SLE clinical associations	Biological pathways
	MHC Class II							
Hamilton et al (1988)[151]	*HLA-DR2/ HLA-DRB1*15*		*HLA-DR2, DQ1*	6p	DR2	anti-Ro/SSA	less LN	
Provost et al (1988)[155]	*HLA-DR3 / HLA-DRB1*03*		*HLA-B8, DR3, DRw6, DR52 (MT2), DQ2 (MB2)*	6p	DR3	anti-Ro/SSA	SCLE, sicca	
Sontheimer et al (1981)[154]	*HLA-DR3 / HLA-DRB1*03*		*HLA-A1, B8, DR3*	6p	DR3		SCLE	
Taylor et al (2011)[176]	*HLA-DR3 / HLA-DRB1*03*	*rs2187668*		6p	DR3	anti-dsDNA	renal disease	
Savi et al (1988)[179]	*HLA-DR7 / HLA-DRB1*0701*		*HLA-DR7*	6p	DR7	aCL (Northern Italian cohort)		
McHugh et al (1989)[152]	*HLA-DR4 / HLA-DRB1*04*		*HLA-DR4*	6p	DR4	aCL GPL (British cohort)		
Galeazzi et al (2000)[157]	*HLA-DR4 / HLA-DRB1*0402, HLA-DR7 / HLA-DRB1*07, HLA-DQB1*0302*		*HLA-DR4, DQ7; HLA-DR7*	6p	DR4, DQ7, DR7	aCL GPL (European cohort)		
Hartung et al (1992)[158]	*HLA-DR4*		*HLA-DR4 , DRw53*	6p	DR4, DR53	aCL MPL		
	HLA-DRw53		*HLA-DR4 / HLA-DR7 / HLA-DR9, DRw53*	6p	DR4/DR7/DR9, DR53	aCL GPL	APS	
Bastian et al (2002)[126]	*HLA-DRB1*13*		*HLA-DRB1*13*	6p	DR13		LN	
	*HLA-DQB1*0201*		*HLA-DQB1*02*	6p	DQ2		less LN	

Authors (year)	Gene	Marker / SNP	Haplotype	Chromosome	Protein	SLE serological associations	SLE clinical associations	Biological pathways
	MHC Class II							
Arnett et al (1991)[159]	*HLA-DQB1*0301*		*DQB1*0301*	6p	DQ7	LA		
Arnett et al (1999)[156]	*DQB1*03, DQB1*0302*		*DQB1*03*	6p	DQ7, DQ8	anti-β_2GPI		
	MHC Class III							
Racila et al (2003)[180], Sontheimer (2005)[143]	*C1qA*	*Gly70GGA*		6p	C1q	C1q deficiency	SCLE	
Pickering & Walport (2000)[141]	*C2*	*C2*Q0*		6p	C2	C2 deficiency		
Deng & Tsao (2010)[140], Pickering & Walport (2000)[141]	*C4*	*C4A*Q0*	*HLA-A1, B8, C4A*Q0, C4B1, DR3, DQ2*	6p	C4	C4 deficiency		
Millard et al (2001)[142]	*TNF-α*	*-308A*		6p	TNF-α		SCLE	
Werth et al (2000)[144]	*TNF-α*	*-308A*		6p	TNF-α	↑TNF production	SCLE	
	Innate immunity							
Harley et al (2008)[160], Graham et al (2009)[166], Hom et al (2008)[177], Rhodes & Vyse (2008)[173]	*ITGAM*	*rs9888739, rs1143679*		16p	α-chain of $\alpha_M\beta_2$-integrin / Mac-1 / CD11b/CD18 / CR3			Mediation of immune cell adhesion, IC processing & apoptosis regulation

Authors (year)	Gene	Marker / SNP	Haplotype	Chromosome	Protein	SLE serological associations	SLE clinical associations	Biological pathways
	Innate immunity							
Taylor et al (2011)[176]	*ITGAM*	*rs9888739*		16p	α-chain of $\alpha_M\beta_2$-integrin / Mac-1 / CD11b/CD18 / CR3	anti-dsDNA	↓arthritis	Mediation of immune cell adhesion, IC processing & apoptosis regulation
Järvinen et al (2010)[174]	*ITGAM*	*rs1143679*		16p	α-chain of $\alpha_M\beta_2$-integrin / Mac-1 / CD11b/CD18 / CR3	anti-Ro/SSA	discoid rash, renal disease	
Graham et al (2009)[166]	*TNFAIP3*	*rs5029937*		6q	A20			IFN & TLR 7/9 signalling
Niewold et al (2008)[169]	*IRF5*	*rs3807306, rs10488631*		7q	IRF5	↑IFN-α activity, anti-Ro/SSA / La/SSB / Sm/RNP, anti-dsDNA		IFN & TLR 7/9 signalling
Harley et al (2008)[160], Graham et al (2009)[166]	*IRF5*	*rs12537284*		7q	IRF5			
	Adaptive immunity							
Graham et al (2009)[166], Hom et al (2008)[177]	*BLK*	*rs13277113*		8p	Blk			B cell receptor signalling & development

Authors (year)	Gene	Marker / SNP	Haplotype	Chromosome	Protein	SLE serological associations	SLE clinical associations	Biological pathways
	Adaptive immunity							
Karassa et al (2003)[181]	*FcγRIII-R*	*F158*		1q	FcγRIIIA-R (CD16)		LN	Fc receptor -IC clearance
Manger et al (2002)[182]	*FcγRIIA-R*	*R131*		1q	FcγRIIA-R		LN	
Harley et al (2008)[160], Graham et al (2009)[166], Remmers et al (2007)[171], Rhodes & Vyse (2008)[173]	*STAT4*	*rs7574865*		2q	STAT4			Mediation of Th1 - type cell cytokine production
Taylor et al (2008)[172], Taylor et al (2011)[176]	*STAT4*	*rs7574865*		2q	STAT4	anti-dsDNA	↑disease severity - LN, age < 34 yrs at diagnosis, ↓oral ulcers	

3. Arthritis in SLE

Joint involvement is a common feature of SLE, occurring in up to 91% of patients[25, 183]. Clinical manifestations range from recurrent, transient polyarthralgia to deforming, rheumatoid-like arthritis, with synovitis affecting metacarpophalangeal (MCP) or proximal interphalangeal (PIP) joints. Jaccoud's arthropathy is a type of deforming, nonerosive arthritis (NEA) first described in patients with recurrent rheumatic fever. This pattern of arthritis was first recognised by Bywaters in patients with SLE[184]. Jaccoud's arthropathy is uncommon, occurring in 3% to 13% of SLE patients[184-187]. It is characterised by the deformities of ulnar deviation at the MCP joints and subluxation of the MCP and PIP joints, which are correctable in the early stage of the arthropathy. Swan-neck, boutonniere and Z-thumb deformities may occur at later stages. Typical RA-like erosions are absent on radiographs, although "hook-like" erosions of the metacarpal heads may be present in late disease. Erosive arthritis (EA) is also uncommon in SLE, affecting 5% - 11% of patients[129, 187-189]. A further clinical subset exists, consisting of patients with radiological RA-like joint erosions, who fulfil both clinical features of RA and SLE. This disease subset has been termed "rhupus"[190]. Apart from polyarthritis, the clinical features that occur more frequently in rhupus patients include malar rash, DLE, photosensitivity, LN, anaemia, leucopenia, and thrombocytopenia[191]. As EA is associated with worse functional outcome and disability, determining a biomarker that can identify the subset of patients at risk of developing EA would enable clinicians to make informed decisions about initiating aggressive disease-modifying therapy for arthritis.

3.1. Antibodies as biomarkers of arthritis in SLE

RFs are polyclonal autoantibodies directed at epitopes within the Fc portion of human IgG. RF is the major antibody associated with RA and its presence is a criterion included in the 1987 ACR and 2010 ACR / European League Against

Rheumatism (EULAR) RA classification criteria[192-194] (see Appendix). The sensitivity of RF for predicting RA is relatively low, as 75% of patients with RA are RF-positive[195]. Furthermore, RF is not specific for RA, as it is also detected in patients with SLE, SS, SSc and inflammatory myositis[195]. Mediwake et al. found that RF was unhelpful in distinguishing RA patients from SLE patients with EA[187].

The anti-cyclic citrullinated peptide antibody (ACPA) has been reported to be a more reliable serological marker for RA than RF. ACPAs are present years before the onset of disease[196]. In patients presenting with early, undifferentiated arthritis, ACPA predicts disease evolution into RA[197, 198]. ACPA has been reported to be highly specific for RA[199], however, it has also been detected in other autoimmune diseases, including psoriatic arthritis (PsA)[200], juvenile idiopathic arthritis (JIA)[201] and SLE[129, 187]. In both RA and PsA, ACPA is associated with EA and radiographic disease progression[200, 202]. Furthermore, we and others have reported the association of ACPA with EA in SLE[129-131, 187, 203] (see chapter 2). These SLE patients with EA and ACPA may represent a subset of rhupus patients.

3.2. Genetic markers of arthritis in SLE

ACPA production is associated with the shared epitope (SE)[204, 205], a highly-conserved, 5-amino acid sequence found in the third hypervariable region of the HLA-DRB1 molecule. The SE binds citrullinated arginine-containing peptides with high affinity, thereby facilitating the generation of antibodies to these peptides from synovium[206]. The SE is encoded by specific MHC class II *HLA-DRB1* alleles that are predictive for progressive, erosive disease in RA[207]. Recently, Huizinga et al. proposed the theory that *SE* alleles may not be specific for RA, but are associated with the production of antibodies to citrullinated peptides which play a pathogenic role in the development of more severe, erosive arthritis[208]. Furthermore, *HLA-DQB1*0302*, which is in linkage disequilibrium with *HLA-DRB1 SE* alleles and associated with RA disease severity, is also

associated with ACPA production[209]. Similarly, we found an association of *HLA-DQB1*0302* with EA in SLE patients[129] (see chapter 2).

Several single nucleotide polymorphisms (SNPs) at the *IRF5, STAT4, BLK* and *TNFAIP3* loci are shared by SLE and RA[171, 210, 211]. Genetic variants at the *IRF5*, *STAT4* and *BLK* loci are also shared by SLE and SSc[140, 210]. Ablation of *TNFAIP3* in the myeloid cells of A20-deficient mice resulted in development of a destructive, erosive polyarthritis which was dependent on TLR4-MyD88 signalling pathways and IL-6, but not dependent on TNF[212]. A recent study of Norwegian patients demonstrated that the *rs2004640* SNP at the *IRF5* locus was shared by patients with SLE and the RF-negative polyarthritis subtype of JIA[213]. The sharing of genetic loci between SLE and RA and/or SSc suggests the existence of common pathways in the pathogenesis of several autoimmune diseases, providing further support for the potential application of biomarkers of other autoimmune diseases to SLE disease subsets such as arthritis.

3.3. Summary

- Clinical manifestations of lupus arthritis include polyarthralgia, transient synovitis, Jaccoud's arthropathy, EA and rhupus.
- EA is uncommon, affect up to 11% of SLE patients.
- RF is not specific for RA.
- ACPA has a much higher specificity for RA but has been detected in other autoimmune diseases, including SLE. ACPA is associated with EA in SLE and may also predict for the rhupus subset.

4. Mortality in SLE

Survival rates of patients with SLE have improved significantly since the 5-year survival rate of less than 50% reported in 1955[214]. Ginzler et al. studied 1,103 patients between 1965 and 1978 and found 5- and 10-year survival rates of 77%

and 71% respectively[215]. Wallace et al. followed 609 private patients between 1950 and 1979 and found 5-, 10- and 15-year survival rates of 88%, 79% and 74% respectively[216]. The same group later reported a significant improvement in the 5-, 10- and 15-year survival rates to 97%, 93% and 83% respectively, for 507 patients followed between 1980 and 1989[183]. In a series of 110 English patients with LN followed between 1963 and 1986, overall 5-, 10- and 15-year survival rates were 84%, 72% and 62% respectively[217]. Survival improved significantly for the cohort diagnosed between 1976 and 1986, compared with the cohort from 1963-1975, with survival rates of 90% vs 78% at 5 years, 81% vs 56% at 10 years and 76% vs 43% at 15 years. In a study of 100 SLE patients attending a tertiary rheumatology clinic in London, the 5- and 10-year survival rates for the period 1978 - 1988 were 88% and 86% respectively[137]. An extension of this study comprising 165 adult-onset and juvenile SLE patients found an improvement in the 5-year survival rate to 93% for the period 1978 - 1993, with the 10-year survival rate unchanged at 86%[218]. During the 1990s, survival rates improved further to 93% - 97% at 5 years, 83% - 92% at 10 years and 78% - 79% at 15 years[50, 218-221]. More recent studies have demonstrated survival rates of 95% - 97% at 5 years and 93% at 10 years[222-224]. Improvement in survival rates over the last 6 decades may be attributed to more careful use of glucocorticoids, the availability of newer immunosuppressive agents, the advent of renal dialysis and transplantation, and advances in general medical therapy, including treatment of infections.

Nevertheless, the mortality rate in SLE remains significantly higher, with up to a 4-fold increased rate compared with age- and sex-matched controls or with the general population[50, 225-228]. In an international, multi-centre cohort comprising 9,547 patients studied between 1970 and 2001, Bernatsky et al. reported an overall standardised mortality ratio (SMR) of 2.4 (95% CI 2.3, 2.5), with a 60% decrease in the SMR from 4.9 between 1970 and 1979, to 2.0 between 1990 and 2001[227]. In a cohort of 1,241 SLE patients attending a tertiary rheumatology service in Toronto, the overall SMR reduced significantly from 12.6 (95% CI 9.1, 17.4) between 1970 and 1979, to 3.5 (95% CI 2.7, 4.4) between 1996 and

2005[229]. Similarly, in a cohort of 300 SLE patients attending a tertiary rheumatology clinic in London and followed between 1978 and 2000, the overall SMR was 4.0 (95% CI 2.8, 5.2)[226].

4.1. Causes of death in SLE

Major causes of death include those related to active SLE disease, complications of therapy, or other co-morbid conditions. In a multi-centre US study of 1,103 SLE patients followed between 1965 and 1978, the major causes of death were active SLE-related organ disease and infection[230]. Active SLE disease as a major primary cause of death is well-documented in other studies[26, 216, 219, 231-235]. Infection also remains a major cause of death, with a SMR of 4.9 - 5.0[217, 227, 228, 235, 236]. In 1976, Urowitz et al. first described the "bimodal mortality pattern" in SLE[231], reporting that deaths early in the course of SLE were mostly due to active disease such as LN, or treatment-related complications such as infection, whereas late deaths were mostly due to atherosclerosis-related MI, at a time when SLE was relatively quiescent. Several authors have since confirmed Urowitz et al.'s findings of early deaths related to active SLE and infections, and late deaths from CV causes[216, 220, 221, 226, 237, 238]. In the last 4 decades, all-cause mortality has declined, together with infection- and renal-related deaths[227], however, the incidence of ischaemic heart disease (IHD) has risen[229] and the risk of MI-related death has also tended to increase, with SMRs between 1.7 and 3.0[227, 236].

Patients with SLE are at increased risk of developing certain types of malignancies, which are significant causes of death in SLE[226, 227, 239]. In Bernatsky et al.'s cohort, the standardised incidence ratio (SIR) of observed-to-expected cancers was 1.2 (95% CI 1.1, 1.3) for all cancers, 2.8 (95% CI 2.1, 3.5) for all haematological malignancies, 3.6 (95% CI 2.6, 4.9) for non-Hodgkin's lymphoma (NHL) and 1.4 (95% CI 1.1, 1.8) for lung cancer[240]. A previous Swedish study of 5,715 SLE patients also found an increased SIR of 2.9 (95% CI 2.0, 4.0) for NHL[241]. In Bernatsky et al.'s cohort, although the overall SMR of

0.8 (95% CI 0.6, 1.0) was lower for cancer, SMRs were higher for NHL and lung cancer, at 2.8 (95% CI 1.2, 5.6) and 2.3 (95% CI 1.6, 3.0) respectively[227].

4.2. Predictors of mortality

Table 1.3 summarises factors predictive of early mortality in patients with SLE, which were mostly determined from cohort studies. The independent predictor factors listed in this table were derived from studies that employed multivariate analyses to adjust for possible confounding due to multiple predictor variables and selection bias.

Non-SLE related predictors of premature death in SLE include older age at disease onset[242-245], male gender[50, 183, 216, 228, 229, 243, 246, 247], non-Caucasian ethnicity[28, 244, 248], and low socioeconomic status[215, 223, 243, 248-250]. As might be expected, patients who are older at disease onset are more likely to die from CV-related causes and age-related co-morbidities[242, 245]. Male patients have more severe disease (such as LN) and increased damage accrual, resulting in a poorer prognosis and a higher than expected male/female age-adjusted mortality ratio when compared with the general population[183, 243, 251-253]. Various authors have related low socioeconomic status to lack of health insurance in USA, poverty, and varying patients' health-related attitudes and behaviours, which are more likely to contribute to early mortality.

SLE-related predictors of poor survival include higher disease activity[223, 254] including higher SLEDAI scores[223, 229, 254, 255], renal disease[26, 50, 183, 215, 216, 228, 256, 257], NP-SLE[256, 258], lung involvement[254], pleurisy[255, 259], haemolytic anemia[243, 258, 259], thrombocytopenia[183, 244, 254, 256, 259], and APS[259]. Organ damage, whether early in the disease course[249, 260], or from accrual of damage (as measured by the Systemic Lupus Erythematosus Disease Activity Index [SLICC DI]) is also a significant predictor of mortality[50, 223, 229, 254, 261-266].

The clinical factors listed above in general reflect subsets of patients with more severe disease and hence a poorer prognosis. Higher disease activity as a predictor suggests that persistent chronic inflammation is important mechanism in the development of organ damage and accelerated atherosclerosis resulting in premature death. In contrast, Ward et al. reported that leucopenia was protective against mortality, a surprising finding given that leucopenia is a manifestation of increased disease activity[256]. They found that this protective effect mainly occurred in Caucasian patients compared with African American patients, which suggests that genetic factors may be involved. This theory is supported by the increased risk of mortality in non-Caucasian patients[28, 244, 248]. One possible explanation for the protective effect of leucopenia is that the immunogenetic mechanisms causing leucopenia may also reduce mortality risk, however when combined with other pathogenic mechanisms that contribute to increased disease activity, the overall effect would be to increase mortality risk. Moreover, leucocytosis is associated with progression of subclinical atherosclerosis in SLE[267], another mechanism for increased mortality risk. Several authors have shown that accumulation of damage (by SLICC-DI) is an important predictor of mortality (see Table 1.3).

Hydroxychloroquine therapy was reported in several studies to have an independent protective effect on survival[70, 229, 268, 269]. Patients with milder disease tend to be treated with hydroxychloroquine, which may have introduced selection bias to this result. Nevertheless, hydroxychloroquine has several mechanisms of action that could contribute to a protective effect against mortality. These effects include an anti-thrombotic effect, possibly through platelet inhibition, improvement of lipid profiles by decreasing total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels, reduction in disease activity and prevention of damage accrual[70, 268].

4.3. Antibodies as predictors of mortality

Anti-Sm was reported to be an independent predictor of mortality in SLE[28]. Two studies reported anti-dsDNA was a predictor of early mortality[28, 228], however, another study found it had a protective effect[255]. Pathogenic anti-Sm and anti-dsDNA antibodies that cause LN may partly account for their association with reduced survival in SLE. Anti-Sm or anti-dsDNA antibodies with specificities for non-renal antigens may not have significant associations with mortality, which may account for the lack of data from other studies.

Similarly, the paucity of data demonstrating associations of aPL with mortality may be due to difficulties identifying pathogenic aPL that cause thrombosis, thrombocytopenia and APS, established predictive factors for mortality[253, 259]. Furthermore, APS is associated with increased damage accrual, another important predictor of mortality[253]. Gómez et al. showed that aCL was associated with increased mortality[270], while Gulko et al. found that only aCL MPL was associated with increased mortality[271].

In a single-centre study of 338 German SLE patients followed for up to 15 years, no deaths were observed in patients who had anti-Ro/SSA and anti-U1RNP at disease onset[50]. As anti-Ro/SSA and anti-U1RNP are markers of patient subsets with less LN, this may explain in part their protective effect against mortality. In contrast, no associations of antibodies with mortality were found in other SLE cohorts[243, 249, 259, 272].

4.4. Summary

Despite recent advances in the medical treatment of SLE, patients still have higher mortality rates compared with the general population, with the SMR between 2.4 and 3.5.

- Major causes of death early in the course of SLE include active disease and treatment-related complications such as infection.

- Late deaths in SLE are mostly due to atherosclerosis-related causes and malignancy.

Table 1.3 presents the associations of independent predictive factors for early mortality in patients with SLE.

- Important non-SLE related factors include older age at disease onset, male gender and low socio-economic status.
- Major SLE-related factors include higher disease activity, organ damage accrual, more severe disease manifestations (e.g. renal and neuropsychiatric involvement), and APS.
- Hydroxychloroquine has a protective effect against mortality.
- There is very little data published on the associations of autoantibodies with mortality.

Table 1.3. Predictors of mortality in SLE

Authors (year)	Study design	Follow-up period (years)	SLE subject sample size	Study site(s)	SLE-related baseline predictor variables*	Non-SLE-related baseline predictor variables*	Multivariate analysis
Cervera et al (1999)[26]	prospective cohort (Euro-lupus)	5	1000	multi-centre, Europe	renal disease		yes
Hitchon & Peshken (2007)[28]	retrospective cohort	21	330	Manitoba, Canada	renal disease, ↑SLICC DI scores, anti-Sm	Native American ethnicity, Asian-Oriental ethnicity, male gender	yes
Manger et al (2002)[50]	retrospective cohort	15	338	Erlangen, Germany	older age at diagnosis, LN, SLICC DI early damage accrual; ***protective*** - anti-Ro/SSA, anti-U1RNP	male gender	yes
Ruiz-Irastorza et al (2006)[70]	prospective cohort	15	232	Bizkaia, Spain	***protective*** - HCQ		yes
Pistiner et al (1991)[183]	retrospective cohort	10	503	Los Angeles CA, USA	LN, thrombocytopenia	male gender	no
Ginzler et al (1982)[215]	retrospective cohort	10	1103	multicentre, USA	anaemia, renal disease	low socioeconomic status	yes
Wallace et al (1981)[216]	retrospective cohort	10	609	Los Angeles CA, USA	renal disease	male gender	no
Pons-Estel et al (2004)[223]	prospective inception cohort (GLADEL)	1.7	1214	multicentre, Latin American countries	↑SLEDAI score, SLICC DI damage accrual	low socioeconomic status	yes

Authors (year)	Study design	Follow-up period (years)	SLE subject sample size	Study site(s)	SLE-related baseline predictor variables*	Non-SLE-related baseline predictor variables*	Multivariate analysis
Campbell et al (2008)[228]	prospective case-control	5	265 + 355 controls	N & S Carolina, USA	LN, anti-dsDNA	male gender	yes
Urowitz et al (2008)[229]	prospective cohort (University of Toronto)	36	1241	Toronto, Canada	↑SLEDAI scores, SLICC DI damage accrual, immunosuppressive use; ***protective*** - HCQ	male gender, IHD	yes
Bertoli et al (2006)[242]	multi-ethnic prospective, nested case-control ; multi-ethnic cohort (LUMINA)	≥ 10	73 + 144 contols	multicentre, USA	older age at disease onset		yes
Kasitanon et al (2006)[243]	prospective cohort (Hopkins)	median 6.1	1378	Baltimore MD, USA	older age at diagnosis, haemolytic anaemia, low C3	male gender, low socioeconomic status	yes
Reveille et al (1990)[244]	retrospective cohort	10	389	Birmingham AL, USA	older age at disease onset, thrombocytopenia	African-American ethnicity	yes
Boddaert et al (2004)[245]	retrospective case-control & pooled case series	mean 5.8 - 8.6	161 + 714 controls	Paris, France	older age at disease onset		no
Alamanos et al (2003)[246]	retrospective cohort	10	185	NW Greece		male gender	yes
Doria et al (2006)[247]	prospective cohort	40	207	Padua, Italy		male gender	yes

Authors (year)	Study design	Follow-up period (years)	SLE subject sample size	Study site(s)	SLE-related baseline predictor variables*	Non-SLE-related baseline predictor variables*	Multivariate analysis
Alarcón et al (2001)[249]	multi-ethnic prospective cohort (LUMINA)	5	288	multi-centre USA	↑SLAM & SLICC DI scores	poverty	yes
Studenski et al (1987)[248]	retrospective cohort	15	411	N Carolina, USA		low socioeconomic status, non-Caucasian ethnicity	yes
Ward (2004)[250]	retrospective- US population-based mortality data review 1994-7		4779	Bethesda MD, USA		low socioeconomic status (≤ 12 yrs education)	no
Abu-Shakra et al (1995)[254]	prospective cohort	20	665	Toronto, Canada	age > 50 at diagnosis, renal damage, thrombocytopenia, SLEDAI ≥ 20 at presentation, lung involvement; ***protective*** - LN		yes
Ward et al (1996)[256]	retrospective cohort	median 11	408	Durham NC, USA	(not baseline) LN, seizures, thrombocytopenia; ***protective*** - leucopenia		yes
Seleznick & Fries (1991)[257]	prospective cohort	12	310	Stanford CA, USA	renal impairment, mouth ulcers	↑systolic BP	yes
Jacobsen et al (1998)[258]	retrospective cohort	mean 8.2	513	multicentre, Denmark	renal impairment, NP-SLE, haemolytic anaemia, myocarditis	hypertension, IHD	yes
Drenkard et al (1994)[259]	ambispective cohort	10	667	Mexico City, Mexico	(not baseline) pleurisy, disease activity, thrombocytopenia, APS		yes

Authors (year)	Study design	Follow-up period (years)	SLE subject sample size	Study site(s)	SLE-related baseline predictor variables*	Non-SLE-related baseline predictor variables*	Multivariate analysis
Ruiz-Irastorza et al (2004)[253]	prospective inception cohort	mean 9.7	202	Bizkaia, Spain	APS, SLICC-DI ≥ 1		yes
Cook et al (2000)[255]	prospective cohort	median 6.6	806	Waterloo & Toronto, Canada	(not baseline) higher SLEDAI scores; SLEDAI organic brain syndrome, retinal changes, cranial nerve involvement, proteinuria, pyuria, pleurisy, fever, thrombocytopenia, leucopenia; ***protective*** -new rash, anti-dsDNA		yes
Rahman et al (2001)[260]	inception cohort	10	263	Toronto, Canada	SLICC DI - early damage, SLICC DI - renal damage		no
Chambers et al (2009)[261]	retrospective cohort	> 10	232	London, UK	SLICC DI damage accrual		no
Danila et al (2009)[262]	multi-ethnic prospective cohort (LUMINA)	> 10	635	multicentre USA	SLICC DI damage accrual, SLICC DI renal damage	poverty	yes
Mok et al (2003)[263]	prospective cohort	3	242	Hong Kong, China	SLICC DI damage accrual		yes
Gladman et al (2000)[264]	prospective cohort (SLICC)	> 10	1297	multicentre, Europe, N America	↑SLICC DI scores		no
Nived et al (2002)[265]	prospective cohort (SLICC)	median 7	80	multicentre, Europe, N America	↑SLICC DI scores 5 yrs after diagnosis, SLICC DI vascular damage		no

Authors (year)	Study design	Follow-up period (years)	SLE subject sample size	Study site(s)	SLE-related baseline predictor variables*	Non-SLE-related baseline predictor variables*	Multivariate analysis
Stoll et al (1996)[266]	retrospective inception cohort	10	80	London, UK	↑SLICC DI scores		no
Alarcón et al (2007)[268]	multi-ethnic prospective nested case-control (LUMINA)	median 3.3	608	multicentre USA	***protective*** - HCQ, less severe disease		yes
Shinjo et al (2010)[269]	multi-ethnic prospective inception cohort (GLADEL)	median 4.6	1480	multicentre, Latin American countries	***protective*** - HCQ		yes
Gómez et al (2006)[270]	retrospective cohort	> 10	363	Asturias, Spain	older age at disease onset, renal disease, aCL		yes
Gulko et al (1993)[271]	retrospective cohort	16	139	Birmingham AL, USA	older age at diagnosis, aCL MPL, HLA-DQ7, major infection, thromboembolic events		yes
Jouhikainen et al (1993)[273]	retrospective case-control	median 22	37 + 37 controls	Helsinki, Finland	LA, DVT, LN		no

* Where possible, factors quoted in the table are independent variables ($p<0.05$) derived from multivariate analyses

5. Atherosclerosis in SLE

Although overall survival in SLE has increased dramatically since the 1950s, atherosclerosis has remained a significant cause of mortality. In Bernatsky et al.'s cohort of 9,547 SLE patients, the incidence of CV-related mortality increased slightly over 3 decades from 1970, despite a fall in the incidence of renal and infection-related deaths[227]. A post-mortem study published in 1975 found that in 42% of SLE patients who had received glucocorticoids for over 1 year, atherosclerotic plaques caused over 50% stenosis in at least one major coronary artery[274]. The estimated incidence of new cardiovascular events (CVEs) attributed to atherosclerosis in patients with SLE is approximately 1.5% per annum[275], with prevalence ranging from 6.6% to 10.9% in 3 North American cohorts[276-278]. In a population-based case-control study, using the UK-based General Practice Research Database, the RR of developing MI was 2.7 for patients with SLE[279]. Similarly, the Nurses' Health Study reported an adjusted RR of 2.3 for CVEs[280]. Other studies have found up to a 10-fold increased risk of MI or stroke in patients with SLE compared with the general population[238, 275, 281]. Furthermore, CVEs occur at an earlier age in patients with SLE[278]. Manzi et al. reported that women with SLE aged between 35 and 44 years had a 52-fold increased risk of MI, compared with an age-matched women from the Framingham Offspring Study[278]. Ward found that women with SLE aged between 18 and 44 had a 2.3-fold increased risk of hospitalisation for MI compared with age-and sex-matched controls, and a 2.1-fold increased risk of hospitalisation for stroke[281]. Shah et al. showed that patients with SLE who were hospitalised for MI were more likely to have prolonged admissions and had a higher risk of inpatient mortality than patients without SLE or diabetes mellitus (DM)[282]. Possible explanations for this increased mortality risk post-MI include a chronic inflammatory and pro-coagulant state due to the presence of aPL, resulting in early coronary artery re-occlusion.

5.1. Risk factors for cardiovascular events in SLE

Prospective studies of patients with SLE have demonstrated that CVEs occur at an average of 6 to 9 years after the initial diagnosis of SLE[83, 84, 277]. Several studies, including 5 large cohort studies (LUMINA[84], Hopkins Lupus Cohort[276], University of Pittsburgh[278], SLICC Registry for Atherosclerosis [SLICC-RAS][283] and University of Toronto inception cohorts[277]) have consistently shown that traditional CV risk factors are independent predictors for CVEs in SLE (Table 1.4). These factors include older age at diagnosis[83, 276, 278], hypertension[275, 276, 284-287], hypercholesterolaemia[275, 276, 278, 284, 287], hypertriglyceridaemia[288] and smoking[84, 277]. Other CV risk factors that have been identified include male gender[283, 286], obesity[276], DM[284], elevated homocysteine and lipoprotein(a) [Lp(a)] levels[289] and post-menopausal status[278]. Elevated levels of circulating oxLDL are significantly associated with IHD in the general population[290, 291]. Elevated oxLDL levels and anti-oxLDL have also been reported in SLE patients with CV disease (CVD)[289, 292].

Although traditional CV risk factors are important, they do not fully account for the increased atherosclerotic risk in patients with SLE[293]. Furthermore, SLE patients with a cardiac event have on average one less traditional risk factor than non-SLE patients with premature IHD[294]. The risk of MI in SLE remains elevated, even after adjustment for conventional CV risk factors, suggesting that SLE-related factors or SLE itself may be independent risk factors for atherosclerosis[275, 279, 288]. Several longitudinal studies have identified non-traditional factors and other SLE-related factors as predictors of CVEs. These factors include longer SLE disease duration[278], longer duration of glucocorticoid use[276, 278], azathioprine use[286], the presence of aPL[83, 84], NP-SLE[277], and vasculitis[277]. However, with regard to glucocorticoid and azathioprine use, it is unclear whether they are truly independent predictive factors or markers of disease severity and/or activity. Glucocorticoids are often used in the treatment of active disease, however they may also have deleterious effects. Karp et al. found that a 10 mg increase in the daily prednisolone - equivalent dose in the preceding year was associated with lower high density lipoprotein-cholesterol

(HDL-C) levels and increased systolic blood pressure (BP), triglyceride (TG) and blood glucose levels, and 2-year coronary heart disease risk[295]. As Azathioprine is used for long-term disease control, it too may be a marker of active disease requiring therapy, rather than a true predictor of CVEs. Markers of inflammation, such as elevated CRP[84], have also been implicated. A recent study found that von Willebrand factor (vWF), a marker of endothelial activation, was independently associated with CVEs[83].

Not only are patients with SLE at increased risk of future CVEs, the prevalence of traditional CV risk factors is also increased in these patients. These CV factors include hypertension, hypercholesterolaemia, obesity[296], DM[297], and the metabolic syndrome (MetS)[298, 299]. The most recent consensus definition for the MetS was published in 2009, in a joint interim statement from the International Diabetes Federation Task Force on Epidemiology and Prevention, the National Heart, Lung, and Blood Institute, the American Heart Association, the World Heart Federation, the International Atherosclerosis Society, and the International Association for the Study of Obesity[300]. The MetS doubles the risk of CVD and the diagnosis is based on the presence of 3 or more of 5 criteria of elevated waist circumference, elevated TG, reduced HDL-C, hypertension or DM[300].

Table 1.4. Factors predictive of cardiovascular events in SLE

Authors (year)	Study design	Follow-up (years)	SLE subject sample size	Study site(s)	CVE(s)	Independent SLE-related predictor variables	Independent CV predictor variables	Multivariate analysis
Petri et al (1992)[276]	prospective cohort (Hopkins)	3	229	Baltimore MD, USA	MI, angina, death	older age at diagnosis, ↑duration of glucocorticoid use	hypertension, ↑TC, obesity	yes
Esdaile et al (2001)[275]	retrospective cohort	mean 8.6	263	multi-centre Canada	MI, angina, CCF, stroke, death		age, ↑systolic & diastolic BP, ↑TC	yes
Urowitz et al (2007)[277]	prospective cohort including inception (University of Toronto)	35	total - 1087, inception - 561	Toronto, Canada	MI, angina, TIA, stroke, PVD, death	***total*** -NP-SLE, vasculitis; ***inception*** - NP-SLE	***total*** - ↑number of CV risk factors; ***inception*** - smoking	yes
Manzi et al (1997)[278]	retrospective cohort (University of Pittsburgh)	14	498 women	Pittsburgh PA, USA	MI, angina	older age at diagnosis, ↑disease duration, ↑duration of glucocorticoid use	↑TC, post-menopause	yes
Gustafsson et al (2009)[83]	prospective cohort	> 20	182	Stockholm, Sweden	MI, angina, TIA, stroke, PVD, death	older age at diagnosis, aPL, vWF; ***protective*** - thrombocytopenia		yes
Toloza et al (2004)[84]	multi-ethnic prospective cohort (LUMINA)	median 6.2	546	multi-centre USA	MI, angina, stroke, PVD	aPL, ↑CRP	older age, smoking, ↑no. of CV risk factors, longer follow-up time	yes
Urowitz et al (2010)[283]	prospective inception cohort (SLICC-RAS)	8	1249	multi-centre, Europe, N & C America	MI, angina, pacemaker insertion, CCF, TIA, stroke, PVD	older age at diagnosis	male gender	yes

Authors (year)	Study design	Follow-up (years)	SLE subject sample size	Study site(s)	CVE(s)	Independent SLE-related predictor variables	Independent CV predictor variables	Multivariate analysis
Goldberg et al (2009)[288]	prospective case-control (University of Toronto)	mean 7.2, median 8	241 + 237 controls	Toronto, Canada	MI, angina, death		older age, TG ≥ 2.8 mmol/L	yes
Gladman & Urowitz (1987)[284]	prospective cohort (University of Toronto)	> 10	507	Toronto, Canada	MI, angina	pericarditis, myocarditis	CCF, hypertension, TC > 7.0 mmol/L, TG > 1.8 mmol/L, glucose > 8.0 mmol/L, DM	not stated
Bessant et al (2006)[285]	retrospective case-control	> 10	29 + 58 controls	London & Birmingham, UK	MI, angina, stroke, PVD		hypertension	yes
Haque et al (2010)[286]	retrospective case-control (LASER)	mean 11	53 + 96 controls	multi-centre UK	MI, angina	azathioprine	age, hypertension, male gender, family history	yes
Mikdashi et al (2007)[287]	prospective cohort	mean 8	238	Baltimore MD, USA	ischaemic stroke		hypertension, ↑TC	yes
Svenungsson et al (2001)[289]	retrospective case-control	mean ≥ 18.5	26 + 52 controls	Stockholm & Huddinge, Sweden	MI, angina, stroke, PVD	↑ESR, ↑CRP, LA, α-1 antitrypsin, ↑cumulative glucocorticoid use, ↑IgG anti-oxLDL	↑VLDL, ↓HDL-C, ↑TG, ↑LDL-TG, ↑homocysteine, ↑Lp(a)	yes
Frostegård et al (2005)[118]	retrospective cohort & controls	mean 12	147 + 60 controls	Stockholm, Sweden	MI, angina, stroke, PVD	↑IgM anti-oxLDL	↑oxLDL	yes

5.2. Pathogenesis of atherosclerosis

It is now well established that atherosclerosis is a chronic inflammatory disease[301]. Moreover, it is likely that the inflammatory and immunological mechanisms of SLE enhance the complex interaction of classic CV risk factors with inflammatory pathways of atherogenesis. All these factors may interact to accelerate the atherosclerotic process within the vasculature of patients with SLE.

The atherosclerotic process is initiated by endothelial dysfunction or damage through a variety of mechanisms such as free radicals caused by cigarette smoke, hypertension, diabetes mellitus and elevated homocysteine concentrations[301]. Impaired endothelial repair mechanisms lead to the subendothelial accumulation of LDL, through binding by apolipoprotein B100 (apoB100) within LDL to proteoglycans in the artery wall[302]. Cytokines, such as TNF-α and IL-1, increase binding of LDL to endothelium and smooth muscle[301]. LDL becomes oxidised by reactive oxygen species or enzymes such as myeloperoxidase or lipoxygenases released from local inflammatory cells[303]. Oxidized PLs (oxPL) and oxLDL then activate endothelial cells to express adhesion molecules and secrete chemokines such as monocyte chemotactic protein-1 (MCP-1), resulting in the recruitment of neutrophils, monocytes and T cells and their subsequent migration into the intima[301, 304]. HDL plays an anti-inflammatory role in this process by inhibiting endothelial cell expression of adhesion molecules and production of MCP-1[305, 306] (Figure 1.1). Monocytes differentiate into macrophages, which internalise oxLDL via scavenger receptors and later transform into foam cells[307]. Early fatty-streak lesions in the vasculature consist of T cells and foam cells loaded with lipids[308]. Successive accumulation of apoptotic cells, debris and cholesterol crystals lead to the formation of a necrotic core within the atheromatous plaque. Smooth muscle cells then proliferate and produce collagen to form a fibrous cap on the plaque. The shoulder regions of the cap are heavily infiltrated by T cells and macrophages, which produce enzymes and pro-inflammatory mediators that contribute to destabilisation and

thinning of advanced plaques and ultimately, to plaque rupture, thrombosis and vessel occlusion[307, 309, 310] (Figure 1.2).

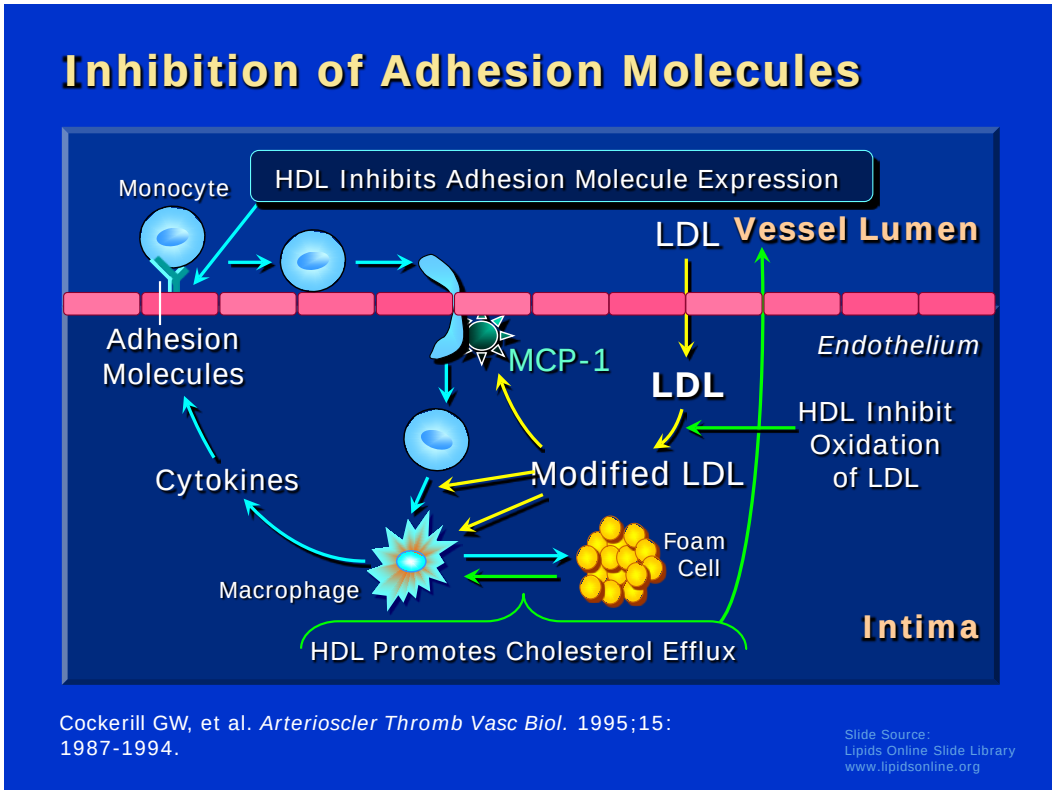


Figure 1.1.

OxPLs and oxLDL/modified LDL activate endothelial cells to express adhesion molecules and secrete chemokines such as MCP-1, resulting in the recruitment of neutrophils, monocytes and T cells and their subsequent migration into the intima. HDL plays an anti-inflammatory role in this process by inhibiting endothelial cell expression of adhesion molecules and production of MCP-1 (see text for references).

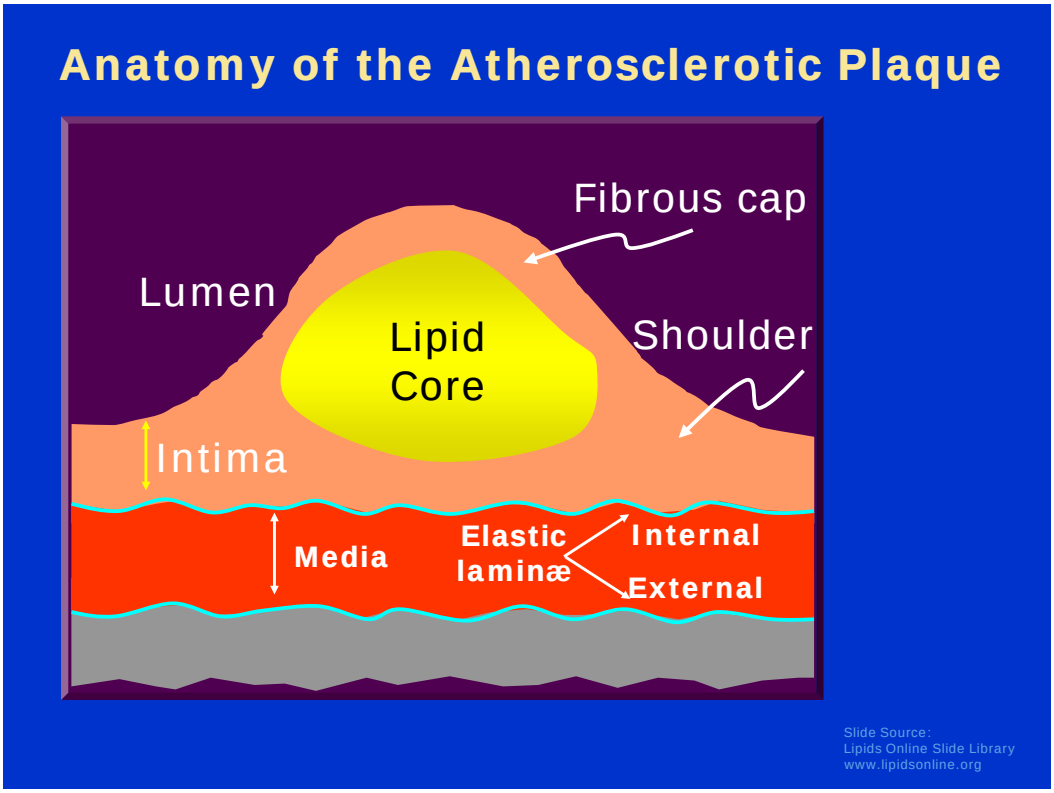


Figure 1.2.

The early fatty-streak lesion consists of T cells and foam cells loaded with lipids. Successive accumulation of apoptotic cells, debris and cholesterol crystals lead to the formation of a necrotic core within the atheromatous plaque. Smooth muscle cells then proliferate and produce collagen to form a fibrous cap on the plaque. The shoulder regions of the cap are heavily infiltrated by T cells and macrophages, which produce enzymes and pro-inflammatory mediators that contribute to destabilisation and thinning of advanced plaques and ultimately, to plaque rupture, thrombosis and vessel occlusion (see text for references).

5.3. Inflammatory mechanisms and autoimmunity in SLE and atherosclerosis

5.3.1. Endothelial dysfunction

It has been proposed that endothelial injury and dysfunction are pivotal steps in the initiation and progression of atherosclerotic CVD[304]. Repair of damaged endothelium or restoration of endothelial function are mediated by migration and proliferation of local endothelial cells (ECs) and recruitment of circulating endothelial progenitor cells (EPCs), which then differentiate into ECs[311]. EPC levels correlate positively with endothelial function[312], and low EPC numbers are associated with increased CVEs and CV-related mortality in patients with IHD[313, 314]. Endothelial dysfunction has been demonstrated in patients with SLE[315-318] and is associated with increased disease activity[318]. Rajagopalan et al. found that elevated levels of apoptotic ECs correlated strongly with elevated tissue factor (TF) levels in women with SLE[315]. As TF is a strong initiator of thrombosis, this provides a possible explanation for endothelial dysfunction as a predictor of CVEs[319, 320]. Recent studies in SLE patients also reported reduced circulating EPC numbers[321-324]. Moreover, EPCs from SLE patients demonstrated impaired endothelial repair function and produced IFN-α, which induced EPC apoptosis[322, 325]. Ferro et al. showed that 70% of aPL positive SLE patients had endothelial perturbation, as defined by elevated plasma levels of vWF and tissue-type plasminogen activator (tPA). Endothelial perturbation was associated with increased disease activity, aPL and anti-endothelial cell antibodies (AECAs). Moreover, in aPL positive SLE patients, a pro-thrombotic state (defined by elevated circulating prothrombin fragments) occurred only in the presence of endothelial dysfunction[326]. The prevalence of AECAs in SLE sera ranges from 15% to 88%, and their levels correlate with SLE disease activity[327]. AECAs from patients with SLE are associated with aCL[328], bind to ECs, inducing EC apoptosis[329], and promote macrophage phagocytosis of apoptotic ECs[330]. These studies suggest that SLE-related inflammatory and immune mechanisms

compromise EC-mediated vascular repair and contribute to a pro-coagulant state, thereby accelerating atherosclerosis.

5.3.2. Innate immunity

There is accumulating evidence that innate immunity plays a role in both the pathogenesis of SLE and in atherosclerosis. TLRs are a major class of pattern recognition receptors (PRRs) that recognise conserved molecular motifs on microbial pathogens, known as pathogen-associated molecular patterns (PAMPs)[331]. The human TLR family currently includes 10 members, each with specificities for important PAMPs[332]. TLR2 and TLR6 recognise peptidoglycan (PGN) on gram-positive bacteria, other bacterial lipoproteins and fungal cell wall components, while TLR4 recognises lipopolysaccharide (LPS) on gram-negative bacteria, heat-shock proteins (HSPs) and some viral proteins. TLR3, TLR7 and TLR9 recognise double-stranded RNA, single-stranded RNA, and unmethylated CpG DNA, respectively[333]. TLR2 and TLR4 are found on the cell surface and TLR3, TLR7 and TLR9 are located within intracellular compartments such as endosomes[334]. The TLR–PAMP interaction triggers an intracellular signalling cascade involving activation of adaptor molecules such as MyD88 that induce NF-κB transcription, resulting in production of pro-inflammatory cytokines and chemokines[333].

In SLE, activation of TLR7 and TLR9 on DCs and B cells is thought to initiate inflammatory pathways leading to the production of type I IFNs and autoantibodies such as ANA[333, 335]. Wong et al. demonstrated increased expression of TLRs in B cells, T cells and monocytes in patients with SLE, compared with controls[336]. Moreover, expression of TLR4 on T cells and TLR6 on B cells correlated positively with disease activity. Other studies have shown increased expression of TLR2, TLR4 and TLR7 in human atherosclerotic plaques[335, 337]. Enhanced TLR4 expression in murine atheromatous plaques is associated with activation of NF-κB, suggesting that TLR4 is an important factor in atherogenesis[338, 339]. Furthermore, DC-derived IFN-α in atheromatous plaques

upregulates TLR4 expression and amplifies the production of cytokines implicated in plaque destabilisation, including TNF-α and IL-12[340]. IFN-α has also been shown to promote macrophage uptake of oxLDL and foam cell formation in patients with SLE[341]. These studies together suggest that innate immune pathways in atherosclerosis are shared with SLE.

5.4. Subclinical atherosclerosis in SLE

Although clinical cardiovascular events occur more frequently in patients with SLE compared with the general population, subclinical atherosclerosis is even more common. External carotid artery intima-media thickness (IMT) and the presence of atherosclerotic plaque may be used as markers for subclinical atherosclerosis and are assessed using the non-invasive technique of B-mode ultrasonography[342, 343]. IMT and carotid plaque are thought to reflect different stages of atherogenesis[344]. IMT is thought to represent an earlier stage of arterial intimal and medial cell hypertrophy in response to lipid infiltration or hypertension, whereas plaque is thought to represent a later stage of atherogenesis[344]. Both the presence of carotid plaque and increased IMT are strong predictors for CVEs in the general population[345-347]. Several groups have reported an increased prevalence of carotid plaque, ranging from 29% to 45% in patients with SLE, compared with 15% to 22% in controls[85, 86, 348-352]. Furthermore, carotid plaque occurs at an earlier age in SLE patients compared with age and sex-matched controls, with a prevalence of 33% to 35% in patients younger than 55 years[85, 86]. Accelerated progression of plaque size and/or number occurs over time in patients with SLE[353]. Carotid IMT is also increased in patients with SLE[354-356] and progresses at an increased rate compared with controls[354, 357]. Svenungsson et al. reported that carotid IMT was increased in SLE patients with CVD than in SLE patients without CVD or healthy controls[289]. They also found higher IgG anti-oxLDL levels in SLE cases compared with SLE controls.

Another method of assessment for subclinical atherosclerosis employs CT scanning to detect coronary artery calcification[87]. The coronary calcium score is a strong predictor for IHD in the general population[346]. Asanuma et al. found coronary calcification in 31% of SLE patients compared with 9% of controls[87].

Endothelial function may be measured by several methods, including Doppler ultrasonography of the brachial artery, and coronary angiography. In response to increased arterial blood flow in the brachial artery, normal endothelium produces nitric oxide (NO), causing flow-mediated dilatation (FMD)[358, 359]. Coronary endothelial dysfunction has been shown to be predictive of atherosclerotic progression and future CVEs in the general population[319, 320]. Patients with SLE have impaired endothelium-dependent FMD of the brachial artery, compared with controls[316, 317, 360]. In one study of women with SLE, endothelial dysfunction was positively correlated with increased carotid IMT, providing further evidence for accelerated subclinical atherosclerosis in SLE[361].

5.4.1 Cardiovascular risk factors

Several cross-sectional studies in patients with SLE have demonstrated independent associations of traditional CV risk factors with carotid ultrasound markers of subclinical atherosclerosis (Table 1.5A). As would be expected, older age was the most important factor associated with the presence of carotid plaque and increased carotid IMT at baseline, as well as progression over time[85, 86, 348-351, 362, 363]. Hypertension and/or anti-hypertensive therapy and dyslipidaemia were other factors with similar vascular associations[267, 349, 362, 364]. Smoking, lower HDL_3 levels and a history of previous CVE were also associated with the presence of carotid plaque[85, 349, 350, 365], while hyperglycaemia, obesity and prevalent IHD were associated with increased carotid IMT[349, 350, 362]. Hyperglycaemia, obesity and dyslipidaemia including low HDL_3 levels are characteristic features of the metabolic syndrome. The associations of these factors with increased carotid IMT may reflect a state of chronic vascular wall inflammation resulting from oxidative stress, endothelial cell dysfunction and

adipose tissue pro-inflammatory cytokine production that occurs in type 2 DM[366]. Interestingly, pro-inflammatory HDL (piHDL), homocysteine and leptin (a pro-inflammatory cytokine secreted by adipose tissue) were also associated with carotid plaque and increased IMT in SLE, supporting this hypothesis[351, 362, 365, 367]. Furthermore, lower HDL concentrations may be marker of increased disease activity[368], itself a predictor for early mortality[223, 229]. A recent report noted IMT regression with rosuvastatin therapy in SLE patients[369], while two studies found no effect of atorvastatin on IMT progression[370, 371].

5.4.2. SLE-related and other risk factors

SLE-related risk factors independently associated with the presence of carotid plaque and/or plaque progression include longer disease duration[86], higher European Consensus Lupus Activity Measurement Index (ECLAM) scores[351], raised ESR, CRP, white cell, neutrophil and lymphocyte counts and complement C3 levels[85, 267, 351] (Table 1.5B). Similarly, predictors of IMT progression included factors associated with chronic inflammation such as longer disease duration[357], elevated creatinine[353], elevated white cell count[267], CRP[267] and C3 or C5a levels[357]. Doria et al. showed that the presence of antibodies to a component of oxLDL, oxidised palmitoyl arachidonoyl phosphocholine (oxPAPC), at the time of the follow-up scan, were associated with IMT progression and the presence of carotid plaque at follow-up[348]. These results provide further support for role of chronic inflammatory mechanisms in the pathogenesis of accelerated atheromatous plaque formation. In contrast, other studies found no significant associations between disease activity measures and plaque prevalence[85, 86, 350, 364]. It is possible that inflammatory processes involved in accelerated atherosclerosis in SLE may differ from those assessed by disease activity outcome measures. This may be an explanation for the associations of elevated C3 levels and leucocytosis with subclinical atherosclerosis, when hypocomplementaemia and leucopenia are typical of active SLE disease.

Several authors have reported that older age at diagnosis to be associated with carotid plaque and/or plaque progression[85, 351, 367]. In contrast, Kiani et al. found a positive association for older age, but a negative association for older age at diagnosis, possibly explained by cohort-related confounding factors[267].

Ahmad et al. found an association of previous history of coronary and/or cerebrovascular events as well as the presence of aPL and/or LA were independent predictors for carotid plaque in Ahmad et al.'s study of 200 women with SLE in North-West England[85]. However a negative association for aCL was found in Roman et al's study[86] and no significant associations were reported in other studies[364, 372]. Jiménez et al. reported a higher prevalence of carotid plaque in SLE patients with APS compared with primary APS patients and controls[351]. Furthermore, SLE patients had significantly greater plaque burden, whereas the plaque burden was similar in primary APS patients and controls. This suggests that other inflammatory processes apart from thrombosis are involved in atherogenesis in patients with SLE and APS. It is possible that aPL with pathogenic effects other than thrombosis may be involved in these atherogenic processes.

Higher SLICC DI scores[86, 349, 351, 364], baseline immunosuppressant use[353], increased or cumulative glucocorticoid use[348, 362] and azathioprine use[85] have also been associated with carotid ultrasound measures of subclinical atherosclerosis. In contrast, Roman et al. reported a negative association of cyclophosphamide use with carotid plaque[86]. As immunosuppressant use may act as a marker of SLE disease severity and/or activity, it is the most likely explanation for the variable associations reported. Similarly the negative association of anti-Sm and/or aCL with the presence of carotid plaque [86] may be due to associations of these antibodies with disease activity or severity factors that may act as confounders.

5.5. Summary

Patients with SLE have up to a 10-fold increased risk of developing MI or stroke and these CVEs tend to occur at an earlier age compared with the general population. The prevalence of CV risk factors is increased in patients with SLE. Despite this fact, traditional risk factors still do not fully account for the increased CV risk in SLE, therefore identification of SLE-related factors that increase CV risk will improve patient management.

Table 1.4 presents traditional and SLE-related factors predictive of CVEs, including death.

- Traditional CV risk factors include older age at diagnosis, hypertension, dyslipidaemia and smoking.
- Other important CV factors include male gender, obesity, DM and post-menopausal status.
- SLE-related factors that predict future CVEs are usually associated with increased disease activity or disease severity, such as longer glucocorticoid use, azathioprine use, NP-SLE or vasculitis. Longer disease duration also increases the risk of future CVEs.
- APL increase thrombotic risk and are also important in predicting future CV disease.

The immunopathogenic mechanisms of SLE most likely enhance the complex interaction of classic CV risk factors with the inflammatory pathways of atherogenesis, thereby accelerating the atherosclerotic process.

- Atherogenesis is initiated by endothelial dysfunction or damage. This is followed by formation of the fatty streak, then the atheromatous plaque with a fibrous cap. The final stage involves plaque destabilisation, rupture and thrombosis with vascular occlusion.
- Endothelial dysfunction is associated with increased disease activity in SLE.

- Activation of TLR4 in SLE is associated with increased disease activity. TLR4 is also found in atherosclerotic plaques, supporting the hypothesis that SLE inflammatory mechanisms enhance atherogenesis.
- HDL plays an anti-inflammatory role in the atherosclerotic process.

Subclinical atherosclerosis is common in SLE, with carotid plaque prevalence of up to 45%. Table 1.5A presents CV factors associated with carotid arterial plaque and IMT which are similar to those identified in Table 1.4.

- Older age is the most important factor associated with carotid plaque and increased IMT both at baseline and with progression. Other important factors are hypertension and dyslipidaemia.
- Smoking, lower HDL_3 levels and a history of previous CVE are associated with carotid plaque
- Hyperglycaemia, obesity and prevalent IHD are associated with increased carotid IMT.

Table 1.5B presents SLE-related factors associated with carotid plaque and IMT.

- Important factors associated with the presence of carotid plaque and/or plaque progression include longer disease duration, increased disease activity, increased damage accrual, raised inflammatory markers (such as leucocytosis and raised CRP), glucocorticoid and azathioprine use.
- Markers of IMT thickening or progression also included longer disease duration, raised inflammatory markers and glucocorticoid use.
- There conflicting results for aPL as marker of subclinical atherosclerosis.

Table 1.5A. Cardiovascular-related risk factors for subclinical atherosclerosis

Authors (year)	Study design	Follow-up (years)	SLE subject sample size	Study site(s)	Carotid plaque	Increased carotid IMT	Multivariate analysis
Manzi et al (1999)[349]	prospective cohort, cross-sectional	-	175 women	Pittsburgh PA, USA	older age, ↑systolic BP, IHD	older age, ↑pulse pressure, IHD	yes
Roman et al (2003)[86]	prospective cohort + controls, cross-sectional	-	197 + controls	New York NY, USA	older age		yes
Selzer et al (2004)[350]	prospective cohort, cross-sectional	-	214 women	Pittsburgh PA, USA	older age, ↑systolic BP, ↓HDL_3	older age, ↑pulse pressure, ↑TC, ↑glucose, ↑CRP	yes
Jiménez et al (2005)[351]	prospective cohort + controls, cross-sectional	-	70 SLE, 25 primary APS + 40 controls	Barcelona, Spain	older age, ↑apoB		yes
Maksimowicz-McKinnon et al (2006)[364]	prospective cohort, cross-sectional (Hopkins)	-	605	Baltimore MD, USA	older age, hypertension		yes
Ahmad et al (2007)[85]	prospective cohort + controls, cross-sectional	-	200 + 100 controls	NW England	smoking, previous CVE		yes
McMahon et al (2009)[362]	prospective cohort, cross-sectional	-	276	Los Angeles, CA, USA	older age, hypertension, dyslipidaemia, mixed race, piHDL	older age, African-American ethnicity, ↑IMT, piHDL,	yes
McMahon et al (2011)[365]	prospective cohort + controls, cross-sectional	-	250 + 122 controls	Los Angeles, CA, USA	older age, hypertension, smoking, piHDL, ↑leptin		yes
de Leeuw et al (2006)[363]	prospective cohort + controls, cross-sectional	-	72 + 36 controls	Groningen, Netherlands		older age, increased coronary risk	yes

Authors (year)	Study design	Follow-up (years)	SLE subject sample size	Study site(s)	Carotid plaque progression	Carotid IMT progression	Multivariate analysis
Doria et al (2003)[348]	prospective cohort, longitudinal	5	78	Padua, Italy	older age	older age, hypertension	yes
Roman et al (2007)[367]	prospective cohort, longitudinal		158	New York NY, USA	↑homocysteine		yes
Thompson et al (2008)[353]	prospective cohort, longitudinal	mean 4.2	217 women	Pittsburgh PA, USA	older age, ↑TG	older age, ↓diastolic BP	yes
de Leeuw et al (2009)[354]	prospective cohort + controls, longitudinal	≥ 1.7	74 + 74 controls	Groningen & Amsterdam, Netherlands		older age	yes
Rua-Figueroa et al (2010)[357]	prospective cohort, longitudinal	2	101	Las Palmas, Spain		older age at diagnosis	yes
Kiani et al (2011)[267]	prospective cohort, longitudinal (Hopkins)	2	187	Baltimore MD, USA	older age, ↑BP, &/or treatment	older age, ↑BP, ↑CRP	yes

Table 1.5B. SLE-related risk factors for subclinical atherosclerosis

Authors (year)	Study design	Follow-up (years)	SLE subject sample size	Study site(s)	Carotid plaque	Carotid IMT thickening	Multivariate analysis
Manzi et al (1999)[349]	prospective cohort, cross-sectional	-	175 women	Pittsburgh PA, USA		↑SLICC DI	yes
Roman et al (2003)[86]	prospective cohort + controls, cross-sectional	-	197 + controls	New York NY, USA	↑disease duration, ↑SLICC DI; ***negative association*** - cyclophosphamide, anti-Sm / aCL		yes
Jiménez et al (2005)[351]	prospective cohort + controls, cross-sectional	-	70 SLE, 25 primary APS + 40 controls	Barcelona, Spain	older age at diagnosis, ↑disease duration, ↑ESR, ↑CRP, ↑ECLAM, ↑SLICC DI, APS		yes
Maksimowicz-McKinnon et al (2006)[364]	prospective cohort, cross-sectional (Hopkins)	-	605	Baltimore MD, USA	↑SLICC DI		yes
Ahmad et al (2007)[85]	prospective cohort + controls, cross-sectional	-	200 + 100 controls	NW England	older age at diagnosis, ↑disease duration, ↑neutrophils, azathioprine use, aCL &/or LA		yes
McMahon et al (2009)[362]	prospective cohort, cross-sectional	-	276	Los Angeles, CA, USA		cumulative glucocorticoid dose $\geq$ 20g	yes

Authors (year)	Study design	Follow-up (years)	SLE subject sample size	Study site(s)	Carotid plaque progression	Carotid IMT progression	Multivariate analysis
Doria et al (2003)[348]	prospective cohort, longitudinal	5	78	Padua, Italy	cumulative glucocorticoid dose	anti-oxPAPC at 2nd scan, cumulative glucocorticoid dose	yes
Roman et al (2007)[367]	prospective cohort, longitudinal		158	New York NY, USA	older age at diagnosis, ↑disease duration		yes
Thompson et al (2008)[353]	prospective cohort, longitudinal	mean 4.2	217 women	Pittsburgh PA, USA	↑C3, baseline immunosuppressant use	↑creatinine	yes
de Leeuw et al (2009)[354]	prospective cohort + controls, longitudinal	≥ 1.7	74 + 74 controls	Groningen & Amsterdam, Netherlands		disease duration > 10 yrs	yes
Rua-Figueroa et al (2010)[357]	prospective cohort, longitudinal	2	101	Las Palmas, Spain		↑C3, ↑C5a	yes
Kiani et al (2011)[267]	prospective cohort, longitudinal (Hopkins)	2	187	Baltimore MD, USA	↑disease duration, ↑white cells ↑lymphocytes, ↑proteinuria; ***negative association*** - older age at diagnosis	↑white cell count	yes

6. Lipid profiles in SLE

The "lupus pattern" of dyslipoproteinaemia is a lipid profile characterised by decreased HDL-C and elevated very low density lipoprotein cholesterol (VLDL-C) and TG[368, 373, 374]. This profile is more marked with increased disease activity, as measured by SLEDAI or the Systemic Lupus Activity Measure (SLAM), and may be accompanied by decreased LDL levels[368, 373, 375]. Raised TG and VLDL-C are positively correlated with increased TNF-α levels[376]. TNF-α stimulates hepatic synthesis of VLDL and also downregulates expression of lipoprotein lipase (LpL), an endothelium-associated enzyme which hydrolyses TGs on chylomicrons and VLDL[377]. Proteinuria exacerbates hypertriglyceridaemia in SLE and is also associated with increased total cholesterol (TC), LDL-C and apolipoprotein B (apoB), a component of VLDL and LDL[378-380]. In the general population, apoB is a stronger predictor for fatal MI than LDL-C[381]. In patients with SLE, hypercholesterolaemia is associated with an increased risk of developing IHD[276, 382]. Glucocorticoid therapy causes increases in TC, HDL-C, LDL-C and apoB concentrations, thereby potentially increasing CV risk in patients with SLE[383-385]. Within the general population, TG was found to be an independent predictor for IHD in some studies, but not in others[386, 387]. This discrepancy may be due to TG concentrations representing both TG-rich highly atherogenic lipoproteins, such as intermediate density lipoproteins (IDL), as well as TG-rich non-atherogenic lipoproteins, such as chylomicrons and large VLDLs[387]. MacGregor et al. studied lipid profiles of 64 SLE patients and found raised TG and apoB concentrations in patients taking prednisolone doses above 10 mg daily in the previous 6 months. However, an increase in vascular events occurred only in the subgroup of SLE patients with raised TG who were also aCL positive[388].

6.1. HDL and ApoA-I

In the Framingham study, reduced HDL-C concentration was the strongest independent predictor for IHD in both men and women[389]. Numerous studies have since confirmed this finding[390, 391]. Furthermore, HDL concentrations show an inverse relation to CV-related mortality[391]. In SLE, low HDL-C levels were detected in 79% of patients with active disease and in 29% of patients with inactive disease[368]. HDL-C is traditionally separated by ultracentrifugation, chemical precipitation, or gradient gel electrophoresis into its two major lipoprotein subfractions, HDL_2-C and HDL_3-C[390, 392]. In the general population, low HDL_2-C concentrations are more strongly predictive of IHD risk than low HDL_3-C concentrations[390]. Apolipoprotein A-I (apoA-I) is the predominant apolipoprotein of HDL, and has been shown to play a protective role against fatal MI in the general population[381]. Ettinger et al. compared lipid profiles of SLE patients with matched control subjects and found similar levels of HDL-C, HDL_3-C and apoA-I in both groups, but lower HDL_2-C levels in SLE patients[383].

HDL plays a critical role in reverse cholesterol transport, which is the primary mechanism for delivering excess cholesterol from peripheral tissues to the liver for disposal[393]. ApoA-I stimulates extracellular efflux of phospholipid and cholesterol via ATP-binding membrane cassette transport protein A1 (ABCA1). ApoA-I binds to PLs and interacts with cholesterol to form nascent discoidal HDL (ndHDL). Lecithin-cholesterol acyltransferase (LCAT) then esterifies cholesterol on the surface of ndHDL. Cholesteryl esters move to the hydrophobic core of HDL, producing a steady gradient for free cholesterol to move out of cells towards HDL. As the amount of esterified cholesterol within the HDL particle increases, it becomes progressively rounder and larger, resulting in the formation of mature HDL. Exchange of cholesteryl esters on HDL for TGs on lipoprotein remnant particles (VLDL or IDL) is mediated by cholesteryl ester transfer protein (CETP). These lipoprotein remnant particles are subsequently cleared by the liver. At the same time, TGs and PLs on HDL undergo hydrolysis by hepatic lipase (HL), a process which converts larger and more buoyant HDL_2 to smaller and denser HDL_3. HDL is then taken up by the liver[393, 394] (Figure 1.3).

In addition to its role in reverse cholesterol transport, HDL has a variety of other anti-inflammatory, anti-oxidant and anti-thrombotic functions which contribute to its protective effect on atherosclerosis. HDL actions on endothelium include stimulation of NO and prostacyclin production, inhibition of adhesion molecule expression, and prevention of endothelial apoptosis. HDL's antioxidant effects are due to reduction of lipid peroxides in LDL by apoA-I and activity of several of its anti-oxidant enzymes, including paraoxonase 1 (PON1), which hydrolyses LDL-associated lipid peroxides and prevents generation of oxLDL[394, 395]. However, during chronic inflammation, the oxidative environment promotes oxidative and enzymatic modification of lipids and proteins within HDL, hence impairing its anti-atherogenic effects. This form of HDL, termed pro-inflammatory HDL (piHDL), has a reduced ability to promote cholesterol efflux, and is associated with IHD[396]. McMahon et al. found pro-inflammatory effects of HDL in 45% of SLE patients compared with 4% of controls[397]. Furthermore, piHDL was associated with plaque and increased carotid IMT in SLE patients[362]. However, there were no associations between PON1 activity and apoA-I levels with piHDL or plaque, suggesting that the pro-atherogenic actions for piHDL are independent of PON1 and apoA-I activity. In SLE, conversion of normal HDL to piHDL may result from inhibition of its anti-oxidant enzyme and apolipoprotein activities by antibodies such as aPL directed against HDL epitopes. IgG anti-HDL and anti-apoA-I are associated with reduced PON1 activity[374, 398]. One study found that antibodies from SLE sera directed against HDL and apoA-I also exhibited cross-reactivity with cardiolipin[399]. Lower TC, HDL-C and apoA-I levels were found in aCL GPL positive patients with SLE, compared with aCL GPL negative patients[400]. The prevalence of IgG anti-apoA-I was reported to be 32.5% in SLE patients and was associated with anti-β_2GPI GPL[401]. The association of anti-apoA-I with aCL and anti-β_2GPI antibodies may be explained by the presence of cardiolipin within HDL, possibly bound to β_2GPI[72]. An increased prevalence of IgG anti-apoA-I has also been found in patients with acute coronary syndromes[402]. There is evidence to support pro-inflammatory roles for anti-HDL and anti-apoA-I in SLE. Higher IgG anti-HDL and anti-

apoA-I titres are associated with increased disease activity and damage, as measured by BILAG and SLICC DI respectively in patients with SLE, and remain elevated with persistent disease activity[374, 403].

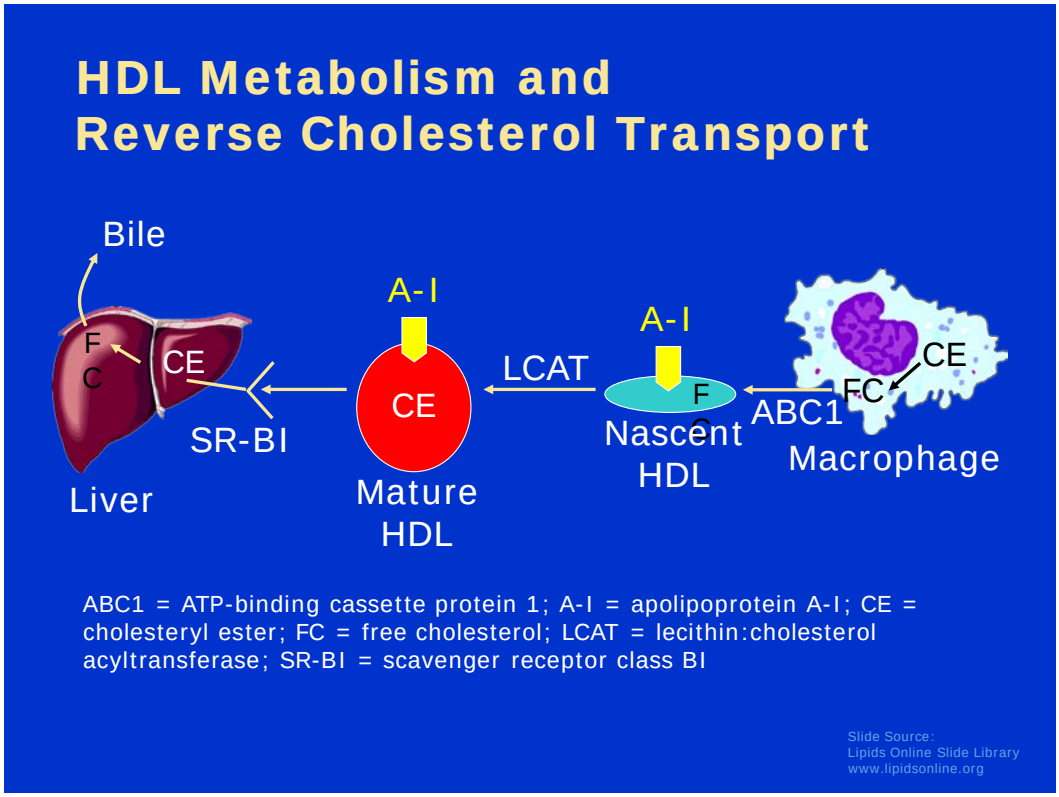


Figure 1.3.

Reverse cholesterol transport is the primary mechanism for delivering excess cholesterol from peripheral tissues to the liver for disposal. A-I stimulates extracellular efflux of phospholipid and cholesterol via ABC1. A-I binds to PLs and interacts with cholesterol to form nascent discoidal HDL. LCAT then esterifies cholesterol on the surface of nascent HDL. Cholesteryl esters move to the hydrophobic core of HDL, producing a steady gradient for free cholesterol to move out of cells towards HDL. As the amount of esterified cholesterol within the HDL particle increases, it becomes progressively rounder and larger, resulting in the formation of mature HDL. HDL is then taken up by the liver via SR-BI. (see text for references).

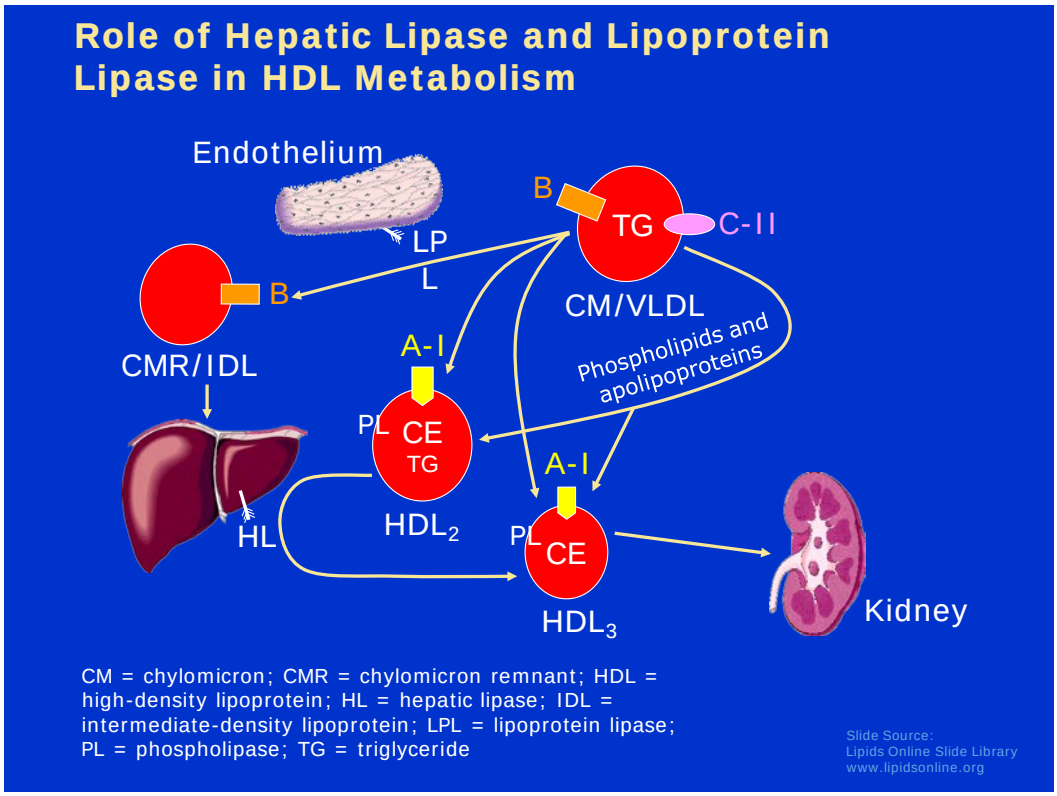


Figure 1.4.

LPL is bound to endothelium in muscle and adipose tissue and hydrolyses lipoprotein-associated TG into free fatty acids. CMs carrying dietary TG absorbed from the intestine compete with VLDL for LPL hydrolysis of TGs. Resultant CMRs are removed from the circulation by the liver. Removal of TG from VLDL results in VLDL remnant particles such as IDL, which are later converted to LDL and subsequently cleared by the liver. Exchange of cholesteryl esters (CE) on HDL for TGs on lipoprotein remnant particles (VLDL or IDL) is mediated by CETP. At the same time, TGs and PLs on HDL undergo hydrolysis by HL, a process which converts larger and more buoyant HDL_2 to smaller and denser HDL_3 (see text for detail and references).

6.2. Lipoprotein lipase

Inhibition of LpL activity may be one of the factors contributing to the elevated TG and VLDL concentrations observed in patients with active SLE[404]. LpL is bound to endothelium in muscle and adipose tissue and hydrolyses lipoprotein-associated TG into free fatty acids. Chylomicrons are lipoproteins carrying dietary TG absorbed from the intestine, which compete with VLDL for LpL hydrolysis of TGs[405]. Resultant chylomicron remnants are removed from the circulation by the liver, using apolipoprotein E (apoE) as a ligand[406]. Removal of TG from VLDL results in VLDL remnant particles such as IDL, which are later converted to LDL[405]. HDL levels are also influenced by LpL activity. Reduced LpL activity results in increased transfer of TGs from VLDL to HDL. TG-rich HDL then acts as a substrate for HL, resulting in smaller, lipid-poor apoA-I, which is then rapidly cleared, thereby accounting for the association of reduced LpL activity with low HDL levels[406] (Figure 1.4). LpL activity is reduced by approximately 50% in patients with SLE compared with healthy individuals[404] and may be a result of inhibition by anti-LpL antibodies. Reichlin et al. detected anti-LpL in 47% of SLE patients[407]. Anti-LpL levels have been positively correlated with TG, apoB and apoE concentrations, providing further evidence for anti-LpL activity[407]. Anti-LpL is also associated with aCL GPL and increased disease activity in SLE[408].

6.3. Lipoprotein(a)

Lipoprotein(a) [Lp(a)] is a LDL-like lipoprotein consisting of apoB100 covalently-linked to a plasminogen-like glycoprotein, apolipoprotein(a) [apo(a)]. Apo(a) interferes with fibrinolysis and promotes thrombosis by inhibiting the function of tissue factor pathway inhibitor (TFPI), a major regulator of TF-mediated coagulation[409, 410]. Lp(a) also promotes monocyte adhesion to endothelium[409], and mediates plaque inflammation and rupture[411, 412]. Lp(a) binds pro-inflammatory oxPLs[291], with apoB100 as the preferential carrier of oxPLs in human plasma. Lp(a), together with oxPLs, are implicated in the

pathogenesis of atherosclerosis within the general population, and higher levels correlate with ultrasound measures of carotid and femoral atherosclerosis[289, 413]. Elevated Lp(a) independently predicts for IHD, ischaemic stroke and coronary mortality, although the effect is relatively weak (adjusted RR 1.1 for all outcomes)[409]. Elevated levels of Lp(a) have also been detected in patients with SLE[414-416]. One study reported serum Lp(a) concentrations were elevated in SLE patients with renal disease and hypoalbuminaemia, and were reduced by treatment with glucocorticoids[416]. Furthermore, SLE patients with elevated Lp(a) concentrations (> 300 mg/L) have higher levels of ICs containing IgM anti-oxLDL, implying involvement of Lp(a) in autoimmune pathways in SLE[417]. In contrast, another group reported that Lp(a) levels were not influenced by glucocorticoids, disease activity or aCL[415].

6.4. Lipoprotein and apolipoprotein ratios

The TC : HDL-C ratio has been used to predict future IHD risk in the general population, with higher ratios predicting increased risk[418]. Recent studies have demonstrated that the apoB : apoA-I ratio may be a more reliable predictor of IHD risk than the TC : HDL ratio[419, 420]. ApoB is considered to be representative of atherogenic lipoproteins, whereas apoA-I represents anti-atherogenic HDL particles[419]. Lipoprotein or apolipoprotein ratios may be more reliable CV risk predictors in the setting of SLE, where lipid profiles may vary according to disease activity.

6.5. Summary

- The "lupus pattern" of dyslipoproteinaemia is characterised by low HDL-C, and elevated VLDL-C and TG and is more marked with increased disease activity.
- Glucocorticoid therapy causes increases in TC, HDL-C, LDL-C and apoB concentrations.

- HDL plays a critical role in reverse cholesterol transport, but also has anti-oxidant effects via PON1 activity.
- Reduced HDL-C is a strong predictor of IHD.
- ApoA-I is the predominant apolipoprotein of HDL.
- SLE patients who are ACL GPL+ve have been shown to have lower TC, HDL-C and apoA-I levels.
- Low HDL levels are associated with lower LpL activity. LpL activity may in turn be inhibited by anti-LpL in SLE.
- Lp(a) is a predictor of IHD and elevated Lp(a) levels have been found in patients with SLE.
- The TC : HDL-C ratio and apoB : ApoA-I ratios are predictors of increased CV risk in the general population and may be useful in assessing CV risk in SLE.

Conclusions

Due to the complex pathogenesis of SLE and the wide spectrum of disease manifestations and clinical outcomes discussed in this review, it is clear that a range of biomarkers would be required to assist clinicians in the management of their lupus patients.

Autoantibodies may be associated with distinct clinical subsets of SLE disease manifestations. These results support the hypothesis that auto-immune inflammatory pathways leading to specific SLE disease manifestations are shared with other auto-immune diseases with similar disease manifestations. Studying biomarkers of other diseases may therefore yield useful biomarkers for clinical subsets of SLE. For example, ACPA may be a useful marker for an erosive subset of lupus arthritis.

Recently discovered genetic markers have provided new insights into SLE disease susceptibility, as well as shared genetic influences on inflammatory

pathways and autoimmune mechanisms in the pathogenesis of SLE. While the majority of these genetic markers are also markers of SLE disease susceptibility, their specific autoantibody and clinical disease associations may provide further prognostic information for patients with established SLE.

Despite recent advances in the medical treatment of SLE, patients still have poorer clinical outcomes and higher mortality rates compared with the general population. Important non-SLE related factors include older age at disease onset, male gender and low socio-economic status. Major SLE-related factors include higher disease activity, organ damage accrual, more severe disease manifestations (e.g. renal and neuropsychiatric involvement), and APS. However, there is very little data published on the associations of autoantibodies with mortality. This may reflect the highly complex nature of autoimmune inflammatory processes, so a single isolated factor such as an autoantibody may not be able to directly predict a future clinical outcome. Nonetheless, research for autoantibodies as markers of future mortality is still warranted, because testing for an autoantibody with predictive value would provide added prognostic accuracy when other predictive factors are taken into account.

This chapter described the multifactorial pathogenesis of premature CVD in SLE, which most likely involves complex interactions of vascular inflammation, lipid oxidation, lipoprotein metabolism, endothelial dysfunction, adverse glucocorticoid effects, hypertension, and aPL-related vascular and thrombotic effects.

As traditional risk factors do not fully account for the increased CV risk in SLE, identification of SLE-related factors that increase CV risk will improve patient management. Traditional CV risk factors include older age at diagnosis, hypertension, dyslipidaemia and smoking. Other important CV factors include male gender, obesity, DM and post-menopausal status. Of note, SLE-related factors that predict future CVEs are usually associated with increased disease activity or disease severity, such as longer glucocorticoid use, azathioprine use,

NP-SLE or vasculitis. Longer disease duration also increases the risk of future CVEs. APL increase thrombotic risk and are also important in predicting future CV disease.

Lower HDL concentrations are associated with subclinical atherosclerosis and may be a marker of increased disease activity, itself a predictor for mortality. SLE-related factors associated with carotid plaque and increased IMT thickness and/or progression are similar to the predictors for CVEs, such as longer disease duration, increased damage accrual, increased disease activity, raised inflammatory markers, glucocorticoid and azathioprine use. However, there are conflicting results for aPL.

The factors above that are associated with CVEs and subclinical atherosclerosis highlight the importance of good control of SLE disease activity for the prevention of long-term CV complications. Moreover, vigilance in identifying and actively treating traditional CV risk factors is an essential component of long-term management of patients with SLE.

The following thesis chapters present the research conducted to identify serological biomarkers for certain clinical aspects of SLE. Markers studied include auto-antibodies and genetic markers of erosive arthritis, markers for the clinical outcomes of cardiovascular events and mortality, as well as markers of subclinical atherosclerosis.

CHAPTER 2

Associations of erosive arthritis with anti-cyclic citrullinated antibodies and MHC class II alleles in SLE

Background

Joint involvement is a common feature of SLE, occurring in up to 91% of patients[25, 183]. Clinical manifestations range from recurrent, transient polyarthralgia to deforming, rheumatoid-like arthritis, with synovitis affecting MCP or proximal PIP joints. Jaccoud's arthropathy is an uncommon type of correctable, deforming NEA that occurs in 3% to 13% of SLE patients[184-187]. Typical RA-like erosions are absent on radiographs, although "hook-like" erosions of the metacarpal heads may develop in late disease. Erosive arthritis (EA) is also uncommon in SLE, and has been reported to affect 5% of patients[187-189]. A further clinical subset, termed "rhupus" describes patients with radiological RA-like joint erosions, who fulfil both clinical features of RA and SLE. As EA is associated with worse functional outcome and disability, determining a biomarker to identify the subset of patients at risk of developing EA would enable clinicians to make informed decisions about initiating aggressive disease-modifying therapy for arthritis.

The presence of RF is one of the ACR/ EULAR classification criteria for RA[192-194] (see Appendix), however, RF is relatively insensitive as a predictor for RA, as only 75% of patients with RA are RF-positive[195]. Moreover, RF is not specific for RA and has been detected in other autoimmune diseases, including SLE[195]. Mediwake et al. found that RF was unhelpful in distinguishing RA patients from SLE patients with EA[187]. ACPA has been reported to be much a more specific serological marker for RA than RF[199]. However, ACPA has also been detected in

other autoimmune diseases, including SLE[129, 187, 421]. In both RA and PsA, ACPA is associated with EA and radiographic disease progression[200, 202].

Antibody production and clinical subsets of SLE are influenced by genetic factors. Several studies have confirmed the association of *HLA-DR3* with the production of both anti-Ro/SSA and anti-La/SSB[147, 148]. Anti-Ro/SSA and anti-La/SSB positive patients with the *HLA-DR3* haplotype are more likely to be older at disease onset, with sicca symptoms and less renal involvement[151]. In RA, disease susceptibility and severity are associated with several MHC Class II alleles encoding protein products collectively termed the shared epitope (SE)[207, 422]. *HLA-DQB1*0302* is inherited in linkage disequilibrium with *HLA-DRB1* SE alleles and is associated with RA disease severity and ACPA production[209]. However, recent evidence suggests that the SE may not be a specific marker for RA, but instead may be a marker for ACPAs involved in the pathogenesis of progressive, erosive joint destruction[208]. In view of the frequent clinical manifestation of arthritis in SLE and the joint damage and functional limitation that is a consequence of EA, ACPA may serve a useful clinical role as a predictor of EA in SLE. This chapter describes in detail our research on the association of ACPA with EA in SLE, a finding which has been confirmed by others[129-131, 187, 203, 423].

Aim

To determine the associations of erosive arthritis with ACPA and MHC class II alleles in patients with SLE.

Methods

Personal contribution by the candidate

This study was designed as a retrospective cohort study by the candidate, under the supervision of Prof Neil McHugh. MHC class II genetic data and serological data were available from a database of SLE patients who were participating in an ongoing serological and genetic study and who had been consecutively recruited from the RNHRD CTD Clinic. Genetic and serological data was also available from a group of local healthy blood donors who were consecutively recruited for a previous study. The SLE patients attended the Connective Tissue Diseases (CTD) Clinic at the Royal National Hospital for Rheumatic Diseases (RNHRD). Radiological reports and clinical data were collected by the candidate for this study. The candidate designed the SLE questionnaire (see Appendix), with advice from Prof McHugh and Dr Eleanor Korendowych. Mrs Charlotte Cavill, the CTD database manager at the Bath Institute for Rheumatic Diseases (BIRD), was responsible for mailing of questionnaires, collation of questionnaire results and tracing of patients who were lost to follow-up. Mrs Juliet Dunphy and Mrs Patricia Owen at BIRD provided training and supervision for the candidate's work on all RF and several ELISAs. The remainder of the ACPA ELISAs were performed by Mrs Dunphy and Owen.

Sample size

Prior to commencement of this study, there was only one published report on the association of ACPA with EA in SLE[187]. In this study, Mediwake et al. utilised the earliest version of the ACPA ELISA, which had a sensitivity of 68%[424], compared with the manufacturer's reported sensitivity of 76% for its later version ACPA-2 ELISA (INOVA Diagnostics, San Diego, CA, USA) that was used in our study. Although a sample size calculation was not performed prior to the study, we expected that a study sample of at least 66 SLE patients (the number of patients tested for ACPA by Mediwake et al.) would have sufficient power to

detect a difference in ACPA positivity between SLE patients with EA and those without EA. Hence we aimed to recruit at least 100 patients for this study.

Patients and controls

We studied 104 subjects with SLE (91 females and 13 males) from a research database of patients attending the RNHRD CTD Clinic, a tertiary rheumatology centre in the UK. All patients fulfilled the updated ACR classification criteria (1997) for SLE[8, 425]. Ethical approval for the study was given by the Bath Local Research Ethics Committee and written informed consent was given by all participants. Clinical data collected for each patient included the documentation of the presence of joint synovitis at any time in the course of the disease. Patients who experienced joint symptoms had radiographs of hands and feet taken during their routine clinic visits, with radiographs repeated at a minimum of yearly intervals (up to 9 year intervals), according to the treating clinician's decision. Available radiographs were reviewed to determine the presence of joint erosions. Patients with synovitis were designated as having erosive arthritis (EA) attributable to an inflammatory arthropathy, or nonerosive arthritis (NEA), according to the presence or absence of joint erosions on radiographs. Each patient with EA was then assigned as having major or minor erosions according to the size and extent of the erosions. Patients with EA were also assessed for RA according to ACR criteria[193]. All remaining patients without joint synovitis were designated as "no arthritis" (NA). Blood samples were collected from all patients for genetic studies and serological tests.

Serum samples from 130 age- and sex-matched healthy local blood donors were selected as serum controls. Seventy-six blood samples from the 130 serum controls were also genotyped and a further 41 samples from sex-matched local blood donors were selected as genetic controls (total n = 117). All serum and genetic control samples were from British Caucasian individuals.

Autoantibody measurement

Serum ACPA and RF autoantibodies from patients and serological controls were measured using commercial ACPA-2 and IgM RF ELISA kits (INOVA Diagnostics). ANA titres were determined by indirect immunofluorescence on HEp-2 cells. Anti-dsDNA antibodies were determined using commercial ELISA kits (Cambridge Life Sciences, Ely, UK). Antibodies to extractable nuclear antigens (including U1RNP, Sm, Ro/SSA, and La/SSB,) were measured by Ouchterlony double diffusion. Patients with anti-U1RNP, anti-Sm, or anti-La/SSB antibodies had these autoantibodies confirmed by western blotting on at least one sample. For ELISAs, all serum samples were tested in duplicate and absorbances were determined using a commercial microplate photometer (Multiskan Ascent, Labsystems, Helsinki, Finland). The intra- and inter-assay coefficients of variation (%CV) for ACPA were 9.0% and 11.3% respectively and intra- and inter-assay %CVs for RF were 8.9% and 20.2% respectively. Cut-off values of $\geq$ 25 Units (U) for ACPA and $\geq$ 6 U for RF were used to indicate a positive result, which were above the 98th and 95th percentiles of control sample results respectively.

HLA-DRB1 and HLA-DQB1 genotyping

All 104 SLE and 117 genetic control whole blood samples were collected into ethylenediamine tetra-acetate (EDTA) tubes and genomic DNA was extracted using a standard salting out procedure. Twenty-six *HLA-DRB1* and 13 *HLA-DQB1* alleles were identified from extracted DNA, using a polymerase chain reaction-based method with sequence specific primers (PCR-SSP), as previously described by McHugh et al.[145] Carriage of an SE allele was documented according to the presence of *HLA-DRB1*0101, *0102, *0401, *0405, *0408* or **1001*[422]. The presence of the SE-associated allele *HLA-DQB1*0302* was also determined, in addition to the SLE-associated alleles *HLA-DRB1*0301* and *HLA-DQB1*0201*.

Statistical analysis

Statistical analysis was performed using the SPSS Statistics 17.0 software package (IBM Corporation, Armonk, NY, USA). Comparisons were made for nominal data using the chi-square test with 2 x 2 contingency tables, and odds ratios (OR) and 95% CI were calculated. Where expected numbers for the contingency tables were less than 5, the Fisher's exact test was used. The Student's t-test was used to compare normally distributed data, with means and standard deviations (SD) quoted. For nonparametric comparisons, Mann-Whitney U and Kruskal-Wallis tests were used, with medians and interquartile ranges (IQR) quoted. Logistic regression was used for comparisons of continuous data to determine ORs. A p value of < 0.05 was considered to represent a significant difference between groups and where appropriate, Bonferroni corrections were made for the number of alleles observed.

Results

Clinical features of SLE patients

We found that 71 of 104 (68%) SLE patients had experienced synovitis during the course of their disease, of whom 12 (11%) patients had EA and 59 (57%) had NEA. The remaining 33 (32%) patients had NA. Patients were followed for a median (IQR) of 13 (13) years. Table 2.1 shows the clinical features of the 3 patient groups. There were no significant differences in age among the groups. Patients from the EA group had a longer median (IQR) disease duration of 14 (10) years, compared with median (IQR) disease durations of 9 (6) and 7 (5.5) years in the NEA and NA groups respectively, although this was not significant ($p_{corrected} = 0.2$ for EA vs NEA and $p_{corrected} = 0.2$ for EA vs NA). There were no significant differences in ethnicity among the groups, as the majority (n = 102, 98%) were of British Caucasian origin. There were two patients of Afro-Caribbean origin who both had NEA. All patients with EA were female, compared with 88.1% of the NEA group and 81.8% of the NA group. There were

no significant differences between groups for SLE-related clinical features, although none of the EA patients had a history of discoid rash or NP-SLE.

Serology results of SLE patients

All 104 SLE patients were ANA positive. Eight (8%) patients were ACPA positive, compared with 2 (1.5%) of 130 ACPA+ serum controls (OR 5.3, 95% CI 1.1, 25.7, p = 0.02). Eighteen (17%) of SLE patients were RF positive, compared with 4 (3%) of RF+ serum controls (OR 6.6, 95% CI 2.2, 20.2, p < 0.0001). ACPA was significantly associated with RF (OR 5.9, 95% CI 1.3, 26.2, p = 0.03). Table 2.2 shows the serology results of the 3 SLE patient groups. Among the 71 patients with a history of synovitis (comprising 12 EA and 59 NEA patients), 6 (11%) were ACPA+ and 5 (15%) were RF+. Compared with other SLE patients, patients with EA were more likely to be ACPA+ [6/12 (50%) in EA vs 2/92 (2%) in other SLE patients, OR 45.0, 95% CI 7.4, 272.5, p < 0.0001]. Similarly, ACPA was more likely to be present in EA patients compared with NEA patients (OR 28.5, $p_{corrected}$ = 0.01). When corrected for multiple comparisons, the association of RF with EA was no longer significant (p = 0.02, $p_{corrected}$ = 0.3). None of the patients with EA had anti-Ro/SSA or anti-La/SSB antibodies. Although more EA patients were anti-U1RNP positive (58% vs 36% of NEA and 33% of NA patients), this was not statistically significant.

Characteristics of SLE patients with EA

Table 2.3 shows the characteristics of the 12 patients with EA, subdivided into 2 groups with major erosions or minor erosions. Six (50%) patients had major erosions on radiographs. The earliest erosions occurred after a mean (SD) of 11.3 (6.8) years for all EA patients, with no differences between those with major or minor erosions. Four of the 6 patients with major erosions (66.7%) were ACPA+ and 3 (50%) were RF+. There were no differences in median ACPA or RF levels between the patients with major or minor erosions. All 6 patients with major

erosions also fulfilled the ACR criteria for RA, compared with only 1 patient with minor erosions ($p = 0.01$).

Characteristics of ACPA positive SLE patients and controls

Table 2.4 shows the characteristics of the 8 ACPA+ SLE patients and 2 ACPA+ serum controls. Both serum controls were also genotyped. Six patients (75%) developed EA, of whom 4 (50%) had major erosions. All 5 *HLA-DQB1*0302* carriers had EA, 4 of whom developed major erosions. Patients 5 and 6 were anti-U1RNP and anti-Sm positive and had LN (patient 5 - class IV diffuse proliferative glomerulonephritis, patient 6 - class II mesangioproliferative glomerulonephritis). Both of these patients had Jaccoud's arthropathy, with one patient developing minor erosions. This patient also met the ACR criteria for RA. Both were carriers of *DRB1*1303,* but neither carried the SE nor *DQB1*0302*. Three patients carried 2 SE alleles and 2 patients were heterozygotes for the SE and the SLE-associated allele *DRB1*1501*. Both ACPA+ controls were carriers of the SE allele *DRB1*0401*.

Frequencies of MHC class II alleles in SLE patients and genetic controls

*HLA-DRB1*0301* was significantly associated with SLE [39/104 (37%) SLE patients vs 24/117 (20%) genetic controls, OR 2.3, 95% CI 1.3, 4.2, $p = 0.005$, $p_{corrected}=0.05$]. There was a similar trend for *DQB1*0201* [49/104 (47%) SLE vs 41/117 (35%) controls, OR 1.6, 95% CI 1.0, 2.8, $p = 0.08$]. Almost all SLE patients and all genetic controls were British Caucasian individuals and as expected, the most common SE allele present was *DRB1*0401* [25/104 (24%) SLE vs 21/117 (18%) controls]. There were no differences between patients and controls for frequencies of other SE alleles or *DQB1*0302* (results not shown).

Associations of arthritis with MHC class II alleles

Table 2.5 shows the associations of arthritis with MHC class II alleles. When corrected for multiple comparisons, *DQB1*0302* was the only SE-related allele significantly associated with EA (OR 8.2, 95% CI 2.2, 30.4, $p_{corrected}$ = 0.01). Furthermore, all 6 patients with major erosions were *DQB1*0302* carriers [6/6 (100%) vs 20/98 (20%) all other SLE, $p < 0.0001$, $p_{corrected}$ = 0.001]. There was a similar but non-significant association of EA with 2 copies of the SE (OR 8.0, 95% CI 1.8, 36.1, $p = 0.01$, $p_{corrected}$ = 0.1).

Associations of MHC class II alleles with ACPA and RF

We also looked for associations of MHC class II alleles with ACPA production. There were trends for *HLA-DQB1*0302* (OR 6.0, 95% CI 1.3, 27.0, $p = 0.02$, $p_{corrected}$ = 0.3), HLA-DRB1*1303 (OR 15.7, 95% CI 1.9, 131.4, $p = 0.03$, $p_{corrected}$ = 0.4) and for 2 SE copies (OR 5.2, 95% CI 1.07, 24.9, $p = 0.06$), but no association for *DRB1*0401* (OR 2.0, 95% CI 0.4, 9.1, $p = 0.4$). There was a trend towards a negative association for *DRB1*0301* with ACPA production [0/39 (0%) vs 8/65 (12%), $p = 0.02$, $p_{corrected}$ = 0.3). There were no genetic associations with RF production.

Table 2.1. Clinical features of patients with SLE (n=104)

	Erosive arthritis (n=12) n (%)*	Nonerosive arthritis (n=59) n (%)*	No arthritis (n=33) n (%)*	*p (corrected)* ‡
Mean (SD) age (years)	45.5 (12.0)	50.2 (14.0)	48.9 (12.9)	0.5
Median (IQR) disease duration (years)	14 (10)[a,b]	9 (6)[c]	7 (5.5)	0.01 (0.1)
Race: British Caucasian	12 (100)	57 (97)[†]	33 (100)	0.3
Female gender	12 (100)	52 (88.1)	27 (81.8)	0.1
Malar rash	8 (66.67)	29 (49.2)	21 (63.6)	0.3
Discoid rash	0	7 (11.9)	4 (12.1)	0.2
Serositis	6 (50)	22 (37.3)	12 (36.4)	0.7
NP-SLE	0 (0)	9 (15.3)	8 (24.2)	0.06
Renal disease	1 (8)	8 (13.6)	9 (27.3)	0.2
Haematological disorder	11 (91.7)	45 (76.3)	27 (81.8)	0.4
Median (IQR) no. of SLE criteria	5 (2.75)	6 (2)	6 (1)	0.1

* variables presented as n (%) unless indicated otherwise

‡ Comparisons made among all 3 groups

† 2 patients (3%) were of Afro-Caribbean descent

[a] Erosive arthritis vs nonerosive arthritis: $p = 0.03$ ($p_{corrected} = 0.2$)

[b] Erosive arthritis vs no arthritis: $p = 0.005$ ($p_{corrected} = 0.2$)

[c] Nonerosive arthritis vs no arthritis: $p = 0.06$

Table 2.2. Antibody profiles of patients with SLE (n=104)

	Synovitis, n = 71						
Antibody	Erosive arthritis n = 12, n (%)	Nonerosive arthritis n = 59, n (%)	OR (95% CI)*	*p (corrected)*	No arthritis n = 33, n (%)	OR (95% CI)†	*p (corrected)*
ACPA +	6 (50)	2 (3)	28.5 (4.7, 173.8)	**<0.0001 (0.001)**	0 (0)	-	**<0.001 (0.001)**
RF +	5 (42)	6 (10)	6.3 (1.5, 26.2)	0.02 (0.3)	7 (21)	2.7 (0.6, 11.0)	0.3
Anti-dsDNA +	10 (83)	48 (81)	1.1 (0.2, 6.0)	1.0	26 (79)	1.3 (0.2, 7.6)	1.0
Anti-U1RNP +	7 (58)	21(36)	2.5 (0.7, 9.0)	0.2	11 (33)	2.8 (0.7, 10.9)	0.2
Anti-Sm +	1 (8)	9 (15)	0.5 (0.1, 4.4)	1.0	6 (18)	0.4 (0.1, 3.8)	0.7
Anti-Ro/SSA +	0 (0)	16 (27)	-	0.06 (0.8)	11 (33)	-	0.02 (0.3)
Anti-La/SSB +	0 (0)	7 (12)	-	0.6	7 (21)	-	0.2

* Erosive arthritis vs nonerosive arthritis

† Erosive arthritis vs no arthritis

Table 2.3. Characteristics of SLE patients with erosive arthritis (n=12)

Clinical feature	Erosive arthritis n = 12, n (%)	Major erosions n = 6, n (%)	Minor erosions n = 6, n (%)	OR (95% CI)*	*p*
ACPA +	6 (50.0)	4 (66.7)	2 (33.3)	4.0 (0.4, 44.1)	0.6
Median (IQR) ACPA (U)	13 (44)	28 (98)	0 (42)	1.0 (0.99, 1.01)	0.4
RF +	5 (42)	3 (50.0)	2 (33.3)	2.0 (0.2, 20.6)	1.0
Median (IQR) RF (U)	0 (19)	3.6 (24)	18 (32)	0.99 (0.9, 1.03)	0.6
Mean (SD) time to earliest erosion (years)	11.3 (6.8)	12.2 (8.5)	10.5 (5.2)	1.04 (0.9, 1.2)	0.7
Mean (SD) no. of ACR SLE criteria	5.5 (1.6)	5.2 (1.2)	5.8 (1.9)	0.7 (0.3, 1.7)	0.5
Fulfils ACR RA criteria	7 (58.3)	6 (100)	1 (16.7)	-	**0.01**

* Comparing major erosions with minor erosions

Table 2.4. Characteristics of ACPA positive SLE patients (n=8) and controls (n=2)

Patient	Disease duration (years)	ACPA value (U)	RF value (U)	Other antibodies	*HLA-DRB1* alleles	*HLA-DQB1* alleles	No. of SE alleles	Type of arthritis	Other clinical features (no. of ACR criteria)	Fulfils 1987 ACR RA criteria
1	17	> 250	29	ANA, DNA, U1RNP	*0401, 1001*	*0302, 0501*	2	erosive (major erosions)	haem, skin (5)	yes
2	20	68	> 100	ANA, DNA, U1RNP	*0101, 1501*	*0501, 0602*	1	erosive (minor erosions)	skin (4)	no
3	12	65	> 100	ANA, DNA, Ro	*0405, 1501*	*0302, 0602*	1	nonerosive (nondeforming)	skin (4)	yes
4	22	47	22	ANA, DNA	*0401H*	*0301, 0302*	2	erosive (major erosions)	skin, haem (5)	yes
5	14	42	0	ANA, DNA, U1RNP, Sm, Ro	*0302, 1303*	*0402, 0301*	0	nonerosive, deforming	serositis, renal (5)	no
6	35	33	0	ANA, U1RNP, Sm	*0901, 1303*	*0303, 0301*	0	erosive, deforming (minor erosions)	skin, oral ulcers, serositis, haem, renal (8)	yes
7	18	30	7	ANA	*0101, 0401*	*0301, 0302*	2	erosive (major erosions)	skin, haem (4)	yes
8	12	26	0	ANA, DNA, U1RNP	*0403, 1501*	*0302, 0602*	0	erosive (major erosions)	skin, haem (5)	yes
Control 1	ND	196	13	ND	*0401, 1401*	*0301, 0503*	1	ND	ND	ND
Control 2	ND	56	9	ND	*0401H*	*0302H*	2	ND	ND	ND

H: homozygote, DNA: anti-dsDNA, U1RNP: anti-U1RNP, Sm: anti-Sm, Ro: antiRo/SSA, haem: haematological disorder, ND: no data available

Table 2.5. Associations of arthritis with MHC Class II alleles

	All erosive arthritis n = 12, n (%)	Major erosions n = 6, n (%)	Nonerosive arthritis n = 59, n (%)	No arthritis n = 33, n (%)	OR (95% CI)*	*p (corrected)*
***DRB1*0301* +**	2 (17)	1 (17)	24 (41)	13 (39)	0.3 (0.1, 1.4)	0.2
***DRB1*0401* +**	6 (50)	4 (67)	13 (22)	6 (18)	3.8 (1.1, 13.3)	0.04 (0.5)
***DQB1*0201* +**	4 (33)	2 (33)	28 (48)	17 (52)	0.5 (0.1, 1.9)	0.4
***DQB1*0302* +**	8 (67)	6 (100)	11 (19)	7 (21)	8.2 (2.2, 30.4)	**0.001 (0.01)**
SE +	8 (67)	5 (83)	28 (47)	13 (39)	2.5 (0.7, 8.8)	0.2
SE, 1 copy	3 (25)	1 (17)	21 (36)	12 (37)	1.2 (0.2, 5.5)‡	1.0
SE, 2 copies	5 (42)	4 (66)	7 (11)	1 (5)	8.0 (1.8, 36.1)‡	0.01 (0.1)

* Comparing erosive arthritis with other groups

‡ Using 1 SE copy (total n = 36) or 2 SE copies (total n = 13), compared with no SE copies (total n = 55)

Discussion

The proportion of patients with EA in our SLE cohort was 11%, which was higher than the prevalence of 4 to 6% reported in other studies[130, 131, 187, 426]. This may reflect differences in the clinical characteristics of our patient population compared with other SLE patient populations. Almost all of our patients were British Caucasian (Table 2.1), whereas in a previous series, 35% were Afro-Caribbean, Asian, or other races[187]. As the mean time from SLE diagnosis to the development of erosions was 11.3 years and our patients were followed up for a long time (median 13 years), our study may have identified more patients in the later stages of arthritis, when erosions are more likely to occur. All patients with EA were women and the 1987 ACR criteria for RA were met for 7 of these patients. Most of the patients with EA (91.7%) had a haematological disorder and over half had skin involvement, serositis and were anti-dsDNA+ and anti-U1RNP+, however, none had NP-SLE. Only one patient with EA had renal involvement, which is consistent with a previous report showing that SLE patients with persistent rheumatoid-like arthritis were less likely to develop LN[427]. As our study was retrospective in nature, it is possible that patient self-selection bias may have influenced the clinical characteristics of our patient cohort. Patients participating in our long-term follow-up study may represent those with less severe major organ disease and more arthritis and skin involvement.

The frequencies of ACPA and RF were low in our SLE cohort (8% and 17% respectively), but higher within the subgroup of patients presenting with synovitis (11% and 25% respectively). As expected, ACPA was significantly associated with RF. Mediwake et al. previously found that 3 of 231 SLE patients (1%) were ACPA+ and that 2 of these patients had EA[187]. These results were based on determinations by ACPA-1 ELISA, which most likely had a lower sensitivity for the detection of ACPAs than the ACPA-2 ELISA used in our study. In support of this explanation is the report from a recent study testing ACPA-2 in 201 SLE

patients, which showed a prevalence of 5.5% for ACPA[421, 428], a result comparable with ours. A more recent study of 267 Chinese patients found a prevalence of 27.3%, however, the cut-off value for ACPA positivity was low (5U)[130].

ACPA antibodies were previously reported to be highly specific for RA[198, 199, 209]. However, ACPA is associated with erosive disease not only in RA, but also in PsA[200, 202]. We found that ACPA was significantly associated with EA in our SLE cohort, as 6 of 12 (50%) EA patients were ACPA+ ($p_{corrected}$ = 0.001, Table 2.2). Moreover, 4 of the 8 ACPA+ patients (50%) had major erosions (Table 2.4). Although 42% of patients with EA were RF+, RF was also found in 21% of patients without arthritis (Table 2.2). Two previous studies found an association of RF with EA in SLE[187, 426], however one of these studies reported that RF was unhelpful in distinguishing RA patients from SLE patients with EA[187]. Similarly, our findings suggest that RF is less useful than ACPA as a marker of EA in SLE. There were no significant differences in median ACPA or RF levels between patients with major or minor erosions in our study, however, our patient numbers were small. Qing et al. reported that higher ACPA levels may be more useful in predicting EA development[130], however, 2 of our patients with major erosions had relatively low ACPA levels (26U and 30U, Table 2.4).

Five of the 8 ACPA+ patients (62.5%) were anti-U1RNP+ (Table 2.4) and anti-U1RNP was present more frequently in patients with EA (7/12, 58%). As the numbers of patients were small, it is difficult to certain about the significance of this observation. Anti-U1RNP is the serological hallmark of mixed connective tissue disease (MCTD). Several different patterns of arthritis have been found in MCTD, ranging from NEA to arthritis mutilans[429]. Piirainen reported that anti-U1RNP was associated with progression to EA in patients with MCTD[430]. However, 35% of MCTD patients in his study also fulfilled criteria for RA. It is noteworthy that all 6 of our SLE patients with major erosions also fulfilled criteria for RA. Four of these patients were ACPA+, with higher median ACPA levels (Table 2.3). All 3 ACPA+ patients who carried 2 copies of the SE had

major erosions. Of the 2 ACPA+ patients who were compound heterozygotes for the SE and the SLE-associated allele *DRB1*1501,* one had minor erosions and the other had non-erosive disease. Patients with overlapping features of both SLE and RA may be defined as belonging to a "rhupus" subset of SLE. Apart from polyarthritis, clinical features that occur more frequently in rhupus patients include malar rash, DLE, photosensitivity, LN, anaemia, leucopenia, and thrombocytopenia[191]. Most of these features were also present in our EA patients. ACPA therefore appears to be a useful marker for the rhupus subset in SLE. Further support for this was provided by Damián-Abrego et al., who found that all 9 rhupus patients in their study were positive for ACPA[423]. Moreover, 2 other studies have reported the presence of ACPA in their rhupus patients[421, 431].

Two ACPA+ patients with Jaccoud's arthropathy had LN and both were carriers of *HLA-DRB1*1303,* an allele associated with LN[126]. One patient with low ACPA levels (26U) had major erosions and was negative for the SE. Recent genetic studies have demonstrated that several SNPs at the *IRF5, STAT4, BLK* and *TNFAIP3* loci are shared by SLE and RA[171, 210, 211]. In mice, interference with the function of the *TNFAIP3* protein product A20 resulted in a destructive, erosive polyarthritis[212]. The *IRF5* locus was also found to be shared by patients with SLE and the RF-negative polyarthritis subtype of JIA[213]. These studies suggest that the pathogenesis of arthritis in SLE involves at least several complex immunological pathways and low level ACPA does not preclude the possibility of developing EA. The shared genetic loci of SLE and RA also suggest that future RA markers may also have potential utility as markers for lupus arthritis.

As we found previously, the most common SLE-associated MHC class II alleles were *HLA-DRB1*0301* and *HLA-DQB1*0201*[145], which are in linkage disequilibrium. As 67% of our patients with EA were SE carriers, it is not surprising that they were seronegative for anti-Ro/SSA and anti-La/SSB (Table 2.2), auto-antibodies known to be associated with *HLA-DRB1*03*[147, 148]. The most common SE allele was *HLA-DRB1*0401,* which was expected in our mainly British Caucasian cohort[432]. *HLA-DQB1*0302* had the strongest genetic

association with EA in our cohort (OR 8.2, $p_{corrected}$ = 0.01, Table 2.5) and all 6 patients with major erosions carried *DQB1*0302*. There were similar trends for associations of *HLA-DRB1*0401* and 2 copies of the SE with major erosions (Table 2.5). These associations were similar to well-known associations of specific MHC Class II alleles with progression of erosions in RA, including the SE and *HLA-DQB1*0302*[433-435]. These results provide further information for the subset of SLE with specific genetic and antibody features, the "rhupus" subset.

A dose effect of the SE on ACPA production is seen in RA populations and the association of the SE with radiographic disease progression is thought to be an indirect effect mediated by antibodies against citrullinated peptides[204, 205]. Citrullination of arginine-containing residues greatly increases the affinity of the MHC class II peptide binding groove for the SE, thereby facilitating antigen presentation and generation of antibodies to citrullinated antigens[206]. This theory is supported by our finding that both ACPA+ controls were carriers of the SE allele *DRB1*0401*.

*HLA-DQB1*0302* is associated with ACPA production in RA[209] and its association with erosive arthritis in SLE may be via similar immunopathogenic mechanisms. We also observed positive associations of ACPA with *DQB1*0302* and 2 SE copies and a negative association of DRB1*0301 with ACPA, however, because of the small numbers of ACPA+ patients in our study, these findings did not reach statistical significance. Larger studies may be able to confirm this effect. *HLA-DR3* is associated with ACPA-negative RA, which runs a less severe course[436]. The presence of *DRB1*0301* in SLE populations may therefore account for the infrequent development of EA, despite the common clinical feature of synovitis.

Conclusions

Synovitis is a common clinical feature of SLE. Our findings suggest that the incidence of EA in SLE may be higher than previously reported. ACPA may be a useful serological marker for EA, particularly among patients with synovitis. Furthermore, ACPA may also be a marker for the rhupus subset of SLE. Future studies of patients with early SLE may show a predictive role for ACPA in the future development of EA. Future studies may also further elucidate the mechanisms by which MHC Class II alleles influence production and the development of a severe arthritis phenotype that is common to several autoimmune diseases.

CHAPTER 3

Associations of anticardiolipin antibodies with cardiovascular events and mortality

Background

Anticardiolipin antibodies (aCL) are associated with arterial thrombosis (including stroke and MI) and venous thrombosis, which are clinical manifestations of the APS. The prevalence of aCL has been reported in up to 44% of patients with SLE[42], and the prevalence of APS in SLE cohorts of 23% to 42%[41-43]. We previously found a prevalence of 23% for aCL GPL and 5% for aCL MPL in our RNHRD SLE cohort[437]. Within the general population, aCL has been associated with thrombotic stroke and MI in some studies, but not in others[56, 79]. Edwards et al. previously measured aCL in patients with acute MI or unstable angina and found no associations[78]. In SLE, aPL have been reported to be predictors for CV events[83, 84]. However, in contrast, Petri et al. found that aCL was a predictor for thrombotic stroke, but not for MI[49, 61]. There is little data published on the influence of aCL on survival in SLE, particularly persistent aCL as predictors. One study demonstrated an adverse effect of aCL MPL on survival[271] and another found reduced survival rates in SLE patients with aCL[270].

Aim

To determine the associations of persistently raised ACL GPL and MPL with cardiovascular outcomes and mortality in patients with SLE.

Methods

Ethical approval for the study was given by the Bath Regional Ethics Committee and informed written consent was given by all study participants. All study subjects were patients with SLE seen at the RNHRD CTD clinic between 1992 and 2006.

Personal contribution by the candidate

This study was designed as a retrospective case-control pilot study by the candidate, under the supervision of Prof Neil McHugh. Serological data was available from a database of SLE patients who were participating in an ongoing serological and genetic study and previously consecutively recruited from the RNHRD CTD Clinic. Patient clinical data was collected by the candidate for this study from review of medical records and SLE questionnaire responses. The candidate designed the SLE questionnaire (see Appendix), with advice from Prof McHugh and Dr Eleanor Korendowych. Mrs Charlotte Cavill, BIRD CTD database manager, was responsible for mailing of questionnaires, collation of questionnaire results, tracing of patients who were lost to follow-up, data extraction from the Office for National Statistics (ONS) annual UK population mortality rates and obtaining patient mortality data from the UK National Health Service primary care mortality database. Advice and assistance on statistical analysis was provided by Prof Satvinder Dhaliwal at Curtin University, Perth, Western Australia.

Sample size

Prior to commencement of this study, there were only two previous published reports on the associations of aCL with mortality in SLE. Both used definitions for predictive factors which differed from our study, hence it was not possible to perform an accurate sample size calculation. In Gómez et al.'s cohort study of 363 SLE patients[270], 28.1% of patients were aCL+, however it was not stated in this study whether the test was positive on least 2 occasions, which was one of

the inclusion criteria for our study. In Gulko et al.'s cohort study of 139 patients[271], all patients were tested at least once for aCL and 72 patients were tested twice for aCL, however mortality associations were published for the total group. Although a sample size calculation was not performed prior to our pilot study, we estimated that a study sample of 130 to 140 SLE patients (as in Gulko et al.'s study) would have sufficient power to detect a difference in survival between aCL+ and aCL- patients.

Autoantibody measurement

Antinuclear antibodies (ANA) were measured by indirect immunofluorescence on HEp-2 cells. Serial serum samples were available on patients over a span of up to 10 years (median three samples; range 1 to 11 samples)[145]. Antibodies to extractable nuclear antigens (including U1RNP, Sm, Ro/SSA, and La/SSB,) were measured by Ouchterlony double diffusion. All patients with anti-U1RNP, anti-Sm, or anti-La/SSB antibodies had these autoantibodies confirmed by western blotting on at least one sample. Anti-dsDNA and aCL were measured by commercial ELISAs (Cambridge Life Sciences, Ely, UK). ACL was defined as positive if GPL was $\geq$ 14U/mL or MPL was $\geq$ 10U/mL, according to the manufacturer's instructions.

Study subjects

SLE patients with 2 or more positive aCL results, at least 6 weeks apart, were identified from a database of serology results at BIRD and matched for age and sex with other SLE patients who were aCL negative from the database of patients attending the CTD clinic. Disease duration was defined as the interval from the date of SLE diagnosis to the date of the first positive aCL result for aCL+ patients and their matched aCL- counterparts. Clinical information was obtained from RNHRD medical records and cause of death data from the UK National Health Service primary care mortality database. Patients' medical records were reviewed for SLE disease features, CV risk factors, previous histories of IHD (defined as

MI and/or angina) or cerebrovascular events (defined as stroke and/or transient ischaemic attacks [TIAs]), as well as subsequent CV events. Additional clinical information was obtained from questionnaires posted to surviving patients in 2006, which included CV risk factors and any history of IHD or cerebrovascular disease (see Appendix). Initial non-responders were posted another copy of the questionnaire after 2 months. Survival of the patients was determined from the date of the second positive aCL for aCL+ patients and for matched aCL- patients. Five and 10-year survival rates were compared with mean age and sex-matched population cohort survival data from the ONS annual UK population mortality rates (online). (Available from: http://www.ons.gov.uk/ons/datasets-and-tables [Accessed 3 March 2012]).

Statistical analysis

Statistical tests employed included chi-square tests, with Fisher's exact test used where expected numbers for contingency tables were less than 5. For nonparametric comparisons, the Mann-Whitney U test was used, with medians and interquartile ranges (IQR) quoted. Due to the limited numbers of patients available for this study (total n = 135), with incomplete data from medical records and SLE questionnaires with respect to dates of onset of CV and SLE-related predictor factors and CVEs, the decision was made by the candidate to utilise unconditional binary logistic regression to compare predictor factors with outcome variables of CVEs and survival. For the same reasons, the χ^2 Goodness-of-fit test was employed to compare survival of SLE subgroups with age- and sex-matched UK population survival data. Backward, stepwise binary logistic regression models were used for multivariate analyses. Variables found to be significant at $p \leq 0.2$ in the univariate analyses, as well as known predictor variables, were included in the multivariate regression models. The predicted probability of each model was used to generate a receiver operator characteristic (ROC) curve. The areas under the ROC curves (AUC ROC) were then used to determine the accuracy of the final models.

Results

This was a retrospective study of 135 patients (18 males and 117 females), of whom 132 met at least 4 of the updated 1997 ACR classification criteria for SLE. All patients were ANA positive. There was missing ACR criteria information for 3 subjects, who had previously been given definite diagnoses of SLE. The group comprised 132 (97.8%) patients of British Caucasian descent, 1 African, 1 Afro-Caribbean and 1 Indian patient. Median (IQR) follow-up time from the time of the second positive aCL for the whole group was 8 (7) years. Ten (7.4%) patients died during the follow-up period. Disease duration was unknown for one of these patients, who died at the age of 83.

Comparisons of aCL positive and aCL negative patients

Seventy (51.9%) patients had persistent aCL antibodies. Of these 70 patients, 53 (75.7%) were GPL+ and 34 (48.6%) were MPL+. The median (IQR) GPL value was 26.5 U (28.7) and median (IQR) MPL value was 14.8 U (28.8) in the aCL+ group. Nineteen (27.1%) patients were positive for both GPL and MPL. Of the aCL positive patients, 12 (17.1%) had a diagnosis of APS at the time of the 2nd positive aCL. Over the total follow-up period, the number of patients with APS increased to 19 (27.1%). Table 3.1 shows characteristics of aCL+ patients compared with the aCL- patients. There were no differences between both groups in terms of age at SLE diagnosis or disease duration. The aCL+ group met more ACR SLE criteria than the aCL- group (median number 6 vs 5 respectively), with trends for aCL+ patients to have more frequent manifestations of discoid rash, serositis, renal disease and NP-SLE. More aCL+ patients had a history of cerebrovascular disease at the time of the second positive aCL, with a similar trend for deep venous thrombosis (DVT) in these patients.

Questionnaire survey results

The overall response rate from the questionnaire survey in surviving patients was 79/126 (62.7%), with no differences between aCL+ and aCL- patients [39/64

(60.9%) responded in the aCL+ group, vs 40/62 (64.5%) in the aCL- group, OR 0.86, 95% CI 0.42, 1.77, p = 0.7]. Table 3.2 shows comparisons of CV risk factors between aCL+ and aCL- patients who responded to the questionnaire. There was a trend for more aCL- patients to be hypertensive and overweight (p = 0.1).

Associations of aCL with CV events

Table 3.3 shows comparisons of aCL+, aCL GPL+, and aCL MPL+ patients with aCL- patients for the outcomes of all CV events, subsequent CV events and death. There were no associations between the presence of aCL with IHD or mortality. Increasing age was associated with IHD in the whole group (OR 1.07, 95% CI 1.02, 1.11, p = 0.003). This association was also found in the aCL- group (OR 1.09, 95% CI 1.03, 1.16, p = 0.005), but not in the aCL+ group (OR 1.04, 95% CI 0.97, 1.10, p = 0.3). The presence of aCL GPL or aCL MPL was significantly associated with all cerebrovascular events. ACL MPL was also associated with subsequent cerebrovascular events, with a similar trend for aCL GPL. There was no association of age with cerebrovascular events (OR 1.00, 95% CI 0.97, 1.04, p = 1.0).

Survival data

Table 3.4 shows the 5- and 10-year survivals for aCL+, aCL GPL+, aCL MPL+ and aCL- groups, compared with expected survivals for age- and sex-matched population cohorts in the UK. The proportions quoted are based on the numbers of patients in each subgroup who were followed up for at least 5 or 10 years. Overall, the SLE group's 5-year survival was not significantly lower than expected (98.2% vs 99.2%, p = 0.2). However, at 10 years, survival was significantly lower than expected (91.7% vs 98.7%, $p < 0.0001$). This was due to the presence of aCL, ($p < 0.0001$) with 10-year survival of 85.2% compared with expected survival of 98.7%. There were similar survivals in the aCL GPL+ and aCL MPL+ patients (84.6% and 85.7% respectively).

Associations of CV events and aCL with mortality

The influence of CV events and APS on mortality was also determined. Table 3.5 shows comparisons of CV events and aCL with mortality. Patients with IHD and/or cerebrovascular events were at significantly increased risk of mortality ($p < 0.0001$). However, there was no association of mortality with cerebrovascular events or with APS. Mortality was significantly associated with IHD ($p < 0.0001$). There were no associations of mortality with aCL. Similarly, IHD was significantly associated with mortality in both aCL+ and aCL- groups. In the aCL+ group, 4 of 7 deceased patients (57.1%) had IHD, compared with none of 63 living patients ($p < 0.0001$). In the aCL- group, 2 of 3 deceased patients (66.7%) had IHD, compared 6 of 62 living patients (9.7%, OR 18.67, 95% CI 1.47, 237.59, $p = 0.04$). Of the 10 deceased patients, 5 died from IHD-related causes and one from a presumed IHD-related cause at the age of 84. Disease duration was unknown for one patient. The other 5 patients who died of IHD-related causes had disease durations of at least 11 years (up to 36 years). Two patients died from malignancies (metastatic epithelioid tumour and metastatic rhabdomyosarcoma), one patient from pneumonia and one patient from end-stage pulmonary fibrosis. The final age- and sex-adjusted multivariate regression model retained IHD as the independent risk factor for mortality ($p < 0.0001$), with the presence of aCL GPL retained as a contributory factor ($p = 0.06$). The area under the ROC curve for this final model was 0.86.

Table 3.1. Comparisons of characteristics of aCL positive and aCL negative SLE patients

Feature	aCL positive (n=70), n (%)	aCL negative (n=65), n (%)	*p* - value
Female	61 (87.7)	56 (86.2)	0.9
Median (IQR) age at time of 1st aCL (years)	39.0 (21.5)	41.0 (22.0)	0.7
Median (IQR) age at SLE diagnosis (years)	32.3 (19.7)	30.9 (8.9)	0.5
Median (IQR) SLE disease duration at time of 1st aCL (years)	4.0 (6.0)	4.0 (9.0)	0.4
Median (IQR) no. of ACR criteria	6 (2)	5 (2)	**0.02**
Malar rash	34 (49.3)	31 (49.2)	1.0
Discoid rash	11 (15.9)	4 (6.3)	0.08
Arthritis	39 (56.5)	36 (55.4)	0.9
Serositis	25 (36.2)	15 (23.8)	0.1
Renal disease	18 (26.1)	10 (15.9)	0.1
NP-SLE	14 (20.3)	7 (11.1)	0.1
Haematological disorder	57 (82.6)	49 (77.8)	0.5
Anti-dsDNA +	58 (82.9)	50 (76.9)	0.4
Anti-U1RNP +	14 (20.0)	19 (29.2)	0.2
Anti-Sm +	7 (10.0)	8 (12.3)	0.7
Anti-Ro/SSA +	21 (30.0)	14 (21.5)	0.3
Anti-La/SSB +	10 (14.3)	5 (7.7)	0.2
Median (IQR) GPL value (U)	26.5 (28.7)	2.0 (7.5)	**< 0.0001**
Median (IQR) MPL value (U)	14.8 (28.8)	0 (4.0)	**< 0.0001**
Previous cerebrovascular disease at time of 2nd aCL	10 (14.3)	2 (3.1)	**0.02**
Previous IHD at time of 2nd aCL	1 (1.4)	5 (7.7)	0.1
Previous DVT at time of 2nd aCL	8 (11.4)	3 (4.6)	0.1

* variables presented as n (%) unless indicated otherwise

Table 3.2. Comparisons of cardiovascular risk factors in aCL+ and aCL- patients who completed questionnaires

CV risk factor	aCL positive (n=39), n (%)	aCL negative (n=40), n (%)	OR (95% CI)	*p* - value
Smoker ever	19 (48.7)	16 (40.0)	1.43 (0.58, 3.48)	0.4
Hypertension	15 (38.5)	22 (55.0)	0.51 (0.21, 1.25)	0.1
Hypercholesterolaemia	10 (25.6)	8 (20.0)	1.38 (0.48, 3.97)	0.6
Diabetes mellitus	2 (5.1)	2 (5.1)	1.00 (0.13, 7.48)	1.0
Overweight (BMI > 25 kg/m^2)	13 (34.2)	20 (51.3)	0.49 (0.20, 1.24)	0.1
Glucocorticoid use ever	34 (87.2)	33 (82.5)	1.44 (0.42, 5.00)	0.6
Oral contraceptive use ever	23 (59.0)	24 (60.0)	0.96 (0.39, 2.35)	0.9
Hormone replacement therapy ever	11 (28.2)	13 (32.5)	0.82 (0.31, 2.13)	0.7

Table 3.3. Comparisons of aCL+ patients and aCL- patients with cardiovascular outcomes and mortality

CV events	aCL - (n=65) n (%)	aCL + (n = 70) n (%)	aCL+ OR (95% CI)*	*p* - value	aCL GPL+ (n = 53) n (%)	aCL GPL+ OR (95% CI)*	*p* - value	aCL MPL+ (n = 34) n (%)	aCL MPL OR (95% CI)*	*p* - value
All IHD / cerebrovascular events[†] (n = 24)	9 (13.8)	15 (21.4)	1.70 (0.69, 4.20)	0.2	13 (24.5)	2.02 (0.79, 5.19)	0.1	10 (29.4)	2.59 (0.94, 7.19)	0.07
All cerebrovascular events[‡] (n = 16)	4 (6.2)	12 (17.1)	3.16 (0.97, 10.34)	0.06	10 (18.9)	3.55 (1.04, 12.05)	**0.03**	9 (26.5)	5.49 (1.55, 19.48)	**0.009**
All IHD[¶] (n = 12)	8 (12.3)	4 (5.7)	0.43 (0.12, 1.51)	0.2	4 (7.5)	0.58 (0.16, 2.05)	0.4	2 (5.9)	0.45 (0.09, 2.23)	0.5
Subsequent IHD / cerebrovascular events (n = 17)	7 (10.8)	10 (14.3)	1.38 (0.49, 3.87)	0.5	8 (15.1)	1.47 (0.50, 4.37)	0.5	7 (20.6)	2.15 (0.69, 6.74)	0.2
Subsequent cerebrovascular events (n = 9)	2 (3.1)	7 (10.0)	3.50 (0.70, 17.51)	0.2	5 (9.4)	3.28 (0.61, 17.65)	0.2	6 (17.6)	6.75 (1.28, 35.54)	**0.02**
Subsequent IHD (n = 10)	7 (10.8)	3 (4.3)	3.71 (0.09, 1.50)	0.2	3 (5.7)	0.50 (0.12, 2.03)	0.3	1 (2.9)	0.25 (0.03, 2.13)	0.3
Death (n = 10)	3 (4.6)	7 (10.0)	2.30 (0.57, 9.29)	0.3	6 (11.3)	2.64 (0.63, 11.1)	0.3	4 (11.8)	2.76 (0.59, 13.10)	0.2

[†] All previous and subsequent cardiovascular events (MI, angina, stroke and TIA)

[‡] All previous and subsequent cerebrovascular events (Stroke and TIA)

[¶] All previous and subsequent IHD events (MI and angina)

* compared with aCL negative patients

Table 3.4. Survival outcomes for SLE patients compared with expected UK survival outcomes*

	Actual 5-year survival n (%)	Expected 5-year survival n (%)	*p*-value	Actual 10-year survival n (%)	Expected 10-year survival n (%)	*p*-value
Total SLE group (n = 110 at 5 yrs, n = 60 at 10 yrs)	108 (98.2)	109.1 (99.2)	0.2	55 (91.7)	59.2 (98.7)	**< 0.0001**
ACL + (n = 56 at 5yrs, n = 27 at 10 yrs)	55 (98.2)	55.6 (99.3)	0.3	23 (85.2)	26.6 (98.7)	**< 0.0001**
ACL GPL + (n = 44 at 5 yrs, n = 26 at 10 yrs)	43 (97.7)	43.8 (99.5)	0.07	22 (84.6)	25.8 (99.2)	**< 0.0001**
ACL MPL + (n = 27 at 5 yrs, n = 7 at 10 yrs)	27 (100)	26.7 (99.0)	-	6 (85.7)	6.8 (97.4)	0.07
ACL - (n = 54 at 5 yrs, n = 33 at 10 yrs)	53 (98.1)	53.5 (99.1)	0.5	32 (97.0)	32.6 (98.8)	0.3

* expected survival rates based on Office of National Statistics cohort life-tables for age- and sex-matched UK general population

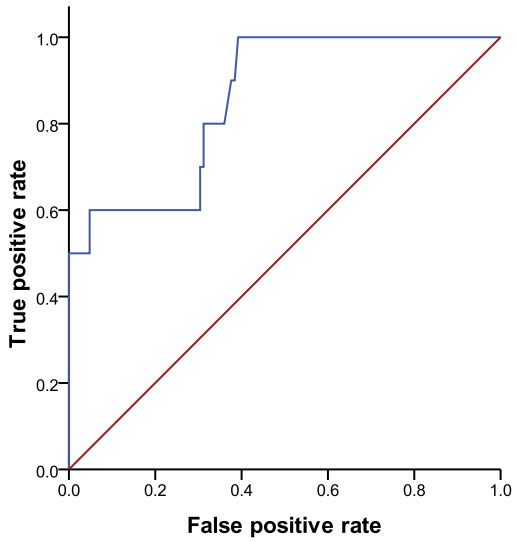
Table 3.5. Comparisons of clinical factors with mortality

Factor	Deceased (n = 10) n (%)	Alive (n = 125) n (%)	OR (95% CI)	*p* - value
Median (IQR) age (years)	59 (46.5)	40 (20.5)	1.05 (0.09)	**0.02**
Antiphospholipid syndrome (n = 19)	2 (20.0)	17 (13.6)	1.58 (0.31, 8.12)	0.6
All IHD / cerebrovascular events (n = 24)	7 (70.0)	17 (13.6)	14.82 (3.49, 62.94)	**< 0.0001**
All cerebrovascular events (n = 16)	2 (20.0)	14 (11.2)	1.98 (0.38, 10.28)	0.6
Ischaemic heart disease (n = 12)	6 (60.0)	6 (4.8)	29.75 (6.59, 134.36)	**< 0.0001**
ACL + (n = 70)	7 (70.0)	63 (50.4)	2.30 (0.57, 9.29)	0.3
ACL GPL + (n = 53)	6 (60.0)	47 (37.6)	2.49 (0.67, 9.28)	0.2
ACL MPL + (n = 34)	4 (40.0)	30 (24.0)	2.11 (0.56, 7.98)	0.4

Table 3.6. Final age- and sex-adjusted model comparing SLE factors and CV events with mortality

Variable	β-coefficient (SE)	Odds Ratio (95% CI)	*p* - value
Age at 2nd positive aCL	0.036 (0.027)	1.04 (0.98, 1.09)	0.2
Female sex	0.753 (1.075)	2.12 (0.26, 17.45)	0.5
Ischaemic heart disease	3.750 (1.008)	42.52 (5.90, 306.56)	**< 0.0001**
ACL GPL +	1.838 (0.963)	6.29 (0.95, 41.47)	0.06

Figure 3.1. ROC curve for the final age- and sex-adjusted model comparing SLE factors and CV events with mortality



Area under the ROC curve = 0.86 (0.75, 0.97)

Discussion

This study showed that 27.1% of aCL+ patients had thrombosis and a diagnosis of APS. As expected, the prevalence of pre-existent cerebrovascular disease at baseline was significantly higher in the aCL+ group than in the aCL- group (14.3% vs 3.1%, $p = 0.02$). These findings are consistent with Pérez-Vázquez et al.'s prevalence of APS in patients with SLE of 23% after 15 -18 years of follow-up[41]. In Love and Santoro's systematic review, 40% of aCL+ SLE patients developed thrombotic complications[42]. McNeil et al. reported a risk of 42% for developing thrombotic complications in aPL-positive SLE patients[43]. The higher frequency of thrombotic complications in previous series compared with our group may be explained by differences in selection of patient cohorts and length of follow-up. The aCL+ group met more ACR SLE criteria than the aCL- group (median number 6 vs 5 respectively), with trends for aCL+ patients to have more frequent manifestations of discoid rash, serositis, renal disease and NP-SLE. These observations are consistent with those of McClain et al., who reported that the presence of aCL in early SLE predicted a more severe clinical course[438]. As measurement of aCL was according to clinical decisions, this may have resulted in a lower detection rate for aCL in this group compared with previous reports.

The questionnaire response rates for the surviving SLE patients in 2006 were similar for both aCL+ and aCL- groups (60.9% vs 64.5%, $p = 0.7$). There is a possibility of non-responder bias in patients' questionnaire responses, which may have resulted to an underestimation of the overall true prevalence of CV risk factors and CV events in this population. However, the similar response rates suggest that the risk of non-responder bias would be similar between both groups. Although there were trends for more hypertension and obesity in the aCL- group, the prevalence of CV risk factors was similar for both groups, suggesting that both groups were similar in terms of their overall CV risk.

There were no differences between aCL+ and aCL- groups for the combined outcome of IHD and cerebrovascular events. ACL GPL and MPL were both significantly associated with cerebrovascular events (p = 0.03 and 0.009 respectively), however, there were no associations with IHD. The positive association of aCL with cerebrovascular events confirms previous studies of aPL as predictors of CV events[83, 84]. The lack of any association of aCL with MI also confirms Petri et al.'s results in the Hopkins Lupus Cohort, which showed significant associations of aCL with stroke and TIA, but no association with MI[49, 61]. The differences in associations of aCL between cerebrovascular disease and ischaemic heart disease in our cohort may be due to the increased frequency of IHD in the aCL- cohort (12.3% vs 5.7%, p = 0.2), which may reflect the association of age with IHD but not with cerebrovascular events. The small numbers of CV events in our study may also have affected our results. Furthermore, as not all aCL are pro-thrombotic, LA may better identify patients with both unstable atherosclerotic plaque and a pro-coagulant state, as demonstrated in the Hopkins Lupus Cohort[49, 61].

The 5-year survival in our SLE cohort was similar to the expected survival for age- and sex- matched cohorts in the general UK population. However, survival of SLE patients was significantly reduced at 10 years. The 5- and 10-year survival results for our cohort (98.2% and 91.7% respectively) are comparable to the survival rates of recent SLE cohorts of 95% - 97% at 5 years and 93% at 10 years[222-224]. Overall, the survival for our cohort was significantly lower compared with the general population, providing confirmation of previous evidence showing that patients with SLE have an increased mortality risk compared with the general population[50, 225-228]. Interestingly, reduced 10-year survival occurred in the aCL+ and aCL GPL+ groups, with a similar trend for aCL MPL. In contrast, survival was not reduced in the aCL- group. This suggests that aCL may have a weak or indirect effect on mortality and this was confirmed in the final multivariate model, where aCL GPL was retained in the model as a contributory factor (p = 0.06). Gomez et al. showed that the presence of aCL was

associated with increased mortality[270], while Gulko et al. found that only aCL MPL was associated with increased mortality[271].

Table 3.5 shows that older age and IHD were predictors of mortality. In the multivariate analysis, IHD was shown to be an independent predictor for mortality ($p < 0.0001$), with age no longer a significant factor ($p = 0.2$). Mortality of our SLE patients with APS was not increased, which contrasts with Drenkard et al.'s study showing APS as an independent predictor of mortality[259]. The difference is most likely due to our small study numbers and possible ethnic differences in our study of mainly British Caucasian subjects, compared with Drenkard et al.'s study of 667 Mexican SLE patients. Five of the 10 deceased patients died from IHD-related causes and one from a presumed IHD cause. This confirms the importance of atherosclerosis as a major cause of death in SLE[227, 229, 236]. Furthermore, 5 of the 10 deceased patients (50%) had disease durations of at least 11 years at the time of death, confirming Urowitz et al.'s "bimodal mortality pattern" of late deaths from CV-related causes[231].

There were no statistically significant associations of aCL with mortality in this study. This may be due to the small numbers in our study, or to a possible selection bias in our study group, where more healthy patients were able to continue in long-term participation (median 8 years) of our study. As this was a retrospective study, there was limited data available for other possible confounding risk factors, such as CV risk factors and medications. However, the clinical information obtained from the combined sources of patient medical records, questionnaires and mortality data was sufficient to generate a final multivariate model with an AUC ROC curve of 0.86, which demonstrated good predictive accuracy for the model.

Conclusions

This study confirms previous reports of the associations of aCL GPL and aCL MPL with cerebrovascular events, including subsequent cerebrovascular events. The 10-year survival in this cohort was significantly reduced at 10 years, compared with expected 10-year survival figures for the general UK population, also confirming other studies' reports of increased mortality risk in patients with SLE. However, although IHD was the major independent predictor of mortality in this study group, aCL GPL was a contributory factor for mortality in the final multivariate model. Moreover, 10-year survival was reduced in aCL+ patients, but not in aCL- patients. These results together suggest that aCL have an indirect influence on mortality, possibly through pathogenic and inflammatory effects on atherosclerosis. In addition, these results provide further evidence of the complexity of the pathogenesis of atherothrombosis in SLE, where multiple interacting factors may modify clinical outcomes.

CHAPTER 4

Extended lipoprotein profiles and anticardiolipin antibodies as predictors of cardiovascular events and mortality

Background

Patients with SLE have an increased risk of developing accelerated atherosclerosis and an increased mortality risk compared with the general population. Moreover, a characteristic pro-atherogenic lipoprotein profile, the "lupus pattern" has been described and comprises reduced HDL-C and elevated VLDL-C and TG concentrations. This pattern is enhanced by active disease[368, 373, 374]. Reduced LpL activity has been demonstrated in patients with SLE[404]. Suppression of LpL activity is associated with increased TG and reduced HDL-C concentrations. ACL from SLE sera have been shown to cross-react with HDL-C and apoA-I[399], and are associated with lower TC, HDL-C and apoA-I concentrations[400]. Lp(a) is an independent predictor of future CVEs[409] and may be a useful marker of CV risk in SLE. Studying extended lipoprotein profiles may yield novel predictors of future CVEs and mortality in SLE.

Aims

1. To compare extended lipoprotein profiles in SLE patients with local healthy controls.
2. To determine the associations of baseline lipoprotein profiles and antibodies with subsequent CVEs and mortality in SLE.

Methods

Personal contribution by the candidate

This study was designed by the candidate as a follow-up study to an earlier case-control pilot study carried out from 1992 to 1993 by Dr Keng Hong Leong, under the supervision of Prof Neil McHugh. During the initial study, SLE patients were consecutively recruited by Drs Leong and McHugh from the RNHRD CTD Clinic, with baseline clinical data collected by Dr Leong. Baseline patient serological assays, including aCL GPL, were performed at BIRD and baseline extended lipoprotein profiles were determined by Ms Chris Stirling at the Wolfsen Centre Clinical Research Unit for Diabetes, Lipid and Endocrinology Research, Royal United Hospital, Bath. A group of healthy volunteers from the surrounding districts of Bath had their lipoprotein profiles determined at the Wolfsen Centre between 1992 and 1993. Age and sex-matched profiles from this cohort were used as controls for the study. Subsequent patient clinical data was collected by the candidate for this study from review of medical records and SLE questionnaire responses. The candidate designed the SLE questionnaire (see Appendix), with advice from Prof McHugh and Dr Eleanor Korendowych. Mrs Charlotte Cavill, BIRD CTD database manager, was responsible for mailing of questionnaires, collation of questionnaire results, tracing of patients who were lost to follow-up, data extraction from the ONS annual UK population mortality rates and obtaining patient mortality data from the UK National Health Service primary care mortality database. Advice and assistance on statistical analysis was provided by Prof Satvinder Dhaliwal (Curtin University).

Sample size

No power calculations were performed prior to commencement of this study. As the original investigators designed this study as a pilot study, they estimated that a case-control sample size of 50 patients and 50 controls would have adequate power to detect a difference in lipoprotein profiles between patients and controls.

Patients and controls

This study was approved by the Bath District Research Ethics Committee. Subjects with SLE were recruited between 1992 and 1993 from a cohort of patients attending the Royal National Hospital for Rheumatic Diseases (RNHRD) Connective Tissue Diseases Clinic. Subjects with SLE were followed until 2006. Patients' auto-antibodies and lipoprotein profiles were measured and their clinical information collected from review of their medical records. Additional clinical information was obtained from results of questionnaires sent to the surviving patients in 2006, which included information about CV risk factors and history of MI and strokes (see Appendix). Lipoprotein profiles from age and sex-matched controls were obtained from a concurrent local population survey. Abnormal lipoprotein concentrations were defined according the Joint British Societies' 2005 guidelines (JBS 2) on prevention of CVD[418]. A subsequent CVE was defined as the development of MI, angina, stroke, transient ischaemic attack (TIA), or peripheral vascular disease (PVD). Patients' mortality data was obtained from the UK National Health Service primary care mortality database. Life expectancy data was derived from the Office for National Statistics annual UK population mortality rates and 2006 period life expectancy tables (online). (Available from: http://www.ons.gov.uk/ons/datasets-and-tables [Accessed 3 March 2012]).

Autoantibody measurement

ACL GPL and anti-dsDNA were determined by ELISA (Cambridge Life Sciences, Ely, UK). ANA was measured by indirect immunofluorescence on HEp-2 cells (The Binding Site, Birmingham, UK). Antibodies to extractable nuclear antigens (U1-RNP, Sm, Ro/SSA and La/SSB) were measured by Ouchterlony double immunodiffusion.

Measurement of lipoproteins

Lipoprotein profiles were determined at the Wolfson Centre for Diabetes, Lipid and Endocrinology Research in Bath. Following an overnight fast of 12 hours, blood samples were obtained from all study subjects and centrifuged within 2 hours of collection. Aliquots of the supernatant were then stored at -20ºC and subsequently analysed in batches. Automated measurements were made using the Abbott VP Super System Autoanalyzer (Abbott, Maidenhead, UK). VLDL, HDL and HDL_3 were prepared by standard precipitation techniques, as previously described by Gidez et al.[439] HDL_2 was calculated by subtraction of HDL_3 from total HDL. TC was measured by cholesterol oxidase - p-amino-antipyrine (CHOD-PAP) and total TG by glycerol phosphate oxidase - p-amino-antipyrine (GPO-PAP) enzymatic colorimetric methods (Boehringer, Mannheim, Germany). Inter-assay coefficients of variation (%CV) were 4% and 5%, and intra-assay %CVs were 3% and 2% respectively. LDL-C and LDL-TG fractions were calculated by subtraction of HDL and VLDL from TC and total TG. ApoA-I and apoB were measured by agarose gel electrophoresis (Sebia, Issy-les-Moulineaux, France). Lp(a) was measured by ELISA (Biopool, Umea, Sweden). Post-heparin LpL and HL lipolytic activities were determined in the following steps: following a single IV heparin injection, blood samples were collected, centrifuged, and incubated with a triolein emulsion. To make the assay specific for HL, LpL was inactivated by incorporating 1 mol NaCl to the mixture. Lipase-mediated free fatty acid release was then measured by the acyl-CoA synthetase - acyl-CoA oxidase - 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (ACS-ACOD-MEHA) enzymatic colorimetric assay (Wako, Neuss, Germany).

Statistical analysis

Data was entered into an electronic Access database and statistical analysis carried out using the SPSS Statistics 17.0 software package (IBM Corporation, Armonk, NY, USA). The chi-square test was employed for comparisons of categorical data. For normally distributed data, the t-test was used and the Mann-Whitney U test for non-parametric data. Pearson's and Spearman's rho

correlations were used for comparisons of parametric and non-parametric continuous data respectively. Statistical significance was set at a p-value of <0.05. Due to the limited numbers of patients available for this study (total n = 105), with incomplete data from medical records and SLE questionnaires with respect to dates of onset of CV and SLE-related predictor factors and CVEs, the decision was made by the candidate to utilise unconditional binary logistic regression to compare predictor factors with outcome variables of CVEs and mortality. For the same reasons, the χ^2 Goodness-of-fit test was employed to compare survival of SLE subgroups with age- and sex-matched UK population survival data. Backward stepwise binary logistic regression analysis was carried out for associations of aCL and other predictor variables with survival and an AUC ROC curve calculated to determine the discrimination ability of the final model.

Results

This was a retrospective study of 54 SLE patients and 51 controls. The patients' mean (SD) age was 45.5 (15.4) years and the controls' mean (SD) age was 47.9 (13.3) years (OR 1.0, 95% CI 0.96, 1.01, p = 0.4). There were 8 males and 46 females in the patient group and 8 males and 43 females in the control group (OR 1.0, 95% CI 0.4, 3.1, p = 0.9). Fourteen (25.9%) SLE patients died during the follow-up period. The response rate from the questionnaires was 24/40 (60%).

Table 4.1 shows the baseline lipoprotein profiles of patients and controls. The majority of controls had abnormally elevated concentrations of TC and LDL-C (74.5% and 84.3% respectively). Although 28 (51.9%) SLE patients had normal TC concentrations, 33 (61.1%) also had abnormally elevated LDL-C. Compared with controls, SLE patients had significantly lower median TC, LDL-C, HDL-C, HDL_2-C, HDL_3-C and apoA-I concentrations. Moreover, 53.7% of patients had low HDL-C concentrations, compared with 31.4% of controls (p = 0.02). There were no differences in the TG components, ApoB, Lp(a) or TC : HDL-C and

ApoB : apoA-I ratios between both groups, although there were fewer SLE patients with elevated apoB concentrations compared with controls (5.6% vs 17.6%). LpL and HL activities were not performed in the control group.

Table 4.2 shows the correlations between baseline aCL GPL levels with lipoprotein concentrations and lipase activity. There were statistically significant negative correlations between aCL GPL levels and TC, HDL_3-C, apoA-I and LpL activity, with similar trends for HDL-C and LDL-C.

Ten (18.5%) patients developed subsequent CVEs. The mean (SD) time to the subsequent CVE was 5.5 (3.8) years (range 1 - 12 years). Eight of these patients developed IHD, 1 patient had a TIA and 1 patient developed PVD. Table 4.3A shows the univariate associations of subsequent CVEs with predictor variables of baseline lipoproteins and CV risk factors that developed during the follow-up period. Table 4.3B shows the associations of baseline antibodies and SLE-related factors with CVEs which developed over the follow-up period. There were no significant associations of age, sex, hypertension, smoking or hypercholesterolaemia with the development of subsequent CVEs, although there was a trend towards for DM (p = 0.07). Both TC : HDL-C and apoB : apoA-I ratios were predictors of future CVEs (p = 0.02 and p = 0.05 respectively). TG was a significant predictor for future CVEs, with LDL-TG as the relevant component. Of the SLE-related factors, longer duration of prednisolone use and renal disease were predictors, with positive trends for longer disease duration and anti-Sm positivity.

Table 4.4 shows the retained factors in the final age and sex-adjusted multivariate analysis model of lipoproteins and risk factors compared with subsequent CVEs. This model included longer disease duration, increased TC : HDL-C ratio and higher aCL GPL levels as significant factors, with male sex as a contributory factor. The AUC ROC curve for the model was 0.95.

Two of the 14 deceased patients died during the first 5 years of the follow-up period (ages at death - 74 and 83 years) and one third died before the end of the 10-year follow-up period (age at death - 59). The overall survival for the 13-year period of follow-up for the cohort was 40/51 (78.4%) compared with an expected survival of 50.5/51 (99.1%) for UK population cohorts matched for age and sex during the same time period ($p < 0.0001$). The 5-year survival for the cohort from year of diagnosis was 50/52 (96.1%), compared with an expected 5-year survival of 51.7/52 (99.4%) for age- and sex-matched UK population cohorts ($p = 0.002$). The 10-year survival of the cohort was 44/51 (86.3%), compared with an expected survival of 50.6/51 (99.1%) for age- and sex-matched UK population cohorts ($p < 0.0001$). Patients who had subsequent CVEs had a significantly increased mortality risk (OR 6.75, 95% CI 1.54, 29.62, $p = 0.01$). Six of the 10 patients (60%) with subsequent CVEs died, compared with 8 of 44 patients (18.2%) who did not develop CVEs. Table 4.5 shows the causes of death for the 14 deceased patients. The cause of death was unknown for 1 patient (aged 83), and disease duration at the time of death was unknown for 3 patients. However, of the remaining 10 deceased patients with at least 10 years' disease duration, 6 (60%) patients died from atherosclerosis-related causes. Two patients died from malignancy and 2 from infection.

Tables 4.6A and 4.6B list the univariate associations of mortality with predictor variables of baseline lipoproteins and antibodies, CV and SLE factors. Older age, as well as elevated TC, LDL-C and LDL-TG were significant predictors of mortality ($p = 0.02$ and $p = 0.03$ respectively), with positive trends for Lp(a) and disease duration ($p = 0.08$ and $p = 0.09$ respectively).

Table 4.7 shows the retained variables in the final age and sex-adjusted multivariate analysis model of lipoproteins and risk factors compared with mortality. The final model included the independent baseline factors of increased TC : HDL-C ratio, increased Lp(a) concentrations, higher aCL GPL levels and longer disease duration. The AUC ROC curve for this model was 0.90.

Table 4.1. Baseline lipoprotein profiles of SLE patients & controls

Lipoprotein concentration / Lipase activity	SLE (n = 54) Median (IQR)	Controls (n = 51) Median (IQR)	*p* - value
TC (mmol/L)	4.97 (2.13)	5.96 (1.90)	**0.0004**
TC ≥ 5.0 mmol/L, n (%)	26.0 (48.1)	38.0 (74.5)	**0.005**
VLDL-C (mmol/L)	0.43 (0.29)	0.44 (0.32)	0.6
LDL-C (mmol/L)	3.39 (1.70)	3.93 (1.87)	**0.007**
LDL-C ≥ 3.0 mmol/L, n (%)	33 (61.1)	43 (84.3)	**0.007**
HDL-C (mmol/L)	1.09 (0.51)	1.32 (0.50)	**0.002**
HDL-C < 1.0 mmol/L (males) or < 1.2 mmol/L (females), n (%)	29 (53.7)	16 (31.4)	**0.02**
HDL_2-C (mmol/L)	0.43 (0.36)	0.57 (0.40)	**0.007**
HDL_3-C (mmol/L)	0.64 (0.28)	0.73 (0.25)	**0.02**
TG (mmol/L)	1.10 (0.64)	1.10 (0.70)	0.2
TG > 1.7 mmol/L, n (%)	8 (14.8)	11 (21.6)	0.4
VLDL-TG (mmol/L)	0.32 (0.48)	0.30 (0.28)	0.4
LDL-TG (mmol/L)	0.53 (0.35)	0.56 (0.49)	0.2
HDL-TG (mmol/L)	0.22 (0.80)	0.22 (0.10)	0.6
ApoB (mg/dL)	62.0 (28.0)	74.0 (32.0)	**0.01**
ApoB > 98 mg/dL, n (%)	3 (5.6)	9 (17.6)	**0.05**
ApoA-I (mg/dL)	117.0 (18.0)	135.0 (35.5)	**0.0001**
Lp(a) (mg/L)	114.5 (301.7)	148.5 (270.0)	0.6
Lp(a) > 300 mg/L, n (%)	16 (32.0)	7 (21.9)	0.3
TC : HDL-C ratio	4.73 (1.87)	4.52 (1.93)	0.3
TC : HDL-C ratio ≥ 6, n (%)	11 (20.4)	6 (11.8)	0.2
ApoB : apoA-I ratio	0.55 (0.23)	0.52 (0.29)	0.5
LpL activity (µmol/mL/hr)	3.46 (3.31)	-	-
HL activity (µmol/mL/hr)	2.05 (1.78)	-	-

Table 4.2. Correlations between baseline aCL GPL levels and lipoproteins

Lipoprotein / Lipase activity	Spearman's		Pearson's	
	ρ	*p*-value	*r*	*p*-value
TC (mmol/L)	- 0.300	0.03	- 0.286	**0.04**
VLDL-C (mmol/L)	0.014	0.9	- 0.104	0.5
LDL-C (mmol/L)	- 0.285	**0.04**	- 0.227	0.1
HDL-C (mmol/L)	- 0.169	0.20	- 0.257	0.06
HDL_2-C (mmol/L)	0.030	0.8	- 0.103	0.5
HDL_3-C (mmol/L)	- 0.363	**0.007**	- 0.292	**0.03**
Total TG (mmol/L)	0.021	0.9	- 0.027	0.9
VLDL-TG (mmol/L)	0.003	1.0	- 0.091	0.5
LDL-TG (mmol/L)	0.030	0.8	0.067	0.6
HDL-TG (mmol/L)	- 0.013	0.9	- 0.013	0.9
ApoB (mg/dL)	- 0.068	0.6	- 0.107	0.5
ApoA-I (mg/dL)	- 0.270	**0.05**	- 0.281	**0.04**
Lp(a) (mg/L)	- 0.157	0.3	- 0.210	0.1
LpL activity (µmol/mL/hr)	- 0.352	**0.01**	- 0.315	**0.03**
HL activity (µmol/mL/hr)	- 0.217	0.1	- 0.144	0.3

Table 4.3A. Univariate associations of CV risk factors and baseline lipoproteins with subsequent cardiovascular events

Variable	OR (95% CI)	*p*-value
Female sex	0.33 (0.06, 1.73)	0.2
Age (years)	1.035 (0.98, 1.09)	0.2
Hypertension	1 (0.19, 5.15)	1.0
Smoker	0.85 (0.14, 5.28)	0.9
Hypercholesterolaemia	2.00 (0.47, 8.56)	0.4
Diabetes mellitus	13.2 (1.00, 173.88)	0.07
TC (mmol/L)	1.39 (0.78, 2.47)	0.3
TC > 5.0 mmol/L, n (%)	3.07 (0.70, 13.46)	0.1
VLDL-C (mmol/L)	5.79 (0.48, 69.60)	0.2
LDL-C (mmol/L)	1.70 (0.84, 3.43)	0.1
LDL-C > 3.0 mmol/L, n (%)	7.50 (0.87, 64.35)	0.07
HDL-C (mmol/L)	0.15 (0.01, 1.48)	0.1
HDL-C < 1.0 mmol/L (males) or < 1.2 mmol/L (females), n (%)	2.33 (0.53, 10.21)	0.3
HDL_2-C (mmol/L)	0.18 (0.01, 3.64)	0.3
HDL_3-C (mmol/L)	0.09 (0.002, 4.16)	0.2
TG (mmol/L)	5.18 (1.35, 19.90)	**0.02**
TG $\geq$ 1.7 mmol/L, n (%)	6.67 (1.31, 34.03)	**0.03**
VLDL-TG (mmol/L)	4.21 (0.43, 41.03)	0.2
LDL-TG (mmol/L)	41.67 (2.65, 655.09)	**0.008**
HDL-TG (mmol/L)	6173.34 (0.02, 2.22 x 10^{-9})	0.2
ApoB (mg/dL)	1.02 (0.99, 1.05)	0.2
ApoA-I (mg/dL)	0.98 (0.95, 1.01)	0.3
Lp(a) (mg/L)	1.001 (0.997, 1.004)	0.7
TC : HDL-C ratio	2.03 (1.10, 3.76)	**0.02**
TC : HDL-C ratio $\geq$ 6.0, (n %)	3.53 (0.79, 15.81)	0.1
ApoB : apoA-I ratio	28.10 (1.05, 750.69)	**0.05**
LpL activity (µmol/mL/hr)	1.06 (0.76, 1.47)	0.8
HL activity (µmol/mL/hr)	0.72 (0.38, 1.36)	0.3

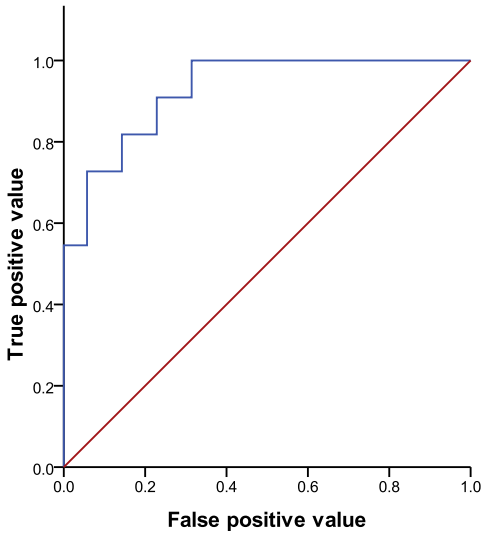
Table 4.3B. Univariate associations of SLE risk factors and baseline antibodies with subsequent cardiovascular events

Variable	OR (95% CI)	*p*-value
Age at diagnosis (years)	1.03 90.97, 1.08)	0.3
Disease duration (years)	1.10 (0.99, 1.22)	0.08
NP-SLE	1.83 (0.29, 11.43)	0.5
Renal disease	6.8 (1.28, 36.26)	**0.03**
Antiphospholipid syndrome	0.63 (0.07, 5.92)	0.7
Duration of prednisolone use (years to 2006)	1.15 (1.04, 1.27)	**0.007**
ACL GPL (U)	1.02 (0.99, 1.04)	0.1
ACL GPL +	1.35 (0.23, 7.91)	0.7
ACL MPL +	4.57 (0.25, 82.25)	0.3
Anti-dsDNA +	1.73 (0.39, 7.66)	0.5
Anti-Ro/SSA +	-	0.2
Anti-La/SSB +	-	0.2
Anti-U1RNP +	2.33 (0.57, 9.58)	0.2
Anti-Sm +	9.75 (0.79, 120.95)	0.07

Table 4.4. Final age and sex-adjusted multivariate model comparing baseline lipoproteins and risk factors with subsequent cardiovascular events

Factor	β-coefficient (SE)	Odds Ratio (95% CI)	*p* - value
Age (years)	0.056 (0.049)	1.06 (0.96, 1.16)	0.3
Male sex	3.045 (1.811)	21.00 (0.60, 731.15)	0.09
Lipoprotein			
TC : HDL ratio	1.937 (0.887)	6.94 (1.22, 39.49)	**0.03**
SLE Factor			
ACL GPL (U)	0.051 (0.021)	1.05 (1.01, 1.10)	**0.02**
Disease duration (years)	0.351 (0.169)	1.42 (1.02, 1.98)	**0.04**

Figure 4.1. ROC curve for the final multivariate model comparing CV and risk factors with subsequent CVEs



Area under the ROC curve = 0.95 (0.87, 1.00)

Table 4.5. Causes of death in SLE patients (n=14)

SLE patient (n=14)	Sex	Age at 1993 (years)	Year of death	Age at death (years)	Life expectancy (years)	Disease duration at death (years)	Cause of death
1	F	41	1999	46	80	9	CCF, SLE
2	F	58	1993	58	81.5	10	MI, CCF, pulmonary fibrosis
3	F	69	2001	77	83.9	11	Pulmonary fibrosis
4	F	69	1999	74	83.9	11	Pulmonary oedema, CCF, IHD
5	F	52	2006	66	80.8	14	Pulmonary embolus, deep vein thrombosis
6	F	18	2006	31	79.5	16	MI, intracerebral haemorrhage, SLE, APS
7	F	22	2006	34	79.5	16	Rhabdomyosarcoma, APS
8	F	71	2004	82	84.5	22	Pneumonia
9	F	51	1998	55	80.7	28	MI
10	F	60	2002	69	81.8	35	Respiratory failure, kyphoscoliosis, osteoporosis, SLE
11	M	49	2003	59	76	36	MI, IHD, SLE
12	F	42	2001	49	80.1	ND	Pneumonia, SLE, peripheral vascular disease
13	F	72	1995	74	82.9	ND	PE, pulmonary hypertension, bronchogenic carcinoma
14	F	82	1994	83	89.1	ND	ND

CCF: congestive cardiac failure, ND: no data available

Table 4.6A. Univariate associations of CV risk factors and baseline lipoproteins with subsequent mortality

Variable	OR (95% CI)	*p*-value
Female sex	2.76 (0.31, 24.67)	0.7
Age (years)	1.05 (1.01, 1.10)	**0.02**
Hypertension	0.68 (0.12, 3.85)	0.7
Smoker	3.00 (0.44, 20.44)	0.3
Hypercholesterolaemia	1.47 (0.40, 5.35)	0.7
Diabetes mellitus	3.40 (0.26, 44.76)	0.4
TC (mmol/L)	1.44 (0.87, 2.39)	0.2
TC > 5.0 mmol/L, n (%)	3.75 (1.00, 14.05)	**0.04**
VLDL-C (mmol/L)	1.14 (0.11, 11.91)	0.9
LDL-C (mmol/L)	1.72 (0.93, 31.90)	0.09
LDL-C > 3.0 mmol/L, n (%)	5.43 (1.07, 27.44)	**0.02**
HDL-C (mmol/L)	1.15 (0.20, 6.74)	0.9
HDL-C < 1.0 mmol/L (males) or < 1.2 mmol/L (females), n (%)	0.82 (0.24, 2.77)	1.0
HDL_2-C (mmol/L)	1.32 (0.14, 14.42)	0.8
HDL_3-C (mmol/L)	0.91 (0.05, 16.68)	0.9
TG (mmol/L)	1.95 (0.64, 5.95)	0.2
TG $\geq$ 1.7 mmol/L, n (%)	1.26 (0.22, 7.33)	1.0
VLDL-TG (mmol/L)	1.06 (0.13, 8.74)	1.0
LDL-TG (mmol/L)	10.62 (1.20, 94.34)	**0.03**
HDL-TG (mmol/L)	91.08 (0.001, 9050089.24)	0.4
ApoA-I (mg/dL)	1.00 (0.98, 1.02)	0.9
ApoB (mg/dL)	1.01 (0.98, 1.04)	0.6
Lp(a) (mg/L)	1.002 (1.000, 1.005)	0.08
TC : HDL-C ratio	1.41 (0.93, 2.12)	0.1
TC : HDL-C ratio $\geq$ 6	3.1 (0.78, 12.7)	0.1
ApoB : apoA-I ratio	3.87 (0.22, 67.48)	0.4
LpL activity (µmol/mL/hr)	1.00 (0.75, 1.34)	1.0
HL activity (µmol/mL/hr)	0.73 (0.43, 1.25)	0.3

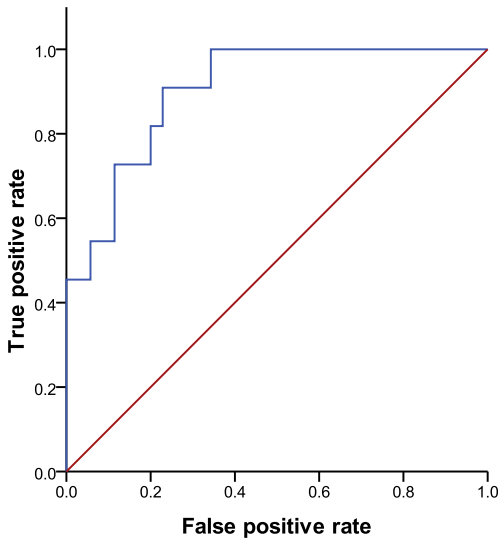
Table 4.6B. Univariate associations of SLE risk factors and baseline antibodies with subsequent mortality

Variable	OR (95% CI)	*p*-value
Age at diagnosis (years)	1.04 (0.99, 1.09)	0.2
Disease duration (years)	1.09 (0.99, 1.21)	0.09
NP-SLE	1.89 (0.30, 11.77)	0.6
Renal disease	3.40 (0.64, 18.13)	0.2
Antiphospholipid syndrome	1.17 (0.20, 6.82)	1.0
ACL GPL (U)	1.02 (0.99, 1.04)	0.2
ACL GPL +	1.70 (0.36, 8.05)	0.5
ACL MPL +	4.0 (0.23, 71.12)	0.4
Anti-dsDNA +	0.60 (0.18, 2.05)	0.5
Anti-Ro/SSA +	2.27 (0.53, 9.65)	0.3
Anti-La/SSB +	3.17 (0.40, 24.96)	0.3
Anti-U1RNP +	0.51 (0.12, 2.12)	0.3
Anti-Sm +	1.46 (0.12, 17.48)	1.0
Cumulative duration of prednisolone use (years to 2006)	1.04 (0.97, 1.12)	0.3

Table 4.7. Final age- and sex-adjusted model comparing baseline lipoprotein and risk factors with mortality

Factor	β-coefficient (SE)	Odds Ratio (95% CI)	*p* - value
Age (years)	0.026 (0.037)	1.03 (0.96, 1.10)	0.5
Male sex	- 1.409 (1.541)	0.24 (0.01, 5.01)	0.4
Lipoprotein			
TC : HDL-C ratio	1.055 (0.471)	2.87 (1.14, 7.22)	**0.03**
Lp(a) (mg/L)	0.005 (0.002)	1.01 (1.00, 1.01)	**0.04**
SLE Factor			
ACL GPL (U)	0.033 (0.016)	1.03 (1.00, 1.07)	**0.04**
Disease duration (years)	0.151 (0.075)	1.16 (1.00, 1.35)	**0.05**

Figure 4.2. ROC curve for the final age and sex-adjusted model comparing baseline lipoproteins and risk factors with mortality



Area under the ROC curve = 0.90 (0.82, 0.99)

Discussion

This study showed that SLE patients had lower TC, LDL-C, HDL-C and apoA-I concentrations compared with age and sex-matched controls. Moreover, 53.7% of patients had low HDL-C concentrations. These findings are consistent with Borba and Bonfá's previous report in 1997 of low HDL-C levels in 79% of patients with active SLE (as measured by SLEDAI) and 29% of SLE patients with inactive disease[368]. The lower proportion of patients with low HDL-C in our group is most likely explained by lower cut-off values for HDL-C used in our study, as recommended in the 2005 JBS 2 guidelines[418]. As both HDL-C subfractions of HDL_2-C and HDL_3-C were lower in our SLE patients compared with controls (p = 0.007 and p = 0.02 respectively), both were contributory factors to the low HDL-C concentrations. As apoA-I is the major lipoprotein component of HDL, it is not surprising that apoA-I concentrations were lower in our SLE patients. Borba and Bonfá also reported elevated TG and VLDL in their SLE cohort, however, these findings were not replicated in our study. Our results also differed slightly from the results of Ettinger et al., which showed lower HDL_2-C levels in SLE patients compared with controls, but similar HDL-C, HDL_3-C and apoA-I levels in both groups[383]. The differences in our study results may be explained by variations in disease activity or glucocorticoid use, however, this baseline information was not available in our study. In Borba and Bonfa's study, lower TC and LDL-C concentrations were present in SLE patients with active disease, compared with patients with inactive disease or controls[368]. This suggests that low TC and LDL-C could act as added markers of disease activity. However, as our study did not have baseline measures of disease activity, this could not be confirmed.

We found that aCL GPL levels were negatively correlated with TC, HDL_3-C, apoA-I, and LpL activity in patients with SLE. Our findings support Lahita et al.'s previous observation of lower TC, HDL-C and apoA-I levels in aCL GPL positive SLE patients[400]. Delgado Alves et al. demonstrated that antibodies

directed against HDL and apoA-I from SLE sera also cross-reacted with cardiolipin[399]. Other studies have shown that higher IgG anti-HDL and anti-apoA-I titres were associated with increased disease activity and damage, and remained elevated during persistent disease activity[374, 403]. Anti-LpL antibodies have been detected in SLE patients[407], and found to be associated with aCL GPL and increased disease activity in SLE[408]. Anti-LpL may play an inhibitory role on LpL and hence account for reduced LpL activity in SLE compared with healthy individuals[404]. As low HDL levels have been associated with reduced LpL activity[406], it is possible that the low HDL-C levels seen in our patients may be an indirect result of aCL inhibition of LpL activity. However, as LpL activity was not performed in controls, we were unable to confirm that LpL activity was indeed lower in SLE patients, as would be expected. Overall, our results provide further evidence for a pathogenic role of aCL in the inflammatory process of SLE, possibly through cross-reaction with PL-binding proteins on apoA-I in HDL, and/or inhibition of LpL activity. Furthermore, in the multivariate analyses, higher aCL GPL levels independently predicted subsequent CVEs and mortality in SLE. High-titre aCL are predictors for future arterial thrombosis[42, 52] including stroke[60], however, the association of aCL with atherosclerotic CVEs, such as MI, is controversial[49, 61, 83, 84] and there have been very few studies that have shown the association of aPL with mortality[247, 270, 271, 273]. Our study provides further support for the utility of aCL GPL as a biomarker for both future atherosclerotic CVEs and mortality.

The TC : HDL ratio is used as an important criterion for CV risk assessment in the general population[418]. In the univariate analysis, the TC : HDL ratio was a significant predictor for future CVEs, confirming its importance as a CV risk factor. Furthermore, in the multivariate analysis, higher TC : HDL-C ratios independently predicted both future CVEs and mortality, with ORs of 6.94 and 4.11 respectively. Although elevated TG was a significant predictor variable for future CVEs in the univariate analysis, it was not retained in the final multivariate regression model adjusted for age and sex. High TG and low HDL-C concentrations may occur together in both SLE (part of the "lupus pattern" of

dyslipidaemia) and the MetS, however different pathogenic mechanisms may be involved in SLE, thereby contributing to the relative importance of HDL over TG as a predictive factor. Furthermore, the independence of TG as a CV risk predictor has been questioned within the general population[387]. The TC : HDL-C ratio may effectively reflect the "lupus pattern" of dyslipidaemia, and hence this ratio may prove to be a reliable marker for CV and mortality risk, particularly in the setting of low or normal TC, LDL-C and TG concentrations, as in our cohort. Moreover, as the ratio is calculated from a fasting lipid profile, it is a simple and cost-effective clinical tool, which could be included in routine clinical monitoring of SLE patients.

Recent studies have demonstrated that the apoB : apoA-I ratio may be a more reliable predictor of IHD risk than the TC : HDL ratio[419, 420]. Although the apoB : apoA-I ratio was a predictor for subsequent CVEs in the univariate analysis, the 95% CI was wide and the ratio was not an independent factor in the multivariate analysis. Larger studies are required to further investigate the apoB : apoA-I ratio as a possible marker for IHD risk in SLE.

With respect to traditional CV risk factors such as smoking, hypertension and older age as predictors of CVEs, we found no significant associations. Since this was a retrospective study in a small sample of patients, it is possible that despite careful review of patient records, missing data would have contributed to these negative results. Nevertheless, we were able to show that male gender was a contributory factor in the final multivariate model, in keeping with findings from other SLE studies[283, 286]. In the univariate analyses, SLE-related factors predicting future CVEs included renal disease and longer duration of prednisolone use, with similar trends for longer disease duration and the presence of anti-Sm at baseline. However, these factors were not independent variables in the multivariate analysis, in contrast to previous reports of longer duration of disease[84, 276, 278] and glucocorticoid use[276, 278] being independent predictors of CVEs. Once again, the most likely explanation for the difference in our results is that we carried out a retrospective study in a small sample of patients.

The overall survival rate from the date of diagnosis for our cohort over the 13-year follow-up period was 78.4%, which is comparable with 15-year follow-up data from the 1990s, with previous studies reporting survival rates of 78 - 79%[50, 218-221]. The 5-year survival rate for our SLE cohort was 96.1%, and the 10-year survival rate was 86.3%. These findings are comparable to other published reports from the 1990s showing survival rates of 93% - 97% at 5 years and 83% - 92% at 10 years[50, 218-221]. The survival rates for our cohort were significantly lower than expected for age- and sex-matched UK population cohorts, which further confirms previous evidence that patients with SLE have an increased mortality risk, compared with the general population[50, 225-228]. Six of the 11 patients who developed subsequent CVEs died, confirming the importance of atherosclerosis as a major cause of death in SLE[227, 229, 236]. Furthermore, of the 10 deceased patients with at least 10 years' disease duration at the time of death, 6 (60%) patients died from atherosclerosis-related causes, confirming Urowitz et al.'s "bimodal mortality pattern" of late deaths from CV-related causes[231].

Predictors of mortality from the univariate analysis were older age, elevated TC, elevated LDL-C and higher LDL-TG levels (p = 0.02, 0.04, 0.02 and 0.03 respectively). There were positive trends for Lp(a) (p = 0.08) and longer disease duration (p = 0.09). In the multivariate analysis, the final age and sex-adjusted model retained the independent variables of longer disease duration, higher TC : HDL ratios, increased Lp(a) and higher aCL GPL levels. Longer disease duration as a risk factor in the final model most likely reflects the reduced survival prognosis of SLE cohorts from earlier decades[183, 218]. Elevated Lp(a) concentrations independently predict for IHD, ischaemic stroke and coronary mortality in general populations, although the effect is relatively weak (adjusted RR 1.1 for all outcomes)[409]. Elevated levels of Lp(a) have been detected in patients with SLE[414-416], however to our knowledge, this is the first study showing Lp(a) as a novel independent risk factor for mortality in SLE. Moreover, as Lp(a) levels are not influenced by disease activity or glucocorticoid

therapy[415], Lp(a) may be a prove to be a reliable predictor of mortality, in combination with other factors.

Conclusions

The findings of this study confirmed previous reports of the "lupus pattern" of dyslipidaemia and showed inverse correlations of TC, HDL_3-C, apoA-I and LpL activity with aCL GPL, supporting the hypothesis that aCL plays a pathogenic role in lipoprotein-associated pathways of atherogenesis. Furthermore, aCL GPL was an independent predictor of both future CVEs and mortality, lending support to its atherogenic role. The TC : HDL ratio was another independent predictor of CVEs and mortality in this study and should be considered for inclusion in the routine clinical monitoring of patients with SLE. To our knowledge, this is the first study showing Lp(a) as an independent predictor of mortality, and further studies to confirm the utility of Lp(a) as a biomarker should be considered.

CHAPTER 5

Associations of antiphospholipid antibodies with subclinical atherosclerosis in SLE - a cross-sectional study

Background

Patients with SLE have a significantly increased risk of developing accelerated atherosclerosis. Traditional CV risk factors do not fully account for this and lupus-specific factors have been implicated. The prevalence of subclinical atherosclerosis is increased in SLE and associated with both classic CV risk factors and SLE-related factors. In a previous cross-sectional study, Ahmad et al. demonstrated that the SLE-related factors of azathioprine therapy, increased neutrophil count, previous coronary and/or cerebral events, and persistent aCL and/or LA were independently associated with the presence of carotid plaque[85]. In contrast, the association of aPL with carotid plaque has not been confirmed in other studies[86, 350, 364, 372]. Furthermore, although the pathogenic actions of aPL such as aCL and anti-β_2GPI have been well-defined, their potential utility as biomarkers of atherosclerosis remain controversial and the effect of other aPL such as anti-AnxA5 and anti-PT are unknown.

Aim

To determine the associations of SLE-related risk factors, including aPL comprising aCL, anti-β_2GPI, anti-PT and anti-AnxA5, with the presence of carotid plaque in female patients with SLE.

Methods

This study was approved by the North West Multi-Centre Research Ethics Committee and written informed consent was obtained from each participant.

Personal contribution by the candidate

This study was designed by the candidate as a cross-sectional study. This ancillary study examined baseline aPL subtypes as additional predictive factors for a longitudinal non-inception SLE cohort study previously designed by Prof Ian Bruce and Dr Yasmeen Ahmad at the Arthritis Research UK Epidemiology Unit in Manchester. In the initial cross-sectional study, study subjects and controls were consecutively recruited between 2000 and 2003 by Dr Yasmeen Ahmad, Prof Bruce and others. Prof Bruce's team collected the study subjects' clinical, serological and carotid ultrasound data and carried out the original cross-sectional data analysis. Between 2006 and 2009, Dr Sahena Haque, Prof Bruce and his team collected the study subjects' follow-up clinical, serological and carotid ultrasound data. In 2008, the candidate retrieved the stored baseline serology samples for this study with the assistance of Dr Allen Yates, Manchester Royal Infirmary (MRI). ACL, anti-β_2GPI, anti-PT and anti-AnxA5 ELISAs were performed by Mrs Dunphy and Mrs Owen at BIRD in Bath. Additional data analysis was performed by the candidate for this substudy using the collected information above stored in an Access database at the University of Manchester and managed by Ms Nicola Dale. Advice and assistance with statistical analysis was provided by Prof Dhaliwal, Curtin University (Perth).

Sample size

In 2003, Roman et al. published a cross-sectional study examining factors associated with the presence of carotid plaque in 197 SLE patients with 197 matched controls[86]. For Prof Bruce's original study, he estimated that a study sample size of 200 female patients SLE and 100 female controls would be similar in number to Roman et al.'s study, and this would provide adequate power for the

study to detect a difference between SLE patients with carotid plaque and those without plaque. As this study was an ancillary study, no power calculations were performed prior to this study.

Patients

Patients with SLE were selected for this study from Ahmad et al.'s original Manchester cohort of 200 female British Caucasian SLE patients[85] who were initially assessed between 2000 and 2003 and later returned for follow-up assessments between 2006 and 2009. Data collected at baseline was used for this study. Other female SLE patients were added to the Manchester cohort during the period between 2006 and 2009, and included younger women with shorter disease durations and women from other ethnic groups. Patients were recruited from rheumatology clinics at Manchester Royal Infirmary (MRI), North Manchester General Hospital, Blackburn Royal Infirmary and other centres in the North-West of England and through Lupus UK, the national patient support group. All patients were over 18 years of age and fulfilled $\geq$ 4 of the 1997 updated ACR criteria for SLE[425]. Patients were on stable therapy for at least 2 months. Women who were pregnant or lactating mothers within 6 months were excluded.

Patients underwent a clinical interview and examination at the MRI Lupus Research Clinic, according to a standard protocol that included demographic information, family history and lifestyle factors. Patients were assessed for the presence of prevalent CVD and a history of prior CVEs, namely MI, angina, stroke, transient ischaemic attacks (TIA), or peripheral vascular disease (PVD). Traditional CV risk factors were also recorded, including hypertension, hyperlipidaemia, diabetes mellitus, smoking history, anthropomorphic measures, family history of premature IHD, and the metabolic syndrome, using standard definitions[300, 418, 440]. SLE-related factors that were assessed included clinical features, previous arterial and venous thromboembolism and the absence and/or presence of APS. SLE disease activity and cumulative damage were measured

on the day of the assessment, using SLEDAI-2K[441] and SLICC DI respectively[442] (see Appendix). Information collected about drug therapy included the use of glucocorticoids and immunosuppressive agents, including antimalarial drugs, as well as antihypertensive and statin therapy.

Controls

Healthy female controls from the same ethnic background and geographical region were recruited using a 'best friend' system. As the prevalence of carotid plaque is very low in healthy young women, older patients were asked to invite a friend (non-relative) to take part in the study. This allowed inclusion of traditional CV factors associated with subclinical atherosclerosis for the controls. Controls were excluded if they had any history of systemic autoimmune disease.

Laboratory assessment (Manchester)

Following avoidance of alcohol for 48 hours and an overnight 12 hour fast, a 50 mL blood sample was drawn for laboratory studies at the baseline time point. As part of the routine clinical care, the following blood samples were assessed: full blood count, blood glucose level, lipid profile and serum creatinine. The estimated glomerular filtration rate (eGFR) was calculated using the modified Cockcroft-Gault formula. Baseline serological tests carried out on SLE serum samples in Manchester included ANA, anti-dsDNA, anti-Ro/SSA, anti-La/SSB, anti-U1RNP, anti-Sm, and complement C3 and C4 levels. Patient positivity for aCL GPL, aCL MPL or LA was also recorded. A patient was defined to be 'ever positive' for aCL if she had a history of two positive tests (> 16 U) at least 6 weeks apart. Similarly, LA was determined by dRVVT and defined as 'ever positive' if present on two occasions at least 6 weeks apart. In addition, apoA-I and apoB concentrations were determined in a subgroup of 78 patients from the original SLE cohort.

Antiphospholipid antibody testing (Bath)

APL testing was performed on baseline serum samples taken at the time of the initial assessment for patients from Ahmad et al.'s original SLE cohort[85] who returned for follow-up assessments. Serum samples were also obtained at the time of assessment from SLE patients and sex-matched controls recruited to the study between 2006 and 2009. Samples were tested using commercially available ELISA kits at the Bath Institute of Rheumatic Diseases (BIRD) in Bath for the following aPL antibodies: aCL GPL and MPL (INOVA Diagnostics Inc., San Diego, CA, USA), anti-β_2GPI GPL and MPL, and anti-AnxA5 GPL and MPL (AESKU.Diagnostics, Wendelsheim, Germany). A subgroup of serum samples from the first consecutive 120 SLE patients and 29 controls studied between 2006 and 2008 were also tested for PS-dependent anti-PT GPL and MPL (AESKU.Diagnostics, Wendelsheim, Germany). The concentration of aPLs in each sample was calculated directly from the absorbency readings by software attached to the plate reader (Multiskan Ascent; Labsystems, Helsinki, Finland). Inter-assay and intra-assay reliability was determined using the coefficient of variation (%CV). ELISA intra-assay %CVs were as follows: aCL GPL - 4.2%, aCL MPL - 13.2%, anti-β_2GPI GPL - 8.1%, anti-β_2GPI MPL - 6.9%, anti-AnxA5 GPL - 12.0%, anti-AnxA5 MPL - 5.7%, anti-PT GPL - 7.6%, and anti-PT MPL - 7.6%. Inter-assay %CVs were as follows: aCL GPL - 15.4%, aCL MPL - 11.6%, anti-β_2GPI GPL - 17.4%, anti-β_2GPI MPL - 17.4%, anti-AnxA5 GPL - 14.3%, and anti-AnxA5 MPL - 12.5%, anti-PT GPL - 1.7%, and anti-PT MPL - 15.3%. A positive result was set as the cut-off value above the 95th percentile for control sample results, and at $\geq$ 20U for anti-β_2GPI GPL, according to the 95th percentile for combined patient and control samples.

Vascular assessment

All study subjects underwent assessment of their carotid arteries at the MRI Vascular Laboratory using a standard protocol with B-mode Doppler ultrasound. Scans were performed with an ATL HDI 5000 scanner equipped with a 7-4 MHz linear array transducer, by operators who were blinded to the subjects' diagnoses.

IMT measurements were made in a longitudinal plane at a point of maximum thickness in the right and left common carotid artery (CCA), along a 1 cm section proximal to the carotid bulb. Maximal IMT measurements were repeated 3 times on each side and all 6 measurements were then used to calculate the mean IMT, as described by Sidhu and Desai[85, 342]. The right and left common carotid artery (CCA), carotid bulb and the first 1.5 cm of the internal and external carotid arteries were examined in the longitudinal and cross-sectional planes for the presence of focal carotid plaques, as defined by Li et al.[343]. Carotid plaque was defined if 2 of the following 3 conditions were met: a distinct area of protrusion > 50% into vessel lumen, increased echogenicity compared with adjacent boundaries, or IMT > 0.15 cm. In a prior study, intra-observer reliability for these scanning techniques was found to be very high, with an intra-class correlation coefficient for repeat assessments of 0.92 (95% CI 0.84, 1.00) for one of the operators[85].

Statistical analysis

The chi-square test was employed for comparisons of categorical data and the Mann-Whitney U test for comparisons of continuous data. Pearson's and Spearman's rho correlations were also used to compare normally-distributed and non-parametric continuous data respectively. Statistical significance was set at a p-value of < 0.05. In univariate analyses, binary logistic regression was used to assess the relationships between predictor variables and the presence of carotid plaque. For multivariate analyses, backward, stepwise logistic regression models were used. Known clinical predictor variables, as well as appropriate variables significant at $p < 0.2$ in the univariate analyses, were included in the multivariate regression models. The predicted probability of each model was used to generate a receiver operator characteristic (ROC) curve. The areas under the ROC curves (AUC ROC curve) were then used to determine the accuracy of the final models. Statistical analysis was performed using the SPSS Statistics 17.0 software package (IBM Corporation, Armonk, NY, USA).

Results

Of the 156 female patients with SLE studied, 120 patients were from Ahmad et al.'s original cohort. The mean (SD) age at the time of assessment was 48.9 (10.2) years. The majority of patients (91.7%) were of British Caucasian descent. The ethnic origins of the remaining patients were African or Afro-Caribbean (n = 5, 3.2%), South Asian (n = 4, 2.6%) and Chinese (n = 1, 0.6%). SLE-related characteristics of the patients are summarised in Table 5.1. Overall, the group had low disease activity (median SLEDAI-2K score 2). Median (IQR) disease duration was 9.5 (14.8) years. Arthritis, rash and serositis were common features, whereas renal disease and NP-SLE were uncommon. A history of APS was present in 17.3% of patients.

Twenty-nine sex-matched controls were included in the study. Table 5.2 shows the characteristics of patients and controls with respect to traditional CV risk factors. Patients had a lower median age compared with controls, as intended in the study design (49 years vs 62 years, $p = 0.02$). Diastolic BP was higher in patients (median 76 mm Hg in patients vs 70 mm Hg in controls, $p = 0.008$). HDL-C and fasting glucose levels were lower in patients ($p = 0.03$ and 0.01 respectively), with a trends for lower TC concentrations and more frequent history of CVD in patients.

Table 5.3 shows the associations of aPL with thrombosis and CVEs. There was a significant association of aCL GPL with APS, as well as for 5 of the other 7 aPL tested. There were significant associations for aCL GPL, anti-β_2GPI GPL, anti-AnxA5 GPL and anti-PT MPL with arterial thrombosis. A similar trend was found for anti-β_2GPI GPL with venous thromboembolism ($p = 0.06$). ACL GPL, anti-β_2GPI GPL, anti-AnxA5 GPL and anti-AnxA5 MPL were significantly associated with cerebrovascular events, however, there were no significant associations of aPL with IHD.

APL correlations with HDL and apoA-I

HDL-C was strongly correlated with apoA-I (r = 0.82, p < 0.001, ρ = 0.80, p < 0.0001). Anti-β_2GPI GPL levels were negatively correlated with apoA-I (ρ = - 0.35, p = 0.002). Other GPLs were also negatively correlated with apoA-I (aCL GPL ρ = - 0.29, p = 0.01; anti-AnxA5 GPL ρ = - 0.31, p = 0.006; anti-PT GPL ρ = - 0.28, p = 0.03). There were no significant correlations between HDL-C concentrations and aPL levels.

Univariate analysis of CV factors compared with carotid plaque

Table 5.4 shows the results from univariate analyses of CV risk factors compared with the presence of carotid plaque. Increasing age, postmenopausal status, history of smoking, hypertension, higher systolic BP at assessment, lower eGFR, and a previous history of IHD and/or cerebrovascular events were significant factors associated with the presence of plaque. Antihypertensive therapy was also a significant factor with a positive effect.

Univariate analysis of SLE factors compared with carotid plaque

Table 5.5 shows significant associations for the presence of plaque with older age at SLE diagnosis, higher SLICC DI scores, previous arterial thrombosis, higher white cell and neutrophil count, lower eGFR, and longer duration of glucocorticoid use. Similar associations approaching significance were found for APS, higher lymphocyte count, and methotrexate use. A history of persistent aCL (including aCL MPL) was also significantly associated with the presence of plaque, with positive but associations approaching significance for persistent aCL GPL, history of aCL and/or LA positivity, and anti-AnxA5 GPL.

Multivariate analyses of factors compared with carotid plaque

Table 5.8 shows the final multivariate model for CV and SLE factors compared with the presence of carotid plaque. The CV factors with independent

associations included a prior history of CVD, history of smoking, and hypertension. There was a trend towards fewer postmenopausal women with baseline carotid plaque (p = 0.06). SLE-related factors with independent positive associations included older age at SLE diagnosis and longer disease duration. Higher daily prednisolone doses in the past 6 months and anti-AnxA5 GPL showed positive associations with carotid plaque that approached significance (p = 0.06 and 0.07 respectively). The AUC ROC curve for the model was 0.89.

Table 5.1. Disease-related features of SLE patients

Variable	n (%)*
British Caucasian ethnicity	143 (91.7)
Median (IQR) age at diagnosis (years)	35.0 (17.8)
Median (IQR) disease duration (years)	9.5 (14.8)
Median no. of (IQR) ACR SLE criteria	5.5 (2.0)
Discoid or malar rash	88 (56.4)
Serositis	60 (38.5)
Arthritis	128 (82.1)
Renal disease	29 (18.6)
NP-SLE	15 (9.6)
Previous arterial thromboembolism	16 (10.3)
Previous venous thromboembolism	17 (10.9)
Ever anti-dsDNA +	99 (63.5)
Ever aCL or LA +	55 (35.3)
Ever aCL GPL +	42 (26.9)
Ever aCL MPL +	26 (16.7)
Ever LA +	32 (20.5)
Antiphospholipid syndrome	27 (17.3)
Median (IQR) SLEDAI-2K	2 (2)
Median (IQR) SLICC DI	0 (2)
Current glucocorticoid therapy	91 (58.3)
Median (IQR) average daily prednisolone dose in past 6 months (mg)	5.0 (8.0)
HCQ therapy, past or present	104 (66.7)
Azathioprine therapy, past or present	60 (38.5)
Methotrexate therapy, past or present	30 (19.2)

*Variables presented as n (%) unless indicated otherwise

Table 5.2. Comparisons of demographic and classic risk factors in SLE patients and controls

Risk factor	SLE patients (n= 156) median (IQR)*	Controls (n=29) median (IQR)*	*p* - value
Age (years)	49 (14)	62 (60)	**0.02**
Post-menopausal, n (%)	85 (54.5)	20 (69.0)	0.2
Total cholesterol (mmol/L)	4.86 (1.54)	5.93 (1.38)	0.06
HDL-C (mmol/L)	1.62 (1.64)	1.77 (0.54)	**0.03**
TC : HDL-C ratio	2.94 (1.25)	2.98 (1.34)	0.6
Triglycerides (mmol/L)	1.07 (0.60)	1.04 (0.78)	0.08
Current smoker, n (%)	22 (14.1)	7 (24.1)	0.2
Smoker (pack-years)	0 (0)	2.2 (16.9)	0.3
Hypertension, n (%)	73 (46.8)	9 (32.1)	0.2
Systolic BP (mm Hg)	126 (25)	133 (28)	1.0
Diastolic BP (mm Hg)	76 (10)	70 (20)	**0.008**
eGFR (mL/min)	77.6 (26.6)	84.5 (17.0)	0.5
Diabetes mellitus, n (%)	4 (2.6)	0 (0)	1.0
Fasting glucose (mmol/L)	4.5 (0.6)	4.8 (0.4)	**0.01**
BMI (kg/m^2)	25.6 (7.2)	26.2 (7.5)	0.3
Metabolic syndrome, n (%)	37 (23.7)	4 (13.8)	0.2
History of CVD, n (%)	26 (16.7)	1 (3.4)	0.08
Family history of premature IHD, n (%)	34 (21.8)	7 (24.1)	0.8

*Variables presented as median and interquartile range unless indicated otherwise

Table 5.3. Associations of aPL with thrombosis and cardiovascular events in SLE patients (n = 156)

aPL*	n (%)	APS OR (95%CI)	*p* - value	Arterial thrombosis OR (95%CI)	*p* - value	Venous thromboembolism OR (95%CI)	*p* - value	Cerebrovascular events OR (95%CI)	*p* - value	IHD OR (95%CI)	*p* - value
aCL GPL+	15 (9.6)	7.34 (2.39, 22.57)	**0.001**	8.67 (2.57, 29.26)	**0.001**	2.27 (0.57, 9.02)	0.2	7.35 (2.33, 23.14)	**0.001**	2.56 (0.49, 13.33)	0.2
aCL MPL+	11 (7.1)	3.03 (0..82, 11.20)	0.1	2.06 (0.41, 10.51)	0.4	0.81 (0.10, 6.72)	0.8	2.49 (0.60, 10.21)	0.2	1.51 (0.17, 13.15)	0.7
Anti-β_2GPI GPL+	9 (5.8)	7.10 (1.77, 28.53)	**0.008**	5.12 (1.14, 22.89)	**0.05**	4.75 (1.07, 21.10)	0.06	5.73 (1.41, 23.35)	**0.02**	1.92 (0.22, 17.05)	1.0
Anti-β_2GPI MPL+	8 (5.1)	5.44 (1.27, 23.30)	**0.03**	3.17 (0.58, 17.20)	0.2	1.18 (0.14, 10.21)	0.9	4.07 (0.90, 18.44)	0.09	2.21 (0.24, 19.93)	0.5
Anti-AnxA5 GPL+	19 (12.2)	5.95 (2.13, 16.63)	**0.001**	5.82 (1.82, 18.59)	**0.006**	1.65 (0.43, 6.36)	0.5	6.39 (2.20, 18.54)	**0.001**	1.90 (0.37, 9.68)	0.4
Anti-AnxA5 MPL+	6 (3.8)	5.25 (1.00, 27.58)	0.07	4.82 (0.81, 28.72)	0.1	1.68 (0.18, 15.25)	0.6	6.90 (1.30, 36.67)	**0.04**	3.13 (0.33, 29.74)	0.3
Anti-PT GPL+	16 (10.3)	6.99 (2.28, 21.48)	**0.001**	3.44 (0.92, 12.92)	0.08	2.41 (0.58, 10.07)	0.2	2.53 (0.70, 9.11)	0.2	0.91 (0.11, 7.97)	0.9
Anti-PT MPL+	8 (5.1)	14.53 (2.72, 77.54)	**0.001**	6.00 (1.24, 28.90)	**0.04**	1.30 (0.15, 11.56)	0.8	4.52 (0.97, 21.18)	0.07	2.12 (0.23, 19.75)	0.5

APS: antiphospholipid syndrome

*Baseline serum samples tested in BIRD, Bath

Table 5.4. Associations of traditional CV risk factors with the presence of carotid plaque

CV risk factor	Plaque presence OR (95% CI)	*p*-value
Age (years)	1.12 (1.07, 1.18)	**< 0.001**
Postmenopausal	3.12 (1.46, 6.66)	**0.003**
TC (mmol/L)	1.24 (0.92, 1.68)	0.2
HDL-C (mmol/L)	1.11 (0.52, 2.36)	0.8
TC : HDL-C ratio	1.19 (0.90, 1.57)	0.2
TG (mmol/L)	1.6 (0.94, 2.72)	0.08
LDL-C (calculated) (mmol/L)	1.18 (0.84, 1.66)	0.3
ApoB (mg/dL)	3.28 (0.43, 24.96)	0.3
ApoA-I (mg/dL)	0.87 (0.14, 5.22)	0.9
ApoB : apoA-I ratio	4.10 (0.43, 38.80)	0.2
Current smoker	2.36 (0.94, 5.94)	0.07
Smoker ever	4.19 (1.95, 8.98)	**< 0.001**
Smoking history (pack-years)	1.06 (1.03, 1.09)	**< 0.001**
Hypertension	5.71 (2.61, 12.49)	**< 0.001**
Systolic BP (mm Hg)	1.03 (1.01, 1.04)	**0.003**
Diastolic BP (mm Hg)	1.03 (0.99, 1.06)	0.1
eGFR (mL/min)	0.98 (0.96, 0.99)	**0.01**
Fasting glucose (mmol/L)	1.47 (0.93, 2.33)	0.1
Diabetes mellitus	0.82 (0.08, 8.08)	0.9
BMI (kg/m^2)	1.03 (0.97, 1.09)	0.3
Metabolic syndrome	1.73 (0.79, 3.77)	0.2
Family history of premature IHD	0.86 (0.37, 2.02)	0.7
Previous history of CVD	10.86 (4.13, 28.56)	**< 0.001**
Previous cerebrovascular event	5.82 (2.23, 15.14)	**< 0.001**
History of IHD	11.78 (2.39, 58)	**0.002**
Antihypertensive therapy	3.39 (1.64, 7.00)	**0.001**

Table 5.5. Associations of SLE-related factors and aPL with the presence of carotid plaque

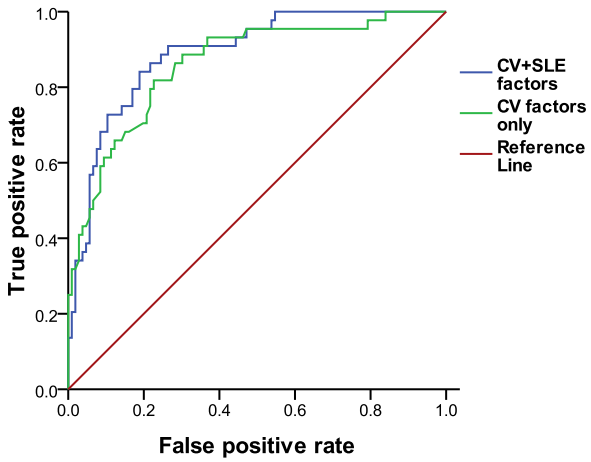
SLE factor	Plaque presence OR (95% CI)	*p*-value
Age at SLE diagnosis (years)	1.05 (1.02, 1.08)	**0.001**
Disease duration (years)	1.02 (0.99, 1.06)	0.2
Rash	0.74 (0.37, 1.48)	0.4
Serositis	1.1 (0.54, 2.23)	0.8
Arthritis	1.27 (0.5, 3.23)	0.6
Renal disease	1.38 (0.59, 3.27)	0.5
NP-SLE	2.37 (0.81, 6.99)	0.1
Haematological disorder	0.68 (0.31, 1.49)	0.3
SLEDAI-2K	0.96 (0.84, 1.09)	0.5
SLICC DI	1.41 (1.08, 1.83)	**0.01**
Ever ACL GPL+	2.06 (0.98, 4.37)	0.06
Ever ACL MPL+	2.47 (1.04, 5.87)	**0.04**
Ever ACL+	2.28 (1.09, 4.77)	**0.03**
Ever LA+	1.16 (0.5, 2.69)	0.7
Ever ACL and/or LA+	1.98 (0.97, 4.04)	0.06
ACL MPL+	1.45 (0.4, 5.22)	0.6
ACL GPL+	11.26 (0.41, 3.92)	0.7
Anti-β_2GPI MPL+	1.51 (0.35, 6.62)	0.6
Anti-β_2GPI GPL+	2.07 (0.53, 8.09)	0.8
Anti-PT MPL+	1.42 (0.32, 6.28)	0.6
Anti-PT GPL+	0.74 (0.22, 2.47)	0.6
Anti-AnxA5 MPL+	2.57 (0.5, 13.25)	0.3
Anti-AnxA5 GPL+	2.52 (0.95, 6.71)	0.06
Antiphospholipid syndrome	1.92 (0.81, 4.55)	0.1
Previous arterial thrombosis	9.64 (2.91, 31.90)	**< 0.001**
Previous venous thromboembolism	1.86 (0.66, 5.24)	0.2
ANA+	1.05 (0.20, 5.66)	0.1
Anti-dsDNA+	0.94 (0.44, 2.01)	0.9
Anti-Ro/SSA+	0.67 (0.31, 1.45)	0.3
Anti-La/SSB+	0.69 (0.24, 2.00)	0.5
Anti-U1RNP+	0.91 (0.33, 2.51)	0.9
Anti-Sm+	-	1.0
White cell count (x 10^{-9}/L)	1.16 (1.01, 1.33)	**0.03**
Neutrophil count (x 10^{-9}/L)	1.23 (1.02, 1.49)	**0.03**
Lymphocyte count (x 10^{-9}/L)	1.5 (0.96, 2.36)	0.08
Complement C3 (g/L)	1.82 (0.55, 6.01)	0.3
Complement C4 (g/L)	1.60 (0.02, 158.62)	0.8
eGFR (mL/min)	0.98 (0.96, 0.99)	**0.01**

SLE factor	Plaque presence Odds ratio (95% CI)	*p*-value
Glucocorticoid therapy ever	1.79 (0.68, 4.74)	0.2
Duration of glucocorticoid therapy (years)	1.04 (1.00, 1.08)	**0.05**
Average daily prednisolone dose in past 6 months (mg)	1.04 (0.99, 1.10)	0.1
Antimalarial therapy ever	0.6 (0.27, 1.33)	0.2
HCQ therapy ever	0.66 (0.32, 1.36)	0.3
Azathioprine therapy ever	1.62 (0.80, 3.26)	0.2
Methotrexate therapy ever	1.86 (0.81, 4.27)	0.1
Cyclophosphamide therapy ever	0.87 (0.29, 2.56)	0.8

Table 5.6. Final multivariate model comparing CV and SLE factors with the presence of carotid plaque

Factor	β-coefficient (SE)	OR (95% CI)	*p*-value
CV Factor			
Previous history of CVD	1.586 (0.600)	4.88 (1.51, 15.82)	**0.008**
Smoker ever	1.442 (0.505)	4.230 (1.57, 11.39)	**0.004**
Hypertension	1.104 (0.525)	3.02 (1.08, 8.44)	**0.04**
Postmenopausal	- 1.357 (0.723)	0.26 (0.06, 1.06)	0.06
SLE Factor			
Age at SLE diagnosis (years)	0.158 (0.044)	1.17 (1.07, 1.28)	**< 0.0001**
Disease duration (years)	0.160 (0.050)	1.17 (1.06, 1.30)	**0.001**
Average daily prednisolone dose in past 6 months (mg)	0.065 (0.034)	1.07 (1.00, 1.14)	0.06
Anti-AnxA5 GPL +	1.445 (0.786)	4.24 (0.91, 19.76)	0.07

Figure 5.1. ROC curve for the final age-adjusted model comparing CV and SLE factors with the presence of carotid plaque



Area under the ROC curve for CV factors only = 0.86 (0.80, 0.93)

Area under the ROC curve for CV + SLE factors = 0.89 (0.84, 0.94)

Discussion

This study was conducted on a cohort of mainly British Caucasian women with SLE. Compared with Ahmad et al.'s original SLE cohort[85], this cohort was similar with respect to the clinical features of median age at SLE diagnosis, previous cerebrovascular and coronary events, those receiving current glucocorticoid therapy, those ever aCL and /or LA positive, and SLEDAI-2K and SLICC DI scores. However, this cohort differed slightly to Ahmad et al.'s cohort in that all patients fulfilled at least 4 updated ACR classification criteria, compared with 96% of Ahmad's cohort. There was also a slightly higher prevalence of APS (17.3% vs 10.5%). Patients were younger in this cohort (median age 49 vs 53 years) and disease duration was shorter (median 9.5 vs 11.7 years), according to the study design. The frequencies of aCL and LA in this group were similar to the overall prevalence reported in other studies[41, 42, 443]. However, the prevalence of APS (17.3%) in this SLE group was slightly lower compared with other studies which have reported a prevalence of between 23% and 42%[41, 42, 443]. The differences may reflect possible selection bias from specialist APS cohorts.

As shown in Table 5.2, compared with the older, sex-matched controls, SLE patients had similar CV risk factors, apart from higher mean diastolic BPs and lower HDL-C and fasting glucose levels. There was also a trend towards lower TC concentrations. The lower HDL-C and possibly lower TC concentrations may reflect the "lupus pattern" of dyslipidaemia, as discussed in detail in chapter 4. There was a higher proportion of SLE patients with a history of CVD in this study (16.7% of patients vs 3.4% of controls, $p = 0.08$). As the SLE group was younger than the control group, this finding supports a previous report of CVEs occurring at an earlier age in SLE[278], as well as results of other studies suggesting that SLE-related factors may be related to CVD in SLE patients[293, 294].

Table 5.3 showed a significant association between aCL GPL with APS, with significant associations for 5 of the other 7 aPL tested. There were significant associations of aCL GPL, anti-β_2GPI GPL, anti-AnxA5 GPL and anti-PT MPL with arterial thrombosis, and a trend for an association of anti-β_2GPI GPL with venous thromboembolism. ACL GPL, anti-β_2GPI GPL, anti-AnxA5 GPL and anti-AnxA5 MPL were significantly associated with cerebrovascular events, but none of the aPL tested were associated with IHD. The lack of association of aPL with IHD in this study is consistent with the lack of association of aCL with IHD reported in chapter 3. APL levels are known to fluctuate, with higher levels occurring at times of increased disease activity[61]. As the aPL we studied were tested at a single time point in this cohort with mainly low disease activity (median SLEDAI-2K score 2), the prevalence of aPL was low, ranging from 3.8% to 12.2%, in contrast to the higher incidence of persistent aCL GPL and MPL (25.9% and 16.7% respectively, Table 5.1). It is likely that with repeated testing over time, more frequent associations of these aPL with thrombo-embolic events may become apparent.

Since apoA-I is a component of HDL, it was not surprising that HDL-C concentrations were strongly correlated with apoA-I concentrations ($p < 0.001$). ACL GPL levels were negatively correlated with apoA-I ($p = 0.01$), confirming the findings in chapter 4 of negative correlations of aCL GPL levels with HDL_3-C and apoA-I concentrations. There were also negative correlations of other GPLs with apoA-I, with anti-β_2GPI GPL showing the strongest correlation. As cardiolipin is a component of HDL and may be bound to β_2GPI within HDL, it is possible that these aPL may have pathogenic effects on apoA-I. Moreover, antibodies from SLE sera directed against HDL and apoA-I have been shown to cross-react with cardiolipin[399], and anti-apoA-I antibodies have been associated with anti-β_2GPI GPL in SLE[401], providing further support for this hypothesis.

The significant associations of known CV risk factors with carotid plaque as shown in Table 5.4 were not unexpected in this cohort of women, of whom 54.5% were postmenopausal.

The univariate analysis of SLE factors compared with the presence of carotid plaque revealed that carotid plaque was associated with older age at SLE diagnosis, higher SLICC DI scores, previous arterial thrombosis, aCL MPL, higher white cell and neutrophil counts, lower eGFR and longer duration of glucocorticoid therapy. These findings are consistent with the univariate analysis from Ahmad et al.'s previous study[85]. The trend for an association of aCL GPL with carotid plaque in this study (p = 0.06) most likely reflects the slight differences in patient characteristics of this cohort compared with Ahmad et al.'s cohort.

The multivariate model comparing CV and SLE factors with the presence of carotid plaque confirmed the importance of the known CV factors of previous CVD, smoking and hypertension as independent risk factors in SLE atherogenesis. This model had a good predictive accuracy (AUC ROC curve 0.89). The SLE factors of older age at disease diagnosis and longer disease duration are consistent with Ahmad et al.'s previous results, however their previous finding of the presence of aCL and/or LA as an independent risk factor was no longer retained in this multivariate model and instead, anti-AnxA5 GPL was retained as a contributory factor (p = 0.07). This result supports Cederholm et al.'s hypothesis that AnxA5 acts as a stabiliser of atherosclerotic plaque as well as their report demonstrating that aCL from SLE sera inhibited AnxA5 binding[444]. The trend towards a reduced likelihood of postmenopausal women developing plaque in the final model (p = 0.06) suggests that the association of postmenopausal status with plaque in the univariate analysis was mainly due to age. When corrected for this factor, it would appear that accelerated atherosclerosis occurs in younger women, confirming Ahmad et al.'s previous findings and those of other authors[85, 278]. The trend for an association of plaque with higher average prednisolone doses during the last 6 months is consistent with a previous report of increased glucocorticoid use being associated with carotid plaque in SLE[348].

In this study, the association of aCL and/or LA with carotid plaque was no longer significant, compared with the previously published report[85], which may be due to the smaller size of the study sample. Other studies have found no significant associations of aPL with carotid plaque[86, 350, 364, 372]. However, these studies defined aCL positivity according to the APS classification criteria of moderate to high levels for aCL or anti-β_2GPI[44]. Where a definite APS diagnosis is required, the APS criteria would be most appropriate, however the recommended cut-off levels for a positive aPL result are likely to be too high for aPL to be a useful biomarker in the setting of atherosclerosis. Ahmad et al. used lower cut-off levels for aCL positivity, which may have contributed to their results, and this study further explored other aPL associations with subclinical atherosclerosis. Another possible explanation for the differences in results is that varying aPL specificities may manifest at different stages of the atherosclerotic process in SLE, resulting in variable results, which may account for the association of anti-AnxA5 GPL with carotid plaque.

Figure 5.1 shows both ROC curves for the final multivariate models of CV factors only, and CV and SLE factors combined, compared with the presence of plaque. Although the ROC curve for traditional CV risk factors only had a good predictive accuracy (AUC ROC curve = 0.86), the addition of SLE-related factors increased the predictive accuracy of the model further (AUC ROC curve = 0.89). The finding that the combination of both traditional CV and SLE-related factors are important factors associated with the presence of carotid plaque supports previous reports that CV factors alone do not fully explain the increased atherosclerotic risk of SLE patients, and that SLE-related factors are also important[275, 279, 288, 293, 294].

Conclusions

This cross-sectional study confirmed the associations of known CV risk factors with the presence of carotid plaque. The trend towards an association of anti-

AnxA5 GPL with the presence of carotid plaque suggests a possible pathogenic role for this antibody, perhaps by inhibiting the stabilising the effect of AnxA5 on plaque surfaces.

CHAPTER 6

Antiphospholipid antibodies as predictors of accelerated atherosclerosis in SLE

Background

The prevalence of subclinical atherosclerosis is increased in SLE and associated with both traditional CV risk factors and SLE-related factors. In a previous cross-sectional study, Ahmad et al. demonstrated that the SLE-related factors of azathioprine therapy, increased neutrophil count , a history of previous coronary and/or cerebral events, and persistent aCL and/or LA were independently associated with the presence of carotid plaque[85]. In contrast, the association of aPL with carotid plaque has not been confirmed in other studies[86, 350, 364, 372]. APL have been shown to be associated with CVEs[83, 84], however, thrombotic events occur only occasionally, despite the persistence of aPL[64], and pro-atherogenic effects of aPL may be involved. The pro-atherogenic effects of anti-β_2GPI have indeed been demonstrated in in-vitro studies[92, 94, 96-99]. However, it is unknown whether aPL with differing specificities may be able to act as markers of subclinical atheromatous progression in SLE.

Aim

To determine the associations of carotid plaque progression with baseline CV and SLE disease-related factors, including aCL, anti-β_2GPI, anti-AnxA5 and anti-PT antibodies.

Methods

Personal contribution by the candidate

This study was designed by the candidate as a substudy examining baseline aPL subtypes as additional predictor factors for plaque progression for Prof Ian Bruce's longitudinal non-inception SLE cohort study described in chapter 5. The personal contribution by the candidate to this study is outlined in the methods section of chapter 5.

Sample size

Prof Bruce's original study recruited a sample size of 200 SLE patients with 100 controls in order to detect a difference in traditional and SLE-related factors in patients with carotid plaque compared with those without plaque. As this study was an ancillary study performed afterwards, no power calculations were performed for this study.

Patients

This was a prospective longitudinal study of subjects from Ahmad et al.'s original cohort of 200 female British Caucasian patients with SLE studied between 2000 and 2003[85], who returned for follow-up assessments between 2006 and 2009. All patients fulfilled at least 4 updated 1997 ACR criteria for SLE in this study. Baseline clinical and laboratory data were collected as described in chapter 5. At the follow-up assessment, clinical information collected included SLE-related clinical and serological factors, traditional CV risk factors, and the development of CVEs during the follow-up period. Baseline aCL GPL and MPL, anti-β_2GPI GPL and MPL, anti-AnxA5 GPL and MPL, and anti-PT GPL and MPL were determined as described in chapter 5.

Vascular assessments

Patients underwent baseline and follow-up B-mode Doppler ultrasound scans of their carotid arteries, using a standard protocol as described in chapter 5. Follow-up scans were performed by the same operators who had performed the baseline scans. Clinical information and the previous carotid scan results for any individual patient were unknown to the operator at the time of the second scan.

Determination of carotid plaque progression

Patients without carotid plaque at baseline and follow-up were defined as having 'no plaque', patients with plaque at baseline and the same number of plaques at follow-up were defined as having 'stable plaque', and patients with plaque at baseline and more plaques at follow-up were defined as having 'more plaque'. Patients with fewer plaques at follow-up compared with baseline were also considered to have stable plaque. Plaque progression was defined as an increase in number of plaques, adjusted for baseline plaque value and time between scans.

Statistical analysis

Statistical analysis was carried out using the SPSS Statistics 17.0 software package (IBM Corporation, Armonk, NY, USA). Comparison of continuous data was carried out using the Mann–Whitney U-test. For categorical data, the chi-square test was employed. Statistical significance was set at a p-value of < 0.05. Logistic regression analysis adjusted for age at baseline was used to determine associations between baseline predictor variables and plaque progression. Univariate associations with significance $p < 0.02$, as well as known risk factor variables, were used to select the covariates for backwards stepwise logistic regression. Multivariate models were adjusted for baseline plaque value.

Results

Of the original 200 patients from Ahmad et al.'s cohort[85], 127 patients (63.5%) were reassessed with follow-up carotid scans by Haque et al[445]. For this study, a further 8 patients were excluded, as 2 patients met 3 of the updated 1997 ACR criteria for SLE, and baseline serum samples were not available for 6 patients. The mean (SD) age of the 119 patients studied was 54.7 (9.3) years at the time of the follow-up assessment and the mean (SD) time interval between baseline and follow-up assessments was 5.2 (0.8) years.

Table 6.1 shows the characteristics of patients who were re-assessed in this study (n = 119) compared with those who were not re-assessed (n = 79). Re-assessed patients were older (median age 49 vs 46, p = 0.04) compared with those who were not reassessed, with a trend for more postmenopausal women (58.8% vs 45.6%, p = 0.07). Re-assessed patients had higher systolic BPs at baseline (median systolic BP 128 mm Hg vs 124 mm Hg, p = 0.02), less malar rash (42.0% vs 58.2%, p = 0.03), with trends for fewer ACR criteria (5 vs 6, p = 0.07) and more anti-malarial use (86.6% vs 77.2%, p = 0.09).

Cardiovascular events during the follow-up period

Eleven of the 119 patients (9.2%) had CVEs following their baseline assessment. Three of these patients had pre-existing CVD at baseline. Two patients had a subsequent MI and 5 patients developed angina. Three patients had a subsequent stroke, with 2 confirmed to be thrombotic in nature. One of the 3 patients with strokes had a TIA 6 months prior to her stroke. One patient developed PVD. There were no associations of subsequent CVEs with the presence of carotid plaque at baseline (OR 2.50, 95% CI 0.71, 8.85, p = 0.2).

Figure 6.1 shows the proportions of patients who developed new carotid plaques over the follow-up period. There were 87 (73.1%) patients who were free of plaque at baseline. New plaques developed in 31 patients over the study period (35.6%, 26.1% of the total cohort). There were 32 (26.9%) patients with plaque at

baseline, and 20 of these patients developed more plaque (62.5%, 16.8% of total cohort). Overall, plaque progressed in 51 (42.9%) patients, with a plaque progression rate of 8.2% per annum. Fifty-six (47.0%) patients remained free of carotid plaque at follow-up. The mean (SD) age at these patients was 49.7 (8.5) years, with mean (SD) age at SLE diagnosis of 33.4 (10.0) years and median (IQR) disease duration of 11.0 (13.5) years. Twenty-three of the patients who remained plaque-free (41.1%) were post-menopausal.

Four (3.4%) patients had plaque regression. These patients had a median (IQR) age of 61 (23) years, with median (IQR) age at SLE diagnosis of 33 (27) years and disease duration of 12.5 (20.2) years at baseline. All 4 patients had a history of hypertension and were taking antihypertensive medications both at baseline and at follow-up. Antihypertensive medications included ACE-inhibitors, calcium-channel antagonists and diuretics, with 3 patients taking a combination of these medications. Two patients were taking aspirin at baseline and neither was taking a statin. The remaining 8 (6.7%) patients had no change in their plaque status at follow up. Baseline antihypertensive therapy was associated with stable plaque (OR 5.38, 95% CI 1.51, 19.23, $p = 0.008$) and plaque regression ($p = 0.008$) at follow-up.

Associations of CV factors with plaque progression

Table 6.2 shows the age-adjusted associations of baseline CV risk factors with plaque progression. Significant factors included older age and lower HDL-C concentrations. There were positive associations that did not reach significance for increased TC : HDL ratio, hypertension, cumulative smoking exposure (cigarette pack-years) and previous cerebrovascular event.

Associations of SLE-related factors with plaque progression

Table 6.3 shows the age-adjusted associations of baseline SLE factors with plaque progression. The only significant factor was the presence of anti-AnxA5

GPL (p = 0.03). There were similar positive associations approaching significance for hydroxychloroquine therapy, the presence of aCL GPL, anti-β_2GPI GPL and anti-dsDNA.

Table 6.4 shows the multivariate model (adjusted for baseline plaque value) comparing baseline CV and SLE factors with plaque progression. The significant CV factors retained in the model included older age, previous cerebrovascular event, lower HDL-C concentrations and a protective effect for anti-hypertensive therapy. Contributory factors retained in the model included statin therapy and smoking history (pack-years). Fewer patients with a family history of premature IHD were likely to develop plaque progression. The significant SLE factors retained in the model included hydroxychloroquine and cyclophosphamide therapy, and the presence of anti-AnxA5 GPL. The presence of a haematological disorder was also retained in the model, although it was not significant.

Figure 6.2 shows the 2 ROC curves for CV factors only and for CV and SLE factors combined. The AUC ROC curve for CV factors was 0.87, with an improvement in the AUC ROC curve to 0.91 after SLE factors were added to the final model.

Table 6.1A. Comparisons of baseline demographics and CV risk factors between SLE patients re-assessed and not re-assessed

CV risk factors	Re-assessed (n=119)*	Not re-assessed (n=79)*	*p*-value
Median (IQR) age at baseline (years)	49 (14)	46 (18)	**0.04**
Postmenopausal	70 (58.8)	36 (45.6)	0.07
Smoker ever	54 (45.4)	42 (53.2)	0.3
Hypertension	39 (32.8)	23 (29.1)	0.6
Median (IQR) systolic BP (mm Hg)	128 (30)	124 (24)	**0.02**
Median (IQR) diastolic BP (mm Hg)	78 (12)	76 (13)	0.3
Diabetes mellitus	4 (3.4)	2 (2.5)	1.0
Median (IQR) fasting glucose (mmol/L)	4.5 (0.6)	4.6 (0.6)	0.3
Median (IQR) TC (mmol/L)	5 (1.7)	5.2 (1.6)	0.5
Median (IQR) HDL-C (mmol/L)	1.6 (0.7)	1.5 (0.7)	0.5
Median (IQR) TG (mmol/L)	1 (0.6)	1.2 (0.8)	0.1
Median (IQR) BMI (kg/m^2)	25.6 (7.3)	26.1 (5.8)	0.6
Family history of IHD	31 (26.9)	26 (32.9)	0.9
Presence of carotid plaque	32 (27.1)	82 (31.7)	0.4

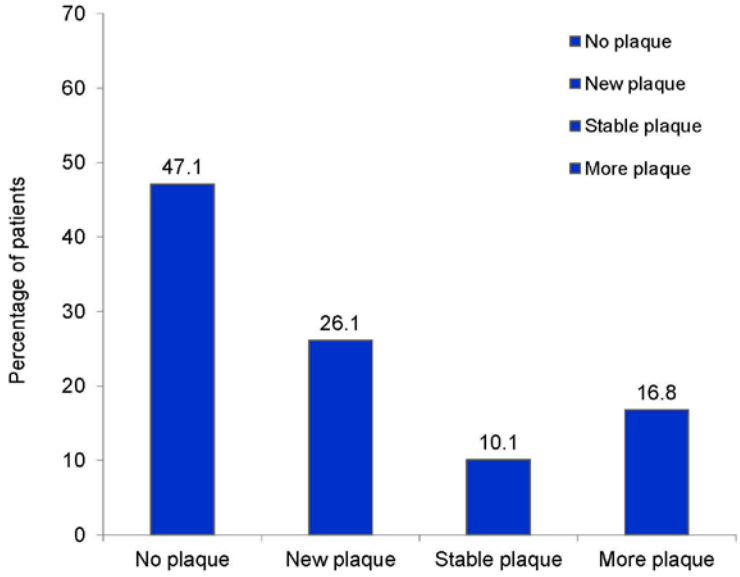
*variables are presented as n (%) unless indicated otherwise

Table 6.1B. Comparisons of baseline SLE-related factors between SLE patients re-assessed and not re-assessed

SLE-related risk factors	Re-assessed (n=119)*	Not re-assessed (n=79)*	*p*-value
Median (IQR) disease duration (years)	10 (14)	8 (12)	0.1
Median (IQR) no. of ACR criteria	5 (2)	6 (2)	0.07
Malar rash	50 (42.0)	46 (58.2)	**0.03**
Discoid rash	9 (7.6)	10 (12.7)	0.2
Serositis	50 (42.0)	31 (39.2)	0.7
Arthritis	102 (85.7)	67 (84.8)	0.9
NP-SLE	8 (6.7)	6 (7.6)	0.2
Renal disease	16 (13.4)	16 (20.3)	0.2
Haematological disorder	99 (83.2)	63 (79.7)	0.5
Median (IQR) eGFR (mL/min)	76.1 (26.1)	82.5 (30.4)	0.2
Previous arterial thromboembolism	12 (10.1)	10 (12.7)	0.6
Previous venous thromboembolism	16 (13.4)	10 (12.7)	0.9
Anti-dsDNA +	68 (57.1)	47 (59.5)	0.7
Anti-Ro/SSA +	43 (36.1)	27 (34.2)	0.8
ACL and/or LA +	42 (35.3)	30 (38.0)	0.7
ACL+	37 (31.1)	24 (30.4)	0.9
Median (IQR) SLEDAI	1 (4)	2 (3.5)	0.9
Median (IQR) SLICC DI	0 (1)	0 (2)	0.5
Glucocorticoid therapy ever	93 (78.2)	66 (83.5)	0.3
Antimalarial therapy ever	103 (86.6)	61 (77.2)	0.09

*variables are presented as n (%) unless indicated otherwise

Figure 6.1. Change in carotid plaque in SLE patients at follow-up assessment



- **No plaque:** no plaque at both baseline and follow up (n = 56, 47.1%))
- **New plaque:** no plaque at baseline, but plaque present at follow up (n = 31, 26.1%)
- **Stable plaque:** plaque present at baseline and not increased at follow up (n = 12, 10.1%)
- **More plaque:** plaque present at baseline, with increased plaque number at follow up (n = 20, 16.8%)

Table 6.2. Age-adjusted associations of CV risk factors with plaque progression

Baseline CV risk factor	Plaque progression age-adjusted OR (95% CI)	*p*-value
Age (years)	1.15 (1.08, 1.22)	**< 0.0001**
Postmenopausal	1.16 (0.36, 3.70)	0.8
TC (mmol/L)	0.92 (0.64, 1.33)	0.7
HDL-C (mmol/L)	0.28 (0.10, 0.79)	**0.02**
TC : HDL-C ratio	1.41 (0.96, 2.07)	0.08
TG (mmol/L)	1.27 (0.63, 2.56)	0.5
LDL-C (calculated) (mmol/L)	1.11 (0.73, 1.69)	0.6
ApoB (mg/dL)	0.70 (0.09, 5.76)	0.7
ApoA-I (mg/dL)	0.27 (0.04, 2.08)	0.2
ApoB : apoA-I ratio	1.79 (0.19, 17.19)	0.6
Current smoker	1.11 (0.33, 3.75)	0.9
Smoker ever	1.99 (0.86, 4.63)	0.1
Smoking history (pack-years)	1.05 (1.00, 1.10)	0.07
Hypertension	0.44 (0.17, 1.16)	0.1
Systolic BP (mm Hg)	1.00 (0.98, 1.03)	0.9
Diastolic BP (mm Hg)	1.00 (0.96, 1.04)	1.0
eGFR (mL/min)	1.01 (0.99, 1.03)	0.6
Fasting glucose (mmol/L)	0.83 (0.52, 1.33)	0.4
Diabetes mellitus	0.72 (0.08, 6.12)	0.8
BMI (kg/m^2)	1.03 (0.95, 1.10)	0.5
Metabolic syndrome	1.04 (0.38, 2.89)	0.9
Family history of premature IHD	0.57 (0.20, 1.66)	0.3
Previous history of CVD	1.51 (0.46, 4.89)	0.5
Previous cerebrovascular event	1.81 (0.57, 5.72)	0.3
History of IHD	1.05 (0.17, 6.61)	1.0
Antihypertensive therapy	0.56 (0.21, 1.46)	0.2
Statin therapy	3.01 (0.72, 12.57)	0.1

Table 6.3. Age-adjusted associations of SLE factors with plaque progression

SLE factor	Plaque progression age-adjusted OR (95% CI)	*p*-value
Age at SLE diagnosis (years)	1.01 (0.97, 1.05)	0.7
Disease duration (years)	0.99 (0.95, 1.04)	0.7
Rash	0.71 (0.31, 1.63)	0.4
Serositis	0.75 (0.32, 1.77)	0.5
Arthritis	0.68 (0.19, 2.39)	0.5
Renal disease	0.85 (0.25, 2.94)	0.8
NP-SLE	1.00 (0.21, 4.78)	1.0
Haematological disorder	2.02 (0.62, 6.62)	0.2
SLEDAI -2K	0.90 (0.73, 1.10)	0.3
SLICC DI	1.10 (0.75, 1.59)	0.6
Ever aCL GPL+	2.30 (0.86, 6.16)	0.1
Ever aCL MPL+	2.23 (0.73, 6.84)	0.2
Ever aCL+	2.13 (0.82, 5.52)	0.2
Ever LA+	1.07 (0.36, 3.17)	0.9
Ever aCL and/or LA+	1.94 (0.77, 4.89)	0.2
ACL MPL+	1.54 (0.24, 9.98)	0.7
ACL GPL+	1.13 (0.24, 5.32)	0.9
Anti-β_2GPI MPL+	0.79 (0.10, 6.30)	0.8
Anti-β_2GPI GPL+	4.54 (0.74, 27.93)	0.1
Anti-PT MPL+	1.05 (0.11, 10.22)	1.0
Anti-PT GPL+	2.29 (0.42, 12.42)	0.3
Anti-AnxA5 MPL+	2.00 (0.18, 22.55)	0.6
Anti-AnxA5 GPL+	4.69 (1.15, 19.16)	**0.03**
Antiphospholipid syndrome	1.27 (0.38, 4.18)	0.7
Previous arterial thromboembolism	2.19 (0.47, 10.18)	0.3
Previous venous thromboembolism	1.87 (0.57, 6.16)	0.3
ANA+	1.41 (0.14, 14.54)	0.8
Anti-dsDNA+	2.11 (0.85, 5.25)	0.1
Anti-Ro/SSA+	1.46 (0.62, 3.41)	0.4
Anti-La/SSB+	1.28 (0.43, 3.85)	0.7
Anti-U1RNP+	1.14 (0.34, 3.85)	0.8
Anti-Sm+	-	1.0
White cell count (x 10^{-9}/L)	1.01 (0.84, 1.22)	0.9
Neutrophil count (x 10^{-9}/L)	1.04 (0.80, 1.34)	0.8
Lymphocyte count (x 10^{-9}/L)	0.92 (0.51, 1.66)	0.8
Complement C3 levels (g/L)	1.65 (0.39, 6.96)	0.5
Complement C4 levels (g/L)	4.81 (0.02, 1160.44)	0.6
eGFR (mL/min)	1.01 (0.99, 1.03)	0.6

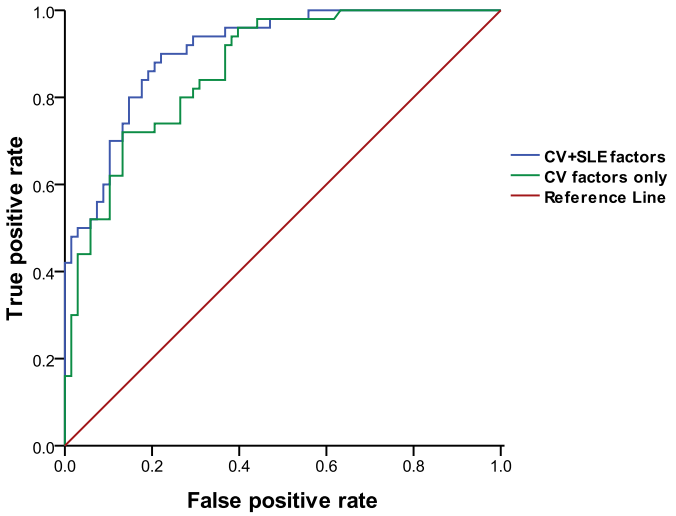
SLE factor	Plaque progression age-adjusted OR (95% CI)	*p*-value
Glucocorticoid therapy ever	0.76 (0.27, 2.13)	0.6
Duration of glucocorticoid therapy (years)	1.02 (0.97, 1.07)	0.5
Average daily prednisolone dose in past 6 months (mg)	1.01 (0.94, 1.09)	0.7
HCQ therapy ever	2.41 (0.94, 6.21)	0.07
Azathioprine therapy ever	1.12 (0.47, 2.69)	0.8
Methotrexate therapy ever	1.19 (0.42, 3.35)	0.7
Cyclophosphamide therapy ever	1.55 (0.45, 5.41)	0.5

Table 6.4. Final multivariate model comparing baseline CV and SLE factors with plaque progression*

Baseline factor	β-coefficient (SE)	OR (95% CI)	*p*-value
CV Factor			
Age	0.245 (0.055)	1.28 (1.15, 1.42)	**< 0.0001**
Previous cerebrovascular event	3.801 (1.530)	44.75 (2.23, 897.13)	**0.01**
HDL-C	- 1.927 (0.789)	0.15 (0.03, 0.68)	**0.02**
Anti-hypertensive therapy	- 1.877 (0.787)	0.15 (0.03, 0.72)	**0.02**
Statin therapy	2.477 (1.288)	11.91 (0.96, 148.56)	**0.05**
Family history of premature IHD	- 1.495 (0.778)	0.22 (0.05, 1.03)	0.06
Smoking history (pack-years)	0.069 (0.036)	1.07 (1.00, 1.15)	0.06
SLE Factor			
HCQ therapy ever	1.670 (0.681)	5.31, (1.40, 20.16)	**0.01**
Cyclophosphamide therapy ever	2.281 (1.037)	9.79 (1.28, 74.74)	**0.03**
Anti-AnxA5 GPL+	2.527 (1.207)	12.52 (1.18, 133.37)	**0.04**
Haematological disorder	1.371 (0.733)	3.94 (0.94, 16.58)	0.06

*adjusted for baseline plaque value

Figure 6.2. ROC curve for both final models comparing plaque progression with CV and SLE factors



Area under the ROC curve for CV factors only = 0.87 (0.81, 0.93)

Area under the ROC curve for CV + SLE factors = 0.91 (0.86, 0.96)

Discussion

Of Ahmad et al.'s original cohort of 200 women with SLE[85], 81(40.5%) were not included in this study. Haque et al. assessed 127 of the original patients from Ahmad et al.'s cohort at a median interval of 5.8 years later[445]. As patients were recruited from a mainly urban area with a significant amount of migration, 42 of the original 73 patients not assessed were lost to follow-up. A further 20 patients declined to participate in the follow-up assessment, largely because of social reasons. Ten patients (5%) died during the follow-up period. Causes of death included malignancy in 4 patients (cervical, intracerebral, lung and liver), 1 ruptured aortic aneurysm, 1 cerebral haemorrhage, 1 gastric haemorrhage and 1 suicide. The cause of death was unknown for 2 patients. This study assessed 119 patients, as baseline serum samples were not available for 6 patients and 2 patients met 3 of the updated ACR criteria for SLE. There is a risk of selection bias occurring with this proportion of patients lost to follow up, however, Haque et al. found no differences in clinical features of the patients who were followed up in their study (n = 127) compared with those who were not followed up (n = 73)[445]. The patients re-assessed in this study were older (median age 49 vs 46, p = 0.04) compared with those who were not reassessed, with a trend for more postmenopausal women (58.8% vs 45.6%, p = 0.07). Re-assessed patients had higher systolic BPs at baseline (median systolic BP 128 vs 124, p = 0.02), less malar rash (42.0% vs 58.2%, p = 0.03), with trends for fewer ACR criteria features (5 vs 6, p = 0.07) and more anti-malarial use (86.6% vs 77.2%, p = 0.09). Given the older age and higher baseline systolic BPs of the re-assessed group, it is possible that re-assessed patients may have had an overall increased CV risk, compared with patients who were not re-assessed. Although the re-assessed group had less malar rash, this factor was unlikely to affect atherosclerotic progression and overall the SLE-related clinical features for both groups were similar, making selection bias less likely. Furthermore, Gladman et al. reported that in their cohort of SLE patients, of whom 39.8% were potentially lost to follow-up, there were no SLE disease characteristics that led to loss of follow up[446].

There was no significant association of the presence of carotid plaque at baseline with subsequent CVEs. Carotid plaque has been shown to be an independent predictor of future CVEs in the general population, where large patient numbers were followed for up to a mean of 7.2 years[345, 346]. The most likely explanation for the lack of association of carotid plaque at baseline with subsequent CVEs in this study is the relatively short time interval between baseline and follow-up scans, and the small numbers of CVEs (11/119, 9.2%) occurring during the follow-up period in this relatively small study sample.

Plaque progression occurred in 51 (42.9%) of the 119 patients, with a plaque progression rate of 8.2% per annum. In comparison, in the EVA study, a French general population-based study of 1010 subjects aged between 59 – 71 years at baseline, plaque progression occurred in 14.8% of women over 4 years, with a plaque progression rate of 3.7% per annum[447]. Although there may be differences in ethnicity and CV risk factors for the EVA study cohort compared with this study cohort, it would appear that the progression of plaque observed in this study was far greater than would be expected in the general population. Moreover, the mean age of the patients in this study at baseline was 49 years, providing further evidence for acceleration of the atherosclerotic process in SLE. There have been 4 other longitudinal studies of plaque progression in SLE. Thompson et al. studied 217 patients with SLE for a mean follow-up duration of 4.2 years and reported a plaque progression rate of 6.4% per annum[353]. Two other North American studies were conducted over an average of 2 years and reported higher plaque progression rates of 10% - 12% per annum[267, 367]. The differences in plaque progression rates may be due to other study cohorts comprising younger patients (mean ages 37 - 45.1 years) with different ethnicities, and including males. Variations in carotid artery measurement protocols may have also accounted for the differences. Overall, our plaque progression rate of 8.2% per annum was comparable to the rates reported in other studies.

Four (3.4%) patients had plaque regression at follow-up and 8 patients (6.7%) had no change in their plaque status at follow-up (stable plaque). All 4 patients with plaque regression had hypertension and were taking antihypertensive medications both at baseline and at follow-up. Antihypertensive medications included ACE-inhibitors, calcium-channel antagonists and diuretics, with 3 patients taking a combination of these medications. Antihypertensive therapy was significantly associated with plaque regression in these patients. These findings are consistent with previous reports of the associations of calcium-channel antagonist therapy with carotid plaque regression and ACE-inhibitors modifying carotid IMT progression in the general population[448-450]. Two patients with plaque regression were also taking aspirin at the baseline assessment. It is possible that operator error accounted for plaque regression readings, however given the high intra-class correlation coefficient for repeat ultrasound assessments, this explanation seems less likely.

In the age-adjusted analyses, plaque progression was associated with known baseline CV risk factors of older age and lower HDL-C concentrations. The classic risk factors of hypertension, cumulative smoking exposure (pack-years), increased TC : HDL ratio and previous cerebrovascular events demonstrated positive associations approaching significance. The only significant age-adjusted baseline SLE-factor was the presence of anti-AnxA5 GPL (p = 0.03). Hydroxychloroquine therapy was the only other factor that approached significance (p = 0.07).

In the multivariate model, older age at baseline, lower HDL-C concentrations, previous cerebrovascular events and antihypertensive therapy were retained as independent risk factors. A reduced HDL-C concentration is a strong independent predictor for future CVD and CV-related mortality in the general population[389-391]. As lower HDL-C concentrations are a typical feature of the "lupus pattern" of dyslipidaemia, a pattern associated with increased disease activity[368, 373, 374], it is possible that SLE patients with low HDL-C may have a higher CV risk compared with their counterparts in the general population.

Furthermore, the inverse association of HDL-C with plaque progression supports the hypothesis that inflammatory mechanisms in SLE promote acceleration of atherogenesis in SLE. HDL-C may therefore prove to be a more useful marker of CV risk for patients with SLE than the commonly recommended TC : HDL ratio. The known independent CV risk factors of older age, previous cerebrovascular events and contributory factor of increased tobacco exposure highlight the requirement for aggressive management of CV risk factors in patients with SLE. The significant protective effect of antihypertensive therapy and weaker protective effect of statin therapy emphasise the benefits of controlling hypertension and hyperlipidaemia in this group of patients with increased CV risk. The finding of plaque progression being associated with fewer patients with a family history of premature IHD (p = 0.06) suggests that SLE-related factors may have more impact on CV risk when compared with certain CV risk factors.

In the final multivariate model, both hydroxychloroquine therapy and cyclophosphamide therapy were retained as independent factors associated with carotid plaque progression. Hydroxychloroquine has been shown to be protective against thrombosis[69] and to have a beneficial effect on survival in SLE[70, 229, 268, 269]. As immunosuppressant therapy is prescribed for SLE patients with active and/or severe disease, both immunosuppressive agents hydroxychloroquine and cyclophosphamide are likely to be markers of a subset of SLE patients with persistent disease activity and/or disease severity, providing further support for pathogenic SLE-related inflammatory or autoimmune mechanisms in accelerated atherosclerosis. The presence of a SLE-related haematological disorder as a weaker predictor of plaque progression also supports the pathogenic role of autoimmune mechanisms in atherogenesis in SLE.

Anti-AnxA5 GPL was found to be an independent predictor of carotid plaque progression in the final multivariate model (OR 12.52, p = 0.04). Although samples were only tested once for anti-AnxA5, its association with cerebrovascular events was still significant in the univariate analyses. It is noteworthy that anti-AnxA5 GPL was an independent baseline predictor of

carotid plaque progression in chapter 6, suggesting a possible mechanism for the association of anti-AnxA5 with cerebrovascular events. Since aPL levels are known to fluctuate[451], one would expect that with repeated testing over time, more frequent associations of novel aPL with thrombo-embolic events may become apparent. In support of this novel finding, anti-AnxA5 GPL was also positively associated with the presence of carotid plaque in chapter 5, although this finding did reach significance (OR 4.24, $p = 0.07$). AnxA5 is thought to act as a stabiliser of atherosclerotic plaque and aCL from SLE sera have been demonstrated to inhibit AnxA5 binding[124]. Future studies may delineate the exact role of anti-AnxA5 GPL in atherogenesis, whether as a low avidity marker of the presence of AnxA5 in association with increasing plaque burden, or as a truly pathogenic antibody through the inhibition of AnxA5 binding to atherosclerotic plaque at sites prone to rupture, as previously demonstrated by Cederholm et al[124]. Furthermore, the association of anti-AnxA5 with plaque progression may explain in part the discordant reports of a positive association of aCL and/or LA with the presence of carotid plaque in Ahmad et al.'s study[85], but negative findings in other studies[86, 350, 364, 372]. The possibility of aPL with differing specificities influencing different stages of atherogenesis remains intriguing, and future studies may provide further information. Our results overall provide further support for the utility of anti-AnxA5 GPL as a biomarker for subclinical atherosclerosis.

Figure 6.2 shows both ROC curves for the final multivariate models of CV factors and CV + SLE factors compared with plaque progression. Although the ROC curve for traditional CV risk factors had a good predictive accuracy (AUC ROC curve = 0.87), the addition of SLE-related factors increased the predictive accuracy of the model further (AUC ROC curve = 0.91). The finding that the combination of both traditional CV and SLE-related factors are important predictors of subclinical atherosclerosis progression supports previous reports that CV factors alone do not fully explain the increased atherosclerotic risk of SLE patients, and that SLE-related factors are also important predictors[275, 279, 288, 293, 294].

Conclusions

In this longitudinal study of British Caucasian women with SLE, subclinical atherosclerosis progressed at a faster rate than would be expected for the general population. This study also supported the findings of recent studies, which overall showed similar rates of carotid plaque progression in patients with SLE. The importance of traditional CV risk factors affecting the progression of subclinical atherosclerosis was highlighted in this study, and HDL-C may be a particularly useful marker in this regard. Furthermore, SLE-related risk factors such as immunosuppressant therapy with hydroxychloroquine and cyclophosphamide in association with plaque progression support the role of inflammatory and autoimmune mechanisms in accelerated atherogenesis. The finding of the novel marker of anti-AnxA5 GPL as an independent predictor of carotid plaque progression supports the pathogenic role of aPL in atherogenesis. Further studies are required to confirm the utility of anti-AnxA5 GPL as a novel marker of subclinical atherosclerosis in SLE.

CHAPTER 7

Conclusions

The previous chapters described the research undertaken to identify potential serological and other biomarkers in SLE. This chapter summarises and discusses the results, conclusions, limitations and implications of the studies, as well as proposals for future research.

1. Summary of results

1.1. ACPA as a marker of "rhupus"

In chapter 2, we found that 12 of 104 patients (11%) had EA, of whom 6 had major erosions and 6 had minor erosions. Seven patients with EA also met the 1987 ACR criteria for RA. Four of the 6 patients with major erosions were ACPA+ and 3 of these patients were homozygous for the *SE*. Most of the patients with EA had a haematological disorder and over half had skin involvement, serositis and were anti-dsDNA+ and anti-U1RNP+. Patients with overlapping features of both SLE and RA may be defined as belonging to a "rhupus" subset of SLE and ACPA was a marker of this in chapter 2. Further support for this was provided by Damián-Abrego et al., who found that all 9 rhupus patients in their study were positive for ACPA[423]. Moreover, 2 other studies have reported the presence of ACPA in their rhupus patients[421, 431]. Apart from polyarthritis, clinical features that occur more frequently in rhupus patients include malar rash, DLE, photosensitivity, LN, anaemia, leucopenia, and thrombocytopenia[191]. Most of these features were also present in our EA patients. As 5 of the 8 ACPA+ patients in this study were carriers of the *SE*, pathogenic ACPAs appear to be due to a dose effect of the *SE*[204, 205], and *HLA-DR* genotyping may provide further predictive information for EA. Furthermore, all 6 patients with major erosions were carriers of the SE-associated allele, *HLA-*

*DQB1*0302*. These associations were similar to the known associations of specific MHC class II alleles (including the SE and *HLA-DQB1*0302*[433-435]).

1.2. TC : HDL-C ratio as a marker of CV risk in SLE

The TC : HDL-C ratio is used as an important criterion for CVD risk assessment in the general population and the JBS 2 guidelines recommend active treatment of CV risk factors if this ratio is $\geq$ 6.0[418]. In chapter 4, the TC : HDL-C ratio was an independent predictor for future CVEs and mortality, confirming its importance as a CV risk factor in SLE. In chapter 5, the TC : HDL-C ratio was not significantly associated with the presence of carotid plaque. In chapter 6, there was a positive association with plaque progression in the univariate analysis, although this did not reach statistically significance (OR = 1.41, 95% CI 0.96, 2.07, p = 0.08). Although our results were overall inconclusive with respect to the TC : HDL-C ratio as a marker of subclinical atherosclerosis, this remains an important CV marker both in the general population and in SLE.

1.3. HDL-C as a marker of CV risk in SLE

In chapter 4, the concentrations of HDL-C and its components HDL_2-C and HDL_3-C were significantly lower in patients with SLE compared with controls. Moreover, the prevalence of below accepted normal concentrations of HDL-C in SLE patients was 53.7%. HDL-C was not significantly associated with CVEs or mortality in the small group of SLE patients studied, although this has been reported in the general population[389-391]. In chapter 5, HDL-C was not associated with the presence of carotid plaque, however, in chapter 6, lower HDL-C concentrations were inversely associated with carotid plaque progression (OR 0.15, p = 0.02), suggesting that it may be more important as a marker of subclinical atherosclerosis.

1.4. ApoA-I and atherosclerosis in SLE

Chapter 4 showed that SLE patients were more likely to have low apoA-I concentrations compared with controls. In chapters 4 and 5, aCL GPL levels were negatively correlated with apoA-I concentrations in both Bath and Manchester SLE cohorts. Our findings support Lahita et al.'s previous observation of lower HDL-C and apoA-I levels in aCL GPL positive patients with SLE[400]. Delgado Alves et al. demonstrated that antibodies directed against HDL and apoA-I from SLE sera also cross-reacted with cardiolipin[399]. Other studies have shown that higher IgG anti-HDL and anti-apoA-I titres were associated with increased disease activity and damage, and remained elevated during persistent disease activity[374, 403]. Moreover, chapter 5 also showed inverse correlations of other GPLs with apoA-I concentrations, with anti-β_2GPI GPL showing the strongest correlation. As cardiolipin is a component of HDL and may be bound to β_2GPI within HDL, it is possible that these aPL may have pathogenic effects on apoA-I. Furthermore, antibodies from SLE sera directed against HDL and apoA-I have been shown to cross-react with cardiolipin[399], and anti-apoA-I antibodies have been associated with anti-β_2GPI GPL in SLE[401], providing further support for this hypothesis.

1.5. The apoB : apoA-I ratio as a marker of CVD risk in SLE

Recent studies have demonstrated that the apoB : apoA-I ratio may be a more reliable predictor of IHD risk than the TC : HDL ratio[419, 420]. As lower apoA-I concentrations occur in SLE, the apoB : apoA-I ratio may be a useful marker of CVD risk in this disease. However, in chapter 4, although this ratio was a predictor for subsequent CVEs in the univariate analysis, it was not an independent predictor in the multivariate analysis. Furthermore, in chapters 5 and 6, there were no significant associations of apoB, apoA-I or the apoB : apoA-I ratio with carotid plaque at baseline or with plaque progression. Our results overall are inconclusive for the apoB : apoA-I ratio as a marker of CV risk.

1.6. APL as markers of cerebrovascular events

In chapter 4, increased aCL GPL levels were associated with future CVEs. In chapters 3 and 5, we found an association of aCL GPL with cerebrovascular events in both the Bath and Manchester cohorts. The positive association of aCL GPL with cerebrovascular events confirms previous studies of aPL as predictors of CV events[83, 84]. However, in both cohorts, there were no associations of aCL with IHD. These results confirm the previous findings of Petri et al. in the Hopkins Lupus Cohort, that showed significant associations of aCL with stroke and TIA, but no association with MI[49, 61]. In chapter 5, anti-β_2GPI GPL, anti-AnxA5 GPL and anti-AnxA5 MPL were significantly associated with cerebrovascular events, however none of the aPL tested were associated with IHD. The lack of associations of anti-β_2GPI, anti-AnxA5 or anti-PT with IHD found in chapter 5 is consistent with previous reports of a lack of association of aCL with IHD in SLE.

1.7. Lp(a) as a predictor of mortality

In chapter 4, Lp(a) was an independent predictor of mortality in patients with SLE. Elevated levels of Lp(a) have been detected in patients with SLE[414-416], however to our knowledge, this is the first study showing Lp(a) as a novel independent predictor for mortality in SLE.

1.8. ACL as predictors of mortality

The 5- and 10-year survival results for both Bath cohorts from chapters 3 and 4 were comparable to the survival rates of recent SLE cohorts[223, 224, 246]. However, 10-year survival for both Bath cohorts was significantly reduced compared with the expected survival for age- and sex- matched cohorts in the general UK population. This confirms previous reports that patients with SLE have an increased mortality risk compared with the general population[50, 225-228]. Of note, in chapter 3, reduced 10-year survival occurred in the aCL+ and aCL GPL+ patient groups, whereas 10-year survival was not reduced in the aCL- group,

suggesting that aCL may have a weak or indirect effect on mortality. Although there was no direct association of aCL with mortality in the univariate analysis in chapter 3, the hypothesis that aCL has an influence on mortality was supported in the final multivariate model, where aCL GPL was retained as a contributory factor (OR 6.29, p = 0.06). Moreover, higher aCL GPL levels were independently associated with mortality in chapter 4 (OR 1.04, p = 0.05).

In chapter 3, 5 of the 10 deceased patients died from IHD-related causes and one patient from a presumed IHD-related cause at the age of 84. In chapter 4, of the known causes of death in 13 deceased patients, 6 were due to IHD-related causes. These results confirm the importance of atherosclerosis as a major cause of death in SLE[227, 229, 236]. Of the patients who died of IHD-related causes, patients from the cohort in chapter 3 had disease durations of at least 11 years and patients from the cohort in chapter 4 had disease durations of at least 9 years at the time of death. These results also confirm Urowitz et al.'s "bimodal mortality pattern" of late deaths from CV-related causes[231].

1.9. Anti-AnxA5 GPL as a predictor of subclinical atherosclerosis

In chapter 5, anti-AnxA5 GPL was associated with the presence of carotid plaque in the univariate analysis, although this finding was not statistically significant (OR 2.52, 95% CI 0.95, 6.71, p = 0.06). However, anti-AnxA5 GPL was retained as a contributory factor in the multivariate model (OR 4.24, 95% CI 0.91, 19.76, p = 0.07), which suggests that this association is of probable clinical significance. In chapter 6, anti-AnxA5 GPL was found to be an independent predictor of carotid plaque progression (OR 12.52, 95% CI 1.18, 133.37, p = 0.04). To our knowledge, this is the first study demonstrating anti-AnxA5 GPL as a predictor of carotid plaque progression. This result also supports Cederholm et al.'s hypothesis that AnxA5 acts as a stabiliser of atherosclerotic plaque, as well as their report demonstrating that aCL from SLE sera inhibited AnxA5 binding[124].

2. Study limitations and strengths

There are several limitations related to the studies presented in this thesis.

2.1. Chapter 2

This study was designed as a pilot study, hence power and study sample calculations were not performed. However, there were more patients tested for ACPA in our study than those previously tested in Mediwake et al.'s report (n = 104 vs n = 66 respectively)[187]. Moreover we employed second generation ACPA ELISAs for our study, compared with Mediwake et al.'s first generation ACPA tests. This reduced the probability of a Type I error (false rejection of the null hypothesis) occurring.

EA is an uncommon feature of lupus arthritis and affected 12 (11%) of our study group of 104 SLE patients. As this was a retrospective study, incomplete clinical information may have affected the study results. In order to reduce bias, we used clinical and x-ray data related to the dates of the samples tested, however it is possible that some serum samples may not have been available for testing. In addition, as x-rays were only requested based on clinical decisions, it is possible that some patients with EA have been missed. Hence the strength of association of ACPA with EA in our study may be conservative.

A major strength of this study is that it identifies a group of patients with characteristics of "rhupus" who are ACPA+ve. Another major strength is that it provides insight into the possible immunogenetic mechanisms of MHC class II associations with ACPA that may be shared between RA and EA in SLE. Recent reports have shown that ACPA predicts for erosive arthritis in RA and that SE alleles are associated with the development of ACPA[205, 452]. The genetic analyses were also performed on small subgroups of patients with specific *HLA-DR* or -*DQ* alleles. Although the probability of a Type I error exists due to the small numbers in our study and we were unable to demonstrate statistically significant

associations between SE alleles and ACPA, our findings are still consistent with these observations in RA and of also potential clinical importance.

Another major strength of this study is that patients were followed for a long period of time. Median [IQR] disease duration for patients with EA was longer (20 [10] years) than for those NEA (9 [6] years) (Table 2.1). The mean [SD] time to the earliest erosion detected on x-ray was 11.3 [6.8] years (Table 2.3). The results of this study are likely indicative of the true prevalence of EA and ACPA in long-standing SLE, and may be important for understanding the progression of minor erosions.

2.2. Chapter 3

Subjects for this retrospective pilot study were derived from a database of patients attending the RNHRD CTD clinic. As no inception cohort was used, survivor bias and/or immortal time bias may have been introduced. It is possible that patients with more severe disease outcomes or events (including death) were prevented from attending the RNHRD CTD clinic and therefore excluded from this study. However, the numbers of such patients would be very small and unlikely to affect our results. Nevertheless, it is possible that both CVEs and mortality were under-reported in this study, which may partly account for the lack of association of aPL with IHD and the non-significant association of aPL with cerebrovascular disease. These results are likely to be conservative, since previous studies have demonstrated significant associations of aPL with CVEs in SLE[83, 84].

Other study limitations include possible non-responder bias arising from the 60% response rate to the questionnaire. For respondents, questionnaire responses may have been affected by their memory (recall bias) and inaccurate responses may have been given to lifestyle questions such as weight or smoking habits (attention bias). This may have resulted in under-reporting of factors or events, thus providing conservative results.

As this study was designed as a pilot study, power and study sample calculations were not performed. There was incomplete data available from medical records and SLE questionnaires with respect to the dates of onset for predictor factors and CVEs. Due to these limitations, it was not possible to perform Kaplan-Meier or Cox-proportional hazards analyses. The decision was therefore made by the candidate to utilise unconditional binary logistic regression to compare predictor factors with the outcome variables of CVEs and mortality. For the same reasons, the χ^2 Goodness-of-fit test was employed to compare survival of SLE subgroups with age- and sex-matched UK population survival data. Unconditional logistic regression analysis reduces the probability of obtaining positive results. Therefore our results demonstrating the associations of aPL with mortality are likely to be conservative.

Another limitation of this study is the small study numbers, which increases the probability of a Type I error occurring with respect to aPL associations with mortality. However, our study results support those of previous studies that showed the associations of aCL and APS with early mortality[259, 270, 271].

The laboratory cut-off levels for aCL were lower than the levels considered clinically significant for a diagnosis of APS ($\geq$ 14U/mL for aCL GPL and $\geq$ 10U/mL for MPL for the study, compared with > 40U GPL or MPL for APS). From the evidence presented in Chapter 1, aPL are also involved in non-thrombotic, auto-immune mechanisms in the pathogenesis of atherosclerosis. Although higher aPL levels are considered important for the diagnosis of APS, this requirement may not be applicable in the setting of atherosclerosis. The decision to use the lower laboratory cut-off levels for aCL was based on the hypothesis that the persistence of aPL is an important factor influencing atherogenesis, a chronic inflammatory condition, thereby increasing CVE and mortality risks. However, the use of lower cut-off levels for aCL in this study may have increased the probability of a Type I error occurring and may also partly explain the lack of association found between aCL and IHD.

The results from this study include the reduced 10-year survival of aCL+ve patients, the association of CVEs with mortality and the contribution of aCL to mortality in the multivariate analyses. Taken together, these results support the hypothesis that aCL-associated pathogenic mechanisms contribute to early mortality, which is the major strength of this study.

2.3. Chapter 4

As this study was designed as a pilot study, power and study sample calculations not performed. The possible biases outlined above for chapter 3 also apply to this chapter, namely survivor bias for the study cohort, and nonresponder, recall or attention bias for the questionnaires. These factors would give conservative results for this study.

As for chapter 3, due incomplete data for the dates of onset of predictor factors and CVEs, it was not possible to perform Kaplan-Meier or Cox-proportional hazards analyses. Therefore unconditional binary logistic regression was used to compare predictor factors with outcome variables of CVEs and mortality.

In this study, SLE patients had median (IQR) TC concentrations of 4.97 (2.13) mmol/L and LDL-C of 3.39 (1.87) mmol/L. In comparison, the healthy control group had median (IQR) TC concentrations of 5.96 (1.9) mmol/L and LDL-C of 3.93 (1.87) mmol/L. Although the control group's TC and LDL-C concentrations would be considered unusually high today, they were not so during the time of the initial study between 1992 and 1993. Control lipoprotein levels were comparable with lipoprotein levels from a Scottish population-based study of 10 359 subjects in 1990[453], which reported mean TC concentrations of 6.1 - 6.5 mmol/L for men and 6.3 - 6.9 mmol/L for women. The British Hyperlipidaemia Association's 1993 guidelines defined severe hyperlipidaemia as TC > 7.8 mmol/L, fasting TG > 4.5 mmol/L, or HDL-C < 1.0 mmol/L. Moreover, the guidelines recommended initiating cholesterol-lowering therapy only at TC > 7.8

mmol/L or LDL-C > 6.0 mmol/L for asymptomatic males or postmenopausal females, the contemporaneous equivalents of our study controls[454]. Taking the above-mentioned criteria for hyperlipidaemia into consideration, it is possible that the higher TC and LDL-C concentrations in the healthy controls may have increased the probability of a Type II error (acceptance of the false null hypothesis), and may partly account for the results of similar VLDL-C levels between SLE and controls, with possibly exaggerated differences between groups for TC, LDL-C and HDL-C. In the SLE patient group, 48.1% had TC $\geq$ 5.0 mmol/L and 61.1% had LDL-C > 3.0 mmol/L. Notwithstanding the healthy control results, SLE patient lipoprotein levels in this study are comparable with the results of Petri et al.'s study in 1992, in which 51% of SLE patients without IHD and 94% with IHD had TC > 5.18 mmol/L[276]. Furthermore, there was a trend towards a higher TC in the Manchester control group in chapter 5 (p = 0.06), which suggests that the results of chapter 4 are not unexpected.

Another limitation of this study is its retrospective nature. There was incomplete data available on patients' glucocorticoid therapy. Glucocorticoid use is associated with raised TC and LDL-C concentrations in SLE[383, 455]. It is possible that absent or low-dose glucocorticoid therapy in this group of SLE patients may have also resulted in lower TC and LDL-C concentrations compared with the healthy controls.

The major strength of this study is the long period of follow-up for this group of SLE patients. It confirms that 10-year survival in SLE patients is significantly reduced compared with the normal UK population and that CV-related mortality is a major cause of death in SLE. Furthermore, the results suggest that TC : HDL-C ratio and Lp(a) may add prognostic value in assessing future mortality risk for patients with SLE.

2.4. Chapters 5 and 6

Power calculations not performed for the studies in chapters 5 and 6, as both were ancillary studies performed according to the design of the original study that was first undertaken between 2000 and 2003. The protocol for the follow-up study undertaken between 2006 and 2009 was the same as the initial study. According to the study design, all SLE patients were female of British Caucasian ethnicity. These results therefore cannot be generalised to other races or males.

Chapter 5 described a cross-sectional study of patients with established SLE. As this study did not have an inception cohort, survivor and/or immortal time bias may have been introduced, which would increase the probability of a Type I error occurring when compared with controls. Ahmad et al.'s original study was designed to include a healthy control group whose mean age (53 years) was higher than that of the SLE group (48 years, $p < 0.01$)[85]. This took into account the fact that the prevalence of carotid plaque in young healthy controls is extremely low. By including older controls in the study, this reduced the probability of a Type I error occurring, but then increased the probability of a Type II error. However, as the study found that the prevalence of carotid plaque was increased in SLE patients in all age groups including younger patients, these results were conservative and therefore both statistically significant and clinically menaingful.

The cut-off levels for aCL for both studies were lower than those considered clinically significant for a diagnosis of APS ($\geq$ 16U aCL GPL and $\geq$ 16U MPL compared with > 40 GPL or MPL for APS). The decision to use the lower laboratory cut-off levels for aCL was based on the evidence to date on the non-thrombotic roles of aPL in atherogenesis, such as the associations of aPL with endothelial perturbation and EC apoptosis[326]. High aCL levels are required for the diagnosis of APS because this increases the likelihood of detecting pathogenic aCL causing a thrombotic event. However, in the setting of atherosclerosis, a chronic inflammatory condition, lower aCL levels may still be useful predictors of subclinical atherosclerosis, given their known effects in the

chronic atherogenic process. The low aCL levels used in the study may have increased the probability of a Type I error occurring. However with respect to the associations of aPL subtypes with CVEs (Table 5.3), the expected associations of APS with aCL, anti-β_2GPI, anti-AnxA5 and anti-PT GPL were still detected, reducing the likelihood of a Type I error.

Patient serum samples were only tested once for anti-AnxA5 GPL. Since aPL levels are known to fluctuate with time[451] and the diagnostic criteria for APS include persistently positive aPL at least 3 months apart (Appendix 1.4), single aPL testing increases the probability of a Type I error occurring. AnxA5 binds endothelium, improving endothelial function[123] and probably stabilises atherosclerotic plaque via the formation of 2D shield that inhibits coagulation[115, 124]. A pathogenic effect of anti-AnxA5 is to inhibit AnxA5 binding to endothelium, thereby causing endothelial dysfunction and an unstable atherosclerotic plaque that is prone to rupture. Chapter 5 suggests a potentially clinically significant association of anti-AnxA5 GPL with the presence of carotid plaque. Likewise, chapter 6 demonstrates a statistically and clinically significant association of anti-AnxA5 GPL with carotid plaque progression, which not only provides proof of the concepts above, but also suggests that it may be a useful clinical prognostic marker for accelerated atherosclerosis. This is the major strength of both studies. Our results are likely to be conservative given our relatively small study number and larger studies would be able to determine the significance of these findings. Repeated testing over time of anti-AnxA5 would also provide further information on the association of anti-AnxA5 with markers of subclinical atherosclerosis.

3. Implications

3.1. ACPA as a marker of "rhupus"

Chapter 2 shows that ACPA, particularly in high titres, predicts major erosive arthritis in SLE. Furthermore, this study's findings support those of others that suggest that ACPA is not as specific a marker for RA as previously thought, but a marker for a phenotype of EA that is mediated by ACPA. Furthermore, ACPA appears to be a useful marker for the rhupus subset in SLE. The major clinical implication of this study is requesting ACPA is worthwhile for a SLE patient presenting with synovitis, since it is an easily accessible test. A positive ACPA result, particularly in a high titre, predicts EA and this information is useful when the physician is considering whether to take more a aggressive approach with respect to disease-modifying therapy for lupus arthritis. Moreover, the genetic markers that are associated with progression of erosions in RA provide further information on the subset of SLE with specific genetic and antibody features, the "rhupus" subset.

3.2. Assessment of CV risk in SLE

SLE patients are at increased risk of developing accelerated atherosclerosis. However, not all subgroups of SLE patients are at risk, as shown in chapter 6, where 47.0% of female SLE patients remained free of carotid plaque at follow-up and 41.1% of these women were post-menopausal. Furthermore, patients with SLE are unlikely to have an increased CV risk compared with the general population if a standard CV risk assessment is undertaken according to current guidelines[293, 418]. Hence a more accurate prediction of CVD risk for a patient with SLE would require an overall CV risk assessment, with the addition of novel risk factors in the assessment.

3.2.1. TC : HDL-C ratio

According to the JBS 2 guidelines[418], the TC : HDL-C ratio is an important factor in CVD risk assessment for the general UK population. In chapter 4, the TC : HDL-C ratio was an independent predictor for future CVEs and mortality, confirming its importance as a CV risk factor in SLE. In chapter 5, the ratio was not significantly associated with the presence of carotid plaque. In chapter 6, the ratio had an association with plaque progression of probable clinical significance in the univariate analysis (OR 1.42, 95% CI 0.96, 2.09, $p = 0.08$), although it was not an independent association in the multivariate analysis. As a marker of subclinical atherosclerosis, the TC : HDL-C ratio appears to be less useful in patients with SLE. Nevertheless, as this ratio is calculated from a fasting lipid profile and is a simple and cost-effective clinical tool, it could easily be included as part of regular CV risk assessment in routine clinical monitoring of SLE patients. Although overall, our results were inconclusive with respect to the TC : HDL-C ratio as a marker of subclinical atherosclerosis, this remains an important CV marker in the general population and should be considered in SLE.

3.2.2. HDL-C

Low HDL-C concentrations reflect the "lupus pattern" of dyslipidaemia, which is enhanced with increased disease activity[368]. The inverse association of HDL-C with plaque progression in chapter 6 supports the hypothesis that inflammatory mechanisms in SLE promote acceleration of atherogenesis in SLE. The negative correlation of HDL_3-C with aCL GPL in chapter 4 provides further support for the pathogenic role of SLE-related autoimmune mechanisms in accelerated atherosclerosis. Hence HDL-C may prove to be a useful marker of CV risk in SLE patients with persistent disease activity and should be included as a risk factor in the overall CV risk assessment of patients with SLE.

3.2.3. Lp(a)

Elevated Lp(a) concentrations independently predict for IHD, ischaemic stroke and coronary mortality in the general population, although the effect is relatively weak (adjusted RR 1.1 for all outcomes)[409]. Elevated levels of Lp(a) have been detected in patients with SLE[414-416]. In chapter 4, Lp(a) was an independent predictor of mortality in patients with SLE. As Lp(a) levels are not influenced by disease activity or glucocorticoid therapy[415], Lp(a) may be a useful factor in combination with other factors in the overall CV risk assessment of SLE patients.

3.2.4. Anti-AnxA5 GPL

In chapter 5, the association of anti-AnxA5 GPL with the presence of carotid plaque approached statistical significance, a finding that is of probable clinical significance. In chapter 6, anti-AnxA5 GPL was an independent predictor of carotid plaque progression. Both these findings provide support for anti-AnxA5 GPL as a pathogenic aPL in atherogenesis. Moreover, these results provide support for the hypothesis that aPL with differing specificities influence the overall clinical effects of aPL at different stages of atherogenesis. This hypothesis may provide an explanation for the discordant reports of a positive association of aCL and/or LA with the presence of carotid plaque in Ahmad et al.'s study[85], but negative findings in other studies[86, 350, 364, 372].

4. Perspectives for future research

The findings from the studies presented in this thesis, including the study limitations discussed above, form the basis for the suggestions outlined below regarding future research.

4.1. Lupus arthritis

Chapter 2 provides insight into the possible immunogenetic mechanisms of MHC class II associations with ACPA that may be shared between RA and EA in SLE. Given the small study numbers in this study, future studies with larger subject numbers may be able to confirm the association of SE alleles with ACPA production in the setting of lupus arthritis.

In chapter 2, one patient with major erosions had low ACPA levels and was also negative for the SE. Recent genetic studies have demonstrated that several SNPs at the *IRF5, STAT4*, *BLK* and *TNFAIP3* loci are shared by SLE and RA[171, 210, 211]. In mice, interference with the function of the *TNFAIP3* protein product A20 resulted in a destructive, erosive polyarthritis[212]. The *IRF5* locus was also found to be shared by patients with SLE and the RF-negative polyarthritis subtype of JIA[213]. These studies suggest that the pathogenesis of arthritis in SLE involves at least several complex immunological pathways that do not involve the MHC or ACPA-related pathways. Future studies could aim to determine the associations of these immunogenetic pathways with arthritis and other clinical subsets of SLE.

4.2. Predictors of CV risk and mortality in SLE

4.2.1. Study populations

Chapters 2, 3 and 4 studied SLE patients from a database of patients attending the RNHRD CTD clinic. Future studies could be extended to include SLE patients from multiple centres, and be designed as prospective inception cohorts of patients with early disease. The studies presented in chapters 5 and 6 were limited to women of mainly British Caucasian descent. Future studies could be extended to include men, more SLE patients with early disease, and individuals of other ethnicities.

4.2.2. Lipoproteins

The studies presented in this thesis found associations of the TC : HDL-C ratio with CVEs and a possible association with carotid plaque progression. HDL-C levels were inversely associated with carotid plaque progression in chapter 6. Future studies with larger patient numbers could further examine whether the TC : HDL-C ratio and HDL-C could be useful as specific markers of subclinical atherosclerosis, and/or markers of future CVEs and mortality. The apoB : apoA-I ratio was a possible predictor of CVEs in chapter 4. As this ratio predicts IHD risk in the general population[419, 420], larger studies could explore the apoB : apoA-I ratio as a potential marker of future CVEs or subclinical atherosclerosis in SLE. Lp(a) was a predictor of mortality in chapter 4. Further studies could also confirm the utility of Lp(a) as a marker of CV risk and mortality in SLE.

4.2.3. ACL GPL

In chapter 4, increased aCL GPL levels were associated with future CVEs and increased mortality. It is difficult to conduct clinical studies to determine auto-antibodies as CV predictive factors, a fact borne out by the very few studies published to date. In atherosclerosis, pathogenic auto-antibodies such as aCL GPL may only play a mechanistic role in complex auto-immune processes involving multiple inflammatory mediators, so that their direct clinical associations may be difficult to appreciate. Future studies examining CVE risk or mortality outcomes could focus on aPL with known actions and specificities in the atherosclerotic process, such as anti-β_2GPI.

4.2.4. Anti-AnxA5 GPL

Anti-AnxA5 GPL was associated with carotid plaque progression in chapter 6 and probably associated with the presence of carotid plaque in chapter 5. Further studies could provide more information on the role of anti-AnxA5 in atherogenesis, whether as a marker of the presence of increased AnxA5 levels in association with increased plaque, or as a truly pathogenic antibody, via

inhibition of AnxA5 binding. Furthermore, future studies could further test the hypothesis that aPL with differing specificities influence the overall clinical effects of aPL at different stages of atherogenesis. Finally, larger studies are required to confirm the utility of anti-AnxA5 GPL as a marker of subclinical atherosclerosis in SLE.

In summary, the challenge for future research remains to find biomarkers with clear pathogenic processes in SLE that can predict future clinical outcomes. Larger studies are required to confirm the preliminary results presented in this thesis.

Conclusion

As SLE is a heterogeneous disease, it is unlikely that any single biomarker will be applicable to all patients. The research carried out for this thesis identified ACPA as a marker of a phenotype of SLE with EA and features of RA - "rhupus". All 6 patients with major erosions were carriers of the *SE*-associated allele, *HLA-DQB1*0302*. This research also identified several serological markers of CV risk - increased TC : HDL-C ratio and anti-AnxA5 GPL. Lower HDL-C concentrations were also a marker of CV risk. Strategies to incorporate these new markers into clinical CV risk assessments may be useful to distinguish the subset of SLE patients most at risk of developing accelerated atherosclerosis. Furthermore, aCL GPL and Lp(a) were identified as markers of mortality risk. Future studies may be able to provide further information on the pathogenic effects of these markers and their potential utility in routine clinical practice.

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APPENDIX

1. Disease Classification Criteria

1. 1. 1997 Update of the 1982 American College of Rheumatology (ACR) revised criteria for classification of systemic lupus erythematosus[8, 425]*

1. Malar Rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions
3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by physician
5. Nonerosive Arthritis	Involving 2 or more peripheral joints, characterised by tenderness, swelling, or effusion
6. Pleuritis or Pericarditis	1. Pleuritis - convincing history of pleuritic pain or rubbing heard by a physician or evidence of pleural effusion *OR* 2. Pericarditis - documented by electrocardiogram or rub or evidence of pericardial effusion
7. Renal Disorder	1. Persistent proteinuria > 0.5 grams per day or > 3+ if quantitation not performed *OR* 2. Cellular casts - may be red cell, haemoglobin, granular, tubular, or mixed
8. Neurologic Disorder	1. Seizures - in the absence of offending drugs or known metabolic derangements; e.g., uremia, ketoacidosis, or electrolyte imbalance *OR* 2. Psychosis - in the absence of offending drugs or known metabolic derangements, e.g., uraemia, ketoacidosis, or electrolyte imbalance
9. Haematologic Disorder	1. Haemolytic anemia - with reticulocytosis *OR* 2. Leucopenia - < 4,000/mm3 on ≥ 2 occasions *OR* 3. Lymphopenia - < 1,500/ mm3 on ≥ 2 occasions *OR* 4. Thrombocytopenia - <100,000/mm3 in the absence of offending drugs
10. Immunologic Disorder	1. Anti-DNA: antibody to native DNA in abnormal titre *OR* 2. Anti-Sm: presence of antibody to Sm nuclear antigen *OR* 3. Positive finding of antiphospholipid antibodies on: 3.1. an abnormal serum level of IgG or IgM anticardiolipin antibodies 3.2. a positive test result for lupus anticoagulant using a standard method *OR* 3.3. a false-positive test result for at least 6 months confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test
11. Positive Antinuclear Antibody	An abnormal titre of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs

***** Available from: http://www.rheumatology.org/practice/clinical/forms [Accessed 9 March 2012].

1.2. 2010 ACR/European League Against Rheumatism (EULAR) classification criteria for rheumatoid arthritis[192, 194]***

	Score
Target population (Who should be tested?): **Patients** **1. who have at least 1 joint with definite clinical synovitis (swelling)*** **2. with the synovitis not better explained by another disease**† **Classification criteria for RA (score-based algorithm: add score of categories A–D;** **a score of ≥ 6/10 is needed for classification of a patient as having definite RA**‡	
A. Joint involvement§	
1 large joint¶	0
2 - 10 large joints	1
1 - 3 small joints (with or without involvement of large joints)#	2
4 - 10 small joints (with or without involvement of large joints)	3
> 10 joints (at least 1 small joint)**	5
B. Serology (at least 1 test result is needed for classification)††	
Negative RF *and* negative ACPA	0
Low-positive RF or low-positive ACPA	2
High-positive RF or high-positive ACPA	3
C. Acute-phase reactants (at least 1 test result is needed for classification)‡‡	
Normal CRP and normal ESR	0
Abnormal CRP or abnormal ESR	1
D. Duration of symptoms§§	
< 6 weeks	0
≥ 6 weeks	1

* The criteria are aimed at classification of newly presenting patients. In addition, patients with erosive disease typical of rheumatoid arthritis (RA) with a history compatible with prior fulfillment of the 2010 criteria should be classified as having RA. Patients with longstanding disease, including those whose disease is inactive (with or without treatment) who, based on retrospectively available data, have previously fulfilled the 2010 criteria should be classified as having RA.

† Differential diagnoses vary among patients with different presentations, but may include conditions such as systemic lupus erythematosus, psoriatic arthritis, and gout. If it is unclear about the relevant differential diagnoses to consider, an expert rheumatologist should be consulted.

‡ Although patients with a score of < 6/10 are not classifiable as having RA, their status can be reassessed and the criteria might be fulfilled cumulatively over time.

§ Joint involvement refers to any *swollen* or *tender* joint on examination, which may be confirmed by imaging evidence of synovitis. Distal interphalangeal joints, first carpometacarpal joints, and first

metatarsophalangeal joints are *excluded from assessment*. Categories of joint distribution are classified according to the location and number of involved joints, with placement into the highest category possible based on the pattern of joint involvement.

[¶] "Large joints" refers to shoulders, elbows, hips, knees, and ankles.

[#] "Small joints" refers to the metacarpophalangeal joints, proximal interphalangeal joints, second through fifth metatarsophalangeal joints, thumb interphalangeal joints, and wrists.

** In this category, at least 1 of the involved joints must be a small joint; the other joints can include any combination of large and additional small joints, as well as other joints not specifically listed elsewhere (e.g., temporomandibular, acromioclavicular, sternoclavicular, etc.).

[††] Negative refers to IU values that are less than or equal to the upper limit of normal (ULN) for the laboratory and assay; low-positive refers to IU values that are higher than the ULN but ≤3 times the ULN for the laboratory and assay; high-positive refers to IU values that are >3 times the ULN for the laboratory and assay. Where rheumatoid factor (RF) information is only available as positive or negative, a positive result should be scored as low-positive for RF. ACPA = anti-citrullinated protein antibody.

[‡‡] Normal/abnormal is determined by local laboratory standards. CRP = C-reactive protein; ESR = erythrocyte sedimentation rate.

[§§] Duration of symptoms refers to patient self-report of the duration of signs or symptoms of synovitis (e.g. pain, swelling, tenderness) of joints that are clinically involved at the time of assessment, regardless of treatment status.

*** Available from: http://www.rheumatology.org/practice/clinical/forms [Accessed 9 March 2012].

1.3. Revised 1987 ACR criteria for the classification of rheumatoid arthritis[193†]

Criterion	Definition
1. Morning stiffness	Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement
2. Arthritis of 3 or more joint areas	At least 3 joint areas simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician. The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints
3. Arthritis of hand joints	At least 1 area swollen (as defined above) in a wrist, MCP, or PIP joint
4. Symmetric arthritis	Simultaneous involvement of the same joint areas (as defined in 2) on both sides of the body (bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry)
5. Rheumatoid nodules	Subcutaneous nodules, over bony prominences, or extensor surfaces, or in juxta-articular regions, observed by a physician
6. Serum rheumatoid factor	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects
7. Radiographic changes	Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localised in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify)

* For classification purposes, a patient shall be said to have rheumatoid arthritis if he/she has satisfied at least 4 or these 7 criteria. Criteria 1 through 4 must have been present for at least 6 weeks. Patients with 2 clinical diagnoses are not excluded. Designation as classic, definite, or probable rheumatoid arthritis is *not* to be made.

† Available from: http://www.rheumatology.org/practice/clinical/forms [Accessed 9 March 2012].

1.4. Revised 2006 classification criteria for the antiphospholipid syndrome[44]

Antiphospholipid antibody syndrome (APS) is present if at least one of the clinical criteria and one of the laboratory criteria that follow are met:*

Clinical criteria

1. Vascular thrombosis[†]

One or more clinical episodes[‡] of arterial, venous, or small vessel thrombosis[§], in any tissue or organ. Thrombosis must be confirmed by objective validated criteria (i.e. unequivocal findings of appropriate imaging studies or histopathology). For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.

2. Pregnancy morbidity

(a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus *OR*

(b) One or more premature births of a morphologically normal neonate before the 34th week of gestation because of:

(i) eclampsia or severe pre-eclampsia defined according to standard definitions, *OR*

(ii) recognised features of placental insufficiency[¶]

OR

(c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.

In studies of populations of patients who have more than one type of pregnancy morbidity, investigators are strongly encouraged to stratify groups of subjects according to a, b, or c above.

Laboratory criteria**

1. Lupus anticoagulant (LA) present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Haemostasis (Scientific Subcommittee on LAs/phospholipid-dependent antibodies).
2. Anticardiolipin (aCL) antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titre (i.e. > 40 GPL or MPL, or > the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA.
3. Anti-β_2 glycoprotein-I antibody of IgG and/or IgM isotype in serum or plasma (in titre > the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA, according to recommended procedures.

* Classification of APS should be avoided if less than 12 weeks or more than 5 years separate the positive aPL test and the clinical manifestation.

† Coexisting inherited or acquired factors for thrombosis are not reasons for excluding patients from APS trials. However, two subgroups of APS patients should be recognised, according to:
(a) the presence, and
(b) the absence of additional risk factors for thrombosis.
Indicative (but not exhaustive) such cases include: age (> 55 in men, and > 65 in women), and the presence of any of the established risk factors for cardiovascular disease (hypertension, diabetes mellitus, elevated LDL or low HDL cholesterol, cigarette smoking, family history of premature cardiovascular disease, body

mass index $\geq$ 30 kg/m^2, microalbuminuria, estimated GFR < 60 mL/min), inherited thrombophilias, oral contraceptives, nephrotic syndrome, malignancy, immobilisation, and surgery. Thus, patients who fulfil criteria should be stratified according to contributing causes of thrombosis.

‡ A thrombotic episode in the past could be considered as a clinical criterion, provided that thrombosis is proved by appropriate diagnostic means and that no alternative diagnosis or cause of thrombosis is found.

§ Superficial venous thrombosis is not included in the clinical criteria.

¶ Generally accepted features of placental insufficiency include:

(i) abnormal or non-reassuring fetal surveillance test(s), e.g. a non-reactive non-stress test, suggestive of fetal hypoxemia,

(ii) abnormal Doppler flow velocimetry waveform analysis suggestive of fetal hypoxemia, e.g. absent end-diastolic flow in the umbilical artery,

(iii) oligohydramnios, e.g. an amniotic fluid index of 5 cm or less, or

(iv) a postnatal birth weight less than the 10th percentile for the gestational age.

** Investigators are strongly advised to classify APS patients in studies into one of the following categories:

I: more than one laboratory criteria present (any combination)

IIa: LA present alone

IIb: aCL antibody present alone

IIc: anti-β_2 glycoprotein-I antibody present alone

2. Disease Activity Measure for Systemic Lupus Erythematosus

2.1. Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K)*

Check box if descriptor is present at the time of visit, or in the proceeding 10 days.

Wt	Present	Descriptor	Definition
8	☐	**Seizure**	Recent onset. Exclude metabolic, infectious or drug cause.
8	☐	**Psychosis**	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucinations, incoherence, marked loose associations, impoverished thought content, marked illogical thinking, bizarre, disorganized, or catatonic behavior. Excluded uremia and drug causes.
8	☐	**Organic Brain Syndrome**	Altered mental function with impaired orientation, memory or other intelligent function, with rapid onset fluctuating clinical features. Include clouding of consciousness with reduced capacity to focus, and inability to sustain attention to environment, plus at least two of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, or increased or decreased psychomotor activity. Exclude metabolic, infectious or drug causes.
8	☐	**Visual Disturbance**	Retinal changes of SLE. Include cytoid bodies, retinal hemorrhages, serious exodate or hemorrhages in the choroids, or optic neuritis. Exclude hypertension, infection, or drug causes.
8	☐	**Cranial Nerve Disorder**	New onset of sensory or motor neuropathy involving cranial nerves.
8	☐	**Lupus Headache**	Severe persistent headache: may be migrainous, but must be nonresponsive to narcotic analgesia.
8	☐	**CVA**	New onset of cerebrovascular accident(s). Exclude arteriosclerosis.
8	☐	**Vasculitis**	Ulceration, gangrene, tender finger nodules, periungual, infarction, splinter haemorrhages, or biopsy or angiogram proof of vasculitis.
4	☐	**Arthritis**	More than 2 joints with pain and signs of inflammation (i.e. tenderness, swelling, or effusion).
4	☐	**Myositis**	Proximal muscle aching/weakness, associated with elevated creatine phosphokinase/adolase or electromyogram changes or a biopsy showing myositis.
4	☐	**Urinary Casts**	Heme-granular or red blood cell casts.
4	☐	**Haematuria**	> 5 red blood cells/high power field. Exclude stone, infection or other cause.
4	☐	**Proteinuria**	> 0.5 gm/24 hours. New onset or recent increase of more than 0.5 gm/24 hours.

Wt	Present	Descriptor	Definition
4	☐	**Pyuria**	> 5 white blood cells/high power field. Exclude infection.
2	☐	**New Rash**	New onset or recurrence of inflammatory type rash.
2	☐	**Alopecia**	New onset or recurrence of abnormal, patchy or diffuse loss of hair.
2	☐	**Mucosal Ulcers**	New onset or recurrence of oral or nasal ulcerations.
2	☐	**Pleurisy**	Pleuritic chest pain with pleural rub or effusion, or pleural thickening.
2	☐	**Pericarditis**	Pericardial pain with at least 1 of the following: rub, effusion, or electrocardiogram confirmation.
2	☐	**Low Complement**	Decrease in CH50, C3, or C4 below the lower limit of normal for testing laboratory.
2	☐	**Increased DNA binding**	> 25% binding by Farr assay or above normal range for testing laboratory.
1	☐	**Fever**	> 38°C. Exclude infectious cause.
1	☐	**Thrombocytopenia**	< 100,000 platelets/mm3.
1	☐	**Leukopenia**	< 3,000 White blood cell/mm3. Exclude drug causes.

***** Available from: http://www.rheumatology.org/practice/clinical/forms [Accessed 9 March 2012].

3. Damage Index for Systemic Lupus Erythematosus

3.1 Systemic Lupus International Collaborating Clinics/ACR Damage Index for Systemic Lupus Erythematosus (SLICC/ACR DI)[a]*

Item	Score
Ocular (either eye, by clinical assessment)	
Any cataract ever	1
Retinal change or optic atrophy	1
Neuropsychiatric	
Cognitive impairment (e.g. memory deficit, difficulty with calculation, poor concentration, difficulty in spoken or written language, impaired performance levels) or major psychosis	1
Seizures requiring therapy for 6 months	1
Cerebrovascular accident ever (score 2 if > 1)	1 (2)
Cranial or peripheral neuropathy (excluding optic)	1
Transverse myelitis	1
Renal	
Estimated or measured glomerular filtration rate<50%	1
Proteinuria ≥3.5 gm/24hours	1
OR	
End-stage renal disease (regardless of dialysis or transplantation)	3
Pulmonary	
Pulmonary hypertension (right ventricular prominence, or loud P2)	1
Pulmonary fibrosis (physical and radiograph)	1
Shrinking lung (radiograph)	1
Pleural fibrosis (radiograph)	1
Pulmonary infarction (radiograph)	1
Cardiovascular	
Angina or coronary artery bypass	1
Myocardial infarction ever (score 2 if > 1)	1 (2)
Cardiomyopathy (ventricular dysfunction)	1
Valvular disease (diastolic murmur, or systolic murmur >3/6)	1
Pericarditis for 6 months, or pericardiectomy	1
Peripheral vascular	
Claudication for 6 months	1
Minor tissue loss (pulp space)	1
Significant tissue loss ever (e.g. loss of digit or limb) (score 2 if > 1 site)	1 (2)
Venous thrombosis with swelling, ulceration, or venous stasis	1

Item	Score
Gastrointestinal	
Infarction or resection of bowel below duodenum spleen, liver, or gall bladder ever, for cause any (score 2 if > 1 site)	1 (2)
Mesenteric insufficiency	1
Chronic peritonitis	1
Stricture or upper gastrointestinal tract surgery ever	1
Musculoskeletal	
Muscle atrophy or weakness	1
Deforming or erosive arthritis (including reducible deformities, excluding avascular necrosis)	1
Osteoporosis with fracture or vertebral collapse (excluding avascular necrosis)	1
Avascular necrosis (score 2 if > 1)	1 (2)
Osteomyelitis	1
Skin	
Scarring chronic alopecia	1
Extensive scarring or panniculum other than scalp and pulp space	1
Skin ulceration (excluding thrombosis) for > 6 months	1
Premature gonadal failure	1
Diabetes (regardless of treatment)	1
Malignancy (exclude dysplasia) (score 2 if > 1 site)	1 (2)

[a]Damage (nonreversible change, not related to active inflammation) occurring since onset of lupus, ascertained by clinical assessment and present for at least **6 months** unless otherwise stated. Repeat episodes must occur at least 6 months apart to score 2. The same lesion cannot be scored twice.

***** Available from: http://www.rheumatology.org/practice/clinical/forms [Accessed 9 March 2012].

4. Royal National Hospital for Rheumatic Diseases SLE Questionnaire

Date: ☐☐/☐☐/☐☐☐☐ CTD ID: ☐☐☐☐☐☐

SYSTEMIC LUPUS ERYTHEMATOSUS QUESTIONNAIRE

Any information you give is confidential and will only be seen by the research team at the Royal National Hospital for Rheumatic Diseases, Bath. The information that you provide will be added to a research database and will contribute to ongoing research by the research team at the Royal National Hospital for Rheumatic Diseases. We would like you to complete this questionnaire as we are interested in finding out about your Lupus as well as your background, general health issues and how you manage your daily activities.

Section 1 - Background data

a) Marital status: ☐ *Married* ☐ *Single* ☐ *Divorced* ☐ *Separated* ☐ *Widowed*

b) Ethnic group:

☐ *British*	☐ *Any other Mixed background*	☐ *African*
☐ *Irish*	☐ *Indian*	☐ *Any other Black background*
☐ *Any other white background*	☐ *Pakistani*	☐ *Chinese*
☐ *White & Black Caribbean*	☐ *Bangladeshi*	☐ *Any other ethnic group*
☐ *White & Black African*	☐ *Any other Asian background*	☐ *I do not wish to answer this question*
☐ *White & Asian*	☐ *Caribbean*	

c) Current employment

☐ *Working full time* ☐ *Working part time* ☐ *Retired due to ill health* ☐ *Retired other reasons* ☐ *Not working by choice* ☐ *Student* ☐ *Other*

When did you last work? ☐☐/☐☐/☐☐☐☐

d) When did your symptoms start? ☐☐☐☐ *Year Symptoms began*

e) When were you diagnosed? ☐☐☐☐ *Year diagnosed with Lupus*

f) Weight ☐☐☐ *kg* ☐ *stone* ☐☐ *lb*

g) Height ☐☐☐ *cm* ☐ *foot* ☐☐ *inches*

Section 2 – Family History

a) Have any of your immediate blood relatives (parents, siblings, children) ever had:
please tick as many as apply and give relationship (E.g. Mother) and age that they were at initial event/diagnosis)

Relation and age at event/diagnosis

E.g. Parental grandfather 56, mother 23, brother 19	**Lupus**	**Stroke**	**Heart attack**
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐

b) Please use space below to provide details of any other family history

Section 3 – Lifestyle

a) Have you ever smoked? ☐ *Current smoker* ☐ *Ex smoker* ☐ *Never smoked*

___ ___ *Years smoked* ___ ___ ___ Average number per day

b) Average time exercising a week? ___ ___ Average number of hours per week

Type of exercise?

c) Have you ever drunk alcohol? ☐ *Currently drink* ☐ *Use to drink* ☐ *Never drunk alcohol*

___ ___ ___ Average number of units per week

Section 4 – Medical History

a) Have you ever had: *(please tick as many as apply)*

☐ *Diabetes* ☐ *High cholesterol* ☐ *High blood pressure*

b) Have you ever had heart problems, blood clots or a stroke? ☐ *Yes* ☐ *No*

if yes please describe below:

c) Have you ever taken:

Steroids? *(If yes, please give details below:)* ☐ *Yes* ☐ *No*

Age when prescribed medication ___ ___

Number of months/ years on medication ___ ___ months ___ ___ Years

Dose if known

Oral contraceptives or had Depot injections? *(If yes, please give details below)* ☐ *Yes* ☐ *No*

Age when prescribed medication ___ ___

Number of months/ years on medication ___ ___ months ___ ___ Years

HRT? *(If yes, please give details below:)* ☐ *Yes* ☐ *No*

Age when prescribed medication ___ ___

Number of months/ years on medication ___ ___ months ___ ___ Years

If you have any queries regarding our research into Lupus, we would be pleased to hear from you. We may contact you again in the future for further studies into lupus if you do not want to receive further correspondence then please let us know. This questionnaire and any questions or queries should be sent to:

Bath Connective Tissue Disease Research Unit
Bath Institute for Rheumatic Diseases
FREEPOST (SN1549)
Bath – 1 Trimbridge
BA1 1XX

Or Tel:01225 448444 or Email: ctd@birdbath.org.uk

THANK YOU FOR YOUR HELP