
A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Medical and Human Sciences

2012

Benjamin Joseph Parker

School of Medicine

Institute of Inflammation and Repair
Contents

List of Tables ................................................................................................................. 10
List of Figures .................................................................................................................. 12
Abstract ............................................................................................................................ 14
Declaration ......................................................................................................................... 15
Acknowledgements ........................................................................................................... 16
Preface ................................................................................................................................. 17
Publications arising from this thesis .................................................................................. 18
List of Abbreviations ........................................................................................................ 20

1 Introduction ....................................................................................................................... 23
  1.1 Systemic Lupus Erythematosus ................................................................................. 23
    1.1.1 Clinical Features of SLE .................................................................................. 23
    1.1.2 Epidemiology of SLE ...................................................................................... 23
    1.1.3 Aetiopathogenesis of SLE ............................................................................... 25
      1.1.3.1 Genetic factors .......................................................................................... 25
      1.1.3.2 Environmental Factors ........................................................................... 26
      1.1.3.3 Immune dysfunction in SLE .................................................................... 27
    1.1.4 Assessment of disease activity and disease damage in SLE ....................... 29
      1.1.4.1 SLEDAI .................................................................................................... 29
      1.1.4.2 BILAG 2004 ............................................................................................ 30
      1.1.4.3 SLICC/ACR-Damage Index ..................................................................... 30
    1.1.5 Management of SLE ......................................................................................... 31
    1.1.6 Mortality in SLE ............................................................................................... 33
  1.2 Atherosclerosis, inflammation and the endothelium. ............................................. 33
  1.3 Premature vascular damage in SLE ......................................................................... 35
    1.3.1 Clinical manifestations of premature vascular damage in SLE ............... 35
    1.3.2 Subclinical manifestations of premature vascular damage in SLE .... 35
  1.4 The role of traditional CHD risk factors in premature vascular damage in SLE .................................................................................................................. 37
    1.4.1 The prevalence of traditional CHD risk factors in SLE .......................... 37
1.4.2 The contribution of traditional CHD risk factors to premature atherosclerosis in SLE ................................................................. 38

1.5 The role of non-traditional risk factors in premature vascular damage in SLE ........................................................................ 40

1.5.1 Type-1 interferon ................................................................. 40

1.5.2 Inflammatory cytokines ....................................................... 40

1.5.3 Complement ......................................................................... 41

1.5.4 Autoantibodies ..................................................................... 41

1.5.4.1 Antiphospholipid antibodies ............................................ 41

1.5.4.2 Other autoantibodies ......................................................... 43

1.5.5 Renal disease ....................................................................... 43

1.5.6 Corticosteroids .................................................................... 44

1.6 Potential strategies to modify CVD risk in SLE ....................... 46

1.6.1 Classic risk factor modification and CVD risk in SLE .......... 47

1.6.1.1 Statins in SLE ................................................................... 47

1.6.1.2 Omega-3 fatty acids in SLE ............................................. 48

1.6.2 Anti-inflammatory therapy and CVD risk in SLE ............... 48

1.6.2.1 Corticosteroid therapy ...................................................... 48

1.6.2.2 Antimalarial therapy ....................................................... 48

1.6.2.3 Immunosuppressive agents ............................................. 49

1.6.2.4 Biological agents ............................................................ 50

1.7 Inflammation and Metabolic Syndrome in SLE .................... 51

1.7.1 The Metabolic Syndrome in the general population .......... 52

1.7.1.1 Defining the Metabolic Syndrome .................................. 52

1.7.1.2 Epidemiology of the Metabolic Syndrome ..................... 54

1.7.1.3 The Metabolic Syndrome and CHD risk prediction ........ 55

1.7.2 The Metabolic Syndrome in SLE ........................................ 55

1.7.2.1 Epidemiology of Metabolic Syndrome in SLE ............... 55

1.7.2.2 Metabolic Syndrome and vascular damage in SLE ......... 56

1.7.2.3 Determinants of Metabolic Syndrome in SLE ............... 56

1.8 Endothelial damage and dysfunction in SLE ....................... 57
3.7.1 Data preparation ..........................................................78
3.7.2 Defining the study period ..............................................78
3.7.3 Defining the study population ......................................78
3.7.4 Selection of exposure variables ...................................79
3.7.5 Identification of confounders ......................................80
3.7.6 Overview of the analysis plan ......................................80
3.7.7 Modelling the time-varying effects of exposures ..........81
  3.7.7.1 Interactions ..........................................................82
  3.7.7.2 Post-estimation analysis ........................................82
3.8 Contribution of candidate ...............................................83

4 Methods (ii): Endothelial dysfunction in active SLE. ............85
  4.1 Study setting .............................................................85
  4.2 Study funding ...........................................................85
  4.3 Study design .............................................................85
  4.4 Ethical approval ........................................................86
  4.5 Patient recruitment ....................................................86
    4.5.1 SLE inclusion and exclusion criteria ....................86
    4.5.2 Recruitment of healthy controls .........................87
  4.6 Clinical assessment ..................................................87
    4.6.1 General clinical assessment ...............................87
    4.6.2 SLE-specific assessment ..................................88
  4.7 Laboratory assessment ..............................................88
  4.8 Assessment of endothelial function ..............................89
    4.8.1 General test conditions .....................................89
    4.8.2 FMD assessment ................................................90
      4.8.2.1 FMD protocol .............................................90
      4.8.2.2 FMD data collection and on-line analysis ............91
    4.8.3 PAT assessment ...............................................92
      4.8.3.1 PAT protocol .............................................92
      4.8.3.2 PAT on-line analysis ..................................92
  4.9 Assessment of endothelial damage using EMPs ..............92
4.9.1 Sample collection .................................................................92
4.9.2 Sample centrifugation and storage ............................................93
4.9.3 Immunolabelling of samples and EMP quantification......................93
  4.9.3.1 Initial EMP protocol development........................................94
  4.9.3.2 Protocol optimisation .....................................................95
4.9.4 EMP enumeration ...................................................................98
4.10 Statistical methodology .................................................................98
  4.10.1 Primary outcome ..................................................................98
  4.10.2 Secondary outcomes .............................................................98
  4.10.3 Sample size calculation ........................................................98
  4.10.4 Statistical analysis ...............................................................99
4.11 Contribution of the candidate ...........................................................100

5 Clinical determinants of the Metabolic Syndrome in an international inception cohort of patients with SLE .................................................................102
  5.1 Description of SLICC-MetS study cohort over first 2 years ...............103
    5.1.1 Defining the SLICC-MetS cohort .............................................103
    5.1.2 Demographic features of SLICC-MetS study cohort ................103
    5.1.3 Clinical, laboratory and immunological features of SLICC-MetS study cohort .................................................................105
    5.1.4 Disease activity and damage in SLICC-MetS cohort ...............107
    5.1.5 Therapeutic exposures in SLICC-MetS cohort ..........................110
      5.1.5.1 Corticosteroids .........................................................110
      5.1.5.2 Immunosuppressive and antimalarial therapies ...............111
    5.1.6 Traditional CHD risk factors in SLICC-MetS .........................112
    5.1.7 Comparison of baseline characteristics of SLICC-MetS study cohort and patients with missing MetS status .................................113
  5.2 Prevalence, persistence and phenotype of MetS .................................116
    5.2.1 Prevalence of metabolic syndrome in SLICC-MetS ..................116
    5.2.2 Incidence and persistence of MetS over time in SLICC-MetS ......118
    5.2.3 MetS phenotype over time in SLICC-MetS ...............................118
    5.2.4 Sensitivity analyses ............................................................119
5.2.4.1 The effect of including statins in the MetS definition. ..........119
5.2.4.2 The effect of including non-fasting blood results MetS definition.
........................................................................................................120
5.3 Determinants of MetS at enrolment into SLICC-MetS .................121
5.3.1 Univariate associations of MetS in SLE at enrolment ..........121
5.3.2 Multivariable associations of MetS in SLE at enrolment ..........122
5.3.3 MetS susceptibility at enrolment and ethnicity ..................124
  5.3.3.1 MetS phenotype by ethnicity ........................................124
  5.3.3.2 Lupus phenotype by ethnicity ....................................124
  5.3.3.3 Determinants of MetS in Korean and Hispanic populations at
           enrolment. ........................................................................126
5.3.4 MetS susceptibility at enrolment and immunosuppressant use ..127
5.3.5 MetS susceptibility at enrolment and active renal disease ......128
5.4 Determinants of MetS over time .........................................129
  5.4.1 Univariate associations of MetS in SLE over time .............129
    5.4.1.1 Variables associated with MetS over time ..................129
    5.4.1.2 Differential effect of timing of exposure on MetS ........130
  5.4.2 Multivariable associations of MetS in SLE over time ..........132
  5.4.3 MetS susceptibility over time and ethnicity ....................132
    5.4.3.1 MetS phenotype over time and ethnicity ....................133
    5.4.3.2 Lupus phenotype over time and ethnicity ...................134
  5.4.4 MetS susceptibility over time and anti-dsDNA positivity ......134
5.5 Summary ............................................................................136
5.6 Discussion ...........................................................................137
  5.6.1 Prevalence and phenotype of MetS in SLE .......................137
  5.6.2 Clinical determinants of MetS in SLE at enrolment ............140
  5.6.3 Clinical determinants of MetS in SLE over time ...............142
  5.6.4 Study strengths and limitations ......................................144
6 Inflammation and endothelial dysfunction in patients with active SLE .....147
  6.1 Validation of study techniques ..........................................148
    6.1.1 Validation of endothelial function assessment ...............148
6.1.1.1 Study design and statistical methods ........................................148
6.1.1.2 FMD and PAT assessment protocol ........................................148
6.1.1.3 Intra-observer variability of FMD ...........................................149
6.1.1.4 Repeatability of FMD .............................................................151
6.1.1.5 Intra-observer variability of endoPAT® ......................................151
6.1.2 Validation of EMP quantification ..................................................152
6.1.3 Discussion ....................................................................................152
6.2 Description of SLE patients and healthy controls ...............................154
6.2.1 Demographic and lifestyle features of SLE patients and controls ......154
6.2.2 Traditional cardiovascular risk factors in SLE patients and controls .....
...............................................................................................................155
6.2.3 Disease-related features in SLE patients ...........................................156
6.2.3.1 Clinical and immunological features of SLE patients ...............156
6.2.3.2 Disease activity and damage in SLE patients ............................158
6.2.3.3 Therapeutic exposures in SLE patients .......................................160
6.2.4 Discussion ....................................................................................160
6.3 Cross-sectional analysis of endothelial function and damage in patients
with active SLE vs. controls ....................................................................162
6.3.1 Methods .......................................................................................162
6.3.2 Endothelial function in SLE vs. controls ........................................162
6.3.2.1 Associations of endothelial dysfunction at baseline ...............163
6.3.3 Endothelial microparticles in SLE vs. controls ...............................164
6.3.3.1 Associations of endothelial damage at baseline .......................165
6.3.4 Endothelial activation markers in SLE vs. controls .......................166
6.4 Longitudinal analysis of effect of inflammation suppression on
endothelial function and damage in active SLE .....................................166
6.4.1 Methods .......................................................................................166
6.4.2 CHD risk factors over time ..........................................................166
6.4.3 Disease activity over time ............................................................167
6.4.4 Endothelial function over time ......................................................168
6.4.5 Endothelial microparticles over time .............................................169
List of Tables

Table 1-1: 1997 ACR revised classification criteria for SLE .............................24
Table 1-2: Summary of MetS definitions ...........................................................53
Table 1-3: IDF 2009 revised criteria for clinical diagnosis of MetS ..........54
Table 1-4: Endothelial microparticles levels in cardiovascular diseases. ...65
Table 5-1: ACR criteria in SLICC-MetS cohort at enrolment ......................106
Table 5-2: SLE phenotype over time using SLEDAI-2K .............................107
Table 5-3: Disease activity and damage indices over follow-up ...............108
Table 5-4: Prevalence of SLICC/ACR-DI items over time .......................109
Table 5-5: Corticosteroid exposures over the first 2 years .......................111
Table 5-6: Immunosuppressive and antimalarial exposures .................111
Table 5-7: Prevalence of traditional CHD risk factors in SLICC-MetS ....112
Table 5-8: Baseline demographics and CHD risk factors of patients with missing MetS status at enrolment ..................................................114
Table 5-9: SLE characteristics of patients with missing MetS status at enrolment .................................................................115
Table 5-10: MetS phenotype over the first 2 years of follow-up ..........119
Table 5-11: Significant univariate associations of MetS at enrolment ........122
Table 5-12: Multivariable model of determinants of MetS at enrolment ....123
Table 5-13: Multivariable model excluding all dose-related corticosteroid variables. .................................................................124
Table 5-14: Lupus phenotype by ethnicity at enrolment .........................126
Table 5-15: MetS and disease phenotype by immunosuppressant use ....128
Table 5-16: Univariate associations of MetS over time .........................130
Table 5-17: Effect of timing of exposures on MetS over time ..............131
Table 5-18: Differential effect of timing of therapy-related exposures on MetS over time .........................................................................131
Table 5-19: Multivariable associations of MetS in SLE over time. ..........132
Table 5-20: MetS phenotype in Korean and Hispanic patients over time ....133
Table 5-21: Therapeutic exposures over time by ethnicity .................133
Table 6-1: Repeatability of brachial artery and FMD measurement ............151
Table 6-2: Repeatability of EMP measurement ........................................152
Table 6-3: Traditional and novel CHD risk factors in SLE patients and controls 156
Table 6-4: ACR classification criteria in SLE patients .................................157
Table 6-5: Immunological features of SLE patients at baseline visit ........157
Table 6-6: Distribution of BILAG-2004 scores at enrolment ...............158
Table 6-7: Prevalence of SLICC/ACR-DI items at enrolment ..................159
Table 6-8: Current therapies at baseline visit into study .....................160
Table 6-9: Endothelial function in SLE and control subjects .................163
Table 6-10: Multivariable analysis of non-lupus associations of endothelial dysfunction (FMD) ..........................................................164
Table 6-11: Multivariable analysis of non-lupus associations of endothelial damage (EMPs) ..............................................................................165
Table 6-12: Endothelial activation markers in SLE vs. controls ..............166
Table 6-13: CHD risk factors over time in SLE patients ..........................167
Table 6-14: Endothelial function over time in SLE patients ....................168
Table 6-15: Endothelial activation markers over time in SLE patients .......169
Table 6-16: Correlation between change in FMD (%) and indices of disease activity and endothelial damage .......................................................170
Table 6-17: Correlation between change in EMP levels (%) and indices of disease activity and endothelial damage .................................171
Table 6-18: Change in disease activity and endothelial function by treatment approach ......................................................................................172
List of Figures

Figure 1-1: Mechanisms of atherogenesis in the general population. ..............34
Figure 1-2: Impact of traditional risk factors on overall risk of CHD in SLE ......39
Figure 1-3: Worldwide prevalence of MetS by definition, sex and age. ...........54
Figure 1-4: Mechanism of microparticle release from cells..........................62
Figure 1-5: CD31+ EMPs levels correlate with invasive and non-invasive measures of endothelial function. .........................................................65
Figure 1-6: Elevated CD31+ EMP levels predict adverse cardiovascular events in coronary artery disease. .................................................................66
Figure 1-7: Elevated E-selectin+ EMP levels in active childhood vasculitis ......67
Figure 3-1: Participating SLICC centres .......................................................74
Figure 4-1: Participant positioning for simultaneous measurement of FMD and PAT............................................................................................................89
Figure 4-2: Position of participant arm, BP cuff and ultrasound probe during FMD assessment.......................................................................................90
Figure 4-3: Automated arterial wall tracking using VIA software. .................91
Figure 4-4: Reactive Hyperaemic Index using EndoPAT 2000©......................92
Figure 4-5: Identification of EMPs ................................................................94
Figure 4-6: Scatter plot of optimised original EMP protocol..........................95
Figure 4-7: Fluorescence pattern of FITC-, PE-, and APC- labelled antibodies...96
Figure 4-8: Fluorescence pattern of efluor450-labelled annexin V. .................96
Figure 4-9: Flow cytometry of PPP using updated optimised protocol.............97
Figure 5-1: SLICC-RAS and SLICC-MetS cohort over the first 2 years ..........103
Figure 5-2: SLICC-MetS recruitment by region. ..............................................104
Figure 5-3: Ethnic distribution of SLICC-MetS cohort ....................................104
Figure 5-4: Ethnicity of SLICC-MetS cohort by region at enrolment ..........105
Figure 5-5: Disease activity and disease damage over time .........................108
Figure 5-6: Therapeutic exposures over time .................................................110
Figure 5-7: Prevalence of MetS over time ......................................................116
Figure 5-8: Prevalence of MetS over time by ethnicity ....................................117
Figure 5-9: Prevalence of MetS by region .....................................................117
Figure 5-10: Persistence of MetS over time in a complete-case analysis .......118
Figure 5-11: MetS prevalence in patients with fasting bloods only vs. SLICC-MetS ........................................................................................................120
Figure 5-12: ROC curve for multivariable model of determinants of MetS ....123
Figure 5-13: MetS phenotype by ethnicity at enrolment ................................125
Figure 6-1: Correlation of brachial artery diameter at two time-points ........149
Figure 6-2: Correlation of FMD (%) at two time-points ................................149
Figure 6-3: Bland-Altman plot of paired measures of brachial artery diameter 150
Figure 6-4: Bland-Altman plot of paired measures of % FMD..........................150
Figure 6-5: Bland-Altman plot of paired measures of PAT ..............................151
Figure 6-6: Age distribution in SLE patients and controls ..............................154
Figure 6-7: Comparison of endothelial-dependent FMD in SLE patients vs. controls. ..................................................................................................................163
Figure 6-8: Comparison of EMPs in SLE patients vs. controls .......................165
Figure 6-9: FMD over time in SLE patients ......................................................168
Figure 6-10: EMPs over time SLE patients ......................................................169
Figure 6-11: Correlation of FMD and RHI in SLE patients .............................173
Figure 6-12: Correlation of FMD and EMP in SLE patients ............................173
Abstract

U niversity of Manchester
Benjamin Joseph Parker
PhD (Medicine)

Determining the relationship between inflammation, therapeutic exposure and cardiovascular risk in systemic lupus erythematosus
August 2012

Introduction
SLE is associated with pro-atherogenic metabolic derangement and an elevated cardiovascular risk. The vascular endothelium may be a key interface between active SLE and premature atherosclerosis. Improved understanding of the contribution of inflammation and its management to cardiovascular risk in SLE will inform personalised treatment decisions in SLE patients.

Methods
Data from an international inception cohort was used to investigate the relationship between inflammatory disease activity, lupus phenotype and corticosteroid exposure and the metabolic syndrome (MetS) over 2 years in SLE patients. The relationship between disease activity (BILAG-2004) and markers of endothelial function (flow-mediated dilatation (FMD) of the brachial artery) and endothelial damage (endothelial microparticles (EMPs)) following a change in anti-inflammatory therapy was investigated in a longitudinal cohort of patients with active SLE.

Results
MetS was common in young SLE patients (12.6-16.0%) over the initial 2 years of disease. Factors independently associated with developing MetS over the 2-year study period were (odds ratio (95% CI)) Hispanic ethnicity (3.47 (1.76, 6.86)), higher initial peak corticosteroid dose (1.02 (1.01,1.03)), and elevated anti-dsDNA antibodies at study entry (1.86(1.19,2.81)). MetS was often persistent and preceding MetS strongly predicted future MetS (4.83 (2.93, 7.87)). Patients with active SLE had reduced FMD (median (IQR) FMD 1.63% (-1.22, 5.32) vs. 5.40% (3.02, 8.57); p = 0.05) and elevated EMPs (157,548/ml (59,906, 272,643) vs. 41,025 (30,179, 98,082); p = 0.003) compared to age-matched controls. Both improved following a change in anti-inflammatory therapy, and correlated moderately with change in disease activity over time.

Conclusions
Inflammatory disease activity and higher doses of corticosteroids in very early disease influence the development of MetS in SLE, which can become persistent. Endothelial dysfunction is common in patients with active SLE but can be improved with better disease control. Therefore even from disease onset, therapeutic regimes should be individually tailored to achieve good disease control whilst minimising corticosteroid doses, to improve cardiovascular risk surrogates in SLE.
Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Copyright Statement

i. The author of this thesis (including any appendices and/or schedules to this thesis) owns certain copyright or related rights in it (the “Copyright”) and s/he has given The University of Manchester certain rights to use such Copyright, including for administrative purposes.

ii. Copies of this thesis, either in full or in extracts and whether in hard or electronic copy, may be made only in accordance with the Copyright, Designs and Patents Act 1988 (as amended) and regulations issued under it or, where appropriate, in accordance with licensing agreements which the University has from time to time. This page must form part of any such copies made.

iii. The ownership of certain Copyright, patents, designs, trade-marks and other intellectual property (the “Intellectual Property”) and any reproductions of copyright works in the thesis, for example graphs and tables (“Reproductions”), which may be described in this thesis, may not be owned by the author and may be owned by third parties. Such Intellectual Property and Reproductions cannot and must not be made available for use without the prior written permission of the owner(s) of the relevant Intellectual Property and/or Reproductions.

iv. Further information on the conditions under which disclosure, publication and commercialisation of this thesis, the Copyright and any Intellectual Property and/or Reproductions described in it may take place is available in the University IPPolicy (see http://www.campus.manchester.ac.uk/medialibrary/policies/intellectual-property.pdf) in any relevant Thesis restriction declarations deposited in the University Library, The University Library’s regulations (see http://www.manchester.ac.uk/library/aboutus/regulations) and in The University’s policy on presentation of Theses.
Acknowledgements

I would like to take this opportunity to express my gratitude to Ian Bruce for the extensive support, advice and guidance he has provided throughout my entire clinical and academic career in rheumatology. I would like to thank him as well for supervising my PhD project and always being available to offer help and advice. I am immensely grateful to Yvonne Alexander for the help, guidance and support she has provided as my PhD and laboratory supervisor for the last 4 years and also to Martin Rutter and Rachelle Donn as my advisors.

My thanks also go to Phil Pemberton and Allen Yates in the Central Manchester Research Laboratory, and to all the staff at the Wellcome Trust Clinical Research Facility and Kelloggren Centre for Rheumatology for hosting and assisting with the study. I am especially grateful to Awal Al-Husain for her continued help and assistance throughout the entire study, and of course to Nicola Dale for her much appreciated and constantly sought-after help, and for whom nothing was too much trouble.

I would also like to thank Murray Urowitz for the opportunity and encouragement to work with SLICC, and to thank all the SLICC members for the experience of being part of such a prestigious international collaboration. My thanks also to all the patients who have generously contributed their time to participate in both studies, without whom this work would not have been possible.

The unconditional support and encouragement of my parents and family has been fundamental to any success I may have had, and I remain forever grateful. Finally, and most importantly, thank you to my wife Gabrielle for supporting everything I have done, and to my two boys – Henry and Solomon – for not playing up too much for the last 3 years.
**Preface**

I graduated from the University of Liverpool in 2000 with an MBChB (Honours) and following my pre-registration house jobs I travelled to Queensland, Australia where I spent an immensely rewarding year working as a junior house officer. I then returned to the UK to undertake a medical SHO rotation at Manchester Royal Infirmary. A developing interest in the rheumatic diseases and particularly connective tissue diseases led me to undertake a rheumatology clinical fellow post with Ian Bruce and Yasmeen Ahmad following my MRCP, which stimulated my interest in lupus research in Manchester. I commenced rheumatology training in the North West deanery in 2006 following a LAT registrar post at Royal Liverpool University Hospital. My on-going pursuit of a research career led to my appointment as a Manchester Biomedical Research Centre Clinical Research Fellow in 2008, and I subsequently obtained an Arthritis Research UK Clinical Research Fellowship in 2009.

My interest in SLE is both clinical and academic, and I hope will form the basis of my post-PhD career. My long-term research goals are to develop the main themes of this thesis, focussing on vascular dysfunction in SLE and related rheumatic conditions. I also intend to develop my interest in clinical therapeutics in SLE, with a long-term aim to deliver personalised and appropriate treatment regimens to patients with SLE at the appropriate time in their disease history.
Publications arising from this thesis

Published manuscripts


Abstracts


- **B Parker**, M Lunt, I.N. Bruce on behalf of SLICC study group. Predictors of MetS at enrolment into a multicentre international inception cohort of patients with SLE. Ann Rheum Dis 2012. 71; (suppl3): 210.


- **B Parker**, M Lunt, I.N. Bruce on behalf of SLICC study group. Variability in the phenotype of MetS over time in a multicentre international inception cohort of patients with SLE. Rheumatology 2012; 51(Suppl 3): iii27-iii38


- S.K. Heathfield, **B. Parker**, L. Zeef, I.N. Bruce and M.Y. Alexander. Certolizumab pegol attenuates the proinflammatory state in endothelial cells in a manner that is atheroprotective. Rheumatology 2012; 51(Suppl 3): iii140-iii184


**Prizes**

- 2010 *Rheumatology* Young Researcher Travel Award
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR</td>
<td>American College of Rheumatology</td>
</tr>
<tr>
<td>AM</td>
<td>Antimalarial</td>
</tr>
<tr>
<td>ANA</td>
<td>Anti-nuclear antibodies</td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>Antibodies to double stranded DNA</td>
</tr>
<tr>
<td>APLa</td>
<td>Antiphospholipid antibodies</td>
</tr>
<tr>
<td>BA</td>
<td>Brachial artery</td>
</tr>
<tr>
<td>BILAG</td>
<td>British Isles Lupus Assessment Group</td>
</tr>
<tr>
<td>BLyS</td>
<td>B lymphocyte stimulating factor</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CS</td>
<td>Corticosteroid</td>
</tr>
<tr>
<td>CV</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>EAM</td>
<td>Endothelial activation markers</td>
</tr>
<tr>
<td>EMP</td>
<td>Endothelial Microparticle</td>
</tr>
<tr>
<td>FMD</td>
<td>Flow-mediated dilatation</td>
</tr>
<tr>
<td>GTN</td>
<td>Glyceryl Trinitrate</td>
</tr>
<tr>
<td>HDL-C</td>
<td>High-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>IDF</td>
<td>International Diabetes Federation</td>
</tr>
<tr>
<td>IFN-α</td>
<td>Type I interferon-α</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Low-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>MetS</td>
<td>Metabolic Syndrome</td>
</tr>
<tr>
<td>MP</td>
<td>Microparticle</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>oxLDL</td>
<td>Oxidised LDL-c</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>PAT</td>
<td>Peripheral arterial tonometry</td>
</tr>
<tr>
<td>pi-HDL</td>
<td>Pro-inflammatory high-density lipoprotein</td>
</tr>
<tr>
<td>PPP</td>
<td>Platelet-poor plasma</td>
</tr>
<tr>
<td>RHI</td>
<td>Reactive hyperaemic index</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SLE</td>
<td>Systemic Lupus Erythematosus</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>Systemic Lupus Erythematosus Disease Active Index</td>
</tr>
<tr>
<td>SLICC</td>
<td>Systemic Lupus International Collaborating Clinics</td>
</tr>
<tr>
<td>SLICC-MetS</td>
<td>SLICC Metabolic Syndrome cohort</td>
</tr>
<tr>
<td>SLICC-RAS</td>
<td>SLICC Registry for Atherosclerosis cohort</td>
</tr>
<tr>
<td>TG</td>
<td>Triglyceride</td>
</tr>
<tr>
<td>VIA</td>
<td>Vascular Image Analysis</td>
</tr>
<tr>
<td>WC</td>
<td>Waist circumference</td>
</tr>
</tbody>
</table>
Chapter 1

An Overview of Vascular Damage in Systemic Lupus Erythematosus

This chapter will provide an introduction to the clinical features, epidemiology, and assessment of systemic lupus erythematosus, and an overview of the approach to management in UK clinical practice. The evidence for the increased risk of clinical and sub-clinical cardiovascular disease, and the traditional and non-traditional factors relevant to this risk, will be reviewed.
1 Introduction

1.1 Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a chronic multisystem inflammatory autoimmune disease predominantly affecting young women, and commonly follows a relapsing-remitting course. SLE is a heterogeneous disease which can manifest a wide variety of clinical and laboratory features. The clinical features, epidemiology, pathogenesis and the approach to management of SLE are discussed below.

1.1.1 Clinical Features of SLE

The clinical presentation of lupus is often insidious and disease manifestations may accumulate over many years, often leading to diagnostic delay. SLE usually follows a relapsing and remitting course, characterised by intermittent disease flares in any organ system. The clinical features of SLE may be constitutional, such as fatigue and weight loss, or result directly from inflammatory activity in any organ system, such as nephritis, arthritis, serositis or vasculitis (1). Commonly, cutaneous and musculoskeletal features congregate in an individual although certain populations are at increased risk of developing major organ involvement, such as nephritis (2).

1.1.2 Epidemiology of SLE

Although the cutaneous manifestations of SLE have been recognised since the mid-nineteenth century it was not until 1971 that the first consensus on classification of the disease was reached by the American College of Rheumatology (ACR), modified most recently in 1997 (3;4) (Table 1-1). To aid standardisation of research into SLE, 4 of these criteria are required to have been present at any time during an individual’s disease duration to be classified as SLE. The accurate estimation of the incidence and prevalence of SLE has only been possible since the advent of these accepted classification criteria. Due to differences in the methods used for case ascertainment and the relapsing nature of the disease, however, there has been considerable variation in estimated prevalence rates. For example, hospital-based studies often underestimate prevalence rates in contrast to self-reporting studies which often over-estimate prevalence rates.
**Table 1-1: 1997 ACR revised classification criteria for SLE**

<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Malar rash</td>
<td>Fixed erythema over the malar eminences</td>
</tr>
<tr>
<td>2. Discoid rash</td>
<td>Erythematous circular raised patches +/- atrophic scarring</td>
</tr>
<tr>
<td>3. Photosensitivity</td>
<td>Exposure to ultraviolet light causes rash (patient or physician observed)</td>
</tr>
<tr>
<td>4. Oral ulcers</td>
<td>Oral or nasopharyngeal ulcers (physician observed)</td>
</tr>
<tr>
<td>5. Non-erosive arthritis</td>
<td>Non-erosive arthritis of two or more peripheral joints, with tenderness, swelling, or effusion</td>
</tr>
<tr>
<td>6. Serositis</td>
<td>Pleuritis or pericarditis documented by ECG or rub or evidence of effusion</td>
</tr>
<tr>
<td>7. Renal Disorder</td>
<td>Proteinuria &gt;0.5g/day or 3+; cellular casts</td>
</tr>
<tr>
<td>8. Neurological disorder</td>
<td>Seizures or psychosis in the absence of drug or metabolic causes</td>
</tr>
<tr>
<td>9. Haematological disorder</td>
<td>Haemolytic anaemia or leucopenia (&lt;4000/mm$^3$) or lymphopenia (&lt;1500/mm$^3$) or thrombocytopenia (&lt;100,000/mm$^3$) in the absence of offending drugs</td>
</tr>
<tr>
<td>10. Immunological disorder</td>
<td>Presence of anti-dsDNA (in abnormal titre) or anti-Sm or antiphospholipid antibody (LAC or ACL)</td>
</tr>
<tr>
<td>11. Positive antinuclear antibody</td>
<td>An abnormal titre of ANA by immunofluorescence or an equivalent assay at any point in time in the absence of drugs known to induce ANAs</td>
</tr>
</tbody>
</table>

*Abbreviations: LAC Lupus Anticoagulant; ACL Anticardiolipin antibody; ANA Antinuclear antibody.

4 or more criteria are required to be present at any point in the disease duration (Adapted from Hochberg 1997(4))

In the UK, 4 key studies have examined the prevalence of SLE using similar robust methodologies, each utilising multiple sources of case ascertainment and capture-recapture techniques to accurately estimate prevalence rates (5-8). Overall, these studies demonstrate a prevalence rate in the UK of 24-27.7 per 100,000. These and other studies have shown that SLE demonstrates a strong female preponderance, with prevalence rates up to 12-fold higher in women. An ethnic gradient has also been noted amongst most epidemiological studies. Individuals of African, Afro-Caribbean, Hispanic and Asian ethnicity have an increased prevalence of SLE and more severe disease than Caucasian populations. For example in the Birmingham cohort the prevalence rate in Afro-
Caribbean’s was more than 5 times that observed in the Caucasian population (197.2 per 100,000 compared to 36.3 per 100,000 women (6)). The study by Patel et al confirmed that non-Caucasian populations also had a higher prevalence of lupus nephritis (2). Worldwide, the prevalence of SLE varies according to the population studied, reflecting the changing prevalence according to ethnicity, the nature of the health care system and methods of reporting disease. For example, in the USA overall prevalence is reported as between 14.6 and 50.8 per 100,000 (9;10) and rates as high as 94 per 100,000 have been reported in Arizona, which has a predominantly Hispanic population (11).

Incidence rates are less well studied because of methodological difficulties, and have been estimated at 4.0 per 100,000 in the UK (7) and 5.56 cases per 100,000 in the US (12), with peak age of onset in the childbearing years (15-40 years).

1.1.3 Aetiopathogenesis of SLE

Like many autoimmune diseases the aetiology of lupus remains obscure. Gene-environment interactions are thought to be at the core of SLE pathogenesis, with disease development likely to result from a combination of genetic susceptibility and cumulative environmental exposures in any given individual. Given the excess cardiovascular mortality in SLE, the mechanisms of disease susceptibility and immunological dysfunction may yield important insights into the development of atherosclerosis in the lupus population.

1.1.3.1 Genetic factors

Familial aggregation of SLE (as demonstrated by a λs of 8-29) and higher concordance in monozygotic compared to dizygotic twins (40% vs. 2-5%) suggests a strong genetic basis to disease susceptibility (13). The association of SLE with complement deficiencies and the HLA region have been known for many years, with both HLA class II variants (including DR2 and DR3) and class III variants (including TNF, C4A and C4B) predisposing individuals to SLE (14). Recent advances in genetic epidemiology, including genome wide association studies (GWAS), have dramatically increased the number of genes considered to be involved in the development of SLE. GWAS and candidate-gene studies have now identified more than 30 robust SLE susceptibility loci to date, across several ethnic populations, many of which are key components of immunological pathways (14). For instance, several functional variants in Fcγ receptor genes, which are intimately associated with immune complex clearance, have been identified and associated with SLE susceptibility. Some of these variants are
population-specific (15;16) and/or organ specific such as FcγR IIa variants in lupus nephritis (17). Genetic polymorphisms in genes involved in T cell signalling, such as PTPN22, and the interferon pathway (IRF5) have also been implicated in lupus susceptibility, and autoimmunity in general (18).

It is therefore probable that multiple genetic loci are involved in lupus susceptibility, each exerting a relatively small individual effect. Genetic susceptibility is likely to interact with a number of important non-genetic factors to initiate and establish pathogenic pathways in the development of lupus.

1.1.3.2 Environmental Factors

No single non-genetic factor has been shown to trigger SLE in any population (other than drug-induced lupus) and many studies of environmental factors in the aetiology of SLE provide inconsistent results. The environmental triggers involved in the gene-environment interaction are therefore likely to be multiple, occurring at different time points, and with an additive effect on an individual's immune system.

The ‘hygiene hypothesis’ of allergy suggests that the developing immune system is sensitive to its environment (19) and exposures during fetal development and infancy may be highly significant in the context of susceptibility to autoimmunity in general. For example, infection with either rubella or mumps during infancy is associated with a positive ANA in adult life (20) and studies of both childhood- and adult-onset lupus have demonstrated significant increases in the rates of EBV seroconversion in cases compared to controls (21;22).

Environmental factors associated with SLE in later life have been more extensively studied although results vary and few findings are consistent, reflecting methodological and population differences, and a heterogeneous disease phenotype. Lifestyle and socioeconomic factors (such as markers of poverty and deprivation) appear to be associated with SLE, although they more closely predict prognosis and outcome rather than susceptibility (23-25) and probably therefore reflect differences in healthcare access. Exposure to ultraviolet (UV) radiation is well recognised to exacerbate both cutaneous and systemic lupus symptoms, referred to as photosensitivity (26). UV radiation (sunlight and artificial light) induces DNA damage and apoptosis, and enhances recruitment and activation of CRP and other opsonins to facilitate clearance of apoptotic cells (27). Type I/II sensitive sun-reactive skin type has been to shown to be associated with the development of SLE in a case-control study from Sweden (28), although the importance of UV light as a trigger for SLE in darker skinned populations is less clear. Deranged and defective apoptotic clearance in SLE may therefore lead to autoantigen exposure and clinical disease. Individual studies of cigarette smoking and the risk of developing SLE are inconsistent,
despite cigarettes exhibiting many biological actions relevant to the development of lupus (29). A meta-analysis of 9 relevant studies however suggested an overall odds ratio (OR) for current smokers developing SLE of 1.5 (95% CI 1.09, 2.08). No increased risk for former smokers and no obvious dose-response relationship were noted (30). Hormonal factors, particularly oestrogens, are of undoubted importance in the development of SLE given the marked female preponderance, although no single marker of oestrogen exposure (apart from gender) has been consistently associated with development of SLE across studies. For example, in a Swedish study of 85 women with SLE, no surrogate for oestrogen exposure (such as age at menarche, age at menopause, use of exogenous oestrogens) was associated with the development of SLE. In contrast, the Carolina Lupus Study of 240 women with SLE demonstrated a positive association between a history of pre-eclampsia and SLE (OR 3.7 (95% CI 1.2, 11.2)) and an apparent protective effect of breastfeeding (OR 0.6 (95% CI 0.4, 0.9)) (31). In this study no association was noted with the use of exogenous oestrogens. Finally, the Nurse’s Health Study from the USA found that post-menopausal exposure to HRT significantly increased the risk of developing SLE (OR 2.1 (95% CI 1.1, 4.0)) (32) in a large US cohort.

Perhaps the most extensively studied environmental exposures studied to date are those related principally to occupation, such as heavy metals, silicates, and hydrocarbons (33;34). Exposure to silica (both high and medium levels) is associated with the development of SLE in several studies (35;36). Whilst high-intensity exposure to SLE tends to occur in male-dominated occupations, women are at risk of low-intensity exposures in roles such as textiles, farming and commercial cleaning (35;37).

1.1.3.3 Immune dysfunction in SLE
The immunopathology of SLE is complex and may relate directly to the vascular dysfunction seen in the course of the disease. Type-1 interferon (IFN-1), a pro-inflammatory cytokine, appears to be central to the immunopathogenesis of SLE (38). The initial description of elevated serum IFN-1 levels in SLE patients by Ytterberg et al and their correlation with disease activity(39), was subsequently supported by the observation that IFN-alpha therapy for diseases such as carcinoid was associated with the development of autoimmunity and SLE (40). Recent investigations have focused on the genetic signature induced in both peripheral blood mononuclear cells (PBMCs) and leucocytes by higher circulating levels of IFN-1. Bennett et al demonstrated an up-regulation of IFN-induced genes in patients with active SLE and this ‘IFN-signature’ appears to correlate with disease activity, and interestingly the IFN-signature is abrogated following treatment with high-dose steroids in vivo (41). Indeed therapeutic blockade of
IFN-1 with IFN-1 antagonists in patients with SLE is currently undergoing early phase clinical trials (42). Whether IFN has a role in the vascular dysfunction is not yet clear and will be discussed in detail later.

A key protein involved in the immunopathology of SLE is B lymphocyte stimulator (BLyS) also known as BAFF (B cell–activating factor). BLyS is a transmembrane protein expressed on monocytes, macrophages, and dendritic cells and its levels are regulated by cytokines, in particular IFN (43). Activation of the BLyS receptors leads to B cell and plasma cell proliferation, differentiation, survival and IgG class switching (44). The importance of BLyS in the immunopathogenesis of SLE was demonstrated initially in animal studies. Elevated BLyS levels promote the development of renal disease in auto-immune prone mice (45), and BLyS-deficient lupus-prone mice show attenuated development of proliferative glomerulonephritis, compared to non-BLyS deficient mice (46). Human clinical studies of SLE reveal that 20–40% of patients have significantly elevated levels of circulating BLyS, and plasma BLyS protein levels appear to correlate with disease activity (47;48). BLyS has recently successfully been targeted therapeutically with a biological BLyS inhibitor (see chapter 1.1.5).

The immunological hallmark of SLE is the presence of autoantibodies directed against nuclear antigens. Autoantibodies often evolve over time and their appearance may precede the clinical onset of the disease (49), reinforcing the theory that cumulative and complex gene-environment interactions are key in the aetiopathogenesis of lupus. The pathogenicity of these autoantibodies varies, with many considered markers of disease presence (such as antinuclear antibodies (ANA)) rather than true mediators of disease manifestations (such as anti-dsDNA antibodies).

Abnormalities in both the innate and adaptive immune response are seen in SLE and failure to clear apoptotic cells, with subsequent exposure to auto-antigens, may underpin loss of self-tolerance and production of autoantibodies (50). Interference with apoptotic pathways has been shown to break self-tolerance in mouse models, with the subsequent development of antinuclear antibodies and a lupus-like glomerulonephritis (51). In human studies of SLE, modification of nuclear antigens by aberrant apoptotic pathways (such as methylation and citrullination) can result in the exposure of modified autoantigens to the immune system, which can then be recognised as non-self (52). Failure of clearance of apoptotic debris and cells has also been shown in mouse models to result in autoantibody production and the development of autoimmunity, and accumulated apoptotic debris has been shown to accumulate in the germinal centres of lymph nodes in patients with SLE, resulting in autoreactive B cells
Microparticles may have a role in the presentation of autoantigens to the immune system in SLE, and are discussed later.

The characteristic clinical manifestations of SLE result from the formation of immune-complexes between autoantibodies and self-antigens, particularly nuclear antigens. Deposition of immune complexes results in increased complement activation and damage to the organ involved, such as is seen in lupus nephritis (54). The process of immune-complex deposition may also have a vital role in the increased cardiovascular mortality seen in SLE, through the interaction with the endothelium. Immune-complexes deposited within the vascular system and endothelium lead to inflammation of vessel walls, and can lead to clinical vasculitis, but may also be the initiating event for subsequent endothelial dysfunction (55-57).

### 1.1.4 Assessment of disease activity and disease damage in SLE

Determining the underlying pathology of a disease manifestation and distinguishing between disease activity secondary to active inflammation (considered reversible) and disease damage (considered irreversible) is a key component of the clinical management of SLE, and is integral to clinical trials of new therapies. There is no simple biomarker to assess the severity of inflammation in SLE, which hampers objective assessment of disease activity. However several biomarkers are utilised to support the clinical assessment of disease activity SLE, such as serum levels of C3 and C4, and serum anti-dsDNA levels. These biomarkers may be predictive of disease flares, particularly renal disease (58;59) but are inconsistent between patients and do not always track changes in disease activity (60;61). The difficulties in objectively and consistently assessing disease activity have led to the development of clinical composite indices of disease activity and damage, instruments that combine clinical and laboratory parameters to evaluate disease activity. Many such indices exist, but few have undergone rigorous validation; of those that have, the most widely used and the indices to be utilised in the present study, are discussed below.

#### 1.1.4.1 SLEDAI

The SLE Disease Activity Index (SLEDAI) score was developed using consensus opinion and has demonstrated construct validity (62;63), reliability (63) and some degree of sensitivity to change (64;65). The index focuses on new or recurrent manifestations of the disease over the preceding 10 days, and may not therefore capture on-going activity efficiently. The maximum potential score is 105 but scores are rarely above 40, and clinical therapeutic trials of usually require scores above 6 in order to be eligible for entry. Many of the validation
studies have involved relatively small numbers of patients, although this has improved with the up-dated version (SLEDAI-2K) which allows for persisting disease activity (Appendix 1) (66). The SLEDAI has also been modified to assess manifestations over the preceding 30 days to harmonize with BILAG (67;68), for use in epidemiological studies of exogenous oestrogens in SLE (the SELENA-SLEDAI) (69). Most recently the SELENA-SLEDAI has been combined with classic BILAG, and a physician global assessment to create a novel response index used in the recent successful randomised clinical trial of belimumab for active SLE (the Systemic lupus erythematosus Responder Index (SRI) (70). Multiple SLEDAI assessments can also be combined to estimate cumulative disease activity over time by calculating the adjusted mean SLEDAI in an individual assessed on 2 or more occasions over a defined period (66). This is a useful tool to quantify exposure to inflammatory disease activity in longitudinal studies.

1.1.4.2 BILAG 2004
The British Isles Lupus Assessment Group Index 2004 (BILAG-2004), an updated version of the revised classic BILAG index (71) was developed on the basis of the physician’s intention to treat, with agreement again based upon consensus opinion. This is a more transitional index than SLEDAI, able to capture the changing severity of clinical manifestations, and reflects changes occurring over the previous 4 weeks. Disease manifestations in all organ systems, as well as constitutional features, are graded A-E based on clinical and laboratory features (Appendix 2) and each category reflects the physician’s intention to treat. For example, category “A” manifestations, such as severe arthritis and active nephritis, require high dose steroids and/or new or additional immunosuppression, category “B” features reflect less severe acute lesions that require a change in systemic therapy (such as lower doses of prednisolone or antimalarials), whilst category “C” manifestations indicate current involvement of an organ system insufficient to score A or B, and for which symptomatic treatment would be considered sufficient. A BILAG “D” score denotes previous resolved organ system involvement, and “E” denotes a system that has never been involved (72). A flare of active lupus for the purpose of research studies and clinical trials is often defined as a new category A or B manifestation, and BILAG 2004 can also be combined into a numerical score to aid analysis (73). Both the classic and 2004 indices have undergone extensive assessment of validity (criterion, reliability, construct and sensitivity to change), although the absence of a gold standard measure does hamper assessment (72;73).

1.1.4.3 SLICC/ACR-Damage Index
A key component of the clinical management of patients with SLE is to distinguish between those symptoms resulting from on-going inflammatory
activity and those due to irreversible organ damage, as this assessment will significantly influence the decision to treat using anti-inflammatory medication. This is true for research studies as well, especially those using disease activity as an outcome, such as therapeutic trials. To standardise this assessment the Systemic Lupus International Collaborating Clinics (SLICC) and American College of Rheumatology (ACR) developed a consensus-based index of disease damage, regardless of cause, known as the SLICC/ACR-Damage Index (SLICC- DI). This index was again subject to assessment of validity (content, face, criterion and discriminant validity) (74). The SLICC-DI is an organ-based system which defines damage as “a clinical feature due to non-reversible change, not related to active inflammation, occurring since the onset of lupus and present for at least 6 months” (Appendix 3), and so, in general, cannot be completed within 6 months of diagnosis with SLE. Although the maximum potential score is 47, scores above 10 are rare, and scores of 2 or more indicate significant organ damage.

1.1.5 Management of SLE

The management of patients with SLE is directed at rapid determination of disease activity, severity and extent, permitting the institution of the appropriate level of therapy for the organ system(s) involved. The overall aim of the treatment chosen is to control inflammatory disease activity, thereby preserving organ function, whilst minimising side effects and maintaining quality of life. More potent immunosuppressive agents are generally reserved for patients with either severe or resistant features.

Mild-to-moderate manifestations, such as predominant musculoskeletal and cutaneous symptoms, are often managed with topical therapies, non-steroidal anti-inflammatory drugs, anti-malarial drugs (such as hydroxychloroquine) and/or low-dose prednisolone (<7.5mg/day). Patients requiring higher doses of corticosteroids may have steroid-sparing agents introduced (such as azathioprine). Moderate or resistant disease manifestations often require the introduction of systemic immunosuppressive therapies, such as azathioprine or mycophenolate mofetil (MMF). Standard management of more severe lupus (such as nephritis, myositis, neuropsychiatric disease and TTP) has traditionally aimed at inducing remission using a combination of high-dose corticosteroids and cytotoxic agents such as cyclophosphamide, followed by ‘stepping-down’ therapy to a maintenance immunosuppressive regime. More recently, less toxic induction regimes using MMF have been introduced in an attempt to decrease the risk of premature ovarian failure from cytotoxic agents, particularly in those with nephritis (75;76).
With the advent of the biological era, several new drugs have been introduced into the management of SLE. Rituximab, a chimeric anti-CD20 monoclonal antibody, induces peripheral CD20-positive B cell depletion leading to removal of auto-reactive B cells from the circulation. (77). Originally developed as a therapy for B cell lymphoma, rituximab is also approved for use in rheumatoid arthritis. Unfortunately, despite several initial positive open-label studies of rituximab (78;79), a recent randomised controlled trial (RCT) failed to achieve its primary end-point in both active non-renal SLE (80) and lupus nephritis (81). These negative rituximab trials have prompted much discussion amongst clinicians about clinical trial design in SLE and the factors that may have contributed to these unexpectedly negative results (82;83). Principle amongst these factors has been the need to achieve complete remission in active disease and how response is best defined in a trial setting. The use of effective background therapy (such as MMF in the case of nephritis) and high doses of steroids in the trials has also made it hard to dissect the impact of additional therapies. However, its use as a treatment of last resort continues based on the extensive open-label evidence of its efficacy in this capacity. Future trials may need to focus on the steroid-sparing qualities of B cell depletion, in addition to its efficacy and safety, to confirm its role in the management of active, resistant SLE. Indeed, it is this quality that may provide much of its future atheroprotective benefits.

Belimumab, a B-lymphocyte-stimulator (BlyS) inhibitor, has achieved positive results in a recent RCT of active seropositive lupus (70), and has recently become the first drug to be approved and licensed for use in SLE in more than half a century. The relative contribution of BlyS to the pathophysiology of SLE is not fully understood, although BlyS is elevated in patients with SLE and an association between plasma BlyS levels and SLE disease activity has been reported (47;48). Belimumab is a human monoclonal antibody specific for BlyS, a B cell survival factor. Belimumab inhibits the survival of B cells, including autoreactive B cells, and reduces the differentiation of B cells into immunoglobulin-producing plasma cells. To date, belimumab has only been approved for use in non-renal lupus.

To date, the only clinical outcome measured in trials of therapies for SLE is their anti-inflammatory effect, i.e. are they effective in controlling active disease? Whilst this is important, in the context of chronic disease management other outcomes are also highly relevant and will increasingly become part of personalised management decisions (as well as funding decisions). These additional outcomes may include the steroid-sparing effects of new/existing therapies, whether an approach to treatment results in the avoidance of serial exposure to immunosuppressive therapies, whether they prevent long term organ damage and disability, and ultimately whether they reduce mortality (both
in acute disease and stable, established disease). Long-term CVD risk is one such outcome, and whether improved management of active SLE and the reduction in inflammation, by any approach, improves cardiovascular risk is key component of this thesis.

1.1.6 Mortality in SLE

Previously associated with significant mortality in the period soon after diagnosis, predominantly due to renal failure, mortality rates in SLE improved significantly with the advent of corticosteroid therapy. Mortality has steadily declined over the years as early diagnosis and more targeted therapy has evolved. However, 10-15 year survival remains reduced at 85% (12), which is of great importance for patients given that peak onset is during child-bearing years. Overall, patients with lupus have a Standardized Mortality Ratio (SMR) of 2.4, which is even higher in younger patients (SMR 19.2 in <40ys) (84). Mortality in SLE is described as following a “bimodal pattern”: early in the course of the disease, deaths are commonly due to disease activity, treatment side-effects, and sepsis; later, deaths are more commonly due to manifestations of cardiovascular disease (CVD), as well as malignancy (85). Whilst mortality in SLE has improved overall over recent decades, there has been no corresponding improvement in mortality from cardiovascular disease despite significant advances in both primary and secondary prevention of cardiovascular events in the general population (84;86). The observation that CVD is a leading cause of death in established SLE is perhaps unexpected and unusual, given the strong female preponderance and relatively young age of affected patients. The reasons for this excess CVD-related mortality are discussed below.

1.2 Atherosclerosis, inflammation and the endothelium.

The endothelium appears to be central to the mechanisms of atherosclerotic plaque development in the general population (Figure 1-1). Current concepts suggest that an initial injury to the endothelial cell monolayer by a variety of stimuli such as hypertensive shear stress, smoking and pro-inflammatory lipids, results in an up-regulation and increased expression of adhesion molecules on their vascular membranes (87). Adhesion molecules permit the capture of white blood cells by endothelial cells that differentiate into tissue macrophages and, following their ingestion of lipoproteins, subsequently form foam cells (88). Cells in early atheroma secrete pro-inflammatory cytokines, reactive oxygen species and a variety of proteases that attract further inflammatory cells and promote the development of the atheromatous lesion. Later in the process smooth muscle
cells migrate from the tunica media to the tunica intima and proliferate, leading to the formation of a cap over the developing plaque. Atherosclerotic plaques cause clinical manifestations either through lumen stenoses and end-organ tissue ischaemia or acute thrombosis and lumen obstruction. Unstable plaques, more prone to acute rupture an thrombosis, are often morphologically different to stable plaque, and inflammatory cells may promote this instability (89).

SLE is an inflammatory disease in which the endothelium plays a key role in many of the immunopathological manifestations, and is associated with a multitude of pro-inflammatory cytokines, cells and pathways. Understanding the interaction between inflammation and endothelial dysfunction which appears to underpin the premature vascular damage seen in SLE, as well as the effects of reducing inflammation therapeutically, may yield important benefits for patients with SLE and will be the focus of this study.

Figure 1-1: Mechanisms of atherogenesis in the general population.

a) Normal artery lined by a single layer of endothelial cells. b) In the early stages of atherogenesis activated endothelial cells express adhesion molecules, permitting capture and migration of circulating white blood cells into the tunica intima that differentiate into tissue macrophages and thence foam cells. c) Progression of the lesion, stimulated by inflammatory cytokines, involves smooth muscle cell migration into the lesion to form a fibrous cap. d) Plaque rupture can lead to acute thrombosis and acute organ ischaemia.

Adapted from Hansson et al, Nature 2011 (87).
1.3 Premature vascular damage in SLE

1.3.1 Clinical manifestations of premature vascular damage in SLE

Many groups have shown that patients with SLE are susceptible to developing vascular damage and atherosclerosis at an earlier age than would be expected, with a substantial risk of progression to clinical disease. Indeed SLE is considered an independent risk factor for accelerated atherosclerosis (90). In a prospective cohort study of 498 women with lupus from the USA, in which rates of incident coronary heart disease (CHD) cases were recorded and compared with the Framingham Offspring Study (a large community-based study of CHD), SLE patients were found to have a 5-6-fold overall increase in CHD risk. The increase in risk was even more marked in women aged 35-44 years at up to 50-fold (91). A similar increase in CVD risk has been demonstrated in the UK, utilising data from the General Practice Research Database. Fischer et al demonstrated that the risk for first-time acute myocardial infarction, adjusted for traditional risk factors, was higher in SLE patients than both controls (adjusted OR (95% CI) 2.67 (1.34, 5.34)), and RA patients (1.47 (1.23, 1.76)) (92). Furthermore, the average age at which the first CV event occurs in patients with SLE is 53 years, significantly earlier than in the non-lupus population (93). Retrospective studies of CV events in patients with SLE confirm the excess of atherosclerotic manifestations. In a Swedish study, hospital discharge information and cause of death data were linked in over 4700 patients to investigate cause of death in SLE patients. Overall, SLE patients had a standardized mortality ratio (SMR) of 3.63 (95% CI 3.49-3.78), and the leading cause of death was CVD (86). Similarly, Manzi et al showed that younger patients (20-39 years) had a SMR from CHD of 15.9 (95% CI 10.4-23.6), which, even allowing for the potential deficiencies in case ascertainment, further highlights the burden of cardiovascular disease in SLE (86).

1.3.2 Subclinical manifestations of premature vascular damage in SLE

The excess risk of observed clinical cardiovascular events is reflected in an increased prevalence of subclinical atherosclerosis, occurring at a younger age in patients with SLE than in the general population. Autopsy studies of patients with SLE have demonstrated significant generalized atherosclerosis in over half of cases, regardless of underlying cause of death (94) and coronary artery atherosclerosis is particularly prevalent (up to 42%) in those patients who have received steroids for at least 1 year (95). Many groups have subsequently
demonstrated the presence of sub-clinical disease in SLE patients in cross-sectional and controlled studies utilising a variety of measures, most commonly the presence of carotid plaque (96-98) but also computerised tomographical (CT) detection of coronary artery calcification (99) and large artery stiffness (100).

In a study by Ahmad et al, 200 Caucasian women (single ethnicity to facilitate further genetic studies) with SLE from the North West of England were compared to 100 healthy female controls, using carotid artery ultrasound to assess the carotid intima media thickness (cIMT) and for the presence of carotid plaque. Carotid plaque was defined if 2 of the following 3 conditions were met: (i) an area of >50% protusion into lumen compared with surrounding area; (ii) increased echogenicity compared to adjacent boundaries; (iii) IMT>0.15cm. Overall, plaque prevalence was 29% in SLE patients and 22% in controls. However, SLE patients <55-years were found to have significantly more plaque than did controls (21% vs. 3%; p <0.001), and traditional Framingham risk factors did not fully predict the presence of plaque in the SLE cohort (96). In a subsequent unpublished study, 127 of these patients underwent repeat carotid ultrasound assessment after a median of 5.8 (5.2, 6.3) years. Control subjects were not re-assessed. At baseline, 34/127 (27%) SLE subjects had at least 1 carotid plaque, and at follow-up this increased to 63/127 (50%). Of these, 33 (26%) subjects developed new plaque, 22 (17.5%) had a higher plaque index and 8 (7%) had stable plaque. Almost half the patients (59/127) never demonstrated plaque and 4 (3%) patients had plaque regression between the 2 visits (Haque – PhD thesis). In another longitudinal study of plaque progression, Roman et al performed serial carotid ultrasound scans on 158 patients with SLE, over a mean time period of 34 months. Interestingly, although 49% of patients had a persistent absence of plaque, a substantial minority of SLE patients (28%) had progression of their carotid plaque, equating to an average of 10% per year. Age at diagnosis, duration of disease and higher homocysteine levels appeared to be independently related to plaque progression (101).

In summary, SLE is associated with an increased risk of developing subclinical atherosclerosis and adverse clinical cardiovascular events than age-matched healthy controls. These events occur at a younger age than would be expected and potential reasons for this will be discussed below.
1.4 The role of traditional CHD risk factors in premature vascular damage in SLE

The contribution of classic Framingham risk factors for CHD to the increased risk of clinical and subclinical atherosclerosis seen in SLE remains incompletely understood. Several groups have examined their prevalence and impact on both the presence and progression of atherosclerosis and this evidence is discussed below.

1.4.1 The prevalence of traditional CHD risk factors in SLE

The increased CHD risk in SLE may simply reflect the increased prevalence of traditional Framingham risk factors (smoking, family history of CHD, hypertension, raised total cholesterol and diabetes mellitus) observed in women with SLE. For example, more than half of the women in the Baltimore lupus cohort had 3 or more CHD risk factors, and those lupus patients with prevalent CHD were more likely to have higher serum cholesterol and hypertension than lupus patients without CHD (102). Further evidence of the increased prevalence of classic CHD risk is provided by Bruce et al who, in a case-control study from Toronto, demonstrated that patients with SLE (but without clinical CHD) were more likely to have dyslipidaemia, hypertension and diabetes compared to healthy controls (103). Similarly, data from the Systemic Lupus International Collaborating Clinics Registry for Atherosclerosis (SLICC-RAS) study group confirmed a high prevalence of traditional risk factors at enrolment into an inception cohort of lupus patients, and on-going significant accumulation of these factors over the first 3 years of follow-up. For example, at enrolment into SLICC (within 15 months of diagnosis) 39.2% of the cohort was hypertensive, rising to 58.3% by year 3, and 3.4% were diabetic at enrolment, rising to 5.0% at year 3 (104). In a retrospective case-control study from the UK, Haque et al observed that those lupus patients with a clinical CHD event were more likely to have been exposed to all traditional risk factors than event-free SLE controls (93) and in an age- and gender-adjusted analysis both hypertension and positive family history of premature CHD were associated with adverse CV events. More recent studies have examined the role of pro-inflammatory (pi) lipids, specifically pi-HDL and oxidised LDL (ox-LDL), in the development of atherosclerosis in SLE. McMahon et al compared pi-HDL and ox-LDL levels in patients with SLE, rheumatoid arthritis and healthy controls. A higher proportion of SLE patients had an elevated pi-HDL, and significantly higher levels of pi-HDL were seen in the SLE cohort.
compared to both the healthy controls and patients with rheumatoid arthritis (105).

In summary, traditional risk factors for CHD are more prevalent in lupus patients than controls, and are often present early in the course of the disease. Clinical vigilance to identify those traditional risk factors that are modifiable, as well as those that may be exacerbated by anti-inflammatory therapies, needs to be maintained.

1.4.2 The contribution of traditional CHD risk factors to premature atherosclerosis in SLE

The impact of traditional CHD risk factors on the risk of developing atherosclerosis in SLE varies across studies and may differ according to the stage of atherosclerosis being investigated. Studies of clinical events in SLE seem to have a stronger association with traditional risk factors than do those of subclinical disease. Hypertension, hypercholesterolaemia, male gender, older age, and smoking have all been positively associated in multivariable models with the development of clinical CHD events in SLE patients (93;106;107). However the difference in frequency and severity of classic Framingham risk factors alone does not entirely explain the disparity in prevalence of clinical cardiovascular disease in all lupus cohorts. Esdaile et al studied a large Canadian cohort with a mean follow-up of 8.6 years and assessed baseline classic risk factor frequency and adverse vascular outcomes. After controlling for the presence of common risk factors (age, sex, total cholesterol, blood pressure, smoking, diabetes and the presence of left ventricular hypertrophy), lupus patients still had a 10-fold increase in relative risk for non-fatal myocardial infarction than would be expected using the Framingham model alone (108). Similarly, in a retrospective analysis of 47 patients with SLE, Bessant et al compared baseline 10-year predicted CV risk (obtained using software based upon the Framingham equation) with actual accumulated events over 10 years of follow-up (109). The cohort was predominantly female and had a mean age of 43 years (range 24-67). Median (IQR) calculated 10-year risk of CHD at baseline was 1.4% (0.2, 3.4) and of stroke was 0.6% (0.4, 1.3). Over the 10-year study period however 4 patients (8.5%) had a CHD event and 5 patients (10.6%) had a stroke, suggesting that conventional risk prediction scores based on traditional risk factors do not perform well in SLE.

Studies of subclinical atherosclerosis in SLE suggest that, although important in the development of atherosclerosis, traditional CHD factors alone do not perform well in multivariable models. Many studies have therefore suggested that lupus-related factors (such as disease duration, disease activity and therapeutic
exposures) exert a significant influence on subclinical disease. For example, in the study by Ahmad et al traditional risk factors performed well in univariable analyses but less so in multivariate models of plaque presence in SLE patients. The estimated area under the ROC curve (AUC) for multivariable models including only traditional CHD risk factors was 0.76 in SLE patients, versus 0.90 in healthy controls. The addition of lupus-related factors to the model significantly improved the prediction model of atherosclerosis in SLE, with an AUC of 0.87 (96). Similarly, in a case-control study of 197 patients with SLE Roman et al found that whilst traditional risk factors (age, systolic blood pressure and serum cholesterol) performed well in univariable analysis, only age at onset and disease duration remained significant predictors of carotid plaque in a multivariable model (98).

It therefore appears that whilst traditional Framingham risk factors for CHD contribute to the development of atherosclerosis in SLE, they do not fully explain the increased risk of development and progression of clinical and subclinical disease seen in lupus patients. SLE is therefore considered an independent risk factor for atherosclerosis, and non-traditional disease-related factors and the lupus phenotype are likely to be important in explaining the increased risk (figure 1-2).

**Figure 1-2: Impact of traditional risk factors on overall risk of CHD in SLE**

Non-traditional risk factors appear to play an important role in CV risk in SLE, either through increasing sensitivity to traditional factors or through other direct effects. (Adapted from Bruce 2005) (90)
1.5 The role of non-traditional risk factors in premature vascular damage in SLE

The mechanisms through which vascular damage accrues in SLE are complex and multifactorial. Whilst it is clear that traditional CHD risk factors do not fully explain the increased risk, it remains unclear which lupus-related factors are important in accelerating the atherosclerotic process. A number of inflammatory pathways central to SLE may contribute to vascular dysfunction, and certain lupus-specific factors and therapeutic exposures are likely to impact upon CHD risk. The evidence for the impact of these non-traditional factors on CHD risk in SLE is discussed below.

1.5.1 Type-1 interferon

The investigation of the role of type-1 interferon (IFN-1) in the development of premature atherosclerosis in SLE remains at an early stage and is focused on its interaction with the endothelium. IFN-1 (both α and β subsets) increases the expression of adhesion molecules, alters chemokine production and appears to increase endothelial cell apoptosis, leading to modulation of endothelial cell function (110). Increased levels of IFN-1, as descibed in SLE, have also been associated with a reduction in number and function of circulating endothelial progenitor cells (EPCs) (111), bone marrow-derived cells involved in endothelial repair (112). EPC numbers are reduced in people with both prevalent CHD and multiple cardiovascular risk factors (113;114). Although results of studies examining EPC numbers in SLE are inconsistent, recent studies suggest they appear to display functional deficiencies that may be a more critical factor in loss of vascular function than their overall number (111;115;116). The excess of IFN-1 seen in SLE may therefore detrimentally affect the balance between endothelial damage and repair, and promote a proatherogenic environment.

1.5.2 Inflammatory cytokines

Tumour Necrosis Factor-alpha (TNF-α) is an inflammatory cytokine that plays a fundamental role in the pathogenesis of many chronic inflammatory conditions, such as rheumatoid arthritis. Higher levels of circulating TNF-α have multiple deleterious effects on the cardiovascular system and TNF α -driven inflammatory activity is now considered to be crucial in the initiation and propagation of atherosclerotic lesions in the general population (117). TNF-α is increased in obesity (118) and is associated with the metabolic derangements characterised by the metabolic syndrome, such as an atherogenic lipid profile and insulin
resistance. In diabetes mellitus endothelial dysfunction is associated with higher circulating levels of TNF-α, which improves when TNF-α is reduced (119). Animal studies have also shown that exposure of the vascular endothelium to TNF-α results in impaired endothelial function, and is improved when its actions are blocked (120) (121). Although TNF-α is not considered a key cytokine in the pathogenesis of SLE, several groups have demonstrated increased circulating TNF-α levels in lupus patients, as well as higher renal TNF-α expression (122;123). Elevated TNF-α has observed in SLE patients with prevalent CVD (124) and is associated with coronary calcium scores (125), and. The pro-inflammatory cytokine IL-17, which is elevated in SLE, may also contribute to vascular damage via an increase in endothelial adhesion molecule expression (126). Jackson et al (127) described an association between reduced transforming growth factor-β (TGF-β) levels in the serum of 32 patients with SLE and higher cIMT and worse damage indices. Other potentially relevant cytokines in the observed vascular damage in SLE include IL-18 and IFN-γ (128).

1.5.3 Complement

The formation of immune-complexes is the hallmark of active SLE. Complement activation by deposited immune-complexes results in inflammation, an effect magnified by the impaired complement clearance inherent to lupus. Complement is the common final inflammatory mediator in SLE and its activation within the vasculature leads to increased endothelial permeability, a prelude to endothelial dysfunction (55). Complement may also have adverse effects on atherosclerotic plaque formation. Immune-complexes bind to endothelial cells via C1q receptors and inhibit cholesterol-27-hydroxylase, resulting in impaired reverse cholesterol transport and thus predisposing to plaque formation (129). Whilst reduced complement levels are common in active SLE, increased consumption may be balanced by increased hepatic production of complement and therefore levels may be normal or increased in SLE. Hypocomplementaemia is associated with the metabolic syndrome in SLE (130), and elevated C3 complement has been associated with plaque progression (55). The exact role of complement in the accelerated vascular damage associated with SLE however remains unclear, although hereditary deficiency of C2 has been associated not only with recurrent severe infection but also with SLE and high rates of clinical atherosclerosis (131).

1.5.4 Autoantibodies

1.5.4.1 Antiphospholipid antibodies

The presence of antiphospholipid antibodies and/or the lupus anticoagulant in the context of vascular thrombosis or recurrent miscarriage is known as the
antiphospholipid syndrome (APS). It was first described in SLE patients (132) and antiphospholipid antibodies (APLa) are present in up to 30% of lupus patients (133). Primary APS (PAPS) refers to the syndrome in the absence of an associated autoimmune disorder, and APLa can also be present in asymptomatic individuals. The laboratory tests for APLa in clinical practice are the anticardiolipin assay and detection of the lupus anticoagulant (LAC). These tests detect the presence of a heterogeneous population of antibodies that bind a variety of antigens, such as cardiolipin, β2-glycoprotein-1 (β2-GP-1) and protein/phospholipid complexes (134). The effect these antibodies have on the process of atherosclerosis in general, and in SLE in particular, remains unclear, and will be discussed briefly below.

Although acute vascular occlusion in APS commonly occurs in histologically ‘normal’ blood vessels (135), APLa are thought to contribute to the atherosclerotic process in both patients and asymptomatic carriers. In the general population for example, the presence of anticardiolipin antibodies has been shown to be greater in patients who subsequently developed an MI (136). In mouse models, passive infusion of APLa (both anti-cardiolipin and anti- β2-GP-1) is associated with increased levels of oxidative stress as measured by nitric oxide (NO) bioavailability and paraoxonase (PON) activity (137), and APLa have been shown to induce impaired endothelial function as assessed by small-vessel myography (138). Anticardiolipin antibodies may exert a pro-atherogenic effect through a variety of mechanisms, including increased uptake of oxidised LDL by macrophages (139;140), increased monocyte adhesion to the endothelium (141), and up-regulation of endothelial cell adhesion molecules (142). However not all APLa are necessarily proatherogenic. Nicolo et al described a reduction in plaque formation in atherosclerosis-prone mice following the passive administration of the monoclonal cardiolipin-reactive antibody FB1 (143).

Clinical studies have also suggested a role for APLa in accelerated atherosclerosis, both in SLE and PAPS. In the context of PAPS, many cross-sectional studies have demonstrated reduced FMD, higher cIMT and higher pulse wave velocity in patients with PAPS compared to controls, as well as reduced NO bioavailability, reduced PON activity, and the presence of pro-atherogenic HDL (144-147). APLa have also been associated with clinical CV events in different SLE cohorts. For example, Petri (148) demonstrated in 380 lupus patients that myocardial infarction occurred more commonly in patients positive for the LAC (16% vs. 6%; p = 0.04), but neither LAC nor anticardiolipin antibodies were associated with measures of subclinical atherosclerosis. Similarly, a Swedish prospective cohort study of 182 patients with SLE reported that the presence of ‘any APLa’ was independently associated with a first CV event (hazard ratio 4.23.
In the LUMINA lupus cohort, baseline APLa-positivity also predicted future CV events (OR 4.7 (1.7, 13.2)) (149). The association between APLa and subclinical disease in SLE is less consistent however. Ahmad et al reported the presence of IgG anticardiolipin antibodies in their lupus cohort of 200 women to be independently associated with the development of carotid plaque (odds ratio 2.26; 95% CI: 1.17-4.36) (96), in contrast to many other groups (98;99;106;150).

In summary, APLa appear to increase the risk of clinical CV events in many lupus cohorts, but may not be associated with an increased risk of subclinical disease. Whether the association with clinical events is mediated through accelerated atherosclerosis, and whether all APLa are equally proatherogenic, however remains unclear.

1.5.4.2 Other autoantibodies
Anti-endothelial cell antibodies are common in SLE (and other autoimmune conditions) and cause endothelial damage in acute lupus-associated vasculitis through the up-regulation of cell adhesion molecules such as VCAM-1, and may increase endothelial cell apoptosis (151). Their role in long-term vascular injury remains unclear, however. Antibodies against HDL (and specifically the antioxidant constituent apoA1) are present in up to a third of patients with SLE (152) and correlate with disease activity (153), perhaps providing another mechanism through which disease activity leads to endothelial damage and dysfunction. Oxidised LDL plays a key role in atherogenesis in the general population, promoting foam cell formation and contributing to the immune activation observed in plaque. Anti-oxLDL antibodies are common in both lupus and non-lupus populations (154;155), but their role in atherogenesis remains unclear and may depend on the immunoglobulin class present. For example, IgG anti-oxLDL is associated with CVD in SLE and appears to promote foam cell formation in vitro (156;157), in contrast to IgM anti-oxLDL. Similarly, antibodies against phosphorylcholine (anti-PC), expressed on the surface of apoptotic cells and a major component of oxLDL, have been shown to be protective against atherosclerosis in the general population (158;159). Anti-PC levels are reduced in SLE, and low levels were shown to be independently associated with carotid plaque in a cohort of 114 Swedish patients with SLE (160).

1.5.5 Renal disease

The impact of chronic renal impairment on cardiovascular health in the general population is well documented, and the prevalence of renal disease in SLE has been estimated at between 22% and 41% (161;162). However, no consistent association has been demonstrated between renal disease and CHD risk in SLE
(98;163), perhaps because of differences in estimating renal function but also because of the presence of confounding variables in those patients with renal disease (e.g. higher steroid use, different immunosuppressive regimes). It is clear however that renal disease is associated with hypertension and several studies have demonstrated an increased prevalence of hypertension (both treated and untreated) in SLE compared to healthy controls (130;164;165). Persistent proteinuria and the nephrotic syndrome also lead to a pro-atherogenic lipid profile and a pro-thrombotic tendency, further affecting vascular risk (166).

**1.5.6 Corticosteroids**

The effect of corticosteroid therapy on cardiovascular risk in SLE remains controversial. Corticosteroids have many anti-inflammatory actions which are beneficial in the management of active SLE, including reduced leucocyte numbers, reduced B cell proliferation and reduced inflammatory cytokine production (e.g. TNF-α, IFN-γ and IL-1) (167). Corticosteroids also have a multitude of potential adverse metabolic effects relevant to CVD, such as weight gain, central obesity, dyslipidaemia, hypertension, impaired glucose tolerance and overt diabetes. Therefore corticosteroids may exert both atherogenic effects (related to their adverse metabolic effects,) and atheroprotective effects (due to their anti-inflammatory activity) on the vasculature of patients with active SLE. These conflicting effects may co-exist within an individual, and the balance may shift as a result of many factors (not all of which can be effectively measured clinically), such as corticosteroid dosage (peak, average and cumulative), pattern of usage, the presence of background metabolic disorders, inherent corticosteroid-sensitivity, rate of response, rate of dose reduction, duration of exposure, and of course patient compliance.

A further reason for the apparent conflicting effects of corticosteroids on CV risk is the fact that the assessment of corticosteroid exposure in observational studies (which accounts for the majority of the data) is often hampered by the presence of confounder factors. The corticosteroid regime used in an individual patient is usually a reflection of the underlying level of disease activity, itself closely associated with vascular dysfunction and damage. This is an example of ‘confounding by indication’ or ‘channelling bias’, both of which are common sources of error and bias in observational studies. This potential source of bias can be addressed to some extent by adjusting for known confounding variables (such as disease activity in this example), such as using multivariable regression analysis, although not all confounders will be known or adequately measured.

Finally, the use of different methods to record corticosteroid exposure also complicates the assessment of the effect of steroids on CV risk. Different
research groups quote multiple variables related to corticosteroid exposure, making comparison between studies difficult. Current corticosteroid use, “ever-use”, past use, intravenous corticosteroids, cumulative corticosteroid dose, peak dose and average daily dose have all been used by researchers to estimate corticosteroid exposure, although detailed collection of all this information is unusual in any single study. Equally, the route and duration of corticosteroid use may influence CV risk in SLE. Chronic low-dose (≤7.5mg/day) oral prednisolone and intravenous pulses of corticosteroids are generally considered to be associated with fewer long-term metabolic adverse effects than have high-dose oral corticosteroids (168). Whether this assumption is true for CV disease risk is unclear, and is a key are for investigation of this thesis.

The evidence for the role of corticosteroids in CVD in SLE comes from prospective cohort and case-control studies of clinical CV events in SLE. Many have reported an association between corticosteroid exposure and adverse CV events, although not all associations remain significant in multivariable analyses. For example, Petri et al assessed the association between several corticosteroid variables and coronary artery disease (CAD - angina, MI or sudden cardiac death) in the John Hopkin’s Lupus Cohort, and only increasing duration of corticosteroid use was associated with CAD in the multivariable analysis (106). In a UK-wide study, Haque et al (93) reported that past exposure to corticosteroids was associated with CHD in their lupus cohort (OR 2.46 (1.03, 5.88)), which became non-significant following age and gender adjustment (OR 2.63 (0.97, 7.16)). Similarly, the prospective Toronto Risk Factor Study found that patients who developed CAD were more likely to have been exposed to corticosteroids (82.4% vs. 52.7%; p = 0.02), although the association did not remain significant on multivariate time-to-event analysis (169). In contrast, several studies have found no such association between corticosteroid exposure and clinical CV events, even on unadjusted analyses (149;170;171). However, most CV outcome studies to date have only investigated limited corticosteroid exposure data (such as current or ever-use) rather than detailed dosage data, and may therefore miss any dose-related effect of corticosteroid on CV risk.

The post-mortem study by Bulkley and Roberts in 1975 was amongst the first to suggest a link between corticosteroid exposure and subclinical atherosclerosis in SLE (95), a finding subsequently confirmed by others. Doria et al found that higher cumulative prednisolone dose was associated with carotid plaque (OR 1.09 (1.03, 1.16)) but not cIMT in their cohort of 78 lupus patients (172).

Similarly, Ahmad reported higher total duration of corticosteroid exposure was associated with the presence of carotid plaque (OR 1.005 (1.00, 1.01)), but higher average dose and current dose were not (96). In the John Hopkin’s cohort, patients receiving higher average daily corticosteroid doses (≥10mg)
were more likely to have elevated total cholesterol (OR 2.87 (2.05, 4.00)) (102). Interestingly, several groups have however found no association between subclinical atherosclerosis and corticosteroid exposure (55;99;150). In a case-control study of 197 lupus patients and controls, lupus patients with carotid plaque had received less corticosteroids overall than those patients without plaque (mean (SD) 5 year daily prednisolone dose 11.9mg (6.9) vs. 6.9 (6.8); p = 0.002) (98).

Overall, the weight of evidence suggests that corticosteroids are associated with an increased risk of clinical and subclinical CVD in SLE. It seems likely however that their effects are mediated as much by the dose, duration and pattern of exposure, as by simple categorical exposure status (e.g. ever/never). Intriguingly, in some patients, corticosteroids may actually exert an atheroprotective role at certain time-points in their disease through their anti-inflammatory effects. A key aim of this thesis is therefore examine the effect of corticosteroid exposure over time on CV risk.

In summary, several inflammatory pathways central to SLE may increase the risk of premature cardiovascular disease, and systemic inflammation may act directly on the endothelium and interact with traditional CHD risk factors. In patients with active SLE therefore, we hypothesise that improving control of inflammatory disease activity will reduce several key inflammatory mediators and improve surrogates of CV risk. This reduction in inflammatory disease activity will result in less endothelial damage and dysfunction, and modify traditional risk factors, which in the longer term may translate into reduced risk of future CHD events.

1.6 Potential strategies to modify CVD risk in SLE

Few studies have examined interventions to improve cardiovascular risk and reduce adverse cardiovascular outcomes in SLE. However, endothelial function is a dynamic process, susceptible to both beneficial and adverse influences, which suggests that the therapeutic options available to clinicians in the management of SLE may be individually tailored according to their CVD risk. This would apply not only to management of classical risk factors, but also to the agent used to reduce inflammation and control disease activity, and therefore potentially lead to a more personalised approach to chronic disease management. Discussed below in more detail are the few studies of therapeutic agents on vascular function in SLE and how they relate to the present study.
1.6.1 Classic risk factor modification and CVD risk in SLE

1.6.1.1 Statins in SLE

Statins are widely prescribed for the primary and secondary prevention of CHD in the general population and have many beneficial effects on CVD risk profile related to their marked lipid-lowering and mild anti-inflammatory effects (173-176). They are therefore hypothesised to be beneficial in SLE patients, given the observed higher prevalence of proatherogenic dyslipidaemia. The anti-inflammatory effects of statins that have been observed in large scale clinical trials, such as reducing hsCRP (177), may also improve disease activity in SLE, and indeed many clinicians feel that statins should be used routinely in SLE.

Initial studies of statins in SLE appeared to corroborate this theory. In a parallel group study by Ferreira et al, 8 weeks of atorvastatin 20mg daily improved endothelial function, as measured by FMD, in a cohort of 64 lupus patients (178). In this study, atorvastatin was associated with a significant (p < 0.001) increase in median (IQR) FMD from 3.8% (2.8,7.9%) to 6.9% (4.2,10.7%), while GTN-mediated dilation remained unaffected. No differences in FMD were seen in the parallel lupus cohort who did not receive atorvastatin. The increase in FMD was observed in patients both with and without conventional Framingham risk factors.

Recently however, several randomised controlled trials (RCT) of statins have not shown any beneficial effects on development or progression of subclinical atherosclerosis in SLE. For example, in a small double-blind RCT of 60 patients with SLE (mean age 41.8 years) atorvastatin 40mg had no effect on reducing coronary artery calcification scores vs. placebo over 12 months, despite improving lipid profiles and reducing hsCRP levels (179). Similar negative results were seen in a RCT of rosuvastatin vs. placebo in 72 patients (mean age 50.8 years) with inactive SLE and proven subclinical atherosclerosis, using cIMT as an outcome over 2 years (180). In the largest study of statin use in SLE to date, 200 SLE patients (mean age 44.7 years; 61% Caucasian) free of clinical CVD were randomised to receive either atorvastatin 40mg or placebo (181). The primary outcome was coronary artery calcification (CAC) score at 2 years, as assessed by helical CT. No significant differences were noted in CAC score between the groups, and atorvastatin had no effect on disease activity or endothelial cell activation markers. Although there was no significant difference in change in cIMT between the placebo and statin groups, a post-hoc analysis did suggest there was a difference in the proportion of patients in whom cIMT worsened in favour of atorvastatin (46% vs. 67%; p = 0.01). Finally, a 3 year RCT of atorvastatin in paediatric-onset SLE also showed no benefit versus placebo in preventing cIMT progression, despite improvements in total cholesterol, LDL-cholesterol and hsCRP (182).
1.6.1.2 Omega-3 fatty acids in SLE

A recent randomized interventional trial of omega-3-polyunsaturated fatty acids on endothelial function (as assessed by FMD) and disease activity was performed in SLE by Wright et al (183). Patients were excluded if they had evidence of severe internal organ involvement or required high dose corticosteroids, and mean (SD) SLAM-R at baseline was 10.2 (3.1) and 9.6 (4.2) in in the treatment and placebo group respectively. Following 24 weeks of therapy, with FMD performed at baseline, 12 and 24 weeks, a significant improvement in endothelial function in SLE patients treated with high-dose fish oils supplements was noted. Median (IQR) FMD improved from 3% (-0.5-8.2%) at baseline to 8.9% (1.3-16.9%) at 24 weeks (p<0.001). Disease activity, as measured by SLAM-R and a version of global BILAG (British Isles Lupus Assessment Group) index, also improved over the study period. This study confirms that endothelial function as measured by FMD can improve over a relatively short period of time in SLE, and agents with an anti-inflammatory effect can improve vascular function.

In conclusion, although statin use is beneficial in preventing adverse cardiovascular events in the non-lupus population, they have not yet been shown to be similarly effective in preventing subclinical manifestations of atherosclerosis SLE cohorts. Whether this remains true for clinical events has yet to be assessed. The lack of effect is perhaps in part related to the different factors that promote atherosclerosis development in SLE, compared to the general population. Traditional risk factors do not fully account for the development of CVD in SLE and so the lipid-lowering effect of statins may be insufficient to impact on its development. Their anti-inflammatory effects are also unlikely to counter the high burden of systemic inflammation present in SLE. The routine use of statins in SLE therefore cannot be recommended based on the available evidence and their potential adverse effects, such as precipitating autoimmunity (184).

1.6.2 Anti-inflammatory therapy and CVD risk in SLE

1.6.2.1 Corticosteroid therapy

The effects of corticosteroids on CV risk in SLE have been considered in 1.5.6.

1.6.2.2 Antimalarial therapy

Antimalarial therapies (AM) such as hydroxychloroquine are commonly recommended for patients with mild-moderately active SLE, especially those with cutaneous and musculoskeletal features. Several studies have shown a beneficial effect of AM therapy on lipid profiles in SLE (185-187) and AM use has been associated with reduced damage accrual overall (188). AM drugs also have anti-thrombotic properties (189;190) and are therefore considered by many to
confer a protective effect against the development of atherosclerosis in SLE. Many retrospective studies in which AM use appears to exert an atheroprotective effect (98;100) are however hampered by channelling bias and confounding by indication (i.e. patients with mild disease are more likely to receive AM therapies), for which it is difficult to fully adjust. A recent nested case-control study however demonstrated that AM drugs were protective against thrombotic events overall (OR (95% CI) 0.32 (0.14, 0.74)) and for arterial events specifically (OR 0.34 (0.12-0.99), even after adjusting for disease severity and duration (191). However, these findings have yet to be confirmed by prospective studies or clinical trials.

1.6.2.3 Immunosuppressive agents
The potential impact of immunosuppression on cardiovascular risk is unclear and inconsistent. Mouse models have examined the effect of immunosuppressive agents on atherosclerosis development in SLE. A recent study was conducted to investigate the atheroprotective effects of mycophenolate mofetil (MMF) in lupus-prone/atherosclerosis-prone mice, a drug with cytostatic effects on lymphocytes commonly used in the management of moderate-severe SLE (192). Atherosclerotic-prone mice, transplanted with bone marrow from lupus-prone mice, were fed a pro-atherogenic Western diet containing placebo, atorvastatin or MMF for 8 weeks, after which they were assessed for atherosclerosis and SLE manifestations and compared to non-lupus prone/atherosclerosis-prone mice. Atorvastatin therapy reduced serum cholesterol in both populations, but decreased total atherosclerotic lesion area only in the non-SLE mice. In contrast, MMF reduced overall plaque burden in both the lupus-prone and non-lupus prone mice compared to controls despite no overt beneficial improvements in SLE activity features (such as anti-dsDNA titres and renal function). MMF therapy also modified the inflammatory-cell content of atherosclerotic plaque, with a reduction in CD4+ve T cells noted. The authors state that reduction of cholesterol levels alone is not atheroprotective in SLE models, and that MMF appears to have a direct protective effect on the inflammatory atherosclerotic lesion, independent of the systemic effects on disease activity.

Many cross-sectional and retrospective clinical studies of predictors of CVD in SLE report immunosuppression use to be associated with the development of CVD surrogates (130), subclinical atherosclerosis (96) and clinical events (93). However, apart from ciclosporin A, the agents regularly used in clinical practice (such as azathioprine and MMF) do not have adverse metabolic or vascular effects in patients (193). Indeed these agents appear to have atheroprotective effects in conditions associated with systemic inflammation, such as rheumatoid arthritis (194;195), and low level immunosuppression may even be
Atheroprotective in the general population (196) and is currently the subject of a large scale RCT (177). Therefore, it is more likely that use of immunosuppression agents in SLE is a marker of more severe disease and higher corticosteroid exposure in the studies performed to date, and do not represent a casual association with CVD. Prospective assessment and more effective disease activity measures and estimation of corticosteroid exposure may reduce the confounding effects of immunosuppression in clinical studies, and will be addressed in this thesis.

In one of the few prospective studies to investigate the impact of immunosuppressive agents on vascular function in SLE, a randomized controlled trial was performed recently by Davies et al (197). This investigated the effect of immunosuppressive agents on endothelial function in patients with SLE, comparing MMF to placebo in patients with mild and stable disease. Participants were treated with 8 weeks of MMF, with FMD performed at baseline and after 2 months. There was no baseline difference in FMD between patients and controls and no significant effect of MMF on FMD was observed. However, the differences between the treatment group and the control group may have been minimized due to the particularly low baseline disease activity in the study population and the relatively short assessment period. Similar negative results were observed in the Baltimore Lupus Cohort using MMF over 2 years, as assessed by CAC scores and cIMT (198).

1.6.2.4 Biological agents
Given the link between inflammation and cardiovascular disease in SLE, the development of more targeted biological therapies is hypothesized to reduce future CHD risk through improved disease control and enhanced steroid-sparing effects. There is little evidence in the current literature for the effects of biologics on vascular function in SLE, but their effects have been examined in the rheumatoid arthritis population. For example, Hurliman et al examined the effect of a 12-week course of anti-TNF therapy on FMD in 11 patients with active RA (199). FMD improved significantly from 3.2% (+/- 0.4) to 4.1% (+/- 0.5), as did disease activity, with infliximab therapy. There is also evidence for a reduction in future CV events in RA patients who respond to anti-TNF drugs from large observational studies (195). Interestingly, no difference was found between the anti-TNF treated group and those who responded to standard disease-modifying therapy, suggesting that the method used to reduce inflammatory activity is not necessarily as important as the level of reduction itself. Methotrexate, the most widely using disease-modifying treatment in RA and also used in SLE, has also demonstrated favourable improvements in both overall mortality (Hazard Ratio 0.4 (0.2, 0.8)) and CV mortality (HR 0.3 (0.2, 0.7)) in a prospective study of
1240 RA patients from the USA (194). A recent systematic review also suggested MTX use was associated with a reduced risk of CVD events in patients with RA (200).

B-cell depletion and inhibition is increasingly employed to control active disease in SLE. B-cell therapies have demonstrated atheroprotective effects in the animal studies and attenuates the development of atherosclerosis in mice (201), although whether this effect results from reduced inflammation or therapy-specific benefits has yet to be demonstrated. The potential vasculoprotective effects of B-cell therapies has yet to be examined in a SLE cohort, but in rheumatoid arthritis a recent study has demonstrated a significant and sustained improvement in FMD over a 6 month period in 6 patients receiving rituximab, who had previously failed conventional therapy, including anti-TNF drugs (202). Conversely, recent data on the reduction in immunoglobulin levels following B-cell depletion may have some relevance to cardiovascular risk (203). Repeated courses of rituximab may suppress antibody-mediated pathways over time and an inability to produce anti-oxidised LDL antibodies and similar atheroprotective antibodies may be detrimental to cardiovascular health (204). Therefore, the long-term effects of B-cell depletion on CV risk require further study.

In conclusion, the literature to date suggests that endothelial dysfunction is modifiable in SLE, at least in patients with active disease. The anti-inflammatory agents used to suppress disease activity in SLE may be atheroprotective by reducing systemic inflammation and limiting steroid exposure. They may also have direct biological actions that may exert both beneficial and detrimental cardiovascular effects. Given the experience of anti-TNF therapies and MTX in RA it is however legitimate to consider whether suppressing inflammation by any effective regime will have similar benefits. This will be addressed in this thesis.

1.7 Inflammation and Metabolic Syndrome in SLE

The metabolic syndrome (MetS) refers to a clustering of risk factors for CHD within an individual that has been shown to predict future adverse CV outcomes in the general population. MetS is driven by obesity and insulin resistance in the general population and is a simple clinical tool to identify individuals at high risk of developing CHD. SLE is associated with insulin resistance and metabolic derangement (205;206), and the inflammatory pathways involved in the disease may predispose lupus patients to developing MetS. Mets may therefore contribute to the enhanced CV risk seen in SLE. The use of MetS as a tool to predict CHD in the general population and its potential role in promoting CVD in SLE is discussed below.
1.7.1 The Metabolic Syndrome in the general population

1.7.1.1 Defining the Metabolic Syndrome

The tendency of CHD risk factors to cluster within an individual has been recognised since the 1920s (207) and the term Syndrome X was coined in 1988 to describe the association of diabetes, obesity and cardiovascular disease (208). MetS was first formally defined in 1998 by the World Health Organisation as part of a wider classification and definition of diabetes mellitus (209) and was considered part of the diabetic spectrum. This initial definition required the demonstration of insulin resistance in an individual, in addition to 2 further criteria and was more of a guideline than a true definition of a new syndrome. Since 1998 there have been 4 further definitions of MetS and one consensus statement, demonstrating the evolution of the syndrome from one anchored by insulin resistance, through one in which obesity was essential, to the most recent in which obesity and hyperglycaemia are not necessary to meet the criteria (Table 1-2).

The most widely used definitions of MetS to date are from The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel (ATP) III) (210) and the 2006 International Diabetes Federation (IDF) (211). Both of these definitions remove the need to demonstrate insulin resistance and use fasting blood glucose as a surrogate, greatly increasing the applicability of the definition to large epidemiological studies. In contrast to NCEP, the IDF 2006 definition views central obesity as an essential component of the syndrome and provides ethnic- and gender-specific normal values.
Table 1-2: Summary of MetS definitions

<table>
<thead>
<tr>
<th>Essential</th>
<th>Insulin Resistance:</th>
<th>Insulin Resistance:</th>
<th>Any 3 of:</th>
<th>Central Obesity</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO 1998</td>
<td>Hypertension: ≥140/90*</td>
<td>Hypertension: 140/90</td>
<td>Hypertension: ≥130/85</td>
<td>Hypertension: ≥130/85</td>
</tr>
<tr>
<td>EIGR 1999</td>
<td>Dyslipidaemia: TG ≥1.7 mmol/l</td>
<td>Dyslipidaemia: TG &gt;2.0 mmol/l</td>
<td>Dyslipidaemia: TG&gt;1.7 mmol/l</td>
<td>Dyslipidaemia: TG≥1.7 mmol/l</td>
</tr>
<tr>
<td>NCEP ATPIII 2001</td>
<td>HDL-C: &lt;0.9 mmol/l</td>
<td>HDL-C: &lt;1.0 mmol/l</td>
<td>Low HDL-C: &lt;1.0 mmol/l</td>
<td>Low HDL-C: &lt;1.03 mmol/l</td>
</tr>
<tr>
<td>IDF 2006</td>
<td>WHR ≥ 0.9</td>
<td>WC ≥94cm</td>
<td>WC ≥102cm</td>
<td>WC ≥88cm</td>
</tr>
<tr>
<td></td>
<td>WHR ≥ 0.85</td>
<td>WC ≥80cm</td>
<td>WC ≥88cm</td>
<td>WC ≥88cm</td>
</tr>
<tr>
<td></td>
<td>BMI ≥30 kg/m²</td>
<td>Microalbuminuria</td>
<td>Fasting Glucose ≥6.1 mmol/l</td>
<td>Fasting Glucose ≥5.6 mmol/l</td>
</tr>
</tbody>
</table>

Abbreviations: WHO World Health Organisation; EIGR European Group for the study of Insulin Resistance; NECP ATPIII National Cholesterol Education Programme; Adult Treatment Panel III; IDF International Diabetes Federation; TG triglycerides; HDL-C HDL-cholesterol; WHR waist-to-hip ratio; BMI Body Mass Index; WC waist circumference; *Initially >160/90

The evolving and contrasting definitions reflect a wider debate about the value of MetS over and above existing risk prediction models, and its status as a separate syndrome has been questioned (212;213). Most recently, a consensus statement from several interested bodies (including IDF, National Heart, Lung and Blood Institute (NHLBI), and American Heart Association) has proposed a harmonised definition of MetS in response to these varied criticisms that requires any 3 from 5 criteria to be met to fulfil the definition (214) (Table 1-3). This definition is simpler to use and has the great advantage over others of using ethnic-specific obesity measures and permitting the exploration of the effect of non-obesity related factors in the development of MetS. However, the criteria and thresholds quoted in these definitions are not always based on prospective data, which is considered by many to reduce their value in CHD risk prediction (212).
Table 1-3: IDF 2009 revised criteria for clinical diagnosis of MetS

<table>
<thead>
<tr>
<th>Measure</th>
<th>Categorical Cut Points</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
</tr>
<tr>
<td>Elevated Waist Circumference (IDF defined)</td>
<td>94cm</td>
</tr>
<tr>
<td>Europid</td>
<td>90cm</td>
</tr>
<tr>
<td>Asian</td>
<td>94cm</td>
</tr>
<tr>
<td>Sub-Saharan Africa</td>
<td>90cm</td>
</tr>
<tr>
<td>Central &amp; South America</td>
<td>90cm</td>
</tr>
<tr>
<td>Elevated blood pressure (or specific drug therapy)</td>
<td>≥130/85 mmHg</td>
</tr>
<tr>
<td>Elevated triglycerides (or specific drug therapy)</td>
<td>≥1.7mmol/l</td>
</tr>
<tr>
<td>Reduced HDL-C (or specific drug therapy)</td>
<td>&lt;1.0mmol/l in males &lt;1.3mmol/l in females</td>
</tr>
<tr>
<td>Elevated fasting glucose (or specific drug therapy)</td>
<td>≥5.6mmol/l</td>
</tr>
</tbody>
</table>

Abbreviations: HDL-C high-density lipoprotein cholesterol (214)

1.7.1.2 Epidemiology of the Metabolic Syndrome
The prevalence of MetS varies according to the definition used and the population studied, although in general it is becoming increasingly prevalent in parallel with the increasing prevalence of obesity (215;216). Figure 1-3 demonstrates the varying prevalence of MetS according to age, gender and country of residence.

Figure 1-3: Worldwide prevalence of MetS by definition, sex and age.

Adapted from Cornier et al, Endocrine Reviews 2008 (217)
In the United Kingdom, several studies have described the prevalence of MetS. In a large prospective cohort study from Norfolk, using the modified ATPIII definition (218), the prevalence of MetS amongst 45-79 year olds was 37.6% in men and 30.2% in women (219). In contrast, the Whitehall Study of male UK civil servants has estimated the prevalence of MetS using ATP III at 13.6%, suggesting a role for socio-economic factors in the prevalence of the syndrome. This study also observed an increase in the prevalence of MetS between 1991-93 and 2003-4, in parallel with an increase in central obesity (220).

1.7.1.3 The Metabolic Syndrome and CHD risk prediction
The purpose of MetS is to identify individuals at greater risk of developing diabetes mellitus and cardiovascular disease, and so can be used as risk prediction tool. The presence of MetS predicts future CHD events in those with and those without prevalent CHD, and future risk is related to the number of criteria present (219;221-224). A recent meta-analysis of 37 studies examining the association of MetS and CV events in 172,000 individuals reported a relative risk ((RR) 95% CI) of CHD events or death of 1.78 (1.58-2.00) in those with MetS, which remained significant even after adjusting for the individual risk factors present (RR 1.58) (225). Not all prospective studies have confirmed the association between MetS and prediction of future CHD events however, and MetS does not appear to perform as well in older cohorts (226).

1.7.2 The Metabolic Syndrome in SLE
The metabolic abnormalities captured by MetS, such as hypertension, dyslipidaemia and insulin resistance, are common in SLE and may be influenced by both inflammation associated with SLE and the therapies used to treat active disease, particularly corticosteroids. MetS may therefore contribute to the risk of premature vascular damage that is characteristic of SLE, and the evidence for this is discussed below.

1.7.2.1 Epidemiology of Metabolic Syndrome in SLE
The prevalence of MetS in SLE varies significantly according to the population studied and to an extent reflects the background population prevalence of MetS. For example, the prevalence has been estimated at 30% in the UK (130), 20% in Spain (164), 38.2% in Puerto Rico (227) and 30% in the USA (165). Studies have also confirmed hyperinsulinaemia in SLE patients and increased insulin resistance (205;206;228). Mouse models of SLE have demonstrated that lupus-prone mice are more likely to be obese, hypertensive and insulin-resistant than controls. It would therefore appear that the SLE phenotype is associated with an intrinsic propensity to insulin resistance and MetS is an ideal surrogate to assess
this in larger cohorts. To date however studies are small and include patients of older age with longer disease duration, impeding comparison and minimising possible effects of inflammation on MetS development.

It is does appear however that the MetS phenotype is different in SLE compared to the general population, characterised less by central obesity and more by hypertension and dyslipidaemia (229). This difference may relate to the inflammation and high prevalence of renal disease in SLE. In a US cohort for example, 51% of lupus patients were hypertensive, compared to 34% of controls (165), and in a UK cohort the proportion meeting the hypertension criteria were 57% and 40% respectively (130). In contrast many studies have shown that despite MetS being more common in SLE, similar rates of elevated waist circumference are observed between patients and controls (130;164;205;230). Many of these studies used MetS definitions anchored by central obesity and so may have overestimated the impact of obesity on MetS in SLE and underestimated the impact of the non-obese components of MetS on its prevalence. Adiposity may also be under-estimated in lupus patients using traditional clinical measures of central obesity, such as BMI and waist circumference, compared to modern imaging methods, such as dual x-ray absorptiometry (231). Several key questions remain regarding the epidemiology an phenotype of MetS in SLE, and this will be a focus of this thesis.

1.7.2.2 Metabolic Syndrome and vascular damage in SLE
The high prevalence of MetS in SLE may in part relate to the adverse metabolic effects of inflammatory disease activity and steroid exposure, and may therefore provide a mechanism through which premature vascular damage accrues in SLE. No prospective study has yet been performed in SLE to determine whether MetS predicts adverse CV outcomes, although there have been several cross-sectional and retrospective studies. Sabio et al demonstrated that SLE patients with MetS had higher aortic pulse wave velocity (aPWV) compared to those without MetS (232), and that higher aPWV was independently associated with MetS, longer disease duration and higher CRP, as well as male gender and increasing age. In a UK study of 200 women with SLE however, MetS was not independently associated with carotid plaque, although meeting the individual criteria for hypertension and hypertriglyceridaemia was predictive of plaque presence (130).

1.7.2.3 Determinants of Metabolic Syndrome in SLE
The underlying mechanisms that predispose an individual to MetS are likely to be complex, with significant interplay between inflammatory disease activity, therapeutic exposures, insulin resistance and traditional CHD risk factors. Cross-sectional studies have sought to examine why SLE patients have an increased propensity to MetS by assessing the impact of disease phenotype and
therapeutic exposures on the risk of developing MetS, with differing results, but no prospective study has yet been performed. For example, in a univariate analysis of 160 Spanish SLE patients, both higher SLICC-DI and ESR were associated with the presence of MetS, and hydroxychloroquine use was ‘protective’ (OR 0.19 (0.06, 0.61)) in a multivariable model (164). Similar results were found in an Argentinian cohort of 147 patients (230), and elevated CRP was also found to be significantly associated with MetS in a US cohort of 102 patients (165). Disease activity (using SLAM-R) and ‘ever’ exposure to corticosteroids were found to be significantly associated with MetS in a Puerto Rican cohort of 204 SLE patients (227). Finally, in a cross-sectional multivariate analysis of a large UK cohort of 200 stable SLE patients, longer disease duration, ‘ever’ exposure to corticosteroids and hypocomplementaemia were found to be significantly associated with MetS, in addition to increasing age (130). It is not entirely clear from the current literature therefore whether inflammation and/or steroid use increase the risk of MetS, although this uncertainty may relate more to the methodological constraints of the studies (small cohorts of stable patients with low corticosteroid doses and minimal disease activity) and their cross-sectional design, than the absence of a true relationship. There is a need therefore for larger longitudinal studies to determine the relationship between disease-related factors and MetS, and is a key focus of this thesis.

In summary, MetS predicts adverse cardiovascular events in the general population and patients with SLE have a higher prevalence of MetS and insulin resistance than control populations. MetS may therefore contribute to the excess vascular damage seen in SLE. Inflammatory disease activity and corticosteroid exposure may be key determinants of MetS susceptibility in SLE, although studies to date have been inconsistent because of their cross-sectional design and small sample size. The contribution of inflammation and corticosteroid exposure to MetS development will be therefore be addressed in this study in a large prospective inception cohort of patients with SLE over the first 2 years of their disease.

1.8 Endothelial damage and dysfunction in SLE

The vascular endothelium is a single layer of cells lining the entire vascular system. It is able to both sense and respond to local and systemic stimuli, releasing various vasoactive substances that ultimately regulate vascular tone – a phenomenon termed endothelial function. Endothelial function is a dynamic process sensitive to both adverse and beneficial interventions (233;234) and endothelial dysfunction is considered to represent the earliest stage in the
atherogenic process. It is therefore a useful surrogate of cardiovascular risk to assess the response of an individual to a specific treatment or clinical intervention. Below are described in more detail the strategies that will be utilised in this study to assess endothelial function, incorporating flow-mediated dilatation, peripheral arterial tonometry, endothelial damage and vascular biomarkers of endothelial activation.

1.8.1 Non-invasive measurement of endothelial function

1.8.1.1 Flow-mediated dilatation
The most commonly used non-invasive measure of endothelial function is detection of endothelium-dependent flow-mediated dilatation (FMD) of a medium-sized artery (commonly the brachial artery) in response to hyperaemia. Originally described by Celermajer et al in 1992 (235), the increasing use of FMD in the research setting led to the development of internationally recognized guidelines for its use in 2002 (236;237).

Flow-mediated dilatation of an artery reflects the endothelium’s ability to release nitric oxide in response to hyperaemia-induced shear stress, and healthy blood vessels respond to an increase in blood flow with dilatation. An increase in blood flow causes endothelial cells to hyperpolarize following the opening of calcium-activated potassium channels. This process activates the enzyme endothelial nitric oxide synthase (eNOS) leading to the generation of nitric oxide, which causes dilatation of the affected vessel (238). A reduction in nitric oxide bioavailability is considered to underpin the pathogenesis of vascular disease (239) and FMD can therefore be considered a marker of the homeostatic and autoregulatory properties of the vascular system, and a surrogate for CV risk.

FMD is expressed as a percentage change in arterial diameter from baseline and a small FMD response is considered to represent reduced bioavailability of nitric oxide, and hence an increased cardiovascular risk.

The relationship between shear stress and FMD, and the mechanisms which underpin it, remain incompletely understood, and nitric oxide may not be the only vasodilator of importance. The phenomenon of FMD still occurs in eNOS-deficient mice (240), suggesting alternative mechanisms or compensatory factors may influence FMD such as prostaglandins and Endothelial Derived Hyperpolarising Factor (EDHF) (241).

1.8.1.2 Peripheral arterial tonometry
The widespread use of FMD as a measure of endothelial function in research and clinical settings is restricted due to the significant technical demands of data collection, the steep learning curve for the individual scanner and the need for specialist equipment (236), as well as significant inter-observer variability.
between centres. Therefore, attempts have been made to introduce a more reproducible measure of endothelial function, prominent amongst which is peripheral arterial tonometry (PAT). The EndoPAT© system for the non-invasive measurement of endothelial function has been developed by Itamar Medical Ltd (Israel) and is an example of one such measure. PAT is a non-invasive plethysmographic system for measuring changes in digital pulse wave amplitude (PWA) that utilizes pneumatic finger probes to record changes in pulsatile volumes. Abnormalities in the PWA have long been described in established CHD (242) but the physiological processes involved and their prognostic implications, as well as the relation to established techniques were until recently unclear. Kuvin et al described a moderate correlation between PAT hyperaemic ratio and FMD ($r^2 = 0.55$, $p <0.001$), and that patients with the lowest FMD had the lowest hyperaemic ratio (and vice versa). However, the performance of PAT in patients with connective tissue diseases (CTD) may be influenced by the presence of Raynaud’s phenomenon and a recent study found no correlation between PAT and FMD (243), and this will be further investigated in the present study.

In contrast to FMD, few studies have investigated the underlying physiology of PAT, or whether the vessels and vasoactive pathways assessed are comparable between the two techniques. Whilst nitric oxide has been presumed to play a key role in PAT, the digital vasculature differs from conduit vessels in several key ways, such as the presence of a high number of arterio-venous anastomoses and the increased role of the sympathetic nervous system in regulating tone (244). In a study of 33 healthy subjects, Nohria et al investigated the effect of inhibiting nitric oxide synthesis, achieved by infusing L-NMMA through a brachial artery cannula, on PWA (using the EndoPAT© system) both at rest and during reactive hyperaemia. They conclude that at least 50% of the increase in PWA during reactive hyperaemia is due to nitric oxide, and that measuring PWA with systems such as the EndoPAT© is a valid measure of endothelial function (245).

1.8.1.3 Endothelial dysfunction and cardiovascular disease

In the general population, FMD-assessed endothelial dysfunction is associated with the presence of traditional CHD risk factors such as hypertension, diabetes mellitus, and smoking (246-248) and FMD of the brachial artery correlates well with invasive coronary artery testing of endothelial function (249). Impaired FMD predicts future coronary events (250), and can be improved with statin therapy, ACE-inhibition and aspirin therapy (251-253). Similarly, PAT-assessed endothelial dysfunction is associated with prevalent CHD risk factors and subclinical atherosclerosis and adverse CV outcomes. For example, Kuvin et al and Hamburg et al both report a correlation between a reduced hyperaemic ratio
and the presence of traditional cardiovascular risk factors (254;255). In a cross-sectional study by Bonetti et al patients with impaired coronary artery endothelial function also had an impaired hyperaemic ratio as assessed by PAT. They report that an index of less than 1.35 had a specificity of 80% and a sensitivity of 85% for predicting the presence of coronary artery endothelial dysfunction. Finally, Rubinshtein et al reported that a low reactive hyperaemic index independently predicted adverse cardiac outcomes in a longitudinal study of 270 symptomatic low-risk prevalent CHD patients followed up over a 7-year period. (256).

1.8.1.4 Endothelial dysfunction in SLE
Several groups have demonstrated impaired endothelial-dependent vasodilation using FMD in patients with SLE, but only one study to date has investigated PAT in SLE patients (243). In a study by El-Magadmi et al, FMD was lower in 62 patients with stable SLE compared to 38 age-matched controls (median (IQR) FMD 3.6% (-6.2, 13.7) vs. 6.9% (-6.6, 17.8); p = 0.001) (257). Wright et al described similar results in 32 SLE patients and 19 healthy controls (median (IQR) FMD 2.4% (-2.1, 10.7) vs. 5.8 (1.9, 14.0); p <0.001) (258). This group also identified morphological differences in the analysis of the pulsed Doppler flow velocity waveform during the reactive hyperaemic stage in SLE patients compared to controls, thought to be due to structural or functional alterations of the forearm microcirculation. They suggested that SLE patients exhibit altered arterial waveforms which reflect downstream microvasculature abnormalities, predominantly affecting the pulsatile properties of the microcirculation (258). These findings could provide important mechanistic clues as to the predominant abnormality in the vasculature of lupus patients. Mak et al (259) recently performed a meta-analysis of 13 studies of FMD in 580 SLE patients and 381 matched controls, and reported a standardised mean difference in SLE patients of -0.832 (95% CI -1.172, -0.492). They also reported that patients with associated antiphospholipid syndrome had reduced endothelial-independent vasodilatation, suggesting impaired vascular smooth muscle function in these patients. Wright et al reported an improvement in FMD in 60 patients with SLE following a 6-month course of omega-3 fatty acids in a randomised placebo-controlled trial, (183) and an 8-week course of statins were also associated with improvement in endothelial function in SLE patients over an 8-week period (178), as discussed in 1.6.1.2 The potential beneficial effects of antimalarial therapies on endothelial function have not been prospectively studied in SLE, although they were associated with a less discordant FMD response in the meta-analysis by Mak et al (259).
1.8.2 Endothelial damage and endothelial microparticles

The presence of subcellular particles in plasma has been recognized for many years and were originally described as ‘platelet dust’ by Wolf in 1967(260). These ‘microparticles’ were thought to consist of cellular debris and considered biologically inactive. The term ‘microparticle’ (MP) refers to sub-cellular vesicles less than 1.5µm, which originate from many cell types including endothelial cells (endothelial microparticles (EMPs)) and platelets (platelet microparticles (PMPs)). Smaller circulating vesicles include exosomes and apoptosomes. The availability of improved techniques for detection and identification of microparticles, specifically flow cytometry, has stimulated interest in the potential biological actions of microparticles in health and disease. Elevated EMP levels have been observed in numerous conditions associated with vascular dysfunction but their levels in SLE remain unknown and will therefore be a focus of this study. EMPs are of particular interest in SLE given the importance of the endothelium in both inflammatory disease manifestations and the role of endothelial dysfunction in the early stages of atherosclerosis. The formation, composition and detection will be discussed below, as well as their potential role in atherosclerosis and inflammatory autoimmune conditions.

1.8.2.1 Formation, release, and composition of EMPs

Disruption of the endothelium can occur following endothelial cell activation or apoptotic cell death. This may be initiated and propagated by cardiovascular risk factors, leading to conformational changes of the plasma membrane and the release of phosphatidylserine-expressing microparticles (261;262). An increase in intracellular calcium leads to cytoskeletal and conformational membrane changes, resulting in the formation of membrane blebs and exposure of phosphatidylserine (PS) results in the release of the microparticle (MP) (Figure 1-4).

Triggers for EMP release in vivo are varied and incompletely understood. As mentioned above they are thought to include cell activation and apoptotic stimuli, such as inflammatory cytokines, reactive oxygen species, CRP, and complement (263;264), many of which are important in SLE. Interestingly, recent cell culture experiments comparing Human Umbilical Vein Endothelial Cells (HUVECs) from African-Americans and Caucasian Americans have suggested that HUVECs of African-American origin may be more sensitive to the deleterious effects of inflammatory cytokines such as TNF-α (265). This observation could be directly relevant to the ethnic gradient that characterises the susceptibility and severity of many lupus manifestations.
The formation of MPs is triggered by a variety of apoptotic and activation stimuli, which in the case of EMPs include traditional CHD risk factors. Both formation and release is a calcium-dependent mechanism, and all MPs express phosphatidylserine on their membranes. 
From Boulanger et al Hypertension 2006 (266)

The composition of MPs may also influence their biological action and may differ according to both their cell of origin and the mechanism and trigger of their formation. To date, proteomic analysis has only been performed on EMPs formed in vitro from HUVECs cultured with TNF-α, and therefore may not accurately reflect the composition of microparticles generated in vivo. However, such analysis has demonstrated that EMPs contain nucleic acid material, express adhesion molecules such as ICAM-1, and that the EMP proteome reflects the mechanism of microparticle formation (267). The surface membrane antigenic composition of MPs formed in vitro may also differ from its cell of origin, depending on the trigger for formation (268).

1.8.2.2 Identification and quantification of EMPs
There is significant variation in the methods used to identify and quantify all MPs. Flow cytometry is however the most commonly used analysis technique, utilizing the antigenic composition of microparticle membranes to allow their characterization. The process of identifying and quantifying EMPs involves several distinct stages - blood collection, centrifugation, antibody detection of cell surface antigens, and flow cytometry – and there is significant variation at all stages, with no international consensus on a standardised protocol yet reached (269-271).
Whole blood is collected through a large-bore needle and centrifuged promptly to avoid cellular damage. Centrifugation of samples to generate platelet-poor-plasma (PPP) is a key step in the analysis of MPs, and the most variable (272). Whilst a consensus has yet to be reached, most groups utilise a 2-step centrifugation process to generate PPP on which MP analysis is performed (269). An alternative centrifugation regime is to use higher speeds for longer to isolate a MP pellet, which is then re-suspended and subsequently analysed (273;274). Analysis of PPP or MP pellets can be immediate or samples can be stored at -80°C.

Immunolabelling of PPP with fluorescent cell surface markers allows identification of the cell of origin of MPs but again no consensus on specific markers has been reached. Most groups utilise annexin V binding to phosphatidylserine (PS) to identify microparticles, with calcium added to promote this reaction (266). This is not universal however. The platelet-specific marker CD42b is widely used to identify MPs of platelet origin (269). Several cell surface antigens have been employed to identify MPs of endothelial cell origin, and most frequently utilised is CD31 (272;274-278). CD31 is however not absolutely specific to endothelial cells, and low-level expression is seen on both platelets and some leucocytes (268). Those groups utilising CD31 generally also use a platelet marker, in addition to annexin V, in the analysis and define EMPs as annexin V-positive/CD31-positive/CD42b-negative. Other groups have utilised alternative endothelial cell markers such as CD105, CD144 and CD146 (274;279-281), although again there is no consensus.

The final step in identifying and quantifying MPs is flow cytometry, a technique used by the vast majority of researchers. In addition to identifying MPs on the basis of size and fluorescence, flow cytometry also allows enumeration of MPs through the use of counting beads (282). Recent studies have examined deficiencies of flow cytometry in identifying and quantifying MPs, specifically the loss of sensitivity and specificity at lower event sizes. For example, Gyorgy et al comprehensively characterised MPs using a variety of techniques including electron microscopy and dynamic light-scattering analysis in a mixed cohort of patients with rheumatoid arthritis, osteoarthritis and healthy controls. They report that MPs are typically 80-400nm in size and smaller events were typically exosomes. Many biophysical properties were also shared between MPs and immune complexes (such as size) and the authors caution that events classified as microparticles using conventional flow cytometry may actually represent immune complexes, an observation which is of significance in SLE patients (283). Other studies have also attempted to standardise PMP enumeration by flow-cytometry and noted significant discrepancies between centres based upon
the characteristics of each flow cytometer and method of calibration (284). However, flow cytometry remains the most widely used and validated technique to detect and quantify microparticles.

1.8.2.3 Role of EMPs in vascular dysfunction

EMPs display paracrine and autocrine actions on cells of the vascular system and growing evidence suggests they act as a mediator in intracellular signalling (285). EMPs express a number of endothelial cell-derived proteins including adhesion molecules, vascular endothelial cadherin and E-selectin. Functional proteins such as vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase (eNOS) have also been identified in EMPs (286;287). The composition of EMPs may reflect as yet unidentified important biological functions in disease pathogenesis and vascular dysfunction, and contribute to the increased cardiovascular risk seen in SLE. Alternatively, endothelial-derived MPs may have vasculoprotective effects on the endothelium in conditions associated with acute vascular stresses, such as septic shock (288;289). With regards to their detrimental vascular effects, EMPs from patients with cardiovascular disease have been shown to impair the release of nitric oxide from vascular cells (274;290) and platelet-derived MPs can act as a source for thromboxane A2, which increases vascular contraction (291). Cell culture-derived EMPs have also been shown to inhibit angiogenesis in mouse models of atherosclerosis (292). Therefore, rather than being inert markers of injury, EMPs may act as downstream delivery systems for pro-inflammatory products that are vasculoprotective in acute inflammatory conditions but which may perpetuate further vascular dysfunction in chronic disease, as well as acting as surrogates of vascular dysfunction (293).

1.8.2.4 EMPs and cardiovascular disease

Circulating levels of EMPs have been shown to be elevated in patients with prevalent cardiovascular disease and traditional risk factors, using a variety of methodologies and cell surface markers (Table 1-4). EMP (CD31+) levels have been shown to correlate with in vivo measures of endothelial function such as flow-mediated dilatation in non-inflammatory conditions (290;294-296) and may therefore act as a surrogate biomarker for endothelial function (Figure 1-5). Circulating CD31+ MPs have also been shown to predict adverse CV outcomes (278), and elevated CD144+ MPs predict adverse outcomes in patients with stable heart failure (281).
Table 1-4: Endothelial microparticles levels in cardiovascular diseases.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Ann V+</th>
<th>EMP definition by antigenic markers</th>
<th>Increase in EMP level vs. controls</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS</td>
<td>√</td>
<td>CD146+</td>
<td>3.2x</td>
<td>(275)</td>
</tr>
<tr>
<td></td>
<td>√</td>
<td>CD31+</td>
<td>7.3x</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>x</td>
<td>CD31+/CD42-</td>
<td>2x in severe HT</td>
<td>(276)</td>
</tr>
<tr>
<td>End Stage Renal Disease</td>
<td>√</td>
<td>CD31+/CD42-</td>
<td>3x</td>
<td>(274)</td>
</tr>
<tr>
<td>Heart Failure</td>
<td>√</td>
<td>CD144+</td>
<td>2.4x in Class III/IV</td>
<td>(281)</td>
</tr>
<tr>
<td>Type 2 DM</td>
<td>x</td>
<td>CD31+/CD42b-</td>
<td>2x</td>
<td>(279)</td>
</tr>
<tr>
<td>MetS</td>
<td>x</td>
<td>CD31+/CD42-</td>
<td>3x</td>
<td>(277)</td>
</tr>
<tr>
<td>Obesity</td>
<td>x</td>
<td>CD31+/CD42-</td>
<td>2x</td>
<td>(296)</td>
</tr>
</tbody>
</table>

Abbreviations: Ann V Annexin V; ACS Acute Coronary Syndrome; DM Diabetes Mellitus.

Figure 1-5: CD31+ EMPs levels correlate with invasive and non-invasive measures of endothelial function.

This figure shows that: (A) CD31+/Annexin V+EMP levels inversely correlate with by quantitative coronary angiography during intracoronary acetylcholine infusion (294); (B) CD31+/Annexin V+EMP levels correlate with endothelial-dependent vasodilatation as assessed by flow-mediated dilatation of the brachial artery in obese women (296).

1.8.2.5 The role of EMPs in predicting adverse vascular outcomes
Recent interest in MPs, and specifically EMPs, relates to their potential role in promoting vascular damage, and in their ability to act as a biomarker for cardiovascular risk and predict adverse outcomes. In a longitudinal study of 200 people with stable coronary artery disease, elevated EMP levels (CD31+/Annexin V+) at study entry were independently associated with a higher risk for
cardiovascular death, revascularisation and adverse cardiovascular event during follow-up than were low EMP levels (Figure 1-6). The inclusion of EMP levels into risk factor models also improved their accuracy (278). A similar prognostic role for EMPs (CD144+) was noted in a study of 169 patients with stable heart failure (281). In this study, elevated EMP levels at baseline were significantly associated with future cardiovascular events but not all cause mortality, again suggesting they may be a useful additional factor in risk stratification scores.

**Figure 1-6: Elevated CD31+ EMP levels predict adverse cardiovascular events in coronary artery disease.**

This figure describes event-free survival in a cohort of patients with stable coronary artery studied longitudinally. Patients with elevated CD31+/Annexin V+ MP levels (above the median value) at baseline had significantly more CV events than patients with lower levels, particularly CV mortality and revascularisation. From Sinning et al European Heart Journal 2011(278).

### 1.8.2.6 EMPs in autoimmune diseases and SLE

Microparticle populations have been assessed in several autoimmune and inflammatory conditions, although the focus of investigation to date has often been their potential as biomarkers of disease activity or their role in immunopathogenesis of disease. For example, Brogan et al investigated EMP levels in childhood systemic vasculitis, and hypothesized that EMPs could act as a non-invasive measure of vascular damage related to disease activity (273). They measured EMP levels (e-selectin+/annexin V+) in 39 children with systemic vasculitis, 24 febrile controls and 43 healthy controls, and found that the median total EMP counts were significantly higher in children with active vasculitis compared with inactive vasculitis and both groups of controls. In a smaller longitudinal study of 5 children with active vasculitis, they also demonstrated that EMP levels reduced significantly following induction of remission (Figure 1-7). In a study of 30 patients with a positive lupus anticoagulant (LAC) CD51+...
EMP levels were higher in those patients with a positive LAC compared to controls, and in those with a history of thrombosis compared to those without (263). A larger follow-up study by the same group also found elevated EMPs in patients with positive antiphospholipid antibodies, some of whom had SLE, compared to those without (297).

**Figure 1-7: Elevated E-selectin+ EMP levels in active childhood vasculitis**

![Diagram showing EMP levels in active and inactive vasculitis]

*E-selectin positive EMPs are elevated in active vasculitis compared to inactive disease and controls, and are reduced following remission induction. (Adapted from Brogan et al 2004 (298))*

To date, few studies have investigated MP levels in SLE. Sellam et al quantified total (annexin V +), platelet-derived (CD61+), and leucocyte-derived (CD45+) MPs levels in a mixed cohort of SLE (n =20), RA (n = 24) and Sjogen’s Syndrome (SS; n = 43) patients and compared them to controls (n = 44) (299). Total MP and platelet-derived MPs were elevated in SLE patients compared to controls (71230/μl vs. 6422/μl and 32290/μl vs. 4229/μl respectively), although leucocyte-derived MPs were not, and levels were higher still in both the RA and SS patients. In the only other study to date addressing MP levels in SLE, Nielsen et al characterised MPs in a cross-sectional study of 70 unselected SLE patients (median age 39 years) and compared them to 29 healthy controls (median age 42 years) (300). Utilising an alternative approach to MP characterisation based principally on particle size rather than antibody binding, they described an additional annexin V-negative population of MPs, both of cellular origin and of unknown origin. Interestingly, and contrary to the studies already discussed, total annexin V-binding MPs were reduced in SLE patients (2.76 x 10^6 MPs/ml vs. 6.88 x 10^6 MPs/ml; p = 0.0001), as were MPs of platelet (CD42a+) and endothelial origin (CD146+). However, the novel population of annexin V non-binding MPs was significantly elevated in the SLE cohort compared to controls (1.03 x 10^6 MPs/ml vs. 0.37 x 10^6 MPs/ml; p = 0.007), and correlated with
disease activity, nephritis and immunosuppressant use. The authors acknowledged their results were contrary to several other studies of MPs in autoimmune conditions, and hypothesised that the differences related to a heterogeneous circulating MP pool in SLE and alternate methodological approaches between groups. Similar annexin V non-binding platelet-derived MPs have also been described by Connor et al, in a small study (n = 5) of healthy volunteers, in which up to 80% of PMPs did not bind annexin V (301). In a follow-up study, despite previously demonstrating reduced MP levels in SLE, Nielsen et al investigated the potential role of MPs as antigenic targets and carriers of immune complexes in SLE (302). Using similar techniques, but focusing only on the annexin V-binding MP population, they demonstrated that circulating annexin V+ MPs carried significantly higher IgG load than healthy controls. Using quantitative mass spectrometry of purified MPs, they also demonstrated increased IgG, IgM and C1q levels in SLE compared to both healthy and RA controls. IgG-positive MPs were associated with autoantibodies (anti-dsDNA, ENA and anti-histone antibodies) and leucopenia, and the authors suggest that MPs may therefore act as a source of auto-antigen presentation in SLE.

In summary, EMPs may act as markers and mediators of vascular damage in many disease states, including autoimmune conditions and SLE. Their role as a surrogate of endothelial damage, and therefore a marker of potential endothelial dysfunction, could provide a useful research tool in SLE and is a key component of this study.

1.8.3 Circulating markers of endothelial activation and vascular risk in SLE

Several vascular biomarkers have been shown to be of potential relevance in assessing vascular injury and protection in SLE patients, although most have only been assessed in cross-sectional studies to date. The evidence for their role in endothelial dysfunction and vascular damage will be discussed below, and the limits of current knowledge with regard to how these markers change over time will be reviewed.

1.8.3.1 Vascular Cell Adhesion Molecule-1
Vascular cell adhesion molecule-1 (VCAM-1) is a cell surface adhesion molecule expressed by vascular endothelial cells, and appears to play an important role in the initiation of atherosclerosis through aiding recruitment of inflammatory cells to an early atherosclerotic lesion. Atherosclerotic-prone mice who are deficient in adhesion molecules appear to develop less plaque, and elevated levels of VCAM-1 can be detected in the serum of patients with coronary artery disease (303).
Expression of VCAM-1 is up-regulated in response to inflammation and is particularly sensitive to increased levels of TNF-α (304), but is inhibited by increased levels of high-density lipoproteins (HDLs) (305). Soluble VCAM-1 correlates well with levels of inflammatory disease activity in SLE patients (306-309), perhaps providing a mechanistic link between active lupus and endothelial dysfunction.

1.8.3.2 Vascular Endothelial Growth Factor
Angiogenesis refers to the growth of new capillaries from existing micro-vessels and is a normal part of normal adult physiology, permitting growth and repair in the vascular system. Vascular endothelial growth factor (VEGF) is a family of proteins, which when bound to its membrane receptor is pro-atherogenic, but when bound to its soluble receptor is angiostatic (mainly through reduced bioavailability). Excessive angiogenesis has been associated with the development of many diseases, including cancer, atherosclerosis and rheumatoid arthritis, whilst insufficient angiogenesis and endothelial dysfunction is characteristic of hypertension, pre-eclampsia and nephropathy. An imbalance in the wear and repair of the endothelium is likely to be central to the development of atherosclerosis in SLE, and may be influenced by angiogenic factors such as VEGF. Elevated circulating VEGF has been demonstrated in both ischaemic diseases (such as CHD) and vasoproliferative diseases (such as proliferative retinopathy), and so may be acting both as marker of ischaemia but also a promoter of angiogenesis.

Elevated VEGF has been observed in several SLE cohorts (125;310-313) and may correlate with disease activity and severity (312;314;315). Elevated VEGF has been implicated in the pathogenesis of nephritis (316) and associated with higher cIMT in SLE patients compared to controls (317). IFN-α has also been shown to reduce VEGF production by EPCs from SLE patients (110). However, not all studies have observed elevated VEGF levels in SLE (318), and VEGF levels does not correlate with angiogenic activity of lupus sera (310) or coronary artery calcium scores (317).

These inconsistencies may relate to the cross-sectional design of many studies and the single time-point assessment of VEGF levels, which won’t delineate its role as either a marker of tissue ischaemia or its potential pro-angiogenic molecule. In the context of active SLE, it is expected that VEGF will be elevated and correlate with endothelial dysfunction, and improve with better disease control, and will be examined in this study.

1.8.3.3 Adiponectin
Adiponectin is an adipose-derived plasma protein that has long been associated with the metabolic syndrome, itself associated with increased cardiovascular
Adiponectin increases peripheral sensitivity to insulin and muscle uptake of glucose, promoting the clearance of free-fatty acids and inhibiting gluconeogenesis. A reduction in the level of adiponectin therefore increases insulin resistance and adversely affects cardiovascular risk (319). However, adiponectin levels are higher in SLE compared to controls (320), an observation that may in part relate to co-existent renal impairment and its anti-inflammatory role (321). The inverse relationship with MetS however remains in SLE, suggesting that a relative reduction in adiponectin within SLE patients is associated with increased insulin resistance. Following treatment of lupus nephritis with MMF, Clancy et al (322) described an increase in adiponectin levels which raises the possibility that low adiponectin may mediate a link between SLE, inflammation and MetS.

1.9 Summary of the literature

SLE is associated with an increased risk of premature clinical and subclinical atherosclerosis compared to the non-lupus population, and SLE is considered an independent risk factor for cardiovascular disease. The reasons for this enhanced CV risk are complex and multifactorial, but both traditional and non-traditional risk factors contribute to the vascular damage in SLE. The vascular endothelium appears to act as a key interface between SLE and atherosclerosis, and systemic inflammatory disease activity and its treatment are likely to be central to the proatherogenic environment of SLE. The effects of inflammation and its treatment on CV risk have yet to be fully characterised in longitudinal studies of sufficient size to allow exploration of the relevant factors. This will be examined in this thesis, with MetS employed as a surrogate for CV risk. Improved control of inflammatory disease activity has been hypothesised to improve vascular function and reduce long-term CV risk but has yet to be tested in prospective studies. This will form the second focus of this study.
Chapter 2

Hypothesis and Objectives of Thesis

This chapter will state the hypothesis of the thesis and delineate the specific aims to be achieved.
2 Hypothesis and Objectives

2.1 Hypothesis

SLE is associated with pro-atherogenic metabolic derangements and endothelial dysfunction that lead ultimately to accelerated atherosclerosis. This thesis will investigate the relative contribution of inflammation, and the anti-inflammatory therapies used to improve disease control, to the observed increased cardiovascular risk in SLE. Therefore, the two key hypotheses to be examined by this thesis are:

1. In an international inception cohort of patients with SLE, the Metabolic Syndrome, a surrogate for enhanced CV risk, is associated with inflammatory disease activity and corticosteroid exposure over time.
2. In a controlled longitudinal study, patients with active SLE demonstrate impaired endothelial function and elevated markers of endothelial damage and activation, compared to healthy controls. Improved control of inflammatory disease activity in this cohort will lead to improvements in these indices of endothelial damage and dysfunction.

2.2 Objectives

With regards hypothesis (1), the aims of the study are to:

1. Describe the demographic and clinical features of a large international inception cohort of SLE patients.
2. Determine the prevalence and persistence of MetS over the first 2 years of follow-up in an inception cohort.
3. Determine the relationship between MetS and inflammatory disease activity, lupus phenotype and corticosteroid exposure at enrolment and over the first 2 years of follow up in the study.

With regards hypothesis (2), the aims of the study are to:

1. Describe the demographic and clinical features of a cohort of SLE patients with active disease and a cohort of healthy controls.
2. Compare indices of endothelial function and damage between SLE patients and control subjects in a cross-sectional analysis.
3. Describe change over time, and explore the relationship between, inflammatory disease activity, endothelial function and endothelial damage, following a change in anti-inflammatory therapy in SLE patients.
Chapter 3

Methods (i): Metabolic Syndrome in SLICC-RAS

This chapter will provide details of the methodologies utilised to analyse the determinants of the metabolic syndrome in the SLICC-RAS cohort.
3 Methods (i): SLICC-RAS methodology

This chapter will describe the methodology applicable to the analysis performed on data from the SLICC cohort. The hypothesis states that in an international inception cohort of patients with SLE, the Metabolic Syndrome, a surrogate for enhanced CV risk, is associated with inflammatory disease activity and corticosteroid exposure over time.

3.1 SLICC Registry for Atherosclerosis

The Systemic Lupus International Collaborating Clinics (SLICC) comprises a group of international collaborating SLE experts, formed in 1991 to design a series of studies comparing disease activity indices in SLE. Following initial work that resulted in the development of the SLICC/ACR-damage index (74) the SLICC group developed an international registry of patients with newly diagnosed SLE to facilitate prospective, longitudinal studies of risk factors for the development of atherosclerosis in SLE – the SLICC Registry for Atherosclerosis (SLICC-RAS) (323). SLICC currently comprises 36 investigators from 32 centres in 11 countries (Figure 3-1) and receives funding from a variety of sources, including the Canadian Institutes of Health Research, the Lupus Foundation of America, Lupus Ontario and Lupus UK, amongst others.

The establishment of a large international inception cohort of SLE patients to facilitate the study of disease-related risk factors for the development of
Atherosclerosis in SLE has many advantages over alternative methodologies. Studying an inception cohort reduces the confounding effects of increasing age, disease duration and disease damage that often hamper historical and retrospective studies of cardiovascular disease involving established lupus cohorts. The study design also provides the statistical power to test many potential disease-related factors and the international nature of SLICC ensures results are both relevant and applicable to a wide range of populations and ethnicities.

3.2 Ethical Approval

The study was approved by the University Health Network Research Institute, Research Ethics Committee, Toronto, Canada and by the institutional research ethics boards of participating centres in accordance with the Declaration of Helsinki’s guidelines for research in humans (Appendix 4). All patients provided written informed consent (Appendix 5).

3.3 Patient recruitment and study design

SLICC-RAS is a prospective longitudinal study of patients with recently diagnosed SLE that have been recruited since 2000, and in whom follow-up is ongoing. Patients are enrolled into SLICC-RAS when four or more of the ACR classification criteria for SLE (4) are confirmed. All patients are enrolled within 15 months of the date of their diagnosis and there are no further exclusion criteria. Each patient is assessed at enrolment and annually thereafter and all data is submitted to the co-ordinating centre at the University of Toronto.

3.4 Data collection

3.4.1 Enrolment assessment

Clinical and laboratory features were recorded according to a standard protocol. Demographic data including age, socioeconomic factors (such as education level and marital status), ethnicity and gender were assessed. A full cardiovascular history detailing any relevant events and risk factors was undertaken and a full medical history was documented. A full history of all general drugs was also noted, and specific note is made of anti-hypertensive agents, lipid-lowering drugs and hypoglycaemic agents. Full anthropomorphic assessment was
performed including blood pressure, height, weight, waist circumference, waist: hip ratio and body mass index (BMI).

SLE-specific details were recorded including age at diagnosis, date of diagnosis, disease duration, and specific ACR criteria present. The presence of active renal disease was documented by the physician with active nephritis defined as: haematuria (>5 red blood cells (rbc) per high-powered field (hpf), excluding other causes); pyuria (> 5 white blood cells (wbc)/hpf, excluding infection); new/recent increase of > 500 mg 24 hour protein; casts including granular or rbc; or a consistent renal biopsy. Nephrotic syndrome was defined as proteinuria > 3 grams / 24 hour, oedema, and increased BP. A clinical examination was performed to assess disease activity using SLEDAI-2K. In individuals diagnosed more than 6 months prior to enrolment, a SLICC/ACR damage index was completed. A full lupus-specific therapeutic history was recorded (Appendix 6).

Details of current corticosteroid use were recorded, including current use, current dose, highest dose since diagnosis, parenteral steroid use (dose, route and frequency), and details of each course of steroid received since diagnosis (dose and duration). Antimalarial dose and duration of use was noted. Use of immunosuppressant drugs was also recorded, detailing name, dose and duration of each drug. All laboratory tests to evaluate disease activity (such as immunology profile, full blood count and ESR), define disease damage (such as renal function) and assess CHD risk factors and define MetS (such as lipid profile and glucose) were performed locally. Not all samples tested were fasting samples, and the effect of this is discussed in chapter 5.

### 3.4.2 Follow-up assessment

Patients were assessed annually after enrolment using an abbreviated version of the enrolment proforma. New disease manifestations, new ACR criteria and incident cardiovascular events were recorded. Changes in therapies were noted, with details of all corticosteroid courses (oral and parenteral) received recorded including average dose, highest dose and course duration. Changes in other medications were also recorded. Anthropomorphic details and blood pressure were repeated annually. SLE disease activity (SLEDAI-2K) and damage (SLICC/ACR-DI) were assessed at each visit. All follow-up laboratory tests required for MetS definition and disease assessment were performed locally.
3.5 Defining the Metabolic Syndrome.

MetS was defined according to the 2009 definition quoted in the Joint Interim Statement from the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity (214). This ‘harmonizing statement’ (hereafter referred to as IDF 2009 definition) requires three or more of the following five criteria to be present (see table 1-3): (1) elevated waist circumference (MetS WC) using population/country specific thresholds; (2) elevated triglycerides (MetS TG) ≥1.7mmol/L (≥150mg/dL) or drug therapy for hypertriglyceridaemia (3) reduced HDL-cholesterol (MetS HDL) < 1.3 mmol/L (<50mg/dL) in females and <1.0 in males or drug therapy for reduced HDL-C; (4) elevated blood pressure (MetS BP) ≥130/85 mmHg or drug therapy for hypertension; and (5) elevated fasting glucose (MetS Glu) ≥ 5.6mmol/L (≥100 mg/dl) or drug therapy for hyperglycaemia.

The IDF 2009 definition of MetS (214) states “the most commonly used drug therapies for low HDL-C and/or raised triglycerides are fibrates and nicotinic acid, and that patients receiving these drugs can be presumed to have high triglycerides and low HDL-C”. The use of statins is not explicitly excluded from this or the 2006 definition, despite the fact that they are the most widely prescribed therapy for pro-atherogenic dyslipidaemia, and indeed recommended as treatment of such in the context of MetS. It was decided therefore for the purpose of this analysis that the use of statin therapy would fulfil the criteria for dyslipidaemia, and a sensitivity analysis was subsequently performed to test the impact of this decision (see chapter 5).

3.6 General statistical methods

All data analysis was performed using STATA® 10 statistical analysis software package. The characteristics of all continuous variables were initially analysed visually using histograms. Those that approximated a normal distribution were presented as means with standard deviation (SD). Variables that were not normally distributed were presented as medians with inter-quartile ranges (IQR). Significance of between group differences was assessed using a two-sided t-test for normally distributed continuous variables and Mann-Whitney-U test for non-
normally distributed variables. The significance of between-group differences of categorical variables was assessed using Chi-squared test.

3.7 Specific statistical methods

3.7.1 Data preparation

All data was explored for inaccuracies and inconsistencies and queries were raised with the coordinating centre in Toronto, who contacted each recruiting centre directly. All laboratory results were converted to the international system of units (SI). Where alternative preparations were used, oral corticosteroid dose was converted to milligrams of prednisolone equivalent. Cumulative prednisolone dose was calculated for each individual at each follow-up visit, using the detailed information on dose and course duration collected on every patient.

3.7.2 Defining the study period

The SLICC-RAS cohort has collected data annually since 2000 and individuals have therefore contributed data for a maximum period of 11 years. However, the number of patients contributing follow-up data steadily declines year-on-year due to attrition of follow-up and on-going recruitment into the study, with a patient recruited in 2008 contributing only 1 or 2 visits, for example. The analysis of MetS in the cohort was therefore restricted to the first 2 years of follow-up (enrolment visit, 1st follow-up and 2nd follow-up) to provide maximum statistical power for the testing of several hypotheses. Thus far, the number of patients with follow-up data declines markedly after the 3rd visit (although continues to accrue), and so longer-term follow-up data was not used in this study. The dataset was censored at December 2009.

3.7.3 Defining the study population

The reference population (and hence the population to which the results of this study are applicable) for the SLICC-RAS cohort study is all patients with recent-onset SLE from each of the eleven contributing countries. The experimental population is all patients enrolled in SLICC-RAS, and the specific MetS study population (SLICC-MetS) is all patients within SLICC-RAS who contributed to the MetS study. The size of the study population for the MetS study was dependent largely on the amount of missing data in the experimental population (SLICC-RAS) related to defining MetS.
Missing data are common in most research projects and longitudinal observational studies such as SLICC-RAS are particularly vulnerable due to loss of follow-up visits over time. Within the SLICC-RAS dataset however, missing data were minimal. For example, blood pressure (BP) was missing in <1% of individuals at a single time-point, and was therefore replaced with the mean BP across the other 2 visits where possible. HDL-cholesterol results were the most frequently missing variable and 55% of patients did not have a result for HDL-cholesterol at baseline. Most of these individuals were from centres in the USA and therefore represented a significant source of selection bias if these individuals were excluded from the analysis. Variables related to exposures of interest were also occasionally missing, most frequently the result of non-completed SLICC/ACR-damage indices. This was due to a lack of sufficient disease duration to complete the assessment in many individuals (i.e. the patient had been diagnosed for less than 6 months), and is a function of the study design. Overall, 57% of patients at enrolment did not have a SLICC-DI, and hence this variable exposure was not included in the baseline analysis, although it was included in the over-time analysis.

Whilst imputation was not performed, the analysis was planned to limit the potential impact of missing data. To minimise potential attrition of patient numbers and to maximise the statistical power of the study, a MetS status (yes or no) was allocated to an individual whenever possible, even if they had relevant MetS component data missing. For example, individuals were classified as having MetS provided they met the criteria for at least 3 individual components, even if data on the other 2 MetS components were missing. Similarly, an individual was classified as not having MetS if they did not meet the criteria based on available data, and could not meet it even if all data was available. Individuals were excluded only if they did not have MetS as assessed by the available data, but could potentially do so if all data were available. This approach ensured that 1494 of the 1686 (81%) patients with enrolment data were allocated a MetS status and were included in the study. The characteristics of patients with missing MetS status were subsequently compared to the study population and are discussed later.

### 3.7.4 Selection of exposure variables

SLE factors implicated in MetS development were defined *a priori*, following a comprehensive review of the literature. The variables selected represented inflammatory disease activity (SLEDAI-2K), disease phenotype (e.g. antibody status, organ involvement) and therapeutic exposures, specifically
corticosteroids, antimalarials and immunosuppressive agents. Disease phenotype information was collected on the study questionnaire (mainly concerning organ involvement such as current or past renal disease) but was also extracted from the individual domains of the SLEDAI-2K index. This records individual organ involvement at the time of assessment, but also documents laboratory features such as elevated anti-dsDNA antibodies, hypocomplementaemia, and thrombocytopenia. It was also hypothesised that the impact of each exposure on MetS prevalence would vary, depending on the timing of the exposure. This was examined by creating variables to distinguish between ‘baseline exposure’ and ‘follow-up exposure’ to an individual variable. For example, exposure to high-dose corticosteroids at baseline might have a greater impact on MetS development over time, compared to subsequent visits, as a result of inducing metabolic changes within an individual that would be slow to reverse, such as central obesity.

### 3.7.5 Identification of confounders

A confounding variable is one that is independently associated with both the outcome and predictor, and can introduce bias into statistical models. There is no agreed method for identifying potential confounders in epidemiological studies, but stating in advance which factors were adjusted for in the analysis is good practice. Potential confounders in this study were selected *a priori* and were restricted to variables unrelated to the disease phenotype, a key area of investigation. The confounder variables chosen were known to influence the prevalence of MetS directly after reviewing the literature, or were independently associated with predictor variables. They were limited in number to avoid the introduction of further potential bias. The prevalence of MetS is closely related to both age and sex (being more common in men and with increasing age) (217) and males with SLE often have more severe disease. Ethnicity is also independently related to both the outcome (MetS) and the exposures of interest (e.g. renal disease) and was therefore treated as confounder in this analysis. Therefore all regression analyses were adjusted for age, gender and ethnicity.

### 3.7.6 Overview of the analysis plan

The analysis was performed in three stages. Firstly, the prevalence and persistence of MetS was described over the first 2 years. Secondly, the association between MetS and the pre-defined disease-related factors was assessed at baseline/enrolment into the study. Univariate logistic regression,
adjusted for age, race and gender, was used to assess the relationship between the presence of MetS at enrolment into SLICC-MetS and individual variables. Those factors associated with MetS on univariate analyses (P<0.2) were entered into a multivariable model and backward stepwise multivariate logistic regression was performed with significance set at 5%. Finally, the association between MetS and disease-related factors over the initial 2 years of follow-up was then assessed, using a random-effects logistic regression analysis. As in the baseline analysis, significant variables in an adjusted univariate analyses (p<0.2) were entered into a random effects model, with significance set at 5%. Additional variables related to baseline exposure, follow-up exposure (see above) and preceding MetS status were included in this analysis. Variables were standardised within individuals and across the cohort. Interactions between predictor variables were tested for, and included within the multivariable model when present.

3.7.7 Modelling the time-varying effects of exposures

The SLICC-RAS dataset is a longitudinal dataset of nearly 1700 individuals contributing data annually, and assessment time-points may not be consistent between or within individuals. Traditional approaches to modelling longitudinal data, such as simple regression analysis, would not be ideal in this study as simple regression analysis does not effectively use all of the available data and is not therefore a true longitudinal analysis. For example, simple regression often excludes subjects with missing data (termed complete-case analysis) or makes assumptions about the missing data (such as inferring that missing data equals negativity), which may not be correct. Simple regression analyses also focus on a single final outcome data-point and do not account for repeated outcomes over time, and so information concerning individuals in whom MetS status changes at each time-point may be lost. Such traditional approaches are also flawed in their assumption that repeatedly observed outcomes (e.g. MetS status) are independent within an individual, which is often incorrect in longitudinal datasets. Whilst the MetS status of an individual may change at each assessment, it is unlikely to be completely independent from the preceding status.

The statistical model chosen to analyse the SLICC-MetS cohort over time was therefore a random-effects regression model, a methodology that has many advantages over traditional models. Firstly, a random-effects model (REM) allows all subjects to contribute data when present, and does not exclude those with missing outcomes at any time point. This avoids introducing selection bias
into the analysis by performing a complete-case analysis (as in simple regression), and maximises the size of the cohort thereby increasing the statistical power of the study. A REM also treats follow-up time as a continuous variable and therefore assessments made at different time-points can be included in the analysis. The technique also permits modelling of both time-varying co-variates (such as age and SLEDAI-2K) and invariate co-variates (such as gender and race). Overall, a REM focuses on changes within an individual over time, rather than across a population average and is a true longitudinal analysis of all available data across all data-points.

3.7.7.1 Interactions
In a longitudinal analysis there may be relationships between individual variables and between a variable and time/follow-up that, if present, may influence the analysis (i.e. an interaction). Such interactions are the result of non-independence of variables over time. For example, an individual’s exposure to corticosteroids at year 2 is unlikely to be truly independent to their exposure at year 1. Therefore, in the SLICC-MetS analysis pre-defined interactions were tested for, including interactions between variables representing exposure and follow-up time, and the outcome variable (MetS) and follow-up time. Where an interaction existed, it was included in the statistical model.

3.7.7.2 Post-estimation analysis
Post-estimation analysis of the multivariable regression model of determinants of MetS at enrolment was performed using a Receiver Operating Characteristics (ROC) curve. The ROC curve estimates the ability of a statistical model to discriminate between groups (in this case, those with and without MetS). It can also be use to determine the accuracy of diagnostic tests. The ROC curve is constructed by measuring the sensitivity (true positive) and 1-specificity (false positive) across all possible threshold values that define the positivity of a condition. The accuracy of a test/statistical model depends on the area under the ROC curve (AUC) and is classified as:

- 0.9-1.0 = excellent
- 0.8-0.9 = good
- 0.7-0.8 = fair
- 0.6-0.7 = poor
- <0.6 = fail
ROC curves cannot be constructed following a random effects analysis. Therefore to test the ‘goodness of fit’ of the multivariable model, and to assess whether the estimates produced were reliable, quadrature approximation was used post-estimation. This method refits the model for different numbers of quadrature points, and compares the different solutions. As a general rule, if the coefficients for each predictor variable do not change by more than a relative difference of 0.01%, then the results of the model may be confidently interpreted. This would suggest that the model chosen was suitable.

### 3.8 Contribution of candidate

The candidate (BP) was not involved in data acquisition for SLICC-RAS, which is an on-going international study with a central data storage centre (Toronto). However, cleaning and preparation of the dataset, planning and execution of the statistical analysis, and interpretation of the results was done solely by BP.
Chapter 4

Methods (ii):
Endothelial Dysfunction in Active SLE

This chapter will provide details of the methodologies utilised to assess endothelial function and damage longitudinally in a cohort of patients with active SLE.
4 Methods (ii): Endothelial dysfunction in active SLE.

This chapter will discuss the methods used to assess endothelial function and damage in active SLE. The hypothesis to be tested states that in a cohort of patients with active SLE endothelial function is impaired and markers of endothelial damage and activation are elevated, compared to healthy controls. In a longitudinal analysis, improved control of inflammatory disease activity in SLE patients will be associated with improvements in endothelial damage and dysfunction.

4.1 Study setting

The study was based in the Arthritis Research UK Epidemiology Unit and Cardiovascular Research Group in the University of Manchester, who acted as main sponsors for the study.

4.2 Study funding

This research was funded through a one-year Clinical Research Fellowship from the Manchester Biomedical Research Centre and a three-year Clinical Research Fellowship from Arthritis Research UK (Appendix 7).

4.3 Study design

This was a prospective observational cohort study, based in a tertiary lupus centre. All participants were assessed at the Wellcome Trust Clinical Research Facility (WTCRF), based at Central Manchester Foundation Hospital Trust (CMFT). The assessment of SLE patients was performed at 2 time points. The first assessment was at baseline, prior to any change in immunosuppressant therapy; the second assessment occurred 4-5 months after the change in therapy. This treatment interval was chosen as it allowed sufficient time for the new drug regimen to exert its anti-inflammatory effect, whilst also remaining feasible to follow-up all participants in the context of a defined fellowship. All laboratory experiments were conducted at the University of Manchester.
4.4 Ethical approval

Ethical approval for the study was obtained from the National Research Ethics Service (Oldham Research Ethics Committee) (Appendix 8). Local approval was obtained from the Research and Development Department of each participating centre, namely Central Manchester Foundation Trust, Pennine Acute Trust, Pennine MSK Partnership, South Manchester University Hospitals Trust and East Lancashire Hospitals NHS Trust. Written informed consent was obtained from each participant, as per Good Clinical Practice recommendations (Appendix 9).

4.5 Patient recruitment

Rheumatology centres across Greater Manchester were approached and asked to identify potential participants for the study. The majority of patients however were recruited from the region’s tertiary lupus clinic based at the Kellgren Centre for Rheumatology, CMFT. Study information was provided to potential participants (Appendix 10), who were then contacted 2-3 days later to discuss the study and arrange a research clinic date. The general practitioners of all recruits were also informed (Appendix 11).

4.5.1 SLE inclusion and exclusion criteria

All SLE patients satisfied the following criteria:

- ≥4 modified 1997 ACR criteria for SLE (4).
- Aged between 16 and 70 years
- Able to provide informed consent
- Had active disease warranting an escalation of anti-inflammatory therapy as decided by their treating rheumatologist that necessitated starting either:
  - Standard immunosuppression – azathioprine, MMF, cyclophosphamide; or
  - Biological therapy - rituximab

SLE patients were excluded from participating if any of the following applied:

- Unwilling or unable to provide informed consent
- Acute infection within preceding 1 month
- Acute cardiovascular event within preceding 1 month
- Current pregnancy
- Any chronic infective process (e.g. bronchiectasis, pyelonephritis)
- Chronic Renal Failure (eGFR <20mls/min)
4.5.2 Recruitment of healthy controls

Recruited participants were invited to suggest a friend or relative to act as a healthy control and where this was not feasible, age- and gender-matched controls were recruited from a database of historical controls used in earlier studies of CV risk in SLE. All controls underwent identical clinical and laboratory assessment to the cases on a single occasion, except for SLE-related components.

4.6 Clinical assessment

4.6.1 General clinical assessment

All assessments were performed at the Wellcome Trust Clinical Research Facility. SLE patients and controls underwent identical cardiovascular assessments. At the second review SLE patients underwent an abbreviated assessment to capture any new events or changes in status (e.g. disease activity, therapy, CVD events, CHD risk factors) that may have occurred in the intervening period. At each assessment all participants underwent extensive clinical assessment using a standardized proforma (Appendix 12). This included the presence of CHD risk factors, previous CVD events, hormonal factors, a full medical history and drug history. Urinalysis was also performed.

CHD risk factors were defined as follows:

- Hypertension: blood pressure >135/85 mmHg or current anti-hypertensive therapy
- Hypercholesterolaeemia: total cholesterol >5.2 mmol/l or LDL-cholesterol >3.2mmol/l or receiving current cholesterol-lowering therapy
- Family history of cardiovascular disease: MI, angina or sudden death in a first degree relative (male <55 years, female <65 years)
- Diabetes Mellitus: fasting plasma glucose >7.0mmol/l or current hypoglycaemic therapy

All participants underwent anthropomorphic assessment including measurement of height, weight, waist circumference, hip circumference, and body mass index (BMI) by appropriately trained nursing staff. Bioelectrical impedance (Tanita BC-418MA) and percentage body fat was assessed at each visit. In women <60 years, body fat should be 21-33%. Metabolic Syndrome (MetS) was defined according to the International Diabetes Federation 2009 harmonising definition (214).
4.6.2 SLE-specific assessment

SLE patients underwent a detailed assessment of their current and past SLE clinical features. Renal disease was defined as persistent proteinuria, nephrotic syndrome, renal insufficiency or any grade of lupus nephritis on renal biopsy, either currently or previously. A full therapeutic history was taken, including a history of current and previous immunosuppressive agents received and details of the planned treatment changes. Details were recorded of recent and current corticosteroid use, dose and duration (oral and parenteral), allowing cumulative corticosteroid dose and average daily dose to be calculated. All details were confirmed by a case-note review. Two composite scores of disease activity were recorded at each visit, aided by a full clinical examination. The updated British Isles Lupus Assessment Group disease activity index (BILAG-2004) (324) and the SLE Disease Activity Index 2000 (SLEDAI-2K) (66) were both performed to objectively assess disease activity in each patient. Cumulative damage was recorded using the SLICC/ACR-Damage Index (74). Two measures of quality of life were performed at each visit: the generic SF-36 quality of life indicator (325) and the lupus-specific measure, LupusQoL© (326).

4.7 Laboratory assessment

To facilitate clinical assessment several routine laboratory measures were measured in CMFT clinical laboratories on each participant at each visit:

- Full blood count
- Renal profile and estimated glomerular filtration rate (eGFR)
- Fasting lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides)
- ESR
- Fasting glucose
- Lupus Anticoagulant (LAC) (SLE patients only)
- Immunological profile, including autoantibodies, immunoglobulins, complement, anticardiolipin antibodies (SLE patients only).

Blood samples were also collected to measure the following, performed by ELISA in CMFT Research laboratory:

- high-sensitivity CRP (antibodies and controls from Abcam (Cambridge, UK).
- Fasting insulin (ultrasensitive solid phase ELISA, DRG (Marburg, Germany))
• Vascular cell adhesion molecule-1 (VCAM-1) (Duoset ELISA development kit, R&D Systems (Abingdon, UK))
• Vascular endothelial growth factor (VEGF) (Duoset ELISA development kit, R&D Systems (Abingdon, UK))
• Adiponectin (Duoset ELISA development kit, R&D Systems (Abingdon, UK))

4.8 Assessment of endothelial function

4.8.1 General test conditions

All subjects were fasted for 12 hours prior to the study, and were requested not to consume tobacco on the morning of the study. All scans were performed between 8:00 and 10:00 am. Where relevant, any vasoactive medications were withheld until after the study was completed. FMD and PAT were assessed simultaneously. Participants were examined in a semi-supine position (head of bed raised to approximately 20-30°) in a temperature-controlled room (21-24°C). The EndoPAT finger probes were positioned and inflated; following which the subject’s right arm was abducted to 90°, rotated, and held resting on a cushioned arm support. A blood pressure cuff was positioned at the level of the mid forearm (Figure 4-1).

Figure 4-1: Participant positioning for simultaneous measurement of FMD and PAT.

The subject is positioned in a semi-supine (20-30°) position with their right arm abducted to 90°, or as far as is comfortable.
4.8.2 FMD assessment

4.8.2.1 FMD protocol
Endothelial-dependent and endothelial-independent flow-mediated dilatation of the brachial artery was assessed using an ultrasound system equipped with B mode ultrasound and a 12 MHz probe (Philips ATL HDI 5000). The ultrasound machine was linked to a personal computer (PC) via a BNC cable (National Instruments). The PC was equipped with a frame grabber (National Instruments PCI 1405), vision deployment engine (National Instruments) and automated edge-tracking software. After a 10-minute period of rest and equilibration, the brachial artery was identified longitudinally, 5-10cm proximal to the antecubital fossa, using the 12Mhz ultrasound probe. The probe was fixed in position using a stereotactic probe-holder to minimise observer-related image-disturbance (Figure 4-2).

Figure 4-2: Position of participant arm, BP cuff and ultrasound probe during FMD assessment

![Position of participant arm, BP cuff and ultrasound probe during FMD assessment](image)

*The arm is held abducted and rotated and the blood pressure cuff is wrapped around the forearm. The brachial artery is located longitudinally and the probe is locked into position using the adjustable probe holder.*

Depth and gain adjustments were made to obtain the best possible wall-lumen interface and a reference image of the chosen section of artery was saved to disk, and referred to on the 2nd visit. Using the ‘Vascular Image Analysis’ software system (VIA; MD Medics), a region-of-interest (ROI) was selected and the walls of the brachial artery automatically tracked. The edge-tracking software continuously tracked the arterial walls within the ROI (Figure 4-3), throughout the test. B mode ultrasound images were processed at 25 frames per
second and the vessel diameter was displayed in real time, allowing minor adjustments to the image quality to be made as necessary. Baseline measures were then recorded for 5 minutes for EndoPAT and at least 2 minutes for FMD.

**Figure 4-3: Automated arterial wall tracking using VIA software.**

The green box represents the ROI selected by the operator, within which the brachial artery walls are tracked (green line) automatically by the VIA software.

The BP cuff was then inflated to 50mmHg above the resting systolic blood pressure, to at least 200mmHg, for 5 minutes. Adequate occlusion was visually checked using the EndoPAT tracing and adjusted as necessary. Following cuff deflation, Doppler ultrasound was used to confirm reactive hyperaemia. Edge-tracking of the brachial artery walls was continued until the arterial diameter had returned to baseline. Finally, endothelial-independent dilatation of the brachial artery was then assessed using sublingual glyceryl trinitrate (GTN) 300mcg. The brachial artery diameter was continuously tracked until brachial artery dilatation had peaked.

### 4.8.2.2 FMD data collection and on-line analysis

VIA has extended functionality and is able to calculate mean brachial artery diameters over each time period, using an on-line data analysis function. Analysis was therefore immediate and not subject to observer bias. Average baseline brachial artery diameter (mm), average diameter at 60 seconds post-deflation (mm), and FMD (%) at 60 seconds were all automatically calculated by VIA. Peak diameter (mm) and peak % FMD time-points were also manually selected by the observer and recorded if this did not coincide with the pre-set 60 second time point.

Percentage flow-mediated dilatation can also be calculated as follows:

\[
\text{\% FMD} = \left(\frac{\text{mean peak diam} - \text{mean baseline diam}}{\text{mean baseline diam}}\right) \times 100\%
\]

FMD was analysed as a continuous variable, but the number of individuals with a FMD <5% was also documented.
4.8.3 PAT assessment

4.8.3.1 PAT protocol
Peripheral arterial tonometry (PAT) was assessed using the EndoPAT 2000 © system (Itamar Medical, Israel). This commercial system employs pneumatic finger probes to assess the pulse wave amplitude before and after cuff inflation, comparing the subject’s test (occluded) arm with the non-test (non-occluded) arm. PAT was assessed simultaneously with FMD and the EndoPAT test protocol was identical to that used for FMD. Data recording for EndoPAT 2000© was continued for a full 5 minutes following cuff deflation.

4.8.3.2 PAT on-line analysis
The PAT result is expressed as a reactive hyperaemic ratio (RHI), calculated on-line by the EndoPAT software. The RHI is the post-occlusion to pre-occlusion ratio of pulse wave amplitude, with the non-occluded arm used to control for non-endothelium dependent changes in the pulse wave signal (Figure 4–4). An RHI of <1.67 is deemed to abnormal by Itamar Medical, and < 1.35 is very specific for the presence of coronary artery dysfunction (327). The RHI was analysed primarily as a continuous variable, but the number of subjects with an RHI of <1.67 was also recorded.

Figure 4-4: Reactive Hyperaemic Index using EndoPAT 2000©

The Reactive Hyperaemic Index (RHI) compares the post-occlusion pulse wave amplitude ratio in the occluded arm (C/D) to the non-occlude arm (A/B) after a 5-minute occlusion using a forearm BP cuff (adapted from Itamar Ltd).

4.9 Assessment of endothelial damage using EMPs

4.9.1 Sample collection
Microparticles (MPs) are membrane-bound vesicles that externalise phosphatidylserine (PS) to their outer membranes (266). The calcium-dependent binding of annexin-V to PS is used to identify MPs (regardless of cell of origin), and the method of blood collection can influence MP levels by affecting the
availability of extra-cellular calcium within samples. Peripheral venous blood was therefore collected from the study participant in a 5ml citrated vacutainer, to minimise calcium chelation (when compared to EDTA), using a 21g needle. The citrated sample was the first to be collected from participants to reduce the risk of inducing cell damage and subsequent MP release during venepuncture. The sample was then prepared for storage within 1 hour of blood being drawn.

4.9.2 Sample centrifugation and storage

There are many variations of the centrifugation process but a two-step process is generally employed. Many groups use an initial centrifugation step of between 1000-2000g for 10-20 minutes to removes cells. This is followed by a 2nd step to generate platelet-poor plasma (PPP) of between 10,000-20,000g for 10-30 minutes. Ultracentrifugation of PPP is reserved for isolating MPs or for assessing MPs of red cell origin (269;282). In this study, PPP was generated using a two-step centrifugation process. The venous sample was initially centrifuged at 1700g for 10 minutes at 4°C to generate plasma. The plasma layer was harvested and centrifuged at 20,000g for a further 10 minutes at 4°C. The PPP was harvested, the platelet pellet discarded, and the final sample frozen in aliquots at -80°C to analyse in batches at a later date. Whilst it has been observed that MP levels may reduce when frozen at -80°C compared to those samples analysed immediately, the duration of storage doesn’t appear influence MP levels (328) and didn’t in this study.

4.9.3 Immunolabelling of samples and EMP quantification

EMPs were identified and quantified using flow cytometry after PPP samples had been labelled with conjugated antibodies. PPP samples were thawed on ice and labelled with antibodies to identify specific cell surface antigens on the surface of MPs relevant to their cell of origin. They were then analysed and quantified using flow cytometry (Figure 4-5).
Figure 4-5: Identification of EMPs

This schematic figure describes the stages of the EMP quantification protocol, involving blood sampling, a 2-step centrifugation protocol, labelling with fluorescent antibodies, and finally flow cytometry to quantify EMP levels.

The choice of antibodies was based on an extensive review of the literature. Annexin V was used to identify all MPs via binding to externalised phosphatidylserine. The endothelial cell marker CD31 was used to identify MPs of endothelial origin and CD42b to identify MPs of platelet origin. Events that were positive for both annexin-V and CD31, and negative for CD42b, were classified as EMPs. Excluding MPs that were positive for the platelet-specific marker CD42b ensured that CD31-positive events were not platelet in origin. This combination of cell markers is also the most commonly reported in the literature, to date. To facilitate enumeration of MPs, a predefined volume of counting beads (containing a known concentration of beads) was added to each sample prior to analysis. The addition of beads to the sample allowed a standardised volume to be measured during flow cytometry, facilitating quantification of EMP per millilitre of plasma.

4.9.3.1 Initial EMP protocol development

The initial protocol developed was as follows: 900μl of phosphate buffer solution (PBS Lonza, UK) was added to each 50μl PPP sample to reduce the sample viscosity sufficiently to facilitate flow cytometry. To this was added 5μl of fluorescin isothiocyanate (FITC)-conjugated annexin V (BD Biosciences, UK), 20μl of phycoerythrin (PE)-conjugated antihuman CD31 (BD Biosciences, UK).
and 20μl of allophycocyanin (APC)-conjugated anti-human CD42b (BD Biosciences, UK) in a consecutive fashion, with a separate 10-minute incubation step for each. Finally, 50μl of 10μm diameter counting beads (Flow-Count fluorospheres; Beckman Coulter, UK), with a known concentration of beads/μl, was added to each sample prior to analysis flow cytometry. Flow cytometry was performed on a Cyan flow-cytometer (Dako Sytems) using a protocol based on optimised fluorescence patterns for each marker and the exclusion of large events.

Using this initial protocol, it was noted that the background fluorescence from both PE-CD31 and APC-CD42b was unacceptably high using manufacturer-recommended concentrations (20μl per final volume), so a series of experiments was performed to optimise the immunolabelling of samples. Optimal results were obtained for CD31 and CD42 antibodies by changing the protocol as follows:

- Reducing the volume of conjugated antibody used per sample from 20μl (manufacturers recommendation) to 5μl.
- Simultaneously incubating all antibodies for 10 minutes in the dark, rather than sequentially.

Figure 4-6 demonstrates a representative result using the optimised protocol. There is no discernible difference between unlabelled PPP (A) and isotype-labelled PPP (B). Therefore positive events using the conjugated-antibodies represent genuine binding to the antigens of interest.

**Figure 4-6: Scatter plot of optimised original EMP protocol**

This figure demonstrates the optimised EMP protocol with reduced background fluorescence on unlabelled (A) and isotype-control labelled (B) PPP, with minimal fluorescence on either sample.

### 4.9.3.2 Protocol optimisation

Despite these optimisation steps the fluorescence pattern of FITC- and PE-labelled antibodies was not sufficiently discriminatory to reliably differentiate between populations of subcellular particles (Figure 4-7). The overlap in
fluorescence necessitated significant compensation during data acquisition on the flow cytometer to enable identification of solely PE-positive and FITC-positive populations, with a subsequent reduction in the validity of results.

**Figure 4-7: Fluorescence pattern of FITC-, PE-, and APC- labelled antibodies.**

The chart demonstrates the significant overlap of FITC (yellow line) and PE (blue line) on the fluorescence spectrum using 488nm laser, and hence the requirement to apply compensation during FACS analysis.

An alternative annexin-V marker was therefore sought to avoid the need for compensation during data acquisition. The efluor450 annexin-V marker (eBioscience, UK) was identified as having minimal fluorescence overlap with either PE-CD31 or APC-CD42, based on its fluorescence with the 488nm laser (Figure 4-8). A commercial buffer solution was provided with the efluor450 marker, and so PBS was substituted.

**Figure 4-8: Fluorescence pattern of efluor450-labelled annexin V.**

The fluorescence of e450-annexin V (purple line) demonstrates no significant overlap with either PE (yellow) or APC (red), and hence there is no need for compensation on FACS analysis.

The protocol was further optimised using the efluor450 annexin V marker and buffer. No difference was noted with the binding of CD31 and CD42b when the commercial buffer solution was used, compared to PBS. Optimisation experiments were performed using 5μl, 10μl and 50μl (the manufacturer recommended volume) of efluor450, and a volume of 10μl efluor450 gave optimal binding with minimal background fluorescence. The final EMP protocol allowed identification and quantification of MPs based on their fluorescence...
pattern using platelet–poor plasma, with the exclusion of large events and artefact (Figure 4-9).

**Figure 4-9: Flow cytometry of PPP using updated optimised protocol**

This figure demonstrates results using the updated and optimised EMP protocol. The forward (FS) and side scatter (SS) histogram is shown (A), with the counting beads in R1 and artefact in R3. Events negative for APC-CD42b (i.e. platelet-derived events) are selected (R4 in B) for analysis. These CD42b-negative events are then assessed for PE and e450 fluorescence (C), and dual-positive events are enumerated (R5 in C). If the dual-positive events are then shown on a FS/SS chart, they form a distinct population at the lowest detectable limit of the flow-cytometer (D).

MPs are small and their detection is at the lower limit of the flow cytometer. Background noise in the results is therefore unavoidable but can be excluded from the analysis (R3 in Figure 4-9A). Analysis was stopped once 1000 beads had been counted (R1 in Figure 4-9A). APC-negative events were selected (R4 in Figure 4-9B). Those events positive for PE and efluor450 were selected and counted (R5 in Figure 4-9C). Absolute EMP counts per millilitre of plasma were then calculated.
4.9.4 EMP enumeration

Final EMP numbers per millilitre of plasma were calculated by first determining the volume of plasma assayed \((v)\) at each data acquisition as follows:

\[
v = \frac{z}{(x/y)/20}
\]

Where \(z\) = total volume of sample; \(x\) = total number beads added; \(y\) = number of beads counted; and 20 is the dilution factor.

Once the volume of plasma analysed was known, the total number of dual positive events was multiplied by \((1000/v)\) to generate a number of EMPs per 1000μl of plasma.

4.10 Statistical methodology

4.10.1 Primary outcome

The primary outcome of this study was change in endothelial function over time in patients with active SLE, as assessed by flow-mediated dilatation of the brachial artery.

4.10.2 Secondary outcomes

Secondary outcomes specified \(a \text{ priori}\) were:

- Difference between cases and controls in endothelial function using FMD and PAT
- Difference between cases and controls in endothelial damage and activation markers
- Change over time in endothelial damage and activation markers in SLE patients
- Relationship between change in disease activity and endothelial function/damage over time
- Correlation of FMD and PAT in the whole cohort
- Correlation of FMD and EMPs in the whole cohort

4.10.3 Sample size calculation

A sample size calculation was performed for the primary outcome (change in FMD\% over time) based upon recommendations from the international guidelines for the study of flow-mediated dilatation (236), as well as data from
the study by Hurlimann et al (199). This study investigated change over time in FMD% in a cohort of RA patients treated with anti-TNF therapy and described a significant improvement in FMD% in 11 patients treated for 12 weeks with infliximab. Data from our preliminary studies of intra-observer variability in FMD were used to calculate the mean (7.01%) and standard deviation (1.77%) of serial FMD measurements in one individual (see chapter 5.2). Based on this data, a sample size of 13 patients would give 80% power at 5% significance to detect a 2% change in FMD in over time. A recruitment target of 30 cases was set to allow for a 10-15% dropout rate and permit provisional analysis of the differential treatment effects between standard and biological therapies. A total of 15 healthy controls would also provide 80% power to detect a 2% difference in FMD% between cases and controls at baseline.

4.10.4 Statistical analysis

All data analysis was performed using STATA© 10 statistical analysis software package. The characteristics of all continuous variables were initially analysed visually using histograms. Those that approximated a normal distribution were presented as means with standard deviation (SD). Those variables that were not normally distributed were presented as medians with inter-quartile ranges (IQR). Significance of between group differences was assessed using a two-sided t-test for normally distributed continuous variables and Mann-Whitney-U test for non-normally distributed variables. The significance of between group differences of categorical variables was assessed using Chi-squared test.

Correlation between variables was assessed using Spearman’s correlation coefficient. Two-sided p values of less than 0.05 were considered significant. Bland-Altman plots (329) were used to assess the extent of agreement between pairs of measurements, as in assessment of intra-observer variability of FMD.

Linear regression analysis was used to determine the strength of association between endothelial function (FMD%) and endothelial damage (EMPs) and individual clinical factors in the whole cohort (SLE patients and controls) at the baseline visit. Univariate analysis was performed initially, followed by a multivariable analysis including factors known to affect endothelial function, such as age, baseline brachial artery diameter, blood pressure and lipid profile and SLE status (patient vs. control). Linear regression was also used to assess the association between change in disease activity over time and change in endothelial function. The analysis was adjusted for key factors known to be associated with FMD, limited in number due to the small sample size.
4.11 Contribution of the candidate

The candidate (BP) jointly conceived the study in conjunction with his supervisors, and was responsible for gaining all regulatory approval. BP established and validated the techniques used to assess FMD, PAT and EMP, and performed all EMP assays. BP was trained to perform FMD but the scans were performed by a colleague (A-AH) to avoid introducing bias into data acquisition and to permit the use of the technique by colleagues. BP was responsible for recruitment and assessed every patient clinically, evaluating disease activity and damage at each visit. BP was solely responsible for all sample size calculations, data preparation, statistical analysis and the interpretation of all results.
Chapter 5

Clinical determinants of the Metabolic Syndrome in an international inception cohort of patients with SLE

The vascular damage and enhanced cardiovascular risk observed in SLE patients is in part related to systemic inflammatory disease and its treatment. This chapter will describe the association of disease activity and therapeutic exposure with the metabolic syndrome in an international inception cohort of patients with SLE.
5 Clinical determinants of the Metabolic Syndrome in an international inception cohort of patients with SLE

Prevalent metabolic syndrome predicts future adverse cardiovascular events in the general population. SLE is associated with significant metabolic derangement and MetS may therefore contribute to the premature vascular damage seen in SLE populations. The inflammatory pathways and therapeutic agents associated with SLE may predispose SLE patients to MetS and provide a mechanistic link between inflammation and vascular damage.

The hypothesis examined by this analysis states that in an international inception cohort of patients with SLE MetS, a surrogate for enhanced CV risk, is associated with inflammatory disease activity and corticosteroid exposure over time.

To test this hypothesis, the specific objectives of this chapter are:

1. To describe the demographic and clinical features of a large international inception cohort of SLE patients.
2. To determine the prevalence, persistence and phenotype of MetS over the first 2 years of follow-up of the inception cohort.
3. To determine the relationship between MetS and inflammatory disease activity, lupus phenotype and corticosteroid exposure at enrolment into the inception cohort.
4. To determine the relationship between MetS and inflammatory disease activity, lupus phenotype and corticosteroid exposure over the first 2 years of follow up in the inception cohort.
5.1 Description of SLICC-MetS study cohort over first 2 years

5.1.1 Defining the SLICC-MetS cohort

The total number of patients enrolled into SLICC-RAS by December 31st 2009 was 1686, of which 1494 (88.6%) had sufficient data to allocate a MetS status, and were termed the SLICC-MetS cohort. Patients with an unassigned MetS status were excluded from the analysis, and this population is described in chapter 5.3. Although the number of patients in the study population declined over time, the proportion of the SLICC-RAS cohort allocated a MetS status remained consistent (Figure 5-1). Overall, there were 720 patients with complete follow-up data over the first 2 years.

Figure 5-1: SLICC-RAS and SLICC-MetS cohort over the first 2 years

Patients with insufficient data to allocate a MetS status are termed “missing”, and not included in the SLICC-MetS cohort.

5.1.2 Demographic features of SLICC-MetS study cohort

The mean (SD) disease duration and age at enrolment into SLICC-RAS was 24.1 (18.0) weeks and 35.2 (13.4) years respectively, as would be expected in an inception cohort. Overall, 1336/1494 (89.4%) of the cohort was female, and
there was a wide ethnic variation, reflecting the geographical distribution of participating centres (Figure 5-2 and Figure 5-3).

**Figure 5-2: SLICC-MetS recruitment by region.**

![SLICC-MetS recruitment by region](image)

*This figure presents the proportion of patients in SLICC-MetS from each recruiting geographical region.*

**Figure 5-3: Ethnic distribution of SLICC-MetS cohort.**

![Ethnic distribution of SLICC-MetS cohort](image)

*This figure shows that Caucasians are the most frequent ethnicity at each visit. Ethnicity is defined as per the 2000 NIH recommendations.*
Almost all patients (165/169 (97.6%)) of Korean ethnicity were from Seoul, South Korea. In total, (192/240 (80%)) of Hispanics were from Mexico, and (39/240 (16.3%)) were from USA. The single Mexican centre (Mexico City) exclusively recruited patients of Hispanic ethnicity, and the single Asian centre (Seoul, South Korea) recruited only patients of Korean ethnicity. The ethnic group “other” included those of Chinese, Japanese, Filipino, Native American, Pacific Islander and mixed ethnicity. The pattern of ethnicity by each recruiting country at enrolment is shown in Figure 5-4.

**Figure 5-4: Ethnicity of SLICC-MetS cohort by region at enrolment**

![Ethnicity graph]

This figure describes the breakdown of ethnicity by recruiting centre at the enrolment visit.

**5.1.3 Clinical, laboratory and immunological features of SLICC-MetS study cohort**

Patients were only enrolled into SLICC-RAS when the 4th ACR criteria was recognised, and therefore all patients satisfied the 1997 modified ACR criteria for SLE (Table 5-1). Overall, 95% of the cohort had a positive antinuclear antibody test.
Extensive immunological laboratory data is not recorded in SLICC-RAS, which prohibits detailed investigation of immunological features. However, the SLEDAI-2K does utilise locally performed serological tests to enable determination of disease activity. Therefore certain disease features can be described to give an overview of the disease phenotype (Table 5-2).

At enrolment, 342/1491 (22.9%) of patients had active renal disease as defined by their physician-stated active nephritis or nephrotic syndrome. This figure reduced over time (15.6% at year 1 and 13.4% at year 2). This accords with the proportion of patients with any active renal parameter scored on SLEDAI (24%, 15.3% and 15.8%, respectively). Overall, 1331 patients had both renal SLEDAI data and a physician-statement of their renal status available at enrolment. Of those patients with physician-stated active renal disease, 245/304 (80.6%) also had active renal SLEDAI component(s). Of those patients with active renal SLEDAI, 245/318 (77.0%) were also had physician-stated active renal lupus. 954/1331 (71.6%) had no evidence of active renal involvement on either criteria. Similar patterns were noted at subsequent visits.

### Table 5-1: ACR criteria in SLICC-MetS cohort at enrolment

<table>
<thead>
<tr>
<th>ACR criteria</th>
<th>N (%) patients (total = 1494)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Malar Rash</td>
<td>542 (36.3)</td>
</tr>
<tr>
<td>2. Discoid Rash</td>
<td>183 (12.3)</td>
</tr>
<tr>
<td>3. Photosensitivity</td>
<td>533 (35.7)</td>
</tr>
<tr>
<td>4. Oral Ulcers</td>
<td>558 (37.4)</td>
</tr>
<tr>
<td>5. Arthritis</td>
<td>1091 (73.0)</td>
</tr>
<tr>
<td>6. Serositis</td>
<td>413 (27.6)</td>
</tr>
<tr>
<td>7. Renal Disorder</td>
<td>416 (27.9)</td>
</tr>
<tr>
<td>8. Neurological Disorder</td>
<td>79 (5.3)</td>
</tr>
<tr>
<td>9. Haematological Disorder</td>
<td>925 (61.9)</td>
</tr>
<tr>
<td>10. Immunological Disorder</td>
<td>1153 (77.2)</td>
</tr>
<tr>
<td>11. ANA</td>
<td>1419 (95.0)</td>
</tr>
</tbody>
</table>

Abbreviation: ANA antinuclear antibody
Table 5-2: SLE phenotype over time using SLEDAI-2K

<table>
<thead>
<tr>
<th>Disease Feature</th>
<th>Enrolment n = 1494</th>
<th>FU 1 n = 1065</th>
<th>FU 2 n = 894</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP SLE *</td>
<td>35/1304 (2.7)</td>
<td>12/985 (1.1)</td>
<td>6/870 (0.7)</td>
</tr>
<tr>
<td>Ophthalmic SLE</td>
<td>10/1303 (0.8)</td>
<td>3/985 (0.3)</td>
<td>1/870 (0.1)</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>34/1305 (2.6)</td>
<td>9/985 (1.0)</td>
<td>7/871 (0.8)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>267/1322 (20.2)</td>
<td>129/987 (13.1)</td>
<td>96/11.0</td>
</tr>
<tr>
<td>Myositis</td>
<td>13/1304 (1.0)</td>
<td>1/985 (0.1)</td>
<td>6/871 (0.7)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>231/1329 (17.4)</td>
<td>90/986 (9.1)</td>
<td>89/865 (10.3)</td>
</tr>
<tr>
<td>Haematuria</td>
<td>162/1320 (12.3)</td>
<td>62/980 (6.3)</td>
<td>62/863 (7.2)</td>
</tr>
<tr>
<td>Pyuria</td>
<td>142/1309 (10.9)</td>
<td>64/976 (6.6)</td>
<td>56/858 (6.5)</td>
</tr>
<tr>
<td>Rash</td>
<td>276/1330 (20.8)</td>
<td>130/989 (13.1)</td>
<td>91/872 (10.4)</td>
</tr>
<tr>
<td>Oral ulcers</td>
<td>144/1316 (10.9)</td>
<td>77/989 (7.8)</td>
<td>67/871 (7.7)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>215/1317 (16.3)</td>
<td>69/988 (7.0)</td>
<td>73/873 (8.4)</td>
</tr>
<tr>
<td>Pericarditis</td>
<td>24/1309 (1.83)</td>
<td>2/985 (0.2)</td>
<td>2/870 (0.2)</td>
</tr>
<tr>
<td>Pleurisy</td>
<td>47/1306 (3.6)</td>
<td>17/987 (1.7)</td>
<td>12/870 (1.4)</td>
</tr>
<tr>
<td>High anti-dsDNA</td>
<td>541/1347 (40.2)</td>
<td>332/996 (33.3)</td>
<td>283/875 (32.3)</td>
</tr>
<tr>
<td>Low Complement</td>
<td>519/1349 (38.5)</td>
<td>342/996 (34.3)</td>
<td>292/887 (33.3)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>44/1313 (3.4)</td>
<td>18/984 (1.8)</td>
<td>19/870 (2.2)</td>
</tr>
<tr>
<td>Fever</td>
<td>49/1309 (3.7)</td>
<td>9/986 (0.9)</td>
<td>9/871 (1.0)</td>
</tr>
<tr>
<td>Leucopenia</td>
<td>99/1311 (7.6)</td>
<td>82/986 (8.3)</td>
<td>77/868 (8.9)</td>
</tr>
</tbody>
</table>

Abbreviations: NP neuropsychiatric
*NP SLE amalgamates seizure activity, psychosis, organic brain syndrome, cranial neuropathy, headache, and non-atherosclerotic cerebrovascular accident.

5.1.4 Disease activity and damage in SLICC-MetS cohort

Disease activity in the SLICC cohort was assessed using the SLEDAI-2K, a composite measure of disease activity. For the purposes of this analysis, a SLEDAI-2K of ≥10 was used to indicate significantly active disease, which would also be sufficient to enter a clinical trial of a therapy for active lupus. As can be seen in Table 5-3, disease activity (SLEDAI-2K) was high at enrolment and decreased over the first 2 years.
**Table 5-3: Disease activity and damage indices over follow-up**

<table>
<thead>
<tr>
<th>Mean (SD) or n (%)</th>
<th>Enrolment n = 1494</th>
<th>FU 1 n = 1065</th>
<th>FU 2 n = 894</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLEDAI-2K 5.5 (5.4)</td>
<td>3.6 (4.0)</td>
<td>3.6 (4.1)</td>
<td></td>
</tr>
<tr>
<td>SLEDAI = 0 297/1491 (19.9)</td>
<td>307/1056 (29.1)</td>
<td>269/887 (30.3)</td>
<td></td>
</tr>
<tr>
<td>SLICC 0.29 (0.72)</td>
<td>0.44 (0.89)</td>
<td>0.55 (1.1)</td>
<td></td>
</tr>
<tr>
<td>SLICC = 0 528/645 (81.9)</td>
<td>764/1054 (72.5)</td>
<td>615/885 (69.5)</td>
<td></td>
</tr>
</tbody>
</table>

Cumulative disease damage was assessed using the SLICC/ACR-damage index (SLICC-DI). The SLICC-DI can only be completed in patients with at least 6 months disease duration, and so only 645/1494 (43.2%) patients had a completed SLICC-DI at enrolment. Cumulative damage using the SLICC-DI increased over time, despite a decrease in disease activity (Figure 5-5). The proportion of patients with a SLICC ≥1 increased from 18.1% at baseline to 30.5% over the study period. The proportion of patients with a SLICC-DI of ≥2 was 7.0% at enrolment, 9.9% at year 1 and 13.8% at year 2. The features that contributed to the SLICC-DI at each visit are shown in Table 5-4. Amongst the damage items, there were 45 cardiovascular events in the 885 (5.1%) patients still in follow-up recorded at year 2. The most common items of damage were scarring alopecia, seizures, cognitive impairment and cataracts.

**Figure 5-5: Disease activity and disease damage over time**

*This figure shows the percentage of patients at each visit with a SLICC-DI ≥1 and a SLEDAI ≥10.*
<table>
<thead>
<tr>
<th>SLICC/ACR-DI item</th>
<th>N (%)</th>
<th>Enrol</th>
<th>FU 1</th>
<th>FU 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ocular</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cataract</td>
<td>9 (1.7)</td>
<td>26 (2.6)</td>
<td>26 (3.0)</td>
<td></td>
</tr>
<tr>
<td>Retinal/optic atrophy</td>
<td>3 (0.6)</td>
<td>14 (1.4)</td>
<td>9 (1.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Neuropsychiatric</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cognitive impairment</td>
<td>10 (1.9)</td>
<td>28 (2.8)</td>
<td>30 (3.4)</td>
<td></td>
</tr>
<tr>
<td>Seizures</td>
<td>9 (1.7)</td>
<td>21 (2.1)</td>
<td>21 (2.4)</td>
<td></td>
</tr>
<tr>
<td>CVA</td>
<td>8 (1.5)</td>
<td>19 (1.9)</td>
<td>22 (2.0)</td>
<td></td>
</tr>
<tr>
<td>Neuropathy</td>
<td>2 (0.4)</td>
<td>20 (2.0)</td>
<td>23 (2.6)</td>
<td></td>
</tr>
<tr>
<td>Transverse Myelitis</td>
<td>3 (0.6)</td>
<td>2 (0.2)</td>
<td>6 (0.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Renal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR &lt;50%</td>
<td>7 (1.3)</td>
<td>17 (1.7)</td>
<td>18 (2.1)</td>
<td></td>
</tr>
<tr>
<td>Proteinuria &gt;3.5g</td>
<td>8 (1.5)</td>
<td>24 (2.4)</td>
<td>28 (3.2)</td>
<td></td>
</tr>
<tr>
<td>ESRF</td>
<td>2 (0.4)</td>
<td>6 (0.6)</td>
<td>9 (1.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Pulmonary</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary Hypertension</td>
<td>6 (1.2)</td>
<td>9 (0.9)</td>
<td>11 (1.3)</td>
<td></td>
</tr>
<tr>
<td>Pulmonary Fibrosis</td>
<td>4 (0.8)</td>
<td>12 (1.2)</td>
<td>11 (1.3)</td>
<td></td>
</tr>
<tr>
<td>Shrinking lung</td>
<td>1 (0.2)</td>
<td>5 (0.5)</td>
<td>3 (0.3)</td>
<td></td>
</tr>
<tr>
<td>Pleural fibrosis</td>
<td>1 (0.2)</td>
<td>3 (0.3)</td>
<td>1 (0.1)</td>
<td></td>
</tr>
<tr>
<td>Pulmonary infarction</td>
<td>2 (0.4)</td>
<td>4 (0.4)</td>
<td>5 (0.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Cardiovascular</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angina/CABG</td>
<td>2 (0.4)</td>
<td>10 (1.0)</td>
<td>13 (1.5)</td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>3 (0.6)</td>
<td>7 (0.7)</td>
<td>7 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>7 (0.7)</td>
<td>6 (0.6)</td>
<td>8 (0.9)</td>
<td></td>
</tr>
<tr>
<td>Valve disease</td>
<td>3 (0.6)</td>
<td>9 (0.9)</td>
<td>5 (0.6)</td>
<td></td>
</tr>
<tr>
<td>Pericarditis</td>
<td>3 (0.6)</td>
<td>2 (0.2)</td>
<td>3 (0.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Peripheral Vascular</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Claudication</td>
<td>2 (0.4)</td>
<td>2 (0.2)</td>
<td>3 (0.3)</td>
<td></td>
</tr>
<tr>
<td>Minor tissue loss</td>
<td>3 (0.6)</td>
<td>8 (0.8)</td>
<td>10 (1.2)</td>
<td></td>
</tr>
<tr>
<td>Major tissue loss</td>
<td>2 (0.4)</td>
<td>2 (0.2)</td>
<td>2 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Venous thrombosis</td>
<td>5 (1.0)</td>
<td>18 (1.8)</td>
<td>14 (1.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Gastrointestinal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organ resection/infarction</td>
<td>7 (1.3)</td>
<td>15 (1.5)</td>
<td>16 (1.6)</td>
<td></td>
</tr>
<tr>
<td>Upper GI stricture/surgery</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.1)</td>
<td></td>
</tr>
<tr>
<td>Pancreatic insufficiency</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Musculoskeletal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrophy/weakness</td>
<td>2 (0.4)</td>
<td>9 (0.9)</td>
<td>12 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Deforming/erosive arthritis</td>
<td>3 (0.6)</td>
<td>17 (1.7)</td>
<td>21 (2.4)</td>
<td></td>
</tr>
<tr>
<td>Osteoporosis with fracture</td>
<td>2 (0.4)</td>
<td>5 (0.5)</td>
<td>11 (1.3)</td>
<td></td>
</tr>
<tr>
<td>AVN</td>
<td>1 (0.2)</td>
<td>10 (1.0)</td>
<td>15 (1.7)</td>
<td></td>
</tr>
<tr>
<td>Ruptured tendon</td>
<td>1 (0.2)</td>
<td>3 (0.3)</td>
<td>3 (0.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Skin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scarring alopecia</td>
<td>15 (2.9)</td>
<td>33 (3.3)</td>
<td>39 (4.5)</td>
<td></td>
</tr>
<tr>
<td>Extensive scarring</td>
<td>5 (1.0)</td>
<td>39 (4.5)</td>
<td>14 (1.6)</td>
<td></td>
</tr>
<tr>
<td>Skin ulceration</td>
<td>4 (0.8)</td>
<td>4 (0.4)</td>
<td>3 (0.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Premature gonadal failure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>50 (3.4)</td>
<td>10 (0.9)</td>
<td>5 (0.6)</td>
<td></td>
</tr>
<tr>
<td>Malignancy</td>
<td>5 (0.9)</td>
<td>12 (1.2)</td>
<td>6 (0.7)</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: CVA cerebrovascular accident; eGFR estimated glomerular filtration rate; ESRF end stage renal failure; MI myocardial infarction; CABG coronary artery bypass graft; AVN avascular necrosis*
5.1.5 Therapeutic exposures in SLICC-MetS cohort

SLICC-RAS collects extensive data on therapeutic exposures, and Figure 5.6 summarises the therapies received at each visit. There was a steady increase in the number of patients taking antimalarial and immunosuppressant therapies over time, and a decline in oral corticosteroid use over time.

**Figure 5-6: Therapeutic exposures over time**

![Therapeutic exposures over time chart]

**Abbreviations:** IS immunosuppressive; AM antimalarial; CS corticosteroids

5.1.5.1 Corticosteroids

With regards to corticosteroid exposures, 69.8% of patients were receiving oral corticosteroids at enrolment into the SLICC-MetS cohort, which remained similar at the first follow-up visit, and declined slightly at the second follow-up visit (61.0%). Patients were receiving moderate-high average oral daily corticosteroid doses at enrolment (mean (SD) daily average dose 24.6mg/day), which reduced over time (Table 5.5). The mean (SD) peak oral corticosteroid dose received in the previous 12 months also reduced over the first 2 years, and cumulative dose gradually increased over time. The frequency and dose of IV pulses of corticosteroids in the preceding 12 months remained stable.
Table 5-5: Corticosteroid exposures over the first 2 years

<table>
<thead>
<tr>
<th>Median (IQR) or n (%)</th>
<th>Enrolment n = 1494</th>
<th>FU 1 n = 1065</th>
<th>FU 2 n = 894</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Average daily CS dose (mg)</td>
<td>20 (10, 30)</td>
<td>10 (7, 16)</td>
<td>7.5 (5, 12)</td>
</tr>
<tr>
<td>*Peak CS dose (mg)</td>
<td>40 (20, 60)</td>
<td>20 (10, 40)</td>
<td>10 (5, 20)</td>
</tr>
<tr>
<td>*Cumulative CS dose (g)</td>
<td>2.6 (1.1, 5.1)</td>
<td>3.9 (2.5, 6.1)</td>
<td>5.8 (3.7, 9.0)</td>
</tr>
<tr>
<td>IV CS</td>
<td>70/1423 (4.9)</td>
<td>78/1053 (7.4)</td>
<td>42/891 (4.7)</td>
</tr>
<tr>
<td>IV CS dose per pulse (mg)</td>
<td>500 (187, 1000)</td>
<td>500 (200, 500)</td>
<td>405 (100, 500)</td>
</tr>
<tr>
<td>Number IV pulses</td>
<td>3 (1, 4)</td>
<td>3 (2, 5)</td>
<td>2.5 (1, 5)</td>
</tr>
</tbody>
</table>

Abbreviations: CS corticosteroid; IV intravenous; * oral dose

5.1.5.2 Immunosuppressive and antimalarial therapies

Immunosuppressive use remained relatively constant over the first 2 years of follow-up in SLICC-MetS at between 40.1-43.4% of patients. Several agents were utilised, the most frequent of which were azathioprine, methotrexate and mycophenolate mofetil (Table 5-6). The use of antimalarial medication, most commonly hydroxychloroquine, was common at entry into SLICC-MetS (Table 5-6), and increased slightly over the first 2 years (from 65.0 to 70.5%).

Table 5-6: Immunosuppressive and antimalarial exposures

<table>
<thead>
<tr>
<th>N (%)</th>
<th>Enrolment n = 599</th>
<th>FU 1 n = 451</th>
<th>FU 2 n = 388</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunosuppressive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azathioprine</td>
<td>262 (43.7)</td>
<td>189 (42.0)</td>
<td>164 (42.3)</td>
</tr>
<tr>
<td>Mycophenolate</td>
<td>98 (16.4)</td>
<td>88 (19.5)</td>
<td>76 (19.6)</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>104 (17.4)</td>
<td>84 (18.6)</td>
<td>82 (21.1)</td>
</tr>
<tr>
<td>Cyclophosphamide (PO)</td>
<td>9 (1.5)</td>
<td>4 (0.8)</td>
<td>6 (1.6)</td>
</tr>
<tr>
<td>Cyclophosphamide (IV)</td>
<td>95 (15.9)</td>
<td>47 (10.4)</td>
<td>38 (9.9)</td>
</tr>
<tr>
<td>Ciclosporin A</td>
<td>21 (3.5)</td>
<td>21 (4.7)</td>
<td>18 (4.8)</td>
</tr>
<tr>
<td>Other</td>
<td>10 (1.7)</td>
<td>18 (4.1)</td>
<td>3 (0.7)</td>
</tr>
<tr>
<td>Antimalarial</td>
<td>N = 971</td>
<td>N = 713</td>
<td>N = 630</td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td>896 (92.3)</td>
<td>642 (90.0)</td>
<td>587 (93.1)</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>66 (6.8)</td>
<td>52 (7.3)</td>
<td>33 (5.2)</td>
</tr>
</tbody>
</table>

Abbreviations: PO oral; IV intravenous
5.1.6 Traditional CHD risk factors in SLICC-MetS

At enrolment, 225 (15.1%) patients were current smokers, with a mean (SD) pack year history of 9.3 (14.6) years. No discernible decline in smoking prevalence was recorded over the first 2 years (14.3% at year 1 and 14.1% at year 2). The vast majority of female patients were pre-menopausal at each visit (1113/1336 (83.3%) at enrolment, 755/942 (80.1%) at year 1, and 639/797 (80.2%) at year 2) and obesity parameters remained stable over time. Traditional CHD risk factors are summarised in Table 5-7. The mean (SD) calculated 5-year Framingham Risk Score at enrolment into SLICC-MetS was 0.57% (1.54) for women and 5.1% (6.78) for men.

Table 5-7: Prevalence of traditional CHD risk factors in SLICC-MetS

<table>
<thead>
<tr>
<th>Mean (SD) or n (%)</th>
<th>Baseline n = 1494</th>
<th>FU 1 n = 1065</th>
<th>FU 2 n = 894</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>25.1 (5.9)</td>
<td>25.7 (6.1)</td>
<td>25.5 (6.2)</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>82.9 (14.0)</td>
<td>84.0 (14.8)</td>
<td>83.1 (14.5)</td>
</tr>
<tr>
<td>BP systolic (mmHg)</td>
<td>119.5 (16.8)</td>
<td>118.3 (16.8)</td>
<td>118.8 (16.8)</td>
</tr>
<tr>
<td>BP diastolic (mmHg)</td>
<td>75.3 (11.0)</td>
<td>74.5 (10.6)</td>
<td>74.3 (11.2)</td>
</tr>
<tr>
<td>AHT therapy</td>
<td>435/1494 (29.1)</td>
<td>356/1065 (33.4)</td>
<td>320/894 (35.8)</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.03 (1.63)</td>
<td>4.82 (1.03)</td>
<td>4.77 (1.08)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3.4%</td>
<td>0.9%</td>
<td>0.6%</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.93 (1.49)</td>
<td>4.61 (1.12)</td>
<td>4.55 (1.09)</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.79 (1.19)</td>
<td>1.49 (1.1)</td>
<td>1.42 (0.94)</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.39 (0.60)</td>
<td>1.43 (0.49)</td>
<td>1.43 (0.47)</td>
</tr>
<tr>
<td>Lipid therapy</td>
<td>171/1494 (11.5)</td>
<td>143/1065 (13.4)</td>
<td>129/894 (14.4)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>225/1491 (15.1)</td>
<td>151/1058 (14.3)</td>
<td>125/889 (14.1)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI body mass index; WC waist circumference; BP blood pressure; AHT anti-hypertensive therapy.
5.1.7 Comparison of baseline characteristics of SLICC-MetS study cohort and patients with missing MetS status

A total of 1686 patients had been recruited into SLICC-RAS and undergone an enrolment assessment when the dataset was censored in December 2009. Of these, 1494 (88.6%) had sufficient data to allocate a MetS status and this group constitute the SLICC-MetS study cohort. A MetS status was not allocated to 192/1686 (11.4%) patients at enrolment into SLICC-RAS as a result of missing data related to the MetS definition. The most frequent missing MetS component was HDL-cholesterol (739/1494 (49.4%)), followed by 171/1494 (11.4%) triglyceride results, 160/1494 (10.7%) waist circumference results and 141/1494 (9.4%) glucose results. Blood pressure was almost universally captured (1492/1494).

The clinical characteristics and demographics of the two groups were compared to investigate whether this group differed to the SLICC-MetS study cohort, and hence provide a potential source of bias in the analysis. Overall, patients with a MetS status had similar age (35.2 (13.4) years vs. 35.1 (12.1) years; p = 1.00)) and gender (89.4% female vs. 88.5%; p = 0.70) as did those without a MetS status at entry into SLICC-RAS, but differed in their ethnic and geographic distribution (Table 5-8). The number of patients with an unallocated MetS status was significantly higher in US centres, most frequently due to missing lipid parameters. No significant differences were noted in traditional CHD risk factors, except for a slightly increased BMI in those with missing MetS status (25.1 vs. 26.3; p = 0.02).
Table 5-8: Baseline demographics and CHD risk factors of patients with missing MetS status at enrolment

<table>
<thead>
<tr>
<th></th>
<th>MetS assigned N = 1496</th>
<th>MetS missing N = 192</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>660/1492 (44.2)</td>
<td>100/191 (52.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Black</td>
<td>228/1492 (15.3)</td>
<td>44/191 (23.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SE Asian</td>
<td>303/1492 (20.3)</td>
<td>24/191 (12.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hispanic</td>
<td>240/1492 (16.1)</td>
<td>17/191 (8.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Other</td>
<td>61/1492 (4.1)</td>
<td>6/191 (3.1)</td>
<td>0.90</td>
</tr>
<tr>
<td><strong>Region</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>358/1477 (24.2)</td>
<td>27/178 (15.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mexico</td>
<td>194/1477 (13.1)</td>
<td>9/178 (5.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>USA</td>
<td>374/1477 (25.3)</td>
<td>97/178 (54.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Asia</td>
<td>168/1477 (11.4)</td>
<td>1/178 (0.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Europe</td>
<td>383/1477 (25.3)</td>
<td>44/178 (24.7)</td>
<td>0.90</td>
</tr>
<tr>
<td><strong>CHD Risk Factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP systolic (mmHg)</td>
<td>119.5 (16.8)</td>
<td>118.7 (16.8)</td>
<td>0.54</td>
</tr>
<tr>
<td>BP diastolic (mmHg)</td>
<td>75.3 (11.0)</td>
<td>73.0 (10.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>Antihypertensive Use</td>
<td>435 (29.1)</td>
<td>49 (25.4)</td>
<td>0.28</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.79 (1.19)</td>
<td>1.50 (0.93)</td>
<td>0.31</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.39 (0.60)</td>
<td>1.51 (0.29)</td>
<td>0.79</td>
</tr>
<tr>
<td>Lipid-lowering therapy</td>
<td>171 (11.5)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.03 (1.63)</td>
<td>5.39 (1.81)</td>
<td>0.25</td>
</tr>
<tr>
<td>Diabetes</td>
<td>50/1475 (3.4)</td>
<td>6/184 (3.3)</td>
<td>0.90</td>
</tr>
<tr>
<td>Smoker current</td>
<td>225/1491 (15.1)</td>
<td>27/191 (14.1)</td>
<td>0.70</td>
</tr>
<tr>
<td>BMI</td>
<td>25.1 (5.9)</td>
<td>26.3 (6.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>82.9 (14.0)</td>
<td>84.2 (14.2)</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Abbreviations: BP blood pressure; BMI body mass index; WC waist circumference; HDL-C high-density lipoprotein cholesterol.
Several disease characteristics differed significantly between the two groups (Table 5-9). For example, those with a missing MetS status had a lower mean SLEDAI-2K, a lower prevalence of renal disease, less immunosuppressant use and were more likely to be receiving anti-malarial therapies at enrolment, compared to the SLICC-MetS cohort. Those excluded from the analysis also had a lower frequency of corticosteroid exposure, albeit at similar doses.

**Table 5-9: SLE characteristics of patients with missing MetS status at enrolment**

<table>
<thead>
<tr>
<th>Mean (SD) or n (%)</th>
<th>MetS assigned</th>
<th>MetS missing</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Disease Severity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration (weeks)</td>
<td>24.1 (18.0)</td>
<td>27.6 (19.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>SLEDAI-2K</td>
<td>5.5 (5.4)</td>
<td>3.78 (4.20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SLICC/ACR-DI</td>
<td>0.29 (0.72)</td>
<td>0.18 (0.46)</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Disease Phenotype</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active renal disease</td>
<td>314 (22.9)</td>
<td>21 (10.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Anti-dsDNA positive</td>
<td>541/1347 (40.2)</td>
<td>38/161 (23.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low Complement</td>
<td>519/1349 (38.5)</td>
<td>50/164 (30.5)</td>
<td>0.05</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>44/1313 (3.4)</td>
<td>2/158 (1.3)</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Therapies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral CS use</td>
<td>1043 (69.8)</td>
<td>122 (63.2)</td>
<td>0.06</td>
</tr>
<tr>
<td>*Average CS dose (mg)</td>
<td>20 (10, 30)</td>
<td>15 (7, 30)</td>
<td>0.001</td>
</tr>
<tr>
<td>*Highest CS dose (mg)</td>
<td>40 (20, 60)</td>
<td>30 (20, 50)</td>
<td>0.08</td>
</tr>
<tr>
<td>*Cumulative CS dose (g)</td>
<td>2.6 (1.1, 5.0)</td>
<td>1.7 (0.7, 4.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>Pulse intravenous CS</td>
<td>70/1423 (4.9)</td>
<td>4/184 (2.2)</td>
<td>0.10</td>
</tr>
<tr>
<td>Immunosuppressant</td>
<td>599/1491 (31.0)</td>
<td>58/187 (40.1)</td>
<td>0.02</td>
</tr>
<tr>
<td>Antimalarial Use</td>
<td>971 (65.0)</td>
<td>144 (74.6)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

*Abbreviations: CS corticosteroid; * oral dose*

Overall, those with missing MetS status at baseline had similar demographics and CV risk profile to the SLICC-MetS cohort, but had a less severe disease phenotype at baseline. The potential implications of these findings are discussed in chapter 5.6. The proportion of SLICC-RAS patients with a missing MetS status remained consistent over the following 2 years (146/1211 (12.1%) at year 1 and 127/1065 (11.9%) at year 2).
5.2 Prevalence, persistence and phenotype of MetS

The first aim of the SLICC-MetS analysis was to describe the changing prevalence of MetS in an inception cohort of patients with SLE over the first two years of follow-up. Secondary pre-planned analyses examined the MetS phenotype over time, as well as its persistence in a complete-case analysis.

5.2.1 Prevalence of metabolic syndrome in SLICC-MetS

The overall prevalence of MetS in the SLICC-MetS cohort at enrolment study was 239/1494 (16%). Figure 5-7 shows the changing prevalence over the subsequent 2 years.

**Figure 5-7: Prevalence of MetS over time**

At enrolment, MetS was more prevalent in men than women (22.2% vs. 15.2%; p = 0.03) and those with MetS were older, (mean (SD) age 36.9 (13.3) years vs. 34.9 (14.7) years; p <0.04) than those without MetS. Patients of Hispanic and Korean ethnicity had the highest prevalence of MetS, compared to the rest of the cohort (31.3% and 30.1% vs. 10.3%; p = <0.001). The prevalence in black patients overall was 7.5%. Over time, MetS remained more common in men than women (13.8% vs. 12.4% at year 1 and 15.5% vs. 13.3% at year 2), and those with MetS remained consistently older than those without. Figure 5-8 and Figure 5-9 describe the changing MetS by ethnicity and country of recruitment overtime. The overall variation in MetS prevalence in SLICC-MetS disguises quite marked variation in some ethnic groups. For example, the prevalence in Koreans was amongst the highest at enrolment (30.1%), but falls dramatically at year 1 and remains lower at year 2 (14.6% and 16%
respectively). The prevalence in Caucasians remains stable over 2 years, but increases in those from the Indian subcontinent and Black Africans (which includes African-Americans). MetS was most common in Hispanics at each time-point.

**Figure 5-8: Prevalence of MetS over time by ethnicity**

![Graph showing prevalence of MetS over time by ethnicity](image)

**Figure 5-9: Prevalence of MetS by region**

![Graph showing prevalence of MetS by region](image)
5.2.2 Incidence and persistence of MetS over time in SLICC-MetS

The incidence and persistence of MetS in SLICC-MetS over the first 2 years of follow up was examined in a complete-case analysis. In total, 720 patients had a MetS status at each visit, in whom the overall prevalence of MetS was 14.9% at enrolment, 13.6% at year 1, and 14.4% at year 2. However, the status of an individual patient often changed between visits, as shown in Figure 5-10. Overall, 194/720 (26.9%) patients had MetS on at least 1 occasion, 31/720 (4.3%) of patients had MetS at every visit and 526/720 (73.1%) never developed MetS. Of those who did not have MetS at enrolment, 87/613 (14.2%) developed incident MetS over the study period.

Figure 5-10: Persistence of MetS over time in a complete-case analysis

<table>
<thead>
<tr>
<th>Enrolment</th>
<th>FU 1</th>
<th>FU 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MetS Yes 107 (14.9%)</td>
<td>MetS Yes 41</td>
<td>MetS Yes 31</td>
</tr>
<tr>
<td>MetS No 66</td>
<td></td>
<td>MetS No 10</td>
</tr>
<tr>
<td>MetS Yes 57</td>
<td></td>
<td>MetS Yes 16</td>
</tr>
<tr>
<td>MetS No 613 (85.1%)</td>
<td></td>
<td>MetS No 50</td>
</tr>
<tr>
<td>MetS No 556</td>
<td></td>
<td>MetS Yes 27</td>
</tr>
<tr>
<td>MetS No 526</td>
<td></td>
<td>MetS No 30</td>
</tr>
</tbody>
</table>

This figure describes the changing MetS status of individuals over time in a complete-case analysis of SLICC-MetS (n = 720).

5.2.3 MetS phenotype over time in SLICC-MetS

The MetS phenotype was analysed at each time point, to investigate the hypothesis that obesity may be under-represented in lupus patients with MetS. The proportion of patients that met the individual MetS components at each visit is shown in Table 5-10. Interestingly, less than 50% of SLE patients with MetS met the criteria for central obesity, which remained stable over the study period. The observed variability in the MetS prevalence over the first 2 years was due
primarily to significant variation in dyslipidaemia and hyperglycaemia, with a steady increase in the proportion of patients with elevated blood pressure.

### Table 5.10: MetS phenotype over the first 2 years of follow-up

<table>
<thead>
<tr>
<th>N (%)</th>
<th>Enrolment</th>
<th>FU 1</th>
<th>FU 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MetS WC</td>
<td>645/1334 (48.4)</td>
<td>467/919 (50.8)</td>
<td>359/728 (49.3)</td>
</tr>
<tr>
<td>MetS BP</td>
<td>686/1491 (46.0)</td>
<td>516/1065 (48.5)</td>
<td>452/894 (50.6)</td>
</tr>
<tr>
<td>MetS TG</td>
<td>619/1342 (46.1)</td>
<td>347/942 (36.8)</td>
<td>311/794 (39.2)</td>
</tr>
<tr>
<td>MetS HDL</td>
<td>486/822 (59.1)</td>
<td>337/617 (54.6)</td>
<td>292/528 (55.3)</td>
</tr>
<tr>
<td>MetS Glucose</td>
<td>271/1344 (20.2)</td>
<td>136/966 (14.1)</td>
<td>108/805 (13.4)</td>
</tr>
<tr>
<td>MetS Total</td>
<td>239/1494 (16.0)</td>
<td>134/1065 (12.6)</td>
<td>121/894 (13.5)</td>
</tr>
</tbody>
</table>

**Abbreviations:** WC waist circumference; BP blood pressure; TG triglycerides; HDL high-density lipoprotein.

At enrolment, 904/1494 (60.5%) met no individual MetS criteria and 351/1494 (23.5%) met 1 or 2 individual criteria. At year 1, 683/1065 (64.1%) met no criteria and 248/1065 (23.3%) met 1 or 2 criteria. At the year-2 follow-up visit, 587/894 (65.6%) met no criteria and 186/894 (20.1%) met 1 or 2 criteria.

### 5.2.4 Sensitivity analyses

#### 5.2.4.1 The effect of including statins in the MetS definition.

Statin use is not explicitly included in the IDF 2009 definition of MetS-associated dyslipidaemia, although it does state that fibrates and nicotinic acid are the most commonly prescribed therapies for low HDL and/or high triglycerides. Statin use was however included in the MetS definition utilised in this study, as it was by far the most commonly prescribed lipid-lowering therapy in the SLICC-MetS cohort. Indeed IDF recommend the use of statins for the atherogenic dyslipidaemia commonly observed in patients with MetS. In the SLICC-MetS cohort 171 patients were on lipid-lowering therapies at enrolment, 152 of which were on statins, 14 were on fibrates and 5 were on “other”. Of those on a statin, 120 (78.9%) had elevated triglycerides and 97 (63.8%) had reduced HDL-C, and so fulfilled the criteria for MetS dyslipidaemia regardless of statin use. When statin use was excluded from the definition, MetS prevalence at enrolment was 15.4% - 11 people moved from having to not having MetS. The impact of using the rule that all lipid-lowering therapies (including statins) are included is therefore negligible, but maximises the size of the cohort and hence the power of the analysis.
5.2.4.2 The effect of including non-fasting blood results MetS definition. All definitions of MetS to date state that the relevant blood tests to determine MetS status should be taken with the patient fasting. However, not all patients manage to have fasting blood tests as part of their SLICC assessment visit, for a variety of undocumented reasons (e.g. afternoon appointments or too unwell). At enrolment, 50.2% of glucose results were non-fasting, 46.1% of cholesterol results were non-fasting and 45.9% of triglyceride results were non-fasting. If only those patients with fasting blood results were included in SLICC-MetS the cohort would number 722 and not 1494. The prevalence of MetS in this reduced cohort was 25.0%. Interestingly, patients with non-fasting blood results had lower mean (SD) triglycerides (1.83 (1.23) mmol/l vs. 1.73 (1.14) and glucose (4.87 (1.12) vs. 5.18 (2.00) mmol/l) than those with fasting results. The ethnic variation of MetS prevalence in patients with fasting blood results and SLICC-MetS is shown in Figure 5-11.

Figure 5-11: MetS prevalence in patients with fasting bloods only vs. SLICC-MetS

This figure demonstrates the impact of using fasting and non-fasting blood results on MetS prevalence by ethnicity. The South Korean and Mexican centres had the fewest number of non-fasting samples overall, and hence the MetS prevalence in Koreans and Hispanics is relatively unchanged. The prevalence of MetS in the other ethnicities is higher when the analysis is restricted to those only with fasting blood results.
Overall, the decision to not restrict the analysis to only those with fasting blood results reduced the prevalence of MetS in the SLICC-MetS from 25% to 16%. Patients with non-fasting blood results had lower glucose, lower cholesterol and lower triglycerides than those without, and so their inclusion in the analysis may only serve to bias the results of this study towards the null hypothesis. Their inclusion in the analysis does however increase the relevance and applicability of the study to SLE cohorts generally, and increase the statistical power of the study.

**5.3 Determinants of MetS at enrolment into SLICC-MetS**

A key aim of the SLICC-MetS study was to test the hypothesis that MetS, a surrogate for cardiovascular risk, is associated with disease features that reflect inflammatory disease activity, disease severity and therapeutic exposures, particularly corticosteroids. The first stage of the analysis was to examine those factors associated with MetS at enrolment into SLICC-RAS.

**5.3.1 Univariate associations of MetS in SLE at enrolment**

Age, ethnicity and gender adjusted univariate analyses were performed to assess the strength of the relationship between the presence of MetS at enrolment and those variables related to inflammation, disease severity, disease phenotype, and therapeutic exposure (Table 5-11). Several disease-related features were not associated with MetS at enrolment on univariate analysis, including longer disease duration, past exposure to corticosteroids, recent intra-venous (IV) corticosteroid use, past exposure to antimalarial therapies, low complement, prevalent arthritis, and neuropsychiatric (NP) lupus. Factors that represent more severe SLE, such as NP disease, were uncommon in the SLICC-MetS cohort at enrolment, and did not therefore reach significance. For example, only 4 patients had lupus-related seizures at enrolment, none of whom had MetS, and 5 had lupus-related psychosis, 2 of whom had MetS.
### Table 5-11: Significant univariate associations of MetS at enrolment

<table>
<thead>
<tr>
<th>Median (IQR) or n (%)</th>
<th>MetS Yes</th>
<th>MetS No</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Current CS</td>
<td>193/239 (80.8)</td>
<td>8250/1255 (67.7)</td>
<td>1.53 (1.05, 2.25)</td>
</tr>
<tr>
<td>*Average CS dose (mg)</td>
<td>30 (15, 45)</td>
<td>20 (10, 30)</td>
<td>1.02 (1.01, 1.04)</td>
</tr>
<tr>
<td>*Highest CS dose (mg)</td>
<td>50 (30, 60)</td>
<td>30 (20, 50)</td>
<td>1.00 (1.00, 1.01)</td>
</tr>
<tr>
<td>*Cumulative CS dose</td>
<td>3.1(1.5, 5.4)</td>
<td>2.3(1.0, 4.0)</td>
<td>1.05 (1.00, 1.09)</td>
</tr>
<tr>
<td>Past IV steroids</td>
<td>18/230 (7.8)</td>
<td>52/1193 (4.4)</td>
<td>3.22 (1.35, 7.68)</td>
</tr>
<tr>
<td>Current AM</td>
<td>123/238 (51.5)</td>
<td>848/1255 (67.6)</td>
<td>0.51 (0.38, 0.67)</td>
</tr>
<tr>
<td>Current IS</td>
<td>140/238 58.8</td>
<td>459/1253 (36.6)</td>
<td>2.21(1.63, 3.00)</td>
</tr>
<tr>
<td>SLICC ≥ 1</td>
<td>26/95 (27.4)</td>
<td>91/550 (16.6)</td>
<td>1.99 (1.16, 3.40)</td>
</tr>
<tr>
<td>SLEDAI-2K mean (SD)</td>
<td>6.79 (6.19)</td>
<td>5.24 (5.25)</td>
<td>1.05 (1.02, 1.07)</td>
</tr>
<tr>
<td>SLEDAI ≥ 10</td>
<td>66/239 (27.6)</td>
<td>221/1252 (17.7)</td>
<td>1.73 (1.22, 2.44)</td>
</tr>
<tr>
<td>High anti-dsDNA</td>
<td>101/216 (46.8)</td>
<td>440/1131 (38.9)</td>
<td>1.32 (1.00, 1.82)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>14/206 (6.8)</td>
<td>30/1107 (2.7)</td>
<td>2.10 (1.03, 4.29)</td>
</tr>
<tr>
<td>Leucopenia</td>
<td>7/203 (3.5)</td>
<td>92/1108 (8.3)</td>
<td>0.33 (0.14, 0.75)</td>
</tr>
<tr>
<td>Active renal disease</td>
<td>94/239 (39.3)</td>
<td>220/1255 (17.5)</td>
<td>2.87 (2.05, 4.02)</td>
</tr>
<tr>
<td>Past renal disease</td>
<td>22/239 (9.2)</td>
<td>69/1255 (5.5)</td>
<td>1.67 (1.00, 2.88)</td>
</tr>
</tbody>
</table>

Abbreviations: CI confidence interval; CS corticosteroid; IV intravenous; Am antimalarial use; IS immunosuppressant use; * oral dose

## 5.3.2 Multivariable associations of MetS in SLE at enrolment

Backward stepwise multivariable logistic regression was used to identify those factors independently associated with MetS at enrolment into SLICC-MetS. The final model indicated higher average oral prednisolone dose (mg), increasing age (years), Korean and Hispanic ethnicity, current renal disease, and current immunosuppressant use were all independently associated with MetS at enrolment (Table 5-12). Figure 5-12 shows the receiver operator curve (ROC) for this model, with an area under the curve (AUC) of 0.78, which suggests the model is ‘fairly’ good at discriminating MetS susceptibility at enrolment.
Table 5-12: Multivariable model of determinants of MetS at enrolment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Average corticosteroid dose (mg)</td>
<td>1.02</td>
<td>1.00, 1.03</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.04</td>
<td>1.03, 1.06</td>
</tr>
<tr>
<td>Korean race</td>
<td>6.33</td>
<td>3.68, 10.86</td>
</tr>
<tr>
<td>Hispanic Race</td>
<td>6.20</td>
<td>3.78, 10.12</td>
</tr>
<tr>
<td>Active Renal Disease</td>
<td>1.79</td>
<td>1.14, 2.80</td>
</tr>
<tr>
<td>Immunosuppression use</td>
<td>1.81</td>
<td>1.18, 2.78</td>
</tr>
</tbody>
</table>

Abbreviations: CI confidence interval. * oral daily dose

This model incorporates all factors significant on univariate analyses, including SLEDAI-2K components, but not SLICC-DI (>60% of which were missing at baseline). Age and corticosteroid dose are continuous variables, with MetS risk increasing by 1 year and 1 mg respectively.

Figure 5-12: ROC curve for multivariable model of determinants of MetS

This figure is the ROC curve for the final model of determinants of MetS at enrolment into SLICC-MetS. The calculated area under the curve is 0.78, suggesting that the model is a fair discriminator of MetS susceptibility at enrolment.

When the model was re-run after excluding the SLEDAI-2K variables, the factors independently associated with MetS remained unchanged, although the point estimates differed slightly (e.g. current immunosuppression use 1.64 (1.13, 2.41). A final model was run to explore the relationship between oral corticosteroid dose and MetS (Table 5-13). In this model all oral corticosteroid
dose-related variables were excluded, and the only corticosteroid variable included was current use (yes/no). In this model previous exposure to intravenous corticosteroids was independently associated with MetS at enrolment, but past oral, current intravenous and current oral use was not. Current exposure to antimalarial therapies became significantly “protective” against MetS, and the AUC of this model was 0.76.

Table 5-13: Multivariable model excluding all dose-related corticosteroid variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>1.04</td>
<td>1.03, 1.05</td>
</tr>
<tr>
<td>Previous IV steroids</td>
<td>2.45</td>
<td>1.01, 5.97</td>
</tr>
<tr>
<td>Current AM</td>
<td>0.66</td>
<td>0.48, 0.90</td>
</tr>
<tr>
<td>Current Immunosuppression</td>
<td>1.62</td>
<td>1.17, 2.23</td>
</tr>
<tr>
<td>Korean race</td>
<td>5.23</td>
<td>3.41, 8.01</td>
</tr>
<tr>
<td>Current Renal Disease</td>
<td>2.19</td>
<td>1.54, 3.12</td>
</tr>
<tr>
<td>Hispanic Race</td>
<td>4.15</td>
<td>2.83, 6.09</td>
</tr>
</tbody>
</table>

Abbreviations: CI confidence interval; IV intravenous; AM antimalarial.

5.3.3 MetS susceptibility at enrolment and ethnicity.

The observed effect of ethnicity on MetS prevalence was not expected a priori. Therefore, exploratory analyses of the two high-risk ethnicities were performed, to explore whether differences in disease phenotype or therapeutic exposures might influence the high prevalence of MetS.

5.3.3.1 MetS phenotype by ethnicity
Korean and Hispanic patients demonstrated distinct and contrasting MetS phenotypes compared to each other and the rest of the cohort (Figure 5-13). Patients of Korean ethnicity had a substantially lower prevalence of central obesity compared to the rest of the cohort (MetS WC 20.1% vs. 51.3%; p <0.0001), with a significantly lower BMI (21.6 (4.3) vs. 25.8 (6.1); p = <0.0001). However, they had a significantly increased prevalence of hyperglycaemia (26.8% vs. 19.7%; p = 0.04) and dyslipidaemia. The excess MetS in the Hispanic cohort was due to significantly more dyslipidaemia than the rest of the SLICC cohort (MetS TG 64.3% vs. 40.3% (p <0.0001) and MetS HDL (65.1% vs. 52.7% (p <0.0001)). This was despite a similar prevalence of central obesity (MetS WC 56.6% vs. 513%; p = 0.15) and a slightly lower BMI overall (mean (SD) 24.5 (5.0) vs. 25.8 (6.1)).
5.3.3.2 Lupus phenotype by ethnicity

The ethnic differences in MetS prevalence and phenotype were hypothesised to be due to more severe and active SLE in Koreans and Hispanics, particularly renal disease. Table 5-14 details the disease features in each group. Korean patients had a lupus phenotype characterised by active serology, with a higher prevalence compared to the rest of the cohort of positive anti-dsDNA antibodies (66% vs. 36.0%; p <0.001), hypocomplementaemia (75.2% vs. 33.1%; p<0.001), and thrombocytopenia (11.2% vs. 2.7%; p <0.001). Oral corticosteroid use in the Korean cohort was almost universal (95.3%), although the average and peak doses were similar to the rest of the cohort. Their shorter disease duration also resulted in a lower cumulative corticosteroid exposure at enrolment (median (IQR) 1.4g (0.4, 3.1) vs. 2.5g (1.2, 4.8)). In contrast, Hispanic patients had more frequent active renal disease at enrolment (40.2% vs. 15.5%; p <0.0001) but similar rates of both hypertension and disease activity biomarkers (i.e. elevated anti-dsDNA antibodies and low complement) to the other ethnicities. Hispanic patients were also exposed to higher average, peak and cumulative oral doses of corticosteroids but less anti-malarial therapies than the rest of the SLICC-MetS cohort.

Abbreviations: WC waist circumference criteria; BP blood pressure criteria; TG triglyceride criteria; HDL high-density lipoprotein criteria; hyperglycaemia criteria;
* Significant when compared to all other ethnicities (p <0.05)
### Table 5-14: Lupus phenotype by ethnicity at enrolment

<table>
<thead>
<tr>
<th>Mean (SD) or N (%)</th>
<th>Korean</th>
<th>Hispanic</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease duration (wks)</td>
<td>18.5 (15.9)*</td>
<td>23.2 (16.9)</td>
<td>25.1 (18.4)</td>
</tr>
<tr>
<td>SLEDAI-2K</td>
<td>7.45 (6.09)*</td>
<td>6.46 (5.75)*</td>
<td>5.0 (5.2)</td>
</tr>
<tr>
<td>SLICC/ACR-DI</td>
<td>0.24 (0.69)</td>
<td>0.28 (0.69)</td>
<td>0.30 (0.74)</td>
</tr>
<tr>
<td>Disease Phenotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active renal disease</td>
<td>49 (29.0)*</td>
<td>97 (40.4)*</td>
<td>168 (15.5)</td>
</tr>
<tr>
<td>Anti-dsDNA positive</td>
<td>105/159 (66.0)*</td>
<td>84/211 (39.8)</td>
<td>352/977 (36.0)</td>
</tr>
<tr>
<td>Low Complement</td>
<td>121/161 (75.2)*</td>
<td>74/208 (35.6)</td>
<td>324/980 (33.1)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>16/143 (11.2)*</td>
<td>2/210 (1.0)</td>
<td>26/960 (2.7)</td>
</tr>
<tr>
<td>Medication (median(IQR))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral CS</td>
<td>161 (95.3)*</td>
<td>211 (87.9)*</td>
<td>671 (61.8)</td>
</tr>
<tr>
<td>§Average CS dose (mg)</td>
<td>20 (10, 35)</td>
<td>30 (15, 42.5)*</td>
<td>20 (10, 30)</td>
</tr>
<tr>
<td>§Highest CS dose (mg)</td>
<td>30 (15, 55)</td>
<td>50 (30, 60)*</td>
<td>40 (20, 60)</td>
</tr>
<tr>
<td>§Cumulative CS dose (g)</td>
<td>1.4 (0.4, 3.1)*</td>
<td>3.9 (1.8, 6.2)*</td>
<td>2.5 (1.2, 4.8)</td>
</tr>
<tr>
<td>Pulse intravenous CS</td>
<td>26 (15.4)*</td>
<td>5/223 (2.2)</td>
<td>39/1031 (3.8)</td>
</tr>
<tr>
<td>Immunosuppressant</td>
<td>86 (50.9)*</td>
<td>146 (60.8)*</td>
<td>367/1082 (33.9)</td>
</tr>
<tr>
<td>Antimalarial</td>
<td>120 (71.0)*</td>
<td>125 (52.1)*</td>
<td>705 (65.0)</td>
</tr>
</tbody>
</table>

* Abbreviations: CS corticosteroid; § oral dose

* Significant when compared to all other ethnicities (p <0.05)

#### 5.3.3.3 Determinants of MetS in Korean and Hispanic populations at enrolment.

A backward stepwise logistic regression model was run to determine those factors independently associated with MetS in Korean and Hispanic patients. As in the main cohort, factors that were significantly associated with MetS on univariate analysis were included in the model. In the Korean population, MetS was independently associated with increasing age (OR (95% CI) 1.06 (1.01, 1.10) and higher peak oral corticosteroid dose (mg) (1.03 (1.01, 1.05)). In the Hispanic group, MetS was associated with haematuria on SLEDAI-2K (3.61 (1.59, 8.21)) and higher peak oral corticosteroid dose received (mg) (1.02 (1.0, 1.03)).
5.3.4 MetS susceptibility at enrolment and immunosuppressant use.

The apparent association between current immunosuppressant use and MetS was also examined. Apart from ciclosporin, these therapies, (which include azathioprine, mycophenolate mofetil (MMF), cyclophosphamide and methotrexate) were not thought to be mechanistically involved in MetS development in SLE, but might rather act as a marker of more severe disease, higher disease activity and/or corticosteroid use. The characteristics of patients receiving immunosuppressant therapies at enrolment were therefore compared to those who were not (Table 5-15). Those receiving immunosuppressant therapies at enrolment were older (36.6 (SD) (13.7) vs. 33.3 (13.1) years; p= <0.001) and more likely to be male (12.5% vs. 9.3%; p =0.05) than those who were not. Overall, the majority of patients receiving immunosuppressive therapies also received oral corticosteroids (91.5%), at higher doses, than those not on immunosuppressive agents. Immunosuppressant users also had higher SLEDAI-2K and SLICC-DI. However, use of immunosuppressive agents remained significantly associated with MetS even after adjusting for all the factors that could be represented by immunosuppressant use, such as SLEDAI-2K, renal disease, corticosteroid use and corticosteroid dose. The fully adjusted odds ratio (95% CI) for current immunosuppressant use at enrolment into SLICC-MetS was 2.15 (1.15, 4.00).
### Table 5-15: MetS and disease phenotype by immunosuppressant use

<table>
<thead>
<tr>
<th>Mean (SD) Or N (%)</th>
<th>Not On IS N = 892</th>
<th>On IS N = 599</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MetS Phenotype</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MetS</td>
<td>98 (11.0)</td>
<td>140 (23.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MetS WC</td>
<td>370/786 (47.1)</td>
<td>274/546 (50.2)</td>
<td>0.26</td>
</tr>
<tr>
<td>MetS BP</td>
<td>331/889 (37.2)</td>
<td>352 (58.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MetS TG</td>
<td>319/808 (39.5)</td>
<td>297/531 (55.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MetS HDL</td>
<td>261/453 (57.6)</td>
<td>224/366 (61.2)</td>
<td>0.30</td>
</tr>
<tr>
<td>MetS Glu</td>
<td>140/791 (17.7)</td>
<td>130/550 (23.6)</td>
<td>0.008</td>
</tr>
<tr>
<td>BMI</td>
<td>25.2 (5.9)</td>
<td>24.9 (5.8)</td>
<td>0.25</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>82.7 (14.2)</td>
<td>83.0 (13.5)</td>
<td>0.70</td>
</tr>
<tr>
<td><strong>SLE Phenotype</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLEDAI-2K</td>
<td>4.72 (4.90)</td>
<td>6.65 (6.00)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SLICC/ACR-DI</td>
<td>0.22 (0.66)</td>
<td>0.39 (0.79)</td>
<td>0.004</td>
</tr>
<tr>
<td>Oral CS</td>
<td>492 (55.2)</td>
<td>548 (91.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>*Average CS dose (mg)</td>
<td>15 (10, 30)</td>
<td>25 (15, 40)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>*Highest CS dose (mg)</td>
<td>30 (15, 50)</td>
<td>50 (30, 60)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>*Cumulative CS dose (g)</td>
<td>1.6 (0.6, 3.4)</td>
<td>3.5 (1.8, 6.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pulse intravenous CS</td>
<td>26/838 (3.1)</td>
<td>44/583 (7.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antimalarial</td>
<td>654 (73.3)</td>
<td>316 (52.8)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: IS immunosuppressant; BMI body mass index; WC waist circumference; CS corticosteroid. * oral dose

#### 5.3.5 MetS susceptibility at enrolment and active renal disease.

The final exploratory analysis was of active renal lupus. Renal disease is a marker of more severe SLE and is associated with more frequent exposure to higher doses of corticosteroids and immunosuppression therapies. Renal disease in general is also associated with the metabolic derangements captured by MetS, such as hypertension and dyslipidaemia. Active renal lupus may therefore be a marker of disease severity and/or corticosteroid exposure, but may also influence MetS pathogenesis directly through its associated metabolic abnormalities.
In the SLICC-MetS cohort, the prevalence of MetS was higher in patients with active renal lupus than those with no current renal disease (29.9% vs. 12.3%; p = <0.0001). Those with renal involvement had significantly more hypertension (MetS BP 72.9% vs. 38.8%; p <0.0001) and hypertriglyceridaemia (MetS TG 74.1% vs. 38.8%; p<0.0001), than non-renal patients. Surprisingly however, central obesity parameters (BMI and MetS WC) were lower in those with renal disease. Overall, 93% of those with active renal disease were on oral corticosteroids, compared to 63.6% of the rest of the cohort, and median (IQR) daily oral dose was almost twice that of non-renal patients (30 (20, 50) mg vs. 16 (10, 30) mg; p = <0.0001). Perhaps surprisingly they were also less likely to be receiving antimalarial therapy (43.6% vs. 70.7%; p = <0.0001).

5.4 Determinants of MetS over time

The second stage of the SLICC-MetS analysis was to examine the factors associated with MetS over the first 2 years of follow-up in the SLICC registry. It was hypothesised that these factors may differ from those associated with MetS at baseline, reflecting cumulative exposure to inflammation and therapy. The detailed methodology for this analysis is described in chapter 3. In brief, random effects regression was used to perform a longitudinal analysis. Univariate regression analyses, adjusted for age, gender ethnicity and follow-up time, were performed initially. Variables were then generated to represent exposure at baseline and exposure over follow-up, and interactions between predictor variables and follow-up time were tested and included in the multivariable model where present. Factors that were significant on univariate analysis (p<0.2) were subsequently entered into a multivariable random effects model. Several permutations of the model were run, which incorporated different combinations of predictor variables.

5.4.1 Univariate associations of MetS in SLE over time

5.4.1.1 Variables associated with MetS over time
In a univariate analysis, adjusted for age, ethnicity, gender and the changing prevalence of MetS, we tested the strength of the association between variables related to disease phenotype, disease severity and therapeutic exposure and MetS over the first 2 years of follow-up in SLICC-MetS (Table 5-16). Higher disease activity scores, higher SLICC/ACR-DI, renal lupus, and higher oral doses of corticosteroids were all associated with MetS over time. Intra-venous corticosteroid use was not associated with MetS over time (OR 1.60 (0.87, 2.97). Antimalarial use was negatively associated with MetS over the first 2 years (OR 0.48 (0.34, 0.67)). The magnitude of the association between MetS and
immunosuppression use was less marked in the longitudinal analysis compared to baseline (OR 1.50 (1.10, 2.05) vs. 2.21 (1.63, 3.00)). In contrast, the effect of disease-damage on SLICC/ACR-DI was greater over time than at enrolment (OR 2.82 (1.79, 4.44) vs. 1.99 (1.16, 3.40)). The strongest association with prevalent MetS however was an individual’s MetS status at their preceding visit (OR 7.93 (5.52, 11.42)).

Table 5-16: Univariate associations of MetS over time

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted Odds Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>1.02</td>
<td>1.01, 1.03</td>
</tr>
<tr>
<td>Previous MetS status</td>
<td>7.93</td>
<td>5.52, 11.42</td>
</tr>
<tr>
<td>Korean ethnicity</td>
<td>3.40</td>
<td>1.95, 5.93</td>
</tr>
<tr>
<td>Hispanic ethnicity</td>
<td>6.34</td>
<td>3.78, 10.62</td>
</tr>
<tr>
<td>SLEDAI-2K &gt;10</td>
<td>1.56</td>
<td>1.13, 2.15</td>
</tr>
<tr>
<td>SLEDAI-2K</td>
<td>1.06</td>
<td>1.03, 1.09</td>
</tr>
<tr>
<td>SLICC/ACR-DI &gt;1</td>
<td>2.82</td>
<td>1.79, 4.44</td>
</tr>
<tr>
<td>High anti-dsDNA</td>
<td>1.65</td>
<td>1.18, 2.32</td>
</tr>
<tr>
<td>Active renal disease</td>
<td>2.75</td>
<td>1.89, 4.03</td>
</tr>
<tr>
<td>Current oral CS</td>
<td>1.56</td>
<td>1.05, 2.34</td>
</tr>
<tr>
<td>Average oral CS dose (mg)</td>
<td>1.04</td>
<td>1.02, 1.05</td>
</tr>
<tr>
<td>Highest oral CS dose (mg)</td>
<td>1.03</td>
<td>1.02, 1.04</td>
</tr>
<tr>
<td>Cumulative oral CS dose (g)</td>
<td>1.03</td>
<td>1.00, 1.05</td>
</tr>
<tr>
<td>Current IS</td>
<td>1.50</td>
<td>1.10, 2.05</td>
</tr>
<tr>
<td>Current AM</td>
<td>0.48</td>
<td>0.34, 0.67</td>
</tr>
</tbody>
</table>

Abbreviations: CS corticosteroids; IS immunosuppression; AM antimalarial.

5.4.1.2 Differential effect of timing of exposure on MetS

The next stage in the analysis explored whether exposure to the predictors of interest had a greater effect on MetS in early disease or cumulatively over time, to investigate the changing MetS status described in Figure 5-10. Only those factors related to disease phenotype and disease severity (Table 5-17), and therapeutic exposure (Table 5-18) were explored; ethnicity and gender do not change over time, and there was no documented preceding MetS status at enrolment. Interestingly, the association between immunosuppressant exposure and MetS was significant at baseline but not over the subsequent 2 years (OR 2.84 (1.92, 4.19) vs. 1.38 (0.92, 2.10)). Cumulative oral corticosteroid dose
became significant over follow-up but was not at baseline (OR 1.09 (1.04, 1.15) vs. 1.01 (0.98, 1.04)). There is also a suggestion that more severe disease at baseline (renal disease and anti-dsDNA positivity) was more strongly associated with MetS, compared to the subsequent 2 years.

Table 5-17: Effect of timing of exposures on MetS over time

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLICC ≥1 baseline</td>
<td>2.21</td>
<td>1.12, 4.34</td>
</tr>
<tr>
<td>SLICC ≥1 over time</td>
<td>3.37</td>
<td>2.18, 5.19</td>
</tr>
<tr>
<td>SLEDAI-2k baseline</td>
<td>1.07</td>
<td>1.04, 1.11</td>
</tr>
<tr>
<td>SLEDAI-2K over time</td>
<td>1.07</td>
<td>1.02, 1.14</td>
</tr>
<tr>
<td>SLEDAI ≥10 baseline</td>
<td>1.82</td>
<td>1.24, 2.66</td>
</tr>
<tr>
<td>SLEDAI ≥10 over time</td>
<td>1.87</td>
<td>1.12, 3.14</td>
</tr>
<tr>
<td>High anti-dsDNA baseline</td>
<td>2.01</td>
<td>1.35, 3.01</td>
</tr>
<tr>
<td>High anti-dsDNA over time</td>
<td>1.70</td>
<td>1.09, 2.66</td>
</tr>
<tr>
<td>Active renal disease baseline</td>
<td>3.98</td>
<td>2.58, 6.14</td>
</tr>
<tr>
<td>Active renal disease over time</td>
<td>3.23</td>
<td>1.75, 5.94</td>
</tr>
</tbody>
</table>

Table 5-18: Differential effect of timing of therapy-related exposures on MetS over time

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current oral CS base</td>
<td>1.82</td>
<td>1.15, 2.89</td>
</tr>
<tr>
<td>Current oral CS over time</td>
<td>1.39</td>
<td>0.88, 2.17</td>
</tr>
<tr>
<td>Highest oral CS dose base (mg)</td>
<td>1.04</td>
<td>1.03, 1.05</td>
</tr>
<tr>
<td>Highest oral CS dose over time (mg)</td>
<td>1.03</td>
<td>1.01, 1.04</td>
</tr>
<tr>
<td>Average oral CS dose base (mg)</td>
<td>1.04</td>
<td>1.02, 1.05</td>
</tr>
<tr>
<td>Average oral CS dose over time (mg)</td>
<td>1.06</td>
<td>1.03, 1.09</td>
</tr>
<tr>
<td>Cumulative oral CS dose base (mg)</td>
<td>1.01</td>
<td>0.98, 1.04</td>
</tr>
<tr>
<td>Cumulative oral CS dose over time (mg)</td>
<td>1.09</td>
<td>1.04, 1.15</td>
</tr>
<tr>
<td>Current IS base</td>
<td>2.84</td>
<td>1.92, 4.19</td>
</tr>
<tr>
<td>Current IS over time</td>
<td>1.38</td>
<td>0.92, 2.10</td>
</tr>
<tr>
<td>Current AM base</td>
<td>0.44</td>
<td>0.30, 0.64</td>
</tr>
<tr>
<td>Current AM over time</td>
<td>0.41</td>
<td>0.27, 0.61</td>
</tr>
</tbody>
</table>

Abbreviations: CS corticosteroids; IS immunosuppressant therapy; AM antimalarial.
5.4.2 Multivariable associations of MetS in SLE over time

A random effects logistic regression model was utilised to perform a longitudinal analysis of the factors associated with MetS over the first 2 years of follow-up in SLICC-MetS. Any variable that was significant on adjusted univariate regression analyses (p<0.2) was included. The final model identified preceding MetS status, elevated anti-dsDNA antibodies at enrolment, increasing age, and Hispanic ethnicity as independently associated with MetS over the first 2 years of follow-up in SLICC-MetS (Table 5-19).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>MetS at last visit (y/n)</td>
<td>4.80</td>
<td>2.93, 7.87</td>
</tr>
<tr>
<td>Peak oral CS dose at enrolment (mg)</td>
<td>1.02</td>
<td>1.01, 1.03</td>
</tr>
<tr>
<td>Elevated anti-dsDNA at enrolment (y/n)</td>
<td>1.86</td>
<td>1.19, 2.91</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.03</td>
<td>1.01, 1.05</td>
</tr>
<tr>
<td>Hispanic Ethnicity</td>
<td>3.47</td>
<td>1.76, 6.85</td>
</tr>
</tbody>
</table>

Abbreviations: CS corticosteroid

A post-estimation assessment of the reliability of this model, tested by checking the sensitivity of quadrature approximation, was performed. No single coefficient for each variable changed by a relative difference of more than 0.01%, suggesting the model can be interpreted with confidence.

To examine whether corticosteroids had a dose-related effect on MetS status over time, all corticosteroid dose-related variables were excluded and only current-use (yes/no) was included. In this model, oral corticosteroid use was not independently associated with MetS, but active renal disease at enrolment became significant (OR 1.71 (1.10, 2.64)), whilst the other factors remained unchanged.

5.4.3 MetS susceptibility over time and ethnicity

To examine why the ethnic-risk differed from that observed in the cross-sectional enrolment analysis, the MetS phenotype, lupus phenotype and therapeutic exposures were all examined longitudinally in Korean and Hispanic patients.
5.4.3.1 MetS phenotype over time and ethnicity

There was considerable ethnic variation in the prevalence and phenotype of MetS over time, as observed at enrolment. The Korean population had stable central obesity parameters over time, a falling prevalence of dyslipidaemia and increasing rates of hypertension. Interestingly their rates of hyperglycaemia fluctuated over time. In contrast the Hispanic cohort had increasing central obesity, relatively stable rates of hypertension and falling rates of dyslipidaemia and hyperglycaemia over the first 2 years (Table 5-20).

Table 5-20: MetS phenotype in Korean and Hispanic patients over time

<table>
<thead>
<tr>
<th>n (%)</th>
<th>Visit</th>
<th>Korean</th>
<th>Hispanic</th>
<th>Whole Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enrol</td>
<td>33/164 (20.1)</td>
<td>129/228 (56.6)</td>
<td>645/1333 (48.4)</td>
</tr>
<tr>
<td></td>
<td>FU 1</td>
<td>30/136 (22.1)</td>
<td>64/101 (63.4)</td>
<td>467/919 (50.8)</td>
</tr>
<tr>
<td></td>
<td>FU 2</td>
<td>19/100 (19.0)</td>
<td>55/88 (62.5)</td>
<td>359/728 (49.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enrol</td>
<td>74/169 (43.8)</td>
<td>117/240 (48.8)</td>
<td>686/1489 (46.1)</td>
</tr>
<tr>
<td></td>
<td>FU 1</td>
<td>64/137 (46.7)</td>
<td>59/118 (50.0)</td>
<td>516/1065 (48.5)</td>
</tr>
<tr>
<td></td>
<td>FU 2</td>
<td>49/100 (49.0)</td>
<td>52/104 (50.0)</td>
<td>452/894 (50.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enrol</td>
<td>100/153 (65.4)</td>
<td>108/168 (64.3)</td>
<td>619/1340 (46.2)</td>
</tr>
<tr>
<td></td>
<td>FU 1</td>
<td>40/77 (52.6)</td>
<td>51/99 (51.5)</td>
<td>347/942 (36.8)</td>
</tr>
<tr>
<td></td>
<td>FU 2</td>
<td>26/52 (50.0)</td>
<td>44/88 (50.0)</td>
<td>311/794 (39.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enrol</td>
<td>110/144 (76.4)</td>
<td>97/149 (65.1)</td>
<td>485/821 (59.1)</td>
</tr>
<tr>
<td></td>
<td>FU 1</td>
<td>48/74 (64.9)</td>
<td>53/86 (61.6)</td>
<td>337/617 (54.6)</td>
</tr>
<tr>
<td></td>
<td>FU 2</td>
<td>29/50 (58.0)</td>
<td>45/81 (55.6)</td>
<td>292/528 (55.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enrol</td>
<td>45/168 (26.8)</td>
<td>41/236 (17.4)</td>
<td>271/1342 (20.2)</td>
</tr>
<tr>
<td></td>
<td>FU 1</td>
<td>26/135 (19.3)</td>
<td>10/101 (9.9)</td>
<td>136/966 (14.1)</td>
</tr>
<tr>
<td></td>
<td>FU 2</td>
<td>25/99 (25.3)</td>
<td>8/85 (9.4)</td>
<td>108/805 (13.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enrol</td>
<td>52/169 (30.8)</td>
<td>75/240 (31.3)</td>
<td>239/1494 (16.0)</td>
</tr>
<tr>
<td></td>
<td>FU 1</td>
<td>20/137 (14.6)</td>
<td>27/118 (22.9)</td>
<td>134/1065 (12.6)</td>
</tr>
<tr>
<td></td>
<td>FU 2</td>
<td>16/100 (16.0)</td>
<td>29/104 (27.9)</td>
<td>121/894 (13.6)</td>
</tr>
</tbody>
</table>

Abbreviations: WC waist circumference; BP blood pressure; TG triglycerides; HDL high-density lipoprotein cholesterol; Glu glucose.

This table describes the contrasting MetS phenotypes of Korean and Hispanic patients over the first two years of SLICC. Whilst both groups have a drop in MetS prevalence after enrolment, the prevalence in Koreans remains substantially lower driven mainly by falls in the prevalence of low HDL-C and high TG. Hispanics have reduced lipids but gradually increasing MetS WC, and a higher persistent prevalence of MetS.
5.4.3.2 Lupus phenotype over time and ethnicity
At enrolment, mean (SD) SLEDAI was 5.5 (5.4) overall, 7.4 (6.1) in Koreans and 6.5 (5.8) in Hispanics. At year 1 this fell to 3.6 (4.0), 3.4 (2.6) and 5.1 (4.9), respectively, and by year 2 was 3.6 (4.1), 4.1 (3.8) and 5.7 (4.6). Patients of Hispanic ethnicity accumulated disease damage more than any other group over the 2 year follow up period: 18.9% had a SLICC/ACR-DI >1 at enrolment, 34.8% at year 1 and 41.2% at year 2. In the whole cohort, the proportion of patients with a SLICC/ACR-DI of >1 was 18.2%, 27.5% and 30.5% at each respective visit. In contrast, only 16% of Korean patients had a SLICC-DI of > 1 by their 2nd follow-up visit. These indices were underpinned by significant ongoing active renal lupus in the Hispanic cohort (42.5% at enrolment, 39.3% at year 1, and 48.1% at year 2), compared to Koreans (29.0%, 12.4%, 10.1%). The therapeutic exposures in each ethnic group are described in Table 5-21.

5.4.4 MetS susceptibility over time and anti-dsDNA positivity
Antibodies to dsDNA are a feature of active lupus and strongly associated with renal disease, and elevated anti-dsDNA antibodies are often used to monitor treatment response. That they remain in the multivariable model when renal disease and SLEDAI-2K do not was not predicted, and so their association with MetS was explored further. MetS was significantly more common in patients with elevated dsDNA antibodies at each visit (e.g. 18.7% vs. 14.3% at enrolment, p = 0.03; 18.2% vs. 10.6% at year 2, p = 0.003). However, central obesity was also less common in those with anti-dsDNA antibodies at each visit (e.g. MetS WC 42.6% vs. 52.7% at enrolment), in part related the high prevalence of elevated dsDNA antibodies in the less obese Korean cohort. They were also, as would be expected, more common in patients with renal disease and those with a higher SLEDAI-2K. However, patients with elevated dsDNA antibodies received similar doses of corticosteroids to those without elevated titres at each visit (e.g. mean (SD) peak dose at enrolment 38.2 (21.8 vs. 37.0 (20.8) mg; p = 0.5). The use of intra-venous corticosteroids was also no more common in those with positive dsDNA antibodies. When the univariate analysis was adjusted for renal disease, disease activity and corticosteroid use, in addition to age, gender, ethnicity and time, elevated dsDNA antibodies at enrolment were no longer significantly associated with MetS in a longitudinal analysis (OR 1.19 (0.42, 3.35).
Table 5-21: Therapeutic exposures over time by ethnicity

<table>
<thead>
<tr>
<th>Mean (SD) or %</th>
<th>Visit</th>
<th>Korean</th>
<th>Hispanic</th>
<th>Whole Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral CS</td>
<td>Enrol</td>
<td>161/169 (95.3)</td>
<td>211/240 (87.9)</td>
<td>1042/1494 (69.8)</td>
</tr>
<tr>
<td></td>
<td>FU 1</td>
<td>131/137 (95.6)</td>
<td>102/118 (86.4)</td>
<td>754/1065 (70.8)</td>
</tr>
<tr>
<td></td>
<td>FU 2</td>
<td>94/100 (94)</td>
<td>76/104 (73.1)</td>
<td>545/894 (61.0)</td>
</tr>
<tr>
<td>Average CS dose (mg)</td>
<td>Enrol</td>
<td>20 (10, 35)</td>
<td>30 (15, 42.5)</td>
<td>20 (10, 33)</td>
</tr>
<tr>
<td></td>
<td>FU 1</td>
<td>10 (7.5, 14.5)</td>
<td>15 (7.5, 24)</td>
<td>10 (7, 16)</td>
</tr>
<tr>
<td></td>
<td>FU 2</td>
<td>7.5 (5, 10.5)</td>
<td>10 (5, 19)</td>
<td>7.5 (5, 12)</td>
</tr>
<tr>
<td>Highest CS dose (mg)</td>
<td>Enrol</td>
<td>30 (15, 52.5)</td>
<td>50 (30, 60)</td>
<td>40 (20, 60)</td>
</tr>
<tr>
<td></td>
<td>FU 1</td>
<td>15 (10, 30)</td>
<td>30 (15, 50)</td>
<td>20 (10, 40)</td>
</tr>
<tr>
<td></td>
<td>FU 2</td>
<td>10 (5, 15)</td>
<td>15 (6, 50)</td>
<td>10 (5, 20)</td>
</tr>
<tr>
<td>IV CS</td>
<td>Enrol</td>
<td>26/169 (15.4)</td>
<td>5/223 (2.2)</td>
<td>70/1421 (4.9)</td>
</tr>
<tr>
<td></td>
<td>FU 1</td>
<td>10/137 (7.3)</td>
<td>3/117 (2.6)</td>
<td>78/1053 (7.4)</td>
</tr>
<tr>
<td></td>
<td>FU 2</td>
<td>4/100 (4.0)</td>
<td>1/104 (1.0)</td>
<td>42/891 (4.7)</td>
</tr>
<tr>
<td>Current IS</td>
<td>Enrol</td>
<td>86/169 (50.9)</td>
<td>146/240 (60.8)</td>
<td>597/1494 (40.1)</td>
</tr>
<tr>
<td></td>
<td>FU 1</td>
<td>67/137 (48.9)</td>
<td>68/118 (57.6)</td>
<td>451/1065 (42.4)</td>
</tr>
<tr>
<td></td>
<td>FU 2</td>
<td>54/100 (54)</td>
<td>59/104 (56.7)</td>
<td>388/894 (43.4)</td>
</tr>
<tr>
<td>Current AM</td>
<td>Enrol</td>
<td>120/169 (71.0)</td>
<td>125/240 (52.1)</td>
<td>970/1494 (65.0)</td>
</tr>
<tr>
<td></td>
<td>FU 1</td>
<td>97/137 (70.8)</td>
<td>61/118 (51.7)</td>
<td>713/1065 (67.0)</td>
</tr>
<tr>
<td></td>
<td>FU 2</td>
<td>81/100 (81)</td>
<td>50/104 (48.1)</td>
<td>630/894 (70.5)</td>
</tr>
</tbody>
</table>

Abbreviations: CS corticosteroid; IV intravenous; IS immunosuppressant; AM antimalarial
5.5 Summary

This chapter describes the varying prevalence and phenotype of MetS in SLE over time in an international inception cohort. The results showed that 16% of the cohort had MetS at enrolment, which varied slightly over the subsequent 2 years. However, within this overall figure there was significant ethnic and geographical variation in MetS prevalence, with Hispanics having the highest prevalence at each follow-up visit. The results also describe a MetS phenotype characterised less by obesity, which remained relatively stable throughout follow-up, and more by dyslipidaemia and hyperglycaemia.

At enrolment, disease- and therapy-related factors independently associated with MetS were exposure to increasing daily oral prednisolone doses, active renal lupus and immunosuppression use. Increasing age, Hispanic ethnicity and Korean ethnicity were also independently associated with MetS.

In a longitudinal analysis, preceding MetS status was strongly associated with the presence of subsequent MetS (odds ratio 4.8), as were the non-modifiable risk factors of age and Hispanic ethnicity. Exposure to higher peak corticosteroid doses prior to the enrolment visit and the presence of elevated anti-dsDNA antibodies at enrolment were also significantly associated with MetS over the subsequent 2 years.

In summary, factors independently associated with MetS over the first 2 years of follow-up in patients with recently diagnosed SLE include non-modifiable markers of MetS susceptibility (ethnicity and age) and potentially modifiable disease-related features (corticosteroid dose, inflammatory disease activity and renal lupus). The adverse MetS phenotype can become persistent in some patients and preceding MetS strongly predicts future MetS.
5.6 Discussion

The SLICC-Register for Atherosclerosis represents the largest inception cohort of patients with SLE. As of December 2009, 1686 patients had been recruited and assessed at least once, and 1024 had contributed follow-up data over at least 2 years. This cohort is young and by design within 15 months of SLE diagnosis. This study design reduces or eliminates many important confounders that affect retrospective analyses of smaller, older and more established cohorts such as older age and varying disease duration. SLICC-RAS provides an ideal opportunity to examine disease-related factors that may contribute to the elevated risk of cardiovascular disease that is characteristic of SLE. The breadth of prospective data permits more detailed examination of potential risk factors associated with CVD in SLE than do many retrospective studies, particularly with regard to corticosteroid exposure.

The metabolic syndrome is associated with an increased risk of future diabetes mellitus and coronary heart disease in the general population, and is used as a surrogate for CV risk. Patients with SLE have an increased prevalence of many of the metabolic abnormalities captured by MetS, such as insulin resistance, hypertension and dyslipidaemia. MetS is therefore a useful model in which to examine why SLE is associated with accelerated atherosclerosis, and to investigate the potential role of inflammation, disease phenotype and corticosteroid exposure. MetS is also a simple tool by which individuals at higher risk of future CVD can be identified and in whom a more personalised approach to management may be employed to modify this risk. The results of the analysis of the SLICC-MetS study are discussed below.

5.6.1 Prevalence and phenotype of MetS in SLE

The first aim of the SLICC-MetS analysis was to describe the changing prevalence and phenotype of MetS over the first 2 years of entry into SLICC-RAS. It was hoped that a better understanding of the epidemiology of MetS in SLE would not only inform the subsequent stages of the analysis, but would also provide important practical information for clinicians regarding the natural history of MetS in SLE.

The overall prevalence of MetS was 16% at enrolment, 12.6% at year 1 and 13.5% at year 2. This represents a significant burden of cardiovascular risk factors in a young, predominantly female, cohort who might reasonably be expected to have low CV risk. The metabolic derangements that might contribute to long-term CVD risk, and that are characterised by MetS, therefore appear
early in the course of SLE. The SLICC-RAS cohort has no control population against which to compare the prevalence of MetS, so direct comparisons cannot be made to healthy populations. However, smaller controlled studies of older, more established cohorts have consistently described higher rates of MetS in SLE than controls (130;130;164;165), and it is likely that this remains true in a younger cohort with very active, recent onset disease.

Within the overall variation in MetS prevalence, significant ethnic variation was observed. For example, Hispanic patients had the highest prevalence of MetS at each visit, although the rate varied over time (31.3% at enrolment, 22.9% at year 1 and 27.9% at year 2). Korean patients had a similarly high prevalence at enrolment (30.8%) but this fell sharply at year 1 and remained stable at year 2 (14.6% and 16%). In contrast, Caucasian patients had a relatively stable MetS prevalence over all visits (12.1%, 12.4% and 11.8%), whilst Black African patients had a variable but increasing prevalence (8.1%, 5.2%, 14.4%). To some degree this variable ethnic prevalence reflects the background prevalence of MetS in different populations (217) however as already stated, age-matched controlled studies consistently confirm the excess prevalence of MetS in SLE.

There was also significant variation in the MetS status of individuals noted over time. For example, in a complete-case analysis of the first 2 years of SLICC-MetS, 73.1% of patients never developed MetS, 4.3% had MetS at every visit, 14.2% developed incident MetS over the follow-up period and 26.9% of patients had MetS on at least 1 occasion. A substantial number of patients were therefore moving between the two phenotypes over time, and in many MetS was persistent. The persistence of MetS in many patients early in their disease course suggests that MetS may become a fixed long-term phenotype in a substantial number of SLE patients. This observation supports the need to identify potentially modifiable contributory factors in the early stages of the disease, particularly in those with existing metabolic derangement. Whether persistent or transitory MetS in a young patient with SLE is associated with future CV events is a key question being investigated within this cohort, and will further validate the use of MetS in SLE.

The literature to date suggests that central obesity may be under-represented in SLE patients with MetS, and that the hypertension and dyslipidaemia components are more prevalent in SLE than in controls with MetS (229). This alternative MetS phenotype may be related less to excess adiposity and more to inflammatory disease activity, although the evidence for this hypothesis is inconsistent. This may be because recent studies investigating clinical associations of MetS in SLE have assessed older SLE cohorts with minimal inflammatory disease activity, longer disease duration, and/or used older MetS
definitions that require central obesity to be present (130;164;165). All of these studies to date have been cross-sectional, and the relationship between obesity, inflammation and MetS in SLE has therefore been difficult to examine. The SLICC-MetS cohort therefore provides an ideal population in which to examine this relationship further as many of the biases that affect established and retrospective cohorts, such as channelling bias, do not apply in a prospective inception cohort. Additionally, the IDF 2009 MetS definition does not require central obesity to be present in order to meet the MetS definition, therefore the impact of factors unrelated to obesity, such as inflammatory dyslipidaemia, can be explored.

The prevalence of central obesity remains stable in the SLICC-MetS cohort over the first 2 years and does not exceed 50.8%, whilst there is a gradual increase in the number of patients meeting the hypertension criteria. The most variable MetS components however, are those related to lipid and glucose profiles, confirming that the observed variation in MetS prevalence over the first 2 years is not due to fluctuating levels of central obesity. It is likely therefore that the glucose, triglyceride and HDL-C components are more sensitive to changes in corticosteroid dose and inflammation over the first 2 years of follow-up, in contrast to blood pressure and obesity parameters.

There is considerable ethnic variation in the MetS phenotype, similar to that seen in MetS prevalence. For example, in Korean patients with MetS rates of central obesity were very low (MetS WC 20.1-22.1%) whilst rates of dyslipidaemia (MetS HDL 64-76.4%) and hyperglycaemia (19-26.8%) were the highest in the cohort at each visit. In Hispanic patients, hyperglycaemia was no more frequent than the rest of the cohort but central obesity was increasingly prevalent over time (MetS WC 56-63.4%). These contrasting MetS phenotypes suggest that different factors are important in different populations in its aetiopathogenesis, and obesity is not a driving factor in all lupus populations. This is further supported by the contrasting lupus phenotypes and therapeutic exposures in the Korean and Hispanic cohorts. For example, corticosteroid use was almost universal in the Korean population with frequent use of pulse IV methylprednisolone but the oral doses used were no higher than the rest of the cohort, in contrast to the Hispanic population. Similarly, both high-risk populations had high disease activity, but Korean patients had active lupus characterised by serologically active disease driven by antibody-positivity and haematological features, whilst the Hispanic cohort had a high prevalence of renal lupus.

In summary, the results of the first phase of the SLICC-MetS analysis suggest that MetS is common in SLE, occurs early in the course of the disease, and is
persistent in a significant number of patients. Certain ethnicities have a much higher prevalence of MetS, related in part to the background prevalence of the syndrome, but also due to a lupus phenotype characterised by high inflammatory disease activity and exposure to high doses of oral and intravenous corticosteroids. Analysis of the MetS phenotype confirms that MetS is not always an obesity-related phenomenon in early SLE, and clinicians should therefore not rely on the presence of central obesity as the sole prompt to screen for other CV risk factors. The results also indicate that dyslipidaemia improves over time, as disease control improves and corticosteroid doses are reduced, which may influence the timing of the introduction of statin therapies. Factors related to disease phenotype, inflammatory disease activity and corticosteroid exposure may therefore contribute to the adverse metabolic profile observed in early SLE, and these were further investigated in the second phase of the analysis.

5.6.2 Clinical determinants of MetS in SLE at enrolment

In a cross-sectional study performed in 1494 patients at enrolment into SLICC-MetS, several disease-related factors were associated with MetS in univariate analyses, adjusted for age, gender and ethnicity. Exposure to intra-venous and oral corticosteroids, at increasing daily and peak doses, was associated with MetS at enrolment. The use of immunosuppressant drugs was strongly associated with MetS, and antimalarial therapy appeared to confer a ‘protective’ effect. Higher disease activity, higher damage indices and active renal lupus were all associated with MetS, as were individual components of the SLEDAI-2K such as elevated dsDNA antibodies, and thrombocytopenia. When all these factors were entered into a multivariable model, those that remained independently associated with MetS were higher daily dose of oral corticosteroid, increasing age, Korean ethnicity, Hispanic ethnicity, active renal disease and the use of immunosuppressant therapies.

The association with corticosteroids appears to be dose-related in early disease, as current oral use did not remain significant in a multivariable model that excluded all dose-related variables. Interestingly however, although higher oral doses are associated with MetS, recent exposure to intravenous pulses was not associated with MetS at enrolment and neither was the dose received. This may relate to the small numbers of patients receiving IV therapy (n = 70) all of whom also received high dose oral prednisolone therapy. Therefore in early active SLE, judicious use of corticosteroids should be a key component of early disease management to minimise their adverse metabolic effects. The use of antimalarials did not remain significant in the multivariable model, as it has in other studies of cohorts with stable, less severe lupus (164;230). This is likely to
be due to the prospective study design of SLICC-RAS that minimises channelling bias.

The strong association of MetS with Korean and Hispanic ethnicities was unexpected and in part reflects an inherently higher background prevalence of MetS in certain populations. For example, a large study from Mexico in 2006 found an overall prevalence of MetS in adults over 20 years of 37-50%, depending on the definition used (330) and 24-36% in adults aged 20-39 years. Similar results were noted from the USA (331). A recent study of South Korean adults described a variable MetS prevalence in women of 16-31% depending on the definition used, with low levels of central obesity as we also found in our study (332). The observed ethnic gradient therefore reflects an increased baseline susceptibility to developing MetS in some populations (particularly Hispanics). Significant differences in the actual MetS phenotype were also observed in these two subsets, as well as differences in the clinical and inflammatory pattern of disease observed. Whether these ethnic differences translate into a differential effect on future cardiovascular end-points is the subject of the main prospective SLICC-RAS study.

The significant association between immunosuppressant use at enrolment and MetS in this cohort is likely to represent confounding rather than a true causal association with MetS. However, the relationship persists even after adjusting for all measured potential confounding factors (such as SLEDAI-2K, corticosteroid use, renal lupus and SLICC/ACR-DI). This suggests that either immunosuppressant drugs do indeed have direct adverse metabolic effects, or the disease-related factors immunosuppressant use represents are inadequately measured in this study. Apart from ciclosporin A, the immunosuppressive agents commonly used in SLE are not generally associated with hypertension, obesity, insulin resistance or dyslipidaemia (193); indeed, immunosuppression may have a role in preventing atherosclerosis (194;196). Therefore, current immunosuppressant use is likely to reflect residual confounding as, for example, inflammatory disease activity is unlikely to be adequately captured by an annual SLEDAI-2K assessment, and additional biomarkers and/or indices of disease activity may improve the estimation of exposure to systemic inflammation.

The results of this cross-sectional analysis therefore indicate that age and ethnicity are important in the development of MetS in very early disease, factors that are however not modifiable. Higher daily doses of corticosteroid and the presence of active and severe disease features (indicated by renal lupus and immunosuppressant use) were also independently associated with MetS at enrolment. Therefore, in very early SLE rapid control of inflammatory disease activity (such as renal lupus) with the lowest doses of corticosteroid possible is
likely to be beneficial to long-term cardiovascular risk, especially in older patients and patients of high-risk ethnicity.

**5.6.3 Clinical determinants of MetS in SLE over time**

The final component of the SLICC-MetS analysis was to examine the determinants of MetS over the first 2 years of follow-up in the SLICC cohort in a longitudinal analysis using random effects logistic regression. The association between individual disease-related variables and MetS was examined univariately initially, adjusting for age, sex, ethnicity and follow-up time (as the MetS prevalence varies over time). Similar associations were noted between MetS and disease-related variables as were observed in the baseline analysis, such as oral corticosteroid use, higher daily and peak oral corticosteroid doses, and higher cumulative dose. Higher SLEDAI-2K and SLICC/ACR-DI were associated with MetS, as was the presence of renal lupus. Again, although peak oral prednisolone dose was associated with MetS, pulsed intra-venous therapy was not. The lack of independent association between intra-venous corticosteroids and MetS is most likely to be due to co-linearity of the dose-related variables, rather than a lack of metabolic effects of intra-venous therapies. Important differences were noted between the univariate cross-sectional and longitudinal analyses, such as a less pronounced association with Korean ethnicity compared to baseline, which is likely to reflect the declining MetS prevalence over time in the Korean population. Similarly, there was a less marked association with immunosuppressant use in the longitudinal univariate analysis than at baseline. This may be due to a loss of residual confounding, as the capture of data reflecting disease activity and corticosteroid exposure became more complete over time.

Subsequent univariate analyses sought to examine why patients were developing incident MetS and why MetS was not persistent in all patients, and so variables reflecting the timing of exposure (baseline vs. follow-up) were generated. This method confirmed that immunosuppressant exposure was associated with MetS at baseline but not over the subsequent 2 years, which reaffirms its probable role as a marker for disease severity and steroid exposure in very early disease, but less so overtime. Not unexpectedly, cumulative corticosteroid dose became significantly associated with MetS over the follow-up period, reflecting the improved capture of steroid-related exposures with time. Finally, the preceding MetS status of an individual (over the follow-up period) was strongly associated with subsequent MetS status.
The inclusion of all significant variables in a multivariable random effects regression analysis resulted in a final model that identified previous MetS status, higher peak prednisolone dose at enrolment, elevated anti-dsDNA at enrolment, increasing age, and Hispanic ethnicity as being independently associated with MetS. As in the baseline analysis, the effects of corticosteroid exposure appear to be dose-related, as simple exposure status (yes or no) is not independently associated with MetS when all dose-related variables are actively excluded from the multivariable model.

Interestingly, antimalarial use did not remain significantly negatively associated with MetS in the longitudinal analysis, contrary to many studies investigating predictors of CV risk (98;100;191) and MetS development in SLE (164;230). The lack of an independent ‘protective’ effect of antimalarial therapies on MetS development both at enrolment and over time observed in this study may be secondary to insufficient follow-up time. Their atheroprotective effects (on lipids and insulin resistance, for example) and positive effects on disease-stability may occur gradually with prolonged use and be more important when corticosteroid doses are much lower. Alternatively, the protective effect of antimalarial therapies identified in smaller, retrospective studies may simply represent confounding by indication, as patients with mild disease (and hence a lower CV risk) are more likely to receive these therapies in clinical practice.

The finding that Korean ethnicity was not independently associated with MetS over time reflected the fall in MetS prevalence in this population following the enrolment visit. This strongly suggests that although MetS susceptibility may in part be pre-determined by genotype (ethnicity, gender) and age, several disease-related factors significantly influence its development in early disease. For example, high-dose oral and intravenous corticosteroids were frequently used at enrolment in Korean patients for serologically active disease, and this seems to have contributed to the high prevalence of dyslipidaemia and hyperglycaemia. As disease activity improved and corticosteroid doses fell over time, so the prevalence of MetS declined, despite near universal oral corticosteroid use at each visit. This observation has important implications for the induction regimes used in recently diagnosed active SLE, and supports the need to identify more effective steroid-sparing approaches to management, especially in higher risk populations. Minimising exposure to higher peak doses of corticosteroids, whilst controlling active disease, is likely to result in longer-term benefits to CV risk.

The most pronounced association with MetS in the longitudinal analysis was an individual’s preceding MetS status, which conferred an almost 5-fold increased risk of having MetS over the study period. The onset of MetS in early SLE is
therefore a strong predictor of future MetS, and early exposure to active inflammatory disease and high corticosteroid doses may result in a fixed adverse metabolic phenotype. This imprinted phenotype is likely to contribute to longer-term CV risk in SLE.

5.6.4 Study strengths and limitations

This is the largest study to date examining MetS in SLE and has many advantages over previous studies. Firstly, it is the only study that has examined the determinants of MetS over time and the prospective nature of the cohort limits many potential sources of bias associated with retrospective studies. Secondly, the cohort is young and has a range of disease activity that allows detailed exploration of the impact of inflammation on MetS development. Also, the most recent definition of MetS has been utilised, which permits non-obese patients to meet the definition. Therefore the effects of non-obesity related factors on MetS development could be explored, unlike studies that use definitions of MetS ‘anchored’ by obesity. The SLICC-MetS cohort is international and recruited from centres in 11 countries, with a range of ethnicities, and therefore the results can be generalised to a wide-range of SLE populations. Finally, and perhaps uniquely, a broad range of data on corticosteroid dosing was captured, which permitted detailed analyses of the effect of corticosteroids on MetS, a major weakness of many existing studies.

The analysis does however have several limitations. Firstly, there is missing MetS data on 11.4% of the cohort at enrolment, which remains stable over follow-up. Whilst the demographics of this cohort are broadly similar they appear to have less severe disease, which may bias the analysis towards disease severity markers. The majority of missing data concerned HDL-cholesterol results, predominantly from US centres, which therefore represents a potential source of bias. The estimate of MetS prevalence may therefore be inaccurate in this population. Secondly, not all centres recorded fasting blood results, a potential source of measurement error. When the analysis was restricted to those with fasting blood results however, the overall prevalence of MetS at enrolment rose to 25%. Perhaps counter-intuitively those with non-fasting blood results had lower glucose, lower cholesterol and lower triglycerides than those without, and so their inclusion in the analysis may only serve to bias the results of this study towards the null hypothesis. The exclusion of patients with non-fasting results might also bias the analysis, as clinicians may not insist on performing fasting samples on patients deemed to have a low CV risk or who were unwell at the time of assessment. The inclusion of all patients in the analysis does however increase the relevance and applicability of the study to
SLE cohorts generally and significantly improves the statistical power of the study. Thirdly, the use of MetS as a CHD risk prediction tool has yet to be validated in SLE, and the alternative MetS phenotype in SLE, with obesity seemingly under-represented, may undermine its role in predicting future CV risk prediction. The SLICC-RAS cohort is however an ideal setting in which to examine this further and is the focus of on-going work. Finally, and perhaps most significantly, there is no control population against which to compare the epidemiology of MetS, which hinders the interpretation of the results. Whilst population level data is available for most participating centres, the general population cohorts are generally older than the SLICC-MetS cohort, and so direct comparisons cannot be made. However, all controlled-studies to date have found that MetS is more common in SLE than age-matched controls.

The results of this study suggest that the risk of developing MetS can be determined from early in the SLE disease course, with certain subsets of patients more prone to MetS. This clustering of CHD risk factors and the observed ethnic variation in MetS susceptibility should help inform risk stratification in individual patients and improve the personalised management of early disease. Inflammatory disease activity and higher doses of corticosteroids in very early disease significantly influence the development of MetS, which is a persistent phenotype in a significant number of patients. Therefore even from disease onset, therapeutic regimes should permit corticosteroid doses to be individually tailored in order to minimise longer-term CV risk, especially in high-risk populations.
Chapter 6

Inflammation and endothelial dysfunction in patients with active SLE

Inflammatory disease activity has many deleterious effects on the vascular endothelium and contributes to the enhanced cardiovascular risk seen in SLE. This chapter will describe the impact of improved control of disease activity and subsequent suppression of inflammatory disease activity on markers of endothelial function and damage in a cohort of patients with active SLE.
6 Inflammation and endothelial dysfunction in patients with active SLE

SLE is associated with an increased risk of premature clinical and subclinical atherosclerosis, and is considered an independent risk factor for cardiovascular disease. The vascular endothelium may act as a key interface between disease activity in SLE and the development of atherosclerosis, and therefore systemic inflammatory disease activity may be central to the proatherogenic environment of SLE. Improved control of inflammatory disease activity has been hypothesised to improve vascular function and reduce long-term CV risk but has yet to be tested in prospective studies. The hypothesis to be tested in this study states that patients with active SLE demonstrate impaired endothelial function and elevated markers of endothelial damage and activation, compared to healthy controls. Improved control of inflammatory disease activity in this cohort will lead to improvements in these indices of endothelial damage and dysfunction.

The specific objectives of this chapter are:

1. To validate the methodologies used in this study and examine the correlation between two measures of endothelial function (FMD and PAT) and measures of endothelial damage (EMPs) and function (FMD).
2. To describe the demographic and clinical features of a cohort of SLE patients with active disease and a cohort of healthy controls.
3. To compare indices of endothelial function and damage between SLE patients and control subjects in a cross-sectional analysis.
4. To determine the change in disease activity over time following a change in anti-inflammatory therapy in SLE patients, and describe its impact on endothelial function and damage.
6.1 Validation of study techniques

6.1.1 Validation of endothelial function assessment

A validation study was conducted to examine the reliability of the technique of flow-mediated dilatation (FMD) as a measure of endothelial function within our group, as per published recommendations (236;237). A single observer assessed FMD, so assessment of inter-observer variability was not required. The first aim of the validation study was to assess intra-observer variability of brachial artery diameter and FMD(%) measurement in healthy subjects. The second aim was to assess the repeatability of FMD(%) in one healthy subject. These validation studies would ensure that both the technique itself and the test conditions in which it was performed were fully optimised, in order to limit any natural variation in FMD(%) and to ensure consistency in choice of brachial artery segment scanned at each visit. Although endoPAT® has been validated by Itamar Medical Ltd., intra-observer variability of the reactive hyperaemic index (RHI) was also assessed.

6.1.1.1 Study design and statistical methods
The metrics used to evaluate the precision of FMD assessment were assessed as per international recommendations (236). To examine intra-observer variability, eight healthy subjects were studied on 2 separate occasions at least 1 week apart. On each occasion FMD and PAT were performed simultaneously under test conditions, as they would be in the main study. The observer had access to each participant’s baseline measurements at the second visit. The intra-observer variability between paired measures of baseline brachial artery diameter and FMD(%) at 60 seconds post-cuff deflation was assessed using Spearman’s correlation coefficient (to test correlation) and Bland-Altman plots (to assess agreement) (329). Repeatability was tested by assessing baseline brachial artery diameter and FMD(%) on multiple occasions (n=8) by one observer on one healthy volunteer over a period of several weeks. Repeatability was assessed using the co-efficient of variation (CV) for multiple measures in the same subject, and expressed as a percentage. There is no recognised and agreed acceptable level of variation in repeated measures of FMD(%); however, because FMD is a percentage-ratio small differences between absolute arterial diameters can cause very large differences in FMD%. Intra-observer variability of paired PAT measures was assessed using Spearman’s Rank and Bland-Altman plots, for correlation and agreement respectively.

6.1.1.2 FMD and PAT assessment protocol
This was performed as described in chapter 4.8.
6.1.1.3 Intra-observer variability of FMD

There was good correlation between paired measures of brachial artery diameter (Figure 6-1) and FMD(%) (Figure 6-2) suggesting minimal intra-observer variability. Good correlation between these measures at 2 time-points is a key validation step when performing longitudinal studies of FMD.

Figure 6-1: Correlation of brachial artery diameter at two time-points.

[Graph showing correlation of brachial artery diameter at two time-points with r = 0.95 and p = 0.0003]

This figure demonstrates a high-degree of correlation between measured brachial artery diameters at two time-points.

Figure 6-2: Correlation of FMD (%) at two time-points.

[Graph showing correlation of FMD (%) at two time-points with r = 0.79 and p = 0.02]

Abbreviations: FMD flow mediated dilatation
This scatter-plot shows a good correlation between paired FMD (%) measurements in healthy subjects using the technique established for this study.
A Bland-Altman plot of the difference between measures against their mean was used to assess the level of agreement between two measures. Figure 6-3 reveals good agreement between paired brachial artery measurements. Figure 6-4 describes slightly less agreement for FMD(%), as would be expected. Small absolute differences in brachial artery diameter response produce large percentage changes in FMD(%).

**Figure 6-3: Bland-Altman plot of paired measures of brachial artery diameter**

Abbreviations: BA brachial artery
This Bland-Altman plot demonstrates a good level of agreement between paired brachial artery measurements in eight subjects.

**Figure 6-4: Bland-Altman plot of paired measures of % FMD**

Abbreviations: FMD flow mediated dilatation
This Bland-Altman plot of paired FMD measurements reveals more variation than is seen in the repeated baseline artery measurements.
6.1.1.4 Repeatability of FMD
Repeatability of the FMD technique was assessed by estimating the coefficient of variation (CV%) for multiple studies (n = 8) in one healthy subject. This was calculated using the following formula:

\[ CV\% = \frac{SD}{\text{mean}} \times 100. \]

The CV% of repeated FMD measures is shown in Table 6-1. There was very little variation in brachial artery diameter but significantly more in FMD(%). When a single outlying (low) value was excluded the CV% improved from 42.2% to 25.2%.

**Table 6-1: Repeatability of brachial artery and FMD measurement**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean (mm)</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline BA diameter</td>
<td>4.203</td>
<td>0.148</td>
<td>3.5%</td>
</tr>
<tr>
<td>Maximum BA diameter</td>
<td>4.459</td>
<td>0.156</td>
<td>3.5%</td>
</tr>
<tr>
<td>% FMD</td>
<td>6.27</td>
<td>2.648</td>
<td>42.2%</td>
</tr>
<tr>
<td>% FMD (no outlier)</td>
<td>7.01</td>
<td>1.77</td>
<td>25.2%</td>
</tr>
</tbody>
</table>

*Abbreviations: SD standard deviation; CV coefficient of variation; BA brachial artery; FMD flow mediated dilatation*

6.1.1.5 Intra-observer variability of endoPAT®
Paired measures of RHI using endoPAT® did not show significant correlation \( r^2 = 0.43, p = 0.40 \), although a Bland-Altman plot demonstrated reasonable agreement between paired RHI measures (Figure 6-5).

**Figure 6-5: Bland-Altman plot of paired measures of PAT**

*Abbreviations: RHI reactive hyperaemic index*
6.1.2 Validation of EMP quantification

Repeatability of EMP quantification was assessed by estimating the coefficient of variation (CV %) of EMP numbers assessed in one individual on multiple (n=10) occasions by a single observer. Commercial bioassays should ideally have a CV% of less than 5%, whilst non-commercial quantitative bioassays should have a CV% of 10% or less to be deemed reliable and acceptable. The results for repeated assay of EMP levels are shown in Table 6-2. Total events and total annexin-V/CD31 positive events were counted ‘online’, and EMP count/ml was calculated ‘offline’. The results reveal acceptable levels of variation in repeated measurements of total and dual-positive events.

Table 6-2: Repeatability of EMP measurement

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total events (n)</td>
<td>39533</td>
<td>1690</td>
<td>4.3%</td>
</tr>
<tr>
<td>Ann V+/CD31+/CD42- events (n)</td>
<td>124.4</td>
<td>12.2</td>
<td>9.8%</td>
</tr>
<tr>
<td>EMP/ml</td>
<td>58956</td>
<td>5771</td>
<td>9.8%</td>
</tr>
</tbody>
</table>

Abbreviations: EMP endothelial microparticle; SD standard deviation; CV coefficient of variation.

6.1.3 Discussion

The results from the FMD validation study confirm minimal intra-observer variability in baseline brachial artery diameter measurement and acceptable levels of variation in the FMD(%) response when used in healthy subjects. This confirms that the aspects related to technique, protocol and environment (such as ultrasound use, image acquisition, edge-tracking software, consistency in arterial segment choice, and control of test conditions) were fully optimised. Despite this however, there remains a degree of variation in FMD response within individuals that cannot be completely eradicated. Other groups have reported similar findings, and factors such as age, baseline brachial artery diameter, phase of the menstrual cycle and mental stress have all been reported as affecting FMD (333-335). This variation alone however is not generally regarded as sufficient to invalidate the technique (336).

The EMP quantification validation study confirms acceptable levels of variation in repeated measures in a single healthy subject, in the hands of a single observer. There was however more variation detected in the fluorescence-based quantification (dual-positive events and EMP counts) compared to forward and side scatter-based quantification only (total event count). This suggests that the
various protocol steps that effect total event number (such as venepuncture, centrifugation, freezing and storage) were adequately optimised. The increased variation in the quantification of dual-positive events and calculated EMP count is most likely related to the very small volumes of antibodies used in the protocol, such as pipetting technique and incubation, which was minimised by the use of only one assessor. However, if EMPs were to be used in larger studies with more than one assessor, further inter-observer validation of the technique will be required.
6.2 Description of SLE patients and healthy controls

6.2.1 Demographic and lifestyle features of SLE patients and controls

A total of 27 patients and 22 healthy control subjects were recruited to the study. All controls underwent assessment of endothelial function (RHI and FMD) and these constitute the control group for the vascular function outcomes (FMD(%) and RHI). Fifteen controls also underwent full cardiovascular assessment (including assessment of EMPs and endothelial activation markers) and constitute the control group for the secondary outcomes assessed in the study. Twenty-five patients were recruited from the lupus clinics at Kellgren Centre for Rheumatology, and 2 were recruited from regional centres. Overall, the control subjects were well matched with the SLE cases, in terms of gender (19/22 (86.3%) vs. 26/27 (96.3%) female; p = 0.96) and age (mean (SD) 38.5 (9.3) years (vs. 41.5 years; p = 0.56). The age range of SLE patients was 19 years to 62 years, and in controls was 24 years to 60 years. Figure 6-6 describes the age frequencies in cases and controls, which were less well distributed in the control group.

Figure 6-6: Age distribution in SLE patients and controls

This figure shows that patients had a range of ages. Despite a similar median age in each cohort, the control cohort had a less evenly distributed age range, compared to the SLE.
The groups were slightly less well matched for ethnicity. Of the SLE patients 17/27 (63%) were Caucasian, 6/27 (22%) were Black African/Afro-Caribbean, 1/27 (3.8%) was mixed ethnicity and 3/27 (12%) were Asian. Of the controls, 19 (90%) were Caucasian, 1 was mixed Afro-Caribbean/Caucasian and 1 was Asian. Median (IQR) time spent in school education was similar (11 (11, 12) years vs. 13 (13,14) years), as was time spent in higher education (3 (3,4) years vs. 2 (0,3) years). Alcohol consumption was higher in the control group (median (IQR) weekly intake 11 (4, 12) units vs. 0 (0,6) units; p = 0.05). With regards hormonal status in the 26 female patients, 11 (42.3%) were nulliparous and 4 (15.4%) had suffered 2 or more miscarriages. Seven (27%) were post-menopausal (3 of whom had undergone hysterectomy and oophorectomy) and 2 (7.7%) were currently receiving hormone replacement therapy, 4 (15.4%) were receiving the oral contraceptive pill and 12 (46.2%) had previously used the oral contraceptive pill. Ten patients were married or in common-law partnerships, 12 were single and 5 were either divorced or widowed. No significant differences were noted between the groups in terms of marital and hormonal status, although only 1 female control had suffered more than 1 pregnancy loss (vs. 3 patients; p = 0.8).

6.2.2 Traditional cardiovascular risk factors in SLE patients and controls

Prevalent traditional coronary heart disease (CHD) risk factors in SLE patients and healthy controls are summarised in Table 6-3. One patient had previously suffered a myocardial infarction and had on-going (stable) angina. Three (11%) patients were current smokers and 6 (22.2%) were ex-smokers. By definition, no control subject had either previous or prevalent cardiovascular disease, and none were current smokers. Cases had significantly higher anti-hypertensive therapy prescription, higher serum triglycerides, and lower fasting glucose compared to controls. Relevant but non-significant differences between cases and controls included lower HDL-cholesterol, more common family history of CVD and a higher high-sensitivity c-reactive protein (hsCRP). Metabolic syndrome (MetS), defined using the 2009 IDF criteria (214), was significantly more common in SLE patients compared to controls. With regards the MetS phenotype in SLE patients, 14/27 (52%) met the criteria for elevated waist circumference, 20/27 (74%) met elevated blood pressure criteria 10/27 (37%) met the elevated triglycerides criteria, 12/27 met the low HDL-cholesterol criteria, and 2 (7.4%) met the elevated blood glucose criteria. Adiponectin was also higher in SLE patients (Table 6-3). Adiposity was assessed using bioelectrical impedance (Tanita BC 418MA). Median (IQR) percentage body fat
was similar in patients and controls (33.3% (24.3, 37.8) vs. 32.6% (27.3, 38.5; p = 0.73) as was median (IQR) bioelectrical impedance at 665 (596, 728) ohms (vs. 654 (573, 700); p = 0.38).

Table 6-3: Traditional and novel CHD risk factors in SLE patients and controls

<table>
<thead>
<tr>
<th>Median (IQR) or n (%)</th>
<th>SLE patients (n = 27)</th>
<th>Controls (n = 15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP systolic (mmHg)</td>
<td>131 (103, 144)</td>
<td>119 (114, 127)</td>
<td>0.78</td>
</tr>
<tr>
<td>BP diastolic (mmHg)</td>
<td>76 (66, 86)</td>
<td>77 (69, 80)</td>
<td>0.84</td>
</tr>
<tr>
<td>AHT therapy</td>
<td>12 (44)</td>
<td>0 (0)</td>
<td>0.002</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.7 (21.5, 28.5)</td>
<td>25.1 (23.1, 30.8)</td>
<td>0.42</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>80 (74, 91.6)</td>
<td>80 (72, 93.5)</td>
<td>0.86</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>5.2 (4.6, 6.7)</td>
<td>5.54 (4.83, 6.87)</td>
<td>0.54</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.33 (1.15, 1.61)</td>
<td>1.66 (1.46, 1.78)</td>
<td>0.06</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.71 (1.92, 3.6)</td>
<td>3.01 (2.70, 3.58)</td>
<td>0.35</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.36 (0.90, 1.87)</td>
<td>0.88 (0.64, 1.00)</td>
<td>0.01</td>
</tr>
<tr>
<td>Lipid-lowering therapy</td>
<td>3 (11)</td>
<td>0 (0)</td>
<td>0.18</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.40 (4.1, 5.0)</td>
<td>4.9 (4.8, 5.1)</td>
<td>0.05</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2 (7.4)</td>
<td>0 (0)</td>
<td>0.28</td>
</tr>
<tr>
<td>Family history CVD</td>
<td>10 (43.5)</td>
<td>4 (18.2)</td>
<td>0.09</td>
</tr>
<tr>
<td>MetS</td>
<td>11 (40.7)</td>
<td>1/15 (6.7)</td>
<td>0.03</td>
</tr>
<tr>
<td>hsCRP (mg/l)</td>
<td>2.4 (0.5, 5.1)</td>
<td>0.51 (0.26, 2.83)</td>
<td>0.09</td>
</tr>
<tr>
<td>Adiponectin (mg/l)</td>
<td>3.57 (2.46, 5.90)</td>
<td>2.93 (2.42, 3.60)</td>
<td>0.12</td>
</tr>
<tr>
<td>Fasting insulin (mU/l)</td>
<td>16.3 (12.4, 22.9)</td>
<td>14.0 (12.2, 20.6)</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Abbreviations: BP blood pressure; AHT anti-hypertensive; BMI body mass index; WC waist circumference; TC total cholesterol; HDL-C high-density lipoprotein cholesterol; LDL-C low-density lipoprotein cholesterol; CVD cardiovascular disease; MetS metabolic syndrome, hsCRP high-sensitivity c-reactive protein.

6.2.3 Disease-related features in SLE patients

6.2.3.1 Clinical and immunological features of SLE patients
All patients satisfied the 1997 ACR criteria over the course of their disease and 22 (81%) met 5 or more criteria (Table 6-4). The median (IQR) disease duration at the baseline assessment was 7.0 (3.5, 12) years. Patients starting rituximab therapy had longer disease duration than those starting standard
Immunosuppression (median (IQR) 11.7 (7.6, 15.0) years vs. 3.2 (0.7, 4.8) years; p <0.001). Fifteen (55.6%) patients had Raynaud’s phenomenon.

**Table 6-4: ACR classification criteria in SLE patients**

<table>
<thead>
<tr>
<th>ACR criteria</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malar rash</td>
<td>16 (59.3)</td>
</tr>
<tr>
<td>Discoid rash</td>
<td>13 (48.2)</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>19 (70.4)</td>
</tr>
<tr>
<td>Oral ulcers</td>
<td>17 (63.0)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>22 (81.5)</td>
</tr>
<tr>
<td>Serositis</td>
<td>8 (29.6)</td>
</tr>
<tr>
<td>Renal disorder</td>
<td>11 (40.7)</td>
</tr>
<tr>
<td>Neurological disorder</td>
<td>6 (22.2)</td>
</tr>
<tr>
<td>Haematological disorder</td>
<td>19 (70.4)</td>
</tr>
<tr>
<td>Immunological disorder</td>
<td>14 (51.8)</td>
</tr>
<tr>
<td>Antinuclear antibody</td>
<td>27 (100)</td>
</tr>
</tbody>
</table>

Immunological testing to assess disease-activity was performed at each visit (Table 6-5). At study entry 23 were ANA-positive (all had a positive ANA previously) and 8 (29.6%) patients had positive anti-cardiolipin antibodies and/or lupus anticoagulant.

**Table 6-5: Immunological features of SLE patients at baseline visit**

<table>
<thead>
<tr>
<th>Feature</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antinuclear antibody</td>
<td>23 (85.2)</td>
</tr>
<tr>
<td>Elevated anti-dsDNA antibody $^1$</td>
<td>7 (25.9)</td>
</tr>
<tr>
<td>Low C3 $^2$</td>
<td>3 (11.1)</td>
</tr>
<tr>
<td>Low C4 $^3$</td>
<td>10 (37.0)</td>
</tr>
<tr>
<td>Anti-cardiolipin antibody $^4$</td>
<td>8 (29.6)</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>1 (3.7)</td>
</tr>
</tbody>
</table>

$^1$Above CMFT laboratory normal range 0-13.9 IU/ml  
$^2$Below CMFT laboratory normal range 0.62-1.6 g/L  
$^3$Below CMFT laboratory normal range 0.14-0.39 g/L  
$^4$Above CMFT laboratory normal range IgM aCl 0-10 IU/ml and/or IgG aCl 0-5.7.
6.2.3.2 Disease activity and damage in SLE patients

All patients were recruited because they had active disease requiring a change in therapy, and disease activity scores were therefore high. The median (IQR) SLEDAI-2K was 6 (5, 13), although two patients had a SLEDAI-2K of zero because of active disease features that did not score on the index (peripheral neuropathy and myelopathy). Overall, 9 (33.3%) patients had active renal disease, 14 (51.9%) had active arthritis, 7 (25.9%) had an inflammatory rash and 5 (18.5%) had oral ulceration. Similarly, the BILAG-2004 index reflected high disease activity (Table 6-6). In total, there were 27 BILAG ‘A’ scores and 16 BILAG’B’ scores, and every patient scored at least one ‘B’ score overall. Median (IQR) global BILAG-2004 score was 14 (12, 22). The two most common primary reasons for changing therapy were disease activity in the musculoskeletal (inflammatory arthritis) and renal systems (nephritis), which occurred in 17/27 (70.4%) patients.

Table 6-6: Distribution of BILAG-2004 scores at enrolment

<table>
<thead>
<tr>
<th>BILAG 2004 Domain</th>
<th>Number (%) of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (3.7)</td>
</tr>
<tr>
<td>Constitutional</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>Mucocutaneous</td>
<td>4 (14.8)</td>
</tr>
<tr>
<td>Neurological</td>
<td>3 (11.1)</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>10 (37.0)</td>
</tr>
<tr>
<td>Cardiorespiratory</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ophthalmic</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Gastro-intestinal</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Renal</td>
<td>9 (33.3)</td>
</tr>
<tr>
<td>Haematological</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

At study enrolment the median (IQR) SLICC/ACR-damage index was 1 (1, 2). Six patients (22.2%) had no damage, 8 (29.6%) had a score of 1, 9 (33.3%) had a score of 2 and 4 (14.8%) had SLICC-DI of 3 or more. The pattern of damage is described in Table 6-7.
<table>
<thead>
<tr>
<th>SLICC/ACR-DI item</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ocular</strong></td>
<td></td>
</tr>
<tr>
<td>Cataract</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Retinal/optic atrophy</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Neuropsychiatric</strong></td>
<td></td>
</tr>
<tr>
<td>Cognitive impairment</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>Seizures</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>CVA</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Transverse Myelitis</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td><strong>Renal</strong></td>
<td></td>
</tr>
<tr>
<td>eGFR &lt;50%</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>Proteinuria &gt;3.5g</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>ESRF</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Pulmonary</strong></td>
<td></td>
</tr>
<tr>
<td>Pulmonary Hypertension</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pulmonary Fibrosis</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>Shrinking lung</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pleural fibrosis</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pulmonary infarction</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Cardiovascular</strong></td>
<td></td>
</tr>
<tr>
<td>Angina/CABG</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>MI</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Valve disease</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>Pericarditis</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Peripheral Vascular</strong></td>
<td></td>
</tr>
<tr>
<td>Claudication</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Minor tissue loss</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>Major tissue loss</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Venous thrombosis</td>
<td>1 (7.4)</td>
</tr>
<tr>
<td><strong>Gastrointestinal</strong></td>
<td></td>
</tr>
<tr>
<td>Organ resection/infarction</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Upper GI stricture/surgery</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pancreatic insufficiency</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Musculoskeletal</strong></td>
<td></td>
</tr>
<tr>
<td>Atrophy/weakness</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Deforming/erosive arthritis</td>
<td>8 (29.6)</td>
</tr>
<tr>
<td>Osteoporosis with fracture</td>
<td>0 (0)</td>
</tr>
<tr>
<td>AVN</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ruptured tendon</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Skin</strong></td>
<td></td>
</tr>
<tr>
<td>Scarring alopecia</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>Extensive scarring</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>Skin ulceration</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td><strong>Premature gonadal failure</strong></td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>2 (7.4)</td>
</tr>
<tr>
<td><strong>Malignancy</strong></td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Abbreviations:  eGFR estimated glomerular filtration rate; ESRF end stage renal failure; MI myocardial infarction; CABG coronary artery bypass graft; AVN avascular necrosis
6.2.3.3 Therapeutic exposures in SLE patients

Twenty-four (88.9%) patients were receiving oral corticosteroids at the baseline assessment, at a median (IQR) daily dose of 12.5 (10, 17.5) mg. Table 6-8 provides details of the therapies received at entry into the study, prior to the institution of the treatment change. Three patients had received rituximab therapy previously, and were to undergo further treatment for a disease flare.

Table 6-8: Current therapies at baseline visit into study

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current oral CS</td>
<td>24 (88.9)</td>
</tr>
<tr>
<td>Oral daily dose (median (IQR)</td>
<td>12.5 (10, 17.5)</td>
</tr>
<tr>
<td>Recent IV CS</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Current AM</td>
<td>20 (74.1)</td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td>17 (85)</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Current IS</td>
<td>12 (44.4)</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>2 (16.7)</td>
</tr>
<tr>
<td>Mycophenolate</td>
<td>6 (50)</td>
</tr>
<tr>
<td>Ciclosporin</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>2 (16.7)</td>
</tr>
<tr>
<td>Leflunomide</td>
<td>1 (8.3)</td>
</tr>
<tr>
<td>Past biologic</td>
<td>3 (11.1)</td>
</tr>
</tbody>
</table>

Abbreviations: CS corticosteroid; IV intravenous; AM antimalarial; IS immunosuppressant

Fourteen (51.9%) patients were due to start standard immunosuppression for active disease, of whom 6 were naïve to immunosuppressant therapy. Thirteen (48.1%) were due to start rituximab, all of whom were either receiving (and had failed) standard immunosuppressant therapy (n = 10) or had received biologic therapy previously (n = 3).

6.2.4 Discussion

The study aimed to recruit SLE patients with active disease opportunistically from lupus clinics across the Greater Manchester region. However, only two patients were recruited from outside the tertiary lupus clinic in the Kellgren
Centre for Rheumatology by the end of the study period, for 2 main reasons. Firstly, despite regional clinic lists being monitored by dedicated research nurse staff and frequent reminders to the participating rheumatologists, there were very few patients with active SLE being managed in district general rheumatology departments during the study period. Secondly, several eligible patients referred from regional clinics declined to travel for assessment. Despite this, the cohort had a broad range of ages, ethnicities and socio-demographic features. The patients recruited to this study were younger (mean (SD) age of 41.5 (14.1) years) than previous studies of endothelial function in SLE (e.g. El-Magadmi et al median (range) age 48 (21-73) years (257), Aizer et al mean (SD) age 48.8 (8.8) years (243); Svenungsson et al mean (SD) age 52.2 (8.2) years (306))

Despite their relatively young age, serum triglycerides were significantly higher than in the control subjects, and there were non-significantly higher levels of blood pressure and LDL-cholesterol, and lower HDL-cholesterol. The high prevalence of traditional CHD risk factors in a young cohort with active disease is consistent with findings from the SLICC-MetS study described in chapter 5, as well as other studies examining CV risk in patients with SLE (102-104).

However, it was not clear from the chart review whether the 8 patients with Raynaud’s phenomenon who were receiving anti-hypertensive therapies did so solely for their vasodilator effects or for concurrent hypertension as well.

All patients met the 1997 ACR classification criteria for SLE, and all had had a documented positive ANA result during the course of their disease. The study design meant that only patients with active disease were recruited and therefore disease activity scores in the cohort were very high. The commonest clinical features that dictated the change in therapy were inflammatory arthritis (37%), lupus nephritis (36%), severe inflammatory rash (14%) and CNS lupus (11.1%). A substantial number of patients also had serologically active disease, with low serum C4 in 37% of patients and elevated anti-dsDNA antibodies in 27% of patients.

At the baseline assessment 12/27 patients were on immunosuppression, and a further 3 had previously been treated with rituximab. Six patients were naïve to immunosuppression, and these had shorter disease duration than patients starting biologics. Almost all patients were on oral corticosteroids at moderately high doses, although none had received intra-venous corticosteroids in the 3 months prior to their baseline visit. Consistent with other studies, 75% of patients were receiving antimalarial therapies, usually hydroxychloroquine.
Due to poor control recruitment using our normal best-friend system most control subjects were recruited from a database of controls that had participated in similar studies. However, the control subjects were well matched for median age, gender, obesity, menopausal status and lifestyle factors, but with differences in age distribution and ethnicity. As expected, the prevalence of traditional CHD risk factor profiles was low in healthy controls. In contrast to the SLE group, there were no current smokers in the control group, but there were similar numbers of ex-smokers in each group (28.5% vs. 22.2%; p = 0.7). The results of the study investigating endothelial dysfunction and damage in active SLE against controls and following a change in therapy are discussed below.

**6.3 Cross-sectional analysis of endothelial function and damage in patients with active SLE vs. controls**

**6.3.1 Methods**

All patients with active SLE underwent assessment of endothelial function and damage at baseline and prior to any change in their anti-inflammatory therapies. All healthy controls (n = 22) underwent identical assessment of endothelial function on one occasion, and 15 controls also underwent full cardiovascular assessment. A full description of the methodology and analysis used in this study is detailed in chapter 4.

**6.3.2 Endothelial function in SLE vs. controls**

Patients with active SLE had significantly reduced endothelial-dependent FMD(%) of the brachial artery compared to controls, when measured at both the recommended 60-second time-point (Figure 6-7), and at the peak FMD response as chosen by the observer. Endothelial-independent dilatation of the brachial artery, as assessed by response to sublingual GTN, was similar in cases and controls (Table 6-9). The median baseline brachial artery diameter, a significant predictor of FMD response, was almost identical in SLE and control subjects. The normal GTN-response confirmed that the observed reduction in FMD(%) was due to endothelial dysfunction and not vascular stiffness. There were no significant differences between cases and controls in reactive hyperaemic ratio (RHI) as measured by the endoPAT® system. Overall, FMD% was below the lower limit of ‘normal’ in 75% of SLE patients and 43% of controls (p = 0.04).
Figure 6-7: Comparison of endothelial-dependent FMD in SLE patients vs. controls.

Abbreviations: FMD flow-mediated dilatation of brachial artery

Table 6-9: Endothelial function in SLE and control subjects

<table>
<thead>
<tr>
<th>Median (IQR)</th>
<th>SLE (n = 27)</th>
<th>Control (n = 22)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline BA diameter (mm)</td>
<td>3.34 (3.10, 3.84)</td>
<td>3.34 (3.12, 4.07)</td>
<td>0.89</td>
</tr>
<tr>
<td>% FMD</td>
<td>1.63 (-1.22, 5.32)</td>
<td>5.49 (3.02, 8.57)</td>
<td>0.04</td>
</tr>
<tr>
<td>% FMD (maximum)</td>
<td>2.86 (0.60, 5.32)</td>
<td>6.81 (3.46, 8.57)</td>
<td>0.03</td>
</tr>
<tr>
<td>% GTN dilatation</td>
<td>15.3 (11.9, 19.1)</td>
<td>12.0 (10.3, 17.3)</td>
<td>0.43</td>
</tr>
<tr>
<td>FMD &lt;5%</td>
<td>75%</td>
<td>43%</td>
<td>0.04</td>
</tr>
<tr>
<td>RHI</td>
<td>1.97 (1.68, 2.54)</td>
<td>2.02 (1.79, 2.44)</td>
<td>0.85</td>
</tr>
<tr>
<td>RHI &lt; 1.67</td>
<td>22%</td>
<td>19%</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Abbreviations: BA brachial artery; FMD flow-mediated dilatation; GTN glyceryl trinitrate; RHI reactive hyperaemic index.

It was noted during the assessments that endoPAT® did not perform well in patients with Raynaud’s phenomenon (in both symptomatic and asymptomatic patients). The visual appearance of the pulse wave trace was usually abnormal in these patients and the result was occasionally uninterpretable. When these patients were excluded there was still no difference in RHI between cases and controls.

6.3.2.1 Associations of endothelial dysfunction at baseline

Previously, El-Magadmi et al had demonstrated that SLE was independently associated with impaired endothelial function in a cohort of 62 patients and 38 controls (257). The association between endothelial function (FMD%) and
traditional CHD risk factors was therefore examined in this (smaller) cohort at baseline using univariate linear regression. The only factor significantly associated with FMD% at baseline was SLE status (i.e. patient or control) with a B coefficient (95% CI) of -3.19 (-6.23, -0.15; p 0.04). There was a non-significant inverse relationship between FMD% and baseline brachial artery diameter (B coefficient -2.54, p = 0.09). However, in a multivariable analysis including factors known to be associated with a reduced FMD% (age, traditional CHD risk factors, smoking and brachial artery diameter) SLE was not independently associated with endothelial dysfunction (Table 6-10).

Table 6-10: Multivariable analysis of non-lupus associations of endothelial dysfunction (FMD)

<table>
<thead>
<tr>
<th>Factor</th>
<th>B coefficient</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>-0.38</td>
<td>-0.22, 0.15</td>
<td>0.6</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>-0.026</td>
<td>-0.26, 0.21</td>
<td>0.8</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/l)</td>
<td>-0.34</td>
<td>-3.60, 2.89</td>
<td>0.8</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>-2.58</td>
<td>-8.60, 3.44</td>
<td>0.4</td>
</tr>
<tr>
<td>HDL cholesterol (mol/l)</td>
<td>0.12</td>
<td>-5.44, 5.70</td>
<td>0.9</td>
</tr>
<tr>
<td>Brachial artery diameter (mm)</td>
<td>-3.00</td>
<td>-8.87, 2.87</td>
<td>0.3</td>
</tr>
<tr>
<td>SLE</td>
<td>-3.91</td>
<td>-9.34, 1.52</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Abbreviations: BP blood pressure; HDL high-density lipoprotein
This multivariable model included factors known to cause an impaired FMD response.

6.3.3 Endothelial microparticles in SLE vs. controls

Endothelial damage was assessed using endothelial microparticles (EMPs). Patients with active SLE had significantly elevated EMPs compared to age-matched healthy controls (157, 548/ml (59,906, 278,775) vs. 41,025/ml (30,179, 98,082); p = 0.001), as demonstrated in Figure 6-8. The difference remained significant even after the patients with the two highest EMP levels were excluded.
6.3.3.1 Associations of endothelial damage at baseline

The association between endothelial damage (EMPs) and non-lupus factors was examined in the whole cohort at baseline using univariate linear regression, as was performed for FMD (%). The same factors known to influence FMD (with eGFR substituted for baseline brachial artery diameter) were included in the multivariable model. Factors significantly associated with EMP count (n/μl) at baseline were SLE status (B coefficient (95% CI) 147 (39, 256; p 0.009), higher plasma glucose (B coefficient 31 (13, 48; p 0.001) and use of antihypertensive therapies (B coefficient 120 (2, 23) p 0.05). The association between EMP count (μ/ml) with SLE and higher plasma glucose (mmol/l) remained independent in a multivariable model including age, blood pressure, renal function and total cholesterol (Table 6-11).

Table 6-11: Multivariable analysis of non-lupus associations of endothelial damage (EMPs)

<table>
<thead>
<tr>
<th>Factor</th>
<th>B coefficient</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>-0.07</td>
<td>-5.07, 3.68</td>
<td>0.7</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>1.58</td>
<td>-1.45, 4.61</td>
<td>0.3</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/l)</td>
<td>-6.1</td>
<td>-50.5, 38.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>29.0</td>
<td>9.45, 48.6</td>
<td>0.005</td>
</tr>
<tr>
<td>eGFR</td>
<td>-0.32</td>
<td>-2.3, 1.65</td>
<td>0.7</td>
</tr>
<tr>
<td>SLE</td>
<td>145</td>
<td>29, 260</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Abbreviations: BP blood pressure; HDL high-density lipoprotein; eGFR estimated glomerular filtration rate
6.3.4 Endothelial activation markers in SLE vs. controls

Table 6-12 summarises the cross-sectional comparison of VEGF and VCAM-1 between patients and controls. Serum VCAM-1 was significantly higher in cases compared to controls, whilst VEGF was non-significantly higher in SLE patients.

Table 6-12: Endothelial activation markers in SLE vs. controls

<table>
<thead>
<tr>
<th>Median (IQR)</th>
<th>SLE (n = 27)</th>
<th>Control (n = 22)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCAM-1 (ng/ml)</td>
<td>488 (348, 555)</td>
<td>289 (272, 317)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>108 (57, 156)</td>
<td>55 (42, 153)</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Abbreviations: VCAM-1 vascular cell adhesion molecule-1; VEGF vascular endothelial growth factor.

6.4 Longitudinal analysis of effect of inflammation suppression on endothelial function and damage in active SLE

6.4.1 Methods

In total, 22/27 (81.5%) patients returned for their follow-up visit. Of those patients that did not complete the study 1 died of unrelated causes, 2 had their therapy stopped due to side effects and 2 did not respond to multiple invites to return. Twelve of the patients who completed the study started standard immunosuppression (azathioprine = 5, MMF = 5, cyclophosphamide = 1 and leflunomide =1) and 10 started rituximab therapy. The median (IQR) time between treatment change and follow-up visit was 22 (16, 24) weeks. A full description of the methodology and analysis used in this study is detailed in chapter 4.

6.4.2 CHD risk factors over time

No significant changes in traditional CHD risk factors over time were observed in the 22 patients with paired visits, and no obvious trend towards improvements were noted, apart from slight improvements in HDL-C and triglyceride levels (Table 6-13). No patient commenced anti-hypertensive or lipid-lowering therapy during the study period.
Table 6-13: CHD risk factors over time in SLE patients

<table>
<thead>
<tr>
<th></th>
<th>Median (IQR) or n (%)</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP systolic (mmHg)</td>
<td>131 (104, 144)</td>
<td>134 (118, 154)</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>BP diastolic (mmHg)</td>
<td>77 (66, 85)</td>
<td>75 (67, 85)</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>26.6 (22.5, 28.5)</td>
<td>26.7 (24.2, 32.7)</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>WC (cm)</td>
<td>84.9 (76, 94)</td>
<td>86 (78.5, 94.7)</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.22 (4.71, 6.32)</td>
<td>5.19 (4.60, 6.37)</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.33 (1.24, 1.59)</td>
<td>1.45 (1.33, 1.63)</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>2.86 (1.90, 3.60)</td>
<td>2.66 (2.33, 3.47)</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.45 (0.9, 1.9)</td>
<td>1.1 (0.9, 1.62)</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.4 (4.1, 5.1)</td>
<td>4.7 (4.1, 4.9)</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>MetS</td>
<td>10 (45.5)</td>
<td>9 (40.9)</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>hsCRP (mg/l)</td>
<td>2.47 (0.96, 5.11)</td>
<td>4.57 (1.36, 7.37)</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Adiponectin (mg/l)</td>
<td>3.60 (2.76, 5.90)</td>
<td>3.80 (2.96, 4.84)</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>Fasting insulin (mU/l)</td>
<td>16.1(12.4, 22.9)</td>
<td>17.2 (11.8, 26.4)</td>
<td>0.77</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BP blood pressure; BMI body mass index; WC waist circumference; HDL high-density lipoprotein; LDL low-density lipoprotein; hsCRP high-sensitivity CRP

6.4.3 Disease activity over time

Disease activity improved significantly over time in the 22 patients with paired data. Median (IQR) SLEDAI-2K fell from 6 (4, 14) to 4 (2, 6) (p<0.001) and median (IQR) global BILAG-2004 score improved from 17 (12, 22) to 3 (2, 9) (p <0.001). All BILAG ‘A’ scores improved to at least a BILAG ‘B’ over the study period. Median (IQR) change in BILAG-2004 score was -11 (-18, -3) and change in SLEDAI was-5 (-9, -2). The number of patients with low complement also fell over time (9 vs. 5; p = 0.23), and fewer patients had elevated dsDNA antibodies at follow up (4 vs. 7; p = 0.44). Only one patient stopped their corticosteroids over the study period. Median (IQR) daily prednisolone dose in the 21 patients who remained on corticosteroid therapy at follow up was unchanged (12.5mg at both visits) with minimal individual change in daily dose of prednisolone over time (median (IQR) change 0 (-2.5, 2.5) mg).
6.4.4 Endothelial function over time

Endothelial function measures in patients with paired data (n = 22) are described in Table 6-14 and Figure 6-9. Overall, both FMD% at 60 seconds and maximum FMD% response improved but the change did not reach statistical significance. The median (IQR) change in endothelial-dependent vasodilatation as measured by FMD was +3.54% (-1.61, 6.2), although FMD(%) was not restored to levels observed in control subjects.

Table 6-14: Endothelial function over time in SLE patients

<table>
<thead>
<tr>
<th>Median (IQR)</th>
<th>Baseline (n = 22)</th>
<th>Follow-up (n = 22)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline BA diameter (mm)</td>
<td>3.53 (3.12, 3.85)</td>
<td>3.38 (3.21, 3.85)</td>
<td>0.81</td>
</tr>
<tr>
<td>% FMD</td>
<td>0.64 (-2.31, 4.47)</td>
<td>3.52 (0.98, 5.50)</td>
<td>0.10</td>
</tr>
<tr>
<td>% FMD (maximum)</td>
<td>1.40 (0.54, 4.47)</td>
<td>4.56 (1.71, 5.87)</td>
<td>0.19</td>
</tr>
<tr>
<td>FMD &lt;5%</td>
<td>86.7%</td>
<td>66.7%</td>
<td>0.20</td>
</tr>
<tr>
<td>RHI</td>
<td>1.91 (1.75, 2.54)</td>
<td>2.17 (1.91, 2.51)</td>
<td>0.45</td>
</tr>
<tr>
<td>RHI &lt; 1.67</td>
<td>16.7%</td>
<td>16.7%</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Abbreviations: BA brachial artery; FMD flow-mediated dilatation; GTN glyceryl trinitrate; RHI reactive hyperaemic index.

Figure 6-9: FMD over time in SLE patients

Abbreviations: FMD flow-mediated dilatation of brachial artery
6.4.5 Endothelial microparticles over time

In patients with paired data (n = 22) the median (IQ) EMP count at baseline was 162,265/ml (59906, 278,775). This improved significantly to 55,655/ml (29,475, 188,659) (p = 0.02) following a change in therapy and reduction in disease activity (Figure 6-11). Overall, median (IQR) change in EMP count was -87998 (-184,433, +4949).

**Figure 6-10: EMPs over time SLE patients**

![Graph showing EMPs over time](image)

**Abbreviations: EMP endothelial microparticles**

6.4.6 Endothelial activation markers over time

Table 6-15 summarises endothelial activation markers over time in SLE patients with paired data (n = 22). There was a non-significant reduction in VEGF and VCAM-1 over time.

**Table 6-15: Endothelial activation markers over time in SLE patients**

<table>
<thead>
<tr>
<th>Median (IQR)</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF (pg/ml)</td>
<td>99 (53, 155)</td>
<td>71 (27, 141)</td>
<td>0.14</td>
</tr>
<tr>
<td>VCAM-1 (ng/ml)</td>
<td>488 (375, 587)</td>
<td>458 (297, 488)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

**Abbreviations: VCAM-1 vascular cell adhesion molecule-1; VEGF vascular endothelial growth factor.**
6.4.7 Relationship between improved disease control and endothelial function/damage over time

In the 22 patients with complete data FMD (%) improved by a median (IQR) of 3.54% (-1.61, +6.2) over the 2 visits. The change in indices of endothelial damage/activation and disease activity, and their correlation with change in FMD, are summarised in Table 6-16. There was a moderate correlation between change in FMD (%) and change in SLEDAI-2K, which almost reached statistical significance ($r^2 = -0.33; p = 0.07$) and a moderate but significant correlation between change in FMD (%) and change in VEGF over time. There was a significant correlation between change in FMD (%) and percentage change in global BILAG 2004 score, but the use of percentage change in the index is not widely used and has not been validated. Change in FMD (%) did not correlate with change in EMP level over time.

### Table 6-16: Correlation between change in FMD (%) and indices of disease activity and endothelial damage

<table>
<thead>
<tr>
<th>Median (IQR) (n = 22)</th>
<th>Change (p)</th>
<th>$R^2$</th>
<th>Change % (p)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLEDAI-2K</td>
<td>-5</td>
<td>-0.33</td>
<td>-62.5</td>
<td>-0.40</td>
</tr>
<tr>
<td>(-9, -2)</td>
<td>(0.07)</td>
<td></td>
<td>(-87.5, -33.3)</td>
<td>(0.16)</td>
</tr>
<tr>
<td>BILAG-2004 score</td>
<td>-11</td>
<td>-0.29</td>
<td>-69.4</td>
<td>-0.71</td>
</tr>
<tr>
<td>(18, -3)</td>
<td>(0.12)</td>
<td></td>
<td>(-85.7, -35.7)</td>
<td>(0.004)</td>
</tr>
<tr>
<td>EMP n/ml</td>
<td>-87,998</td>
<td>-0.06</td>
<td>-63.8</td>
<td>-0.11</td>
</tr>
<tr>
<td>(-184,433, +4949)</td>
<td>(0.84)</td>
<td></td>
<td>(-79, 6, +1.6)</td>
<td>(0.71)</td>
</tr>
<tr>
<td>VEGF</td>
<td>-14</td>
<td>-0.37</td>
<td>-17.5</td>
<td>-0.41</td>
</tr>
<tr>
<td>(-46, +5)</td>
<td>(0.04)</td>
<td></td>
<td>(-44.9, +5.2)</td>
<td>(0.12)</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>-18</td>
<td>-0.38</td>
<td>-4.3</td>
<td>-0.31</td>
</tr>
<tr>
<td>(-116, -6)</td>
<td>(0.27)</td>
<td></td>
<td>(23.4, -1.2)</td>
<td>(0.27)</td>
</tr>
</tbody>
</table>

Abbreviations: $R^2$ correlation coefficient; FMD flow-mediated dilatation; EMP endothelial microparticles; VEGF vascular endothelial growth factor; VCAM-1 vascular cell adhesion molecule 1.

In the 22 patients with complete data EMPs (n/ml) improved by a median (IQR) of -87,998/ml (-184,433, +4949) over time. The changes in indices of endothelial activation and disease activity, and their correlation with percentage change in EMPs are summarised in Table 6-17. There was a moderate correlation between change in EMP (%) and change in global BILAG 2004 score ($r^2 = 0.40$),...
which just failed to reach statistical significance (\(p = 0.08\)). Change in EMP level (\%) did not correlate with markers of endothelial activation.

<table>
<thead>
<tr>
<th>Median (IQR) (n = 22)</th>
<th>Change</th>
<th>(R^2) (p)</th>
<th>Change</th>
<th>(R^2) (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLEDAI-2k (-9, -2)</td>
<td>-5</td>
<td>0.05</td>
<td>-62.5</td>
<td>0.06</td>
</tr>
<tr>
<td>BILAG-2004 score (18, -3)</td>
<td>-11</td>
<td>0.40</td>
<td>-69.4</td>
<td>0.17</td>
</tr>
<tr>
<td>VEGF (-46, +5)</td>
<td>-14</td>
<td>-0.07</td>
<td>-17.5</td>
<td>-0.04</td>
</tr>
<tr>
<td>VCAM-1 (-116, -6)</td>
<td>-18</td>
<td>-0.24</td>
<td>-4.3</td>
<td>-0.19</td>
</tr>
</tbody>
</table>

**Table 6-17: Correlation between change in EMP levels (%) and indices of disease activity and endothelial damage**

*Abbreviations: \(R^2\) correlation coefficient FMD flow-mediated dilatation; EMP endothelial microparticles; VEGF vascular endothelial growth factor; VCAM-1 vascular cell adhesion molecule 1.*

The association between change in FMD (%) and change in disease activity was further examined in regression analyses, restricted by the relatively small numbers in the study. Change in FMD (%) was not associated with change in global BILAG score in a univariate analysis (B coefficient (95% CI) -0.08 (-0.33, 0.16); \(p = 0.47\)), even after adjusting for traditional CHD risk factors (B coefficient -0.47 (-1.55, 0.61); \(p = 0.31\)). However, the lack of association with change in SLEDAI-2K on unadjusted analysis (B coefficient -0.32 (-0.87, 0.23); \(p = 0.23\)) improved after adjusting for changes in traditional risk factor over time (B coefficient -0.77 (-1.64, 0.10); \(p = 0.08\)). Although there was a trend towards an association between improved EMP count and improved disease control, this did not reach statistical significance on unadjusted (B coefficient 9.23 (-8.77, 27.24); \(p = 0.30\)) or adjusted (for changes in traditional risk factors) analyses (B coefficient 11.5 (-11.6, 34.5); \(p = 0.30\)).
6.4.8 Effect of treatment approach on outcomes over time

Of the 22 patients with complete follow-up, 10 received rituximab therapy and 12 received standard immunosuppressive therapies. Patients receiving rituximab had non-significantly higher median (IQR) BILAG-2004 (21 (12, 29) vs. 14 (6.3, 21.5); p = 0.45) and SLEDAI (7 (6, 16) vs. 6 (6, 13); p = 0.61) indices at baseline. They had similar median (IQR) FMD% at baseline (0.34% (-0.7, 4.67) vs. 1.01% (-2.86, 4.1); p = 0.56). Over time, there was a trend for disease activity and endothelial function and damage indices to improve more in those receiving rituximab, but these differences did not reach statistical significance (Table 6-18).

Table 6-18: Change in disease activity and endothelial function by treatment approach

<table>
<thead>
<tr>
<th>Median (IQR)</th>
<th>Rituximab (n = 10)</th>
<th>Standard therapy (n = 12)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change BILAG score</td>
<td>-13 (-25, -10)</td>
<td>-5 (-16, 0)</td>
<td>0.18</td>
</tr>
<tr>
<td>Change SLEDAI-2K</td>
<td>-6 (-10, -4)</td>
<td>-2.5 (-7, 0)</td>
<td>0.12</td>
</tr>
<tr>
<td>Change FMD %</td>
<td>+4.76 (+3.54, +6.3)</td>
<td>+1.76 (-1.61, +3.89)</td>
<td>0.28</td>
</tr>
<tr>
<td>Change EMP</td>
<td>-107,549 (-184,433, 50,000)</td>
<td>-81886 (-189393, -14654)</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Abbreviations: FMD flow-mediated dilatation; EMP endothelial microparticles

6.5 Correlation of measures of endothelial damage and dysfunction

6.5.1 Correlation of two measures of endothelial function

The correlation between the two measures of endothelial function was assessed using Spearman’s correlation coefficient. Using all patients and control subjects with paired FMD and EndoPAT® data (n = 46), there was no correlation between the two measures ($r^2 = 0.10; p = 0.50$). Correlation was similarly low when examined in SLE patients only ($r^2 = 0.03; p = 0.89$), although did improve when the 8 patients with Raynaud’s phenomenon were excluded ($r^2 =0.45; p =0.2$) (Figure 6-11).
Figure 6-11: Correlation of FMD and RHI in SLE patients

Abbreviations: RHI reactive hyperaemic index; FMD flow-mediated dilatation

Correlation between FMD and RHI was poor in all SLE patients (A) but did improve when those with Raynaud’s phenomenon were excluded (B).

6.5.2 Correlation of endothelial function and damage measures

The correlation between endothelial function (FMD%) and endothelial damage (EMPs) was examined (Figure 6-14). In all subjects with paired results there was a moderate correlation between the two measures, which was statistically significant ($r^2 = 0.40; p = 0.006$). The correlation in SLE patients only was similar ($r^2 = 0.42; p = 0.008$).

Figure 6-12: Correlation of FMD and EMP in SLE patients

Abbreviations: EMP endothelial microparticles; FMD flow-mediated dilatation

Endothelial function (FMD%) and damage (EMPs) correlated moderately both in the whole cohort (A) and in SLE patients only (B).
6.5.3 Correlation of endothelial function/damage and activation measures

In all subjects with paired data, there was no correlation between FMD (%) and VCAM-1 levels ($r^2 = -0.10; p = 0.49$) or VEGF levels ($-0.06; p = 0.67$). There was a weak correlation between EMP count (n/ml) and VCAM-1 ($r^2 = 0.28; p = 0.03$), which was lost in SLE patients only ($r^2 = 0.24; p = 0.10$). There was no correlation between EMP counts and VEGF, either in the whole cohort or in SLE patients only.

6.5.4 Correlation of endothelial damage and disease activity

Many manifestations of active SLE are the consequence of complement activation mediated by immune-complex deposition at the vascular endothelium. Endothelial damage markers such as EMPs may therefore be a potential novel biomarker for disease activity as well as cardiovascular risk in SLE, and so the correlation between disease activity indices and EMP levels was examined. Overall, there was no correlation between EMPs and SLEDAI-2K ($r^2 = 0.16; p = 0.27$) or BILAG-2004 score ($r^2 = 0.19; p = 0.20$).
6.6 Summary

This chapter describes the validation of endothelial function assessment using flow-mediated dilatation of the brachial artery and peripheral arterial tonometry. The results confirmed that the FMD and PAT techniques and test protocols were fully optimised, with acceptable levels of variability in FMD% and RHI. Similarly, the optimised technique used to quantify EMP levels confirmed good repeatability of the protocol. Peripheral arterial tonometry did not perform well in patients with Raynaud's phenomenon, and overall did not correlate well with FMD%.

However, there was a significant moderate correlation observed between endothelial damage (EMPs) and function (FMD%).

A cohort of patients with active SLE was recruited predominantly from a tertiary lupus clinic in the North West of England, and compared to an age- and gender-matched cohort of healthy controls. The SLE patients represented a broad range of ages, ethnicities and disease manifestations and all had very active disease requiring a change in therapy. The most common disease manifestations that prompted the change in therapies were renal disease and inflammatory arthritis. There was a high prevalence of traditional CHD factors in the SLE patients, compared to controls.

At baseline, patients with active SLE had significantly impaired endothelial function compared to healthy controls. Three-quarters of the SLE cohort had reduced FMD% compared to less than half of controls. SLE patients also had significantly higher indices of endothelial damage and activation at baseline, especially EMPs, compared to the control population.

Disease activity improved substantially over time in the SLE patients following a change in therapy. This was associated with a non-significant improvement in endothelial function and a significant improvement in EMP levels. There was a trend towards a correlation between change in FMD% and EMPs over time and improvements in disease activity.
6.7 Discussion

It was hypothesised that SLE patients with active disease would have significant endothelial dysfunction and damage compared to healthy controls, in part related to inflammatory disease activity, and that improved control of inflammation would result in improvement in these indices. Endothelial dysfunction is considered the earliest stage of the atherosclerotic pathway and reflects an imbalance between endothelium-derived relaxing and constricting factors that ultimately promotes a pro-atherogenic environment (337). The importance of the vascular endothelium in many of the immunopathological features of active SLE suggests that endothelial dysfunction may result from active inflammation and provide a mechanistic link between inflammation and atherosclerosis in SLE. The hypothesis was examined in a cohort of 27 patients with active SLE who required a change in therapy and 22 age-matched controls. Twenty-two patients were subsequently followed-up for a median of 22 weeks after starting either standard immunosuppressive therapy or biological therapy (rituximab). The control cohort was well matched to the SLE patients although a higher prevalence of traditional risk factors in the SLE patients was noted, particularly raised triglycerides and metabolic syndrome. The two most common manifestations that prompted a change in therapy were lupus nephritis and inflammatory arthritis, and overall disease activity was very high.

6.7.1 Cross-sectional analysis

The first aim of this study was to compare indices of endothelial function and damage in SLE patients compared to controls. Despite similar brachial artery diameters and endothelial-independent vasodilatation, SLE patients with active inflammatory disease had significantly impaired endothelial function as measured by FMD% compared to age-matched controls. This has been shown in several older, stable SLE cohorts (257;259;338). However, a greater proportion of patients in this study had low FMD% (defined as <5%) than has been observed previously. For example, El-Magadmi et al reported that 54.8% of their cohort had low FMD, compared to 75% in this study, despite the SLE patients being older (48 years vs. 41.5 years). This may in part relate to the much lower disease activity and damage scores observed in their cohort (median SLEDAI of 2 and SLICC-DI of 0). SLE was associated with low FMD% on unadjusted univariate regression analysis, but did not remain so in an adjusted multivariable model that included age, blood pressure and brachial artery diameter. It is likely
that the sample size was too small to detect an independent association with SLE in a cross-sectional analysis, and unlike the study by El-Magadmi et al (257) this was not the primary outcome of the current study.

The maximum observed FMD% post-cuff deflation was higher in both groups than FMD% measured at the traditional 60-second time-point, although remained lower in SLE patients compared to controls. This phenomenon has been reported previously by Black et al, who noted that 42% of healthy subjects had a peak post-cuff deflation arterial diameter recorded outside of the traditional 60-second time-point, generally occurring earlier in younger subjects and later in older subjects (334). Delayed peak endothelial-dependent vasodilation may also represent endothelial dysfunction, and recording both peak and 60-second brachial artery diameters is now recommended by the most recent methodological guidelines for FMD (237).

Despite the observed reduced FMD response in SLE patients, there was no difference in endothelial function between the groups as assessed by PAT. This has been reported previously by Aizer et al (243). The lack of consistency between FMD and PAT in SLE cohorts probably relates to both inherent differences in the physiological processes measured by each technique, and the poor quality pulse amplitude signal observed in patients with Raynaud’s phenomenon.

EMP levels are elevated in a variety of cardiovascular, autoimmune and inflammatory conditions and may have prognostic importance in patients with stable coronary artery disease (278). EMPs are released from the vascular endothelium during apoptosis and activation, triggered by a variety of signals inflammatory mediators (263) and were therefore hypothesised to be elevated in active SLE and to possibly correlate with other measures of endothelial activation and damage. SLE patients had significantly higher indices of endothelial damage and activation than did control subjects at the baseline assessment, and even within a small cohort there was a degree of correlation between EMP count and plasma VCAM-1. This is the first time such a relationship has been described in SLE. To date, other groups have focussed on the potential role of microparticles as a source of autoantibodies against nuclear antigens in SLE (302) or as a biomarker of disease activity in vasculitis (298), rather than as a biomarker of endothelial dysfunction in inflammatory diseases. EMPs may therefore represent a simpler and less observer-dependent measure of endothelial dysfunction than FMD in SLE, and could potentially be utilised in multi-centre studies of cardiovascular disease in SLE.
6.7.2 Longitudinal analysis

Disease activity improved significantly over time in the 22 SLE patients who completed the study, following a change in therapy. There was a suggestion that treatment response was better in patients treated with rituximab compared to patients receiving standard therapy, but the small numbers in each group meant that the differences were not statistically significant. The improved efficacy of B cell depleting therapy in active SLE was not unexpected, despite recent negative clinical trials of rituximab (80;81). Extensive open-label use of rituximab supports its use in refractory SLE, and the patients who received rituximab in this study had longer disease duration and higher damage and activity indices than those receiving other therapies. The use of biological agents was also hypothesised to confer additional cardiovascular benefits through improved steroid-sparing effects. However, only 1 patient stopped corticosteroid therapy over the study period, and the overall daily dose remained unchanged at 12.5mg per day. It is likely that there was insufficient follow-up of SLE patients to assess beneficial effects on corticosteroid exposure, and longer-term follow-up is required to investigate this further.

Both median FMD(%) and the proportion of patients with low FMD improved over time, although statistical significance was not achieved, and post-treatment FMD response remained lower than control subjects. The median change in FMD (%) was +3.54%, and patients receiving rituximab therapy again appeared to have a more pronounced improvement in FMD (%) (4.76% vs. 1.76%, p = 0.28). Again however, the FMD response did not restore to ‘normal’. A substantial and significant fall in EMP levels over time was also observed in SLE patients, following a change in therapy. At follow-up, median EMP levels were similar to those observed in the control subjects and overall EMP levels fell by almost two-thirds. Finally, a non-significant improvement in VEGF and VCAM-1 levels was observed over time. However no correlation was noted between change in EMP levels and FMD (%) over time, perhaps in part related to the variation within both these indices that may be improved by a larger sample size.

The relationship between disease activity indices and change in FMD and EMP count over time was inconsistent. Whilst no association was observed between global BILAG 2004 score and FMD (%), a moderate negative association with SLEDAI-2K that was almost significant was noted (r² = -0.33; p = 0.07). In contrast, there was no association between SLEDAI-2K and change in EMP count (%) over time, but a non-significant moderate correlation between EMP count and global BILAG 2004 score was observed (r² = 0.40; p = 0.08). Although the
change in disease activity measured by percentage change in BILAG-2004 score correlated well with the change in FMD% between visits (r² = -0.71, p = 0.004), the change in global BILAG score is not routinely expressed as a percentage and has not been validated for use in this way. The change in SLEDAI-2K has however been expressed as a percentage change in a recent phase II study of the safety and efficacy of belimumab in SLE (339), although the outcome used in the subsequent phase III study incorporated absolute change in SLEDAI-2K in the composite end-point. Provisional regression analyses (limited by sample size) suggested there was no obvious relationship between change in FMD and change in global BILAG-2004 score, but an association between SLEDAI-2K and FMD may exist, after adjusting for changes in traditional CHD risk factors over time.

The inconsistencies noted between vascular outcomes and disease activity indices in this study reflect wider issues of measuring treatment response in SLE using composite indices that have hampered effective study design of clinical trials. In a small sample size, 2 patients with a SLEDAI-2K of zero had a BILAG-2004 ‘A’ score and a global BILAG score of at least 8. Therefore inflammatory disease activity was not accurately captured in some patients and a better biomarker of disease activity in SLE may improve outcome measures. Similarly, despite a large change in global BILAG score between the 2 visits, the overall change in SLEDAI-2K was minimal. This reflected large falls in SLEDAI-2K in some patients, but much smaller changes in others as serological features appeared to lag behind clinical features of disease activity. This has been a criticism of the SLEDAI-2K index previously. Similarly, the variation within global BILAG score was greater (range 6-37) than that seen in SLEDAI-2K (range 0-18), which is likely to influence the relationship between outcome and intervention.

Whilst other groups have studied the effects of statins, omega-3-fatty acids and anti-oxidant therapies on endothelial function over time (178;183;340), this is the first study to date that has prospectively examined the effects of improved disease control on endothelial function in SLE. Indices of endothelial function and damage both improved over time, as did disease activity, in the absence of any change in traditional CHD risk factors. There was a suggestion that improved disease control influenced the observed improvement endothelial function and damage, and this supports the hypothesis that reducing inflammatory disease activity may translate into improved longer-term cardiovascular risk in patients with active SLE. Interestingly, the suggestion of a more pronounced improvement in endothelial function markers with rituximab therapy provides further support for a more personalised approach to the management of active
lupus. For example, the presence of endothelial damage or dysfunction in a patient with active and/or refractory disease could be used as an additional indication for biological therapies, especially in the context of other cardiovascular risk factors.

6.7.3 Correlation studies

The final aim of this study was to investigate the correlation between two measures of endothelial function (FMD and PAT) and the correlation between endothelial function and damage (FMD and EMP). It remains debatable whether PAT assesses the same physiological processes as FMD, given the different nature of the vessels studied. FMD is a measure of responsiveness of a large conduit vessel to distal ischaemia, whilst PAT monitors much smaller digital resistance vessels. Whilst both are likely to be mediated largely by nitric oxide (238;245), they may actually represent different physiological processes as, for example, digital vessels are much more susceptible to the influences of the sympathetic nervous system than are conduit vessels such as the brachial artery (244). However PAT does appear to correlate with FMD in the general population, with a correlation coefficient of 0.55 (p =<0.001) reported in a study by Kuvin et al (254). However, there were several methodological issues with the comparison study by Kuvin et al including the use of multiple observers to measure FMD, variation in FMD protocols and using categorical rather than continuous data.

The results of the validation study described above suggested that despite an automated protocol there was still variation in paired endoPAT© results. The correlation between PAT and FMD was poor in both healthy subjects and in lupus patients, and performed particularly badly in patients with Raynaud’s phenomenon. It might be expected that analysis of the digital pulse wave amplitude would be difficult in patients with Raynaud’s phenomenon due to poor signal quality secondary to vasospasm, but the only other study to date that has examined PAT in SLE patients found the opposite to be true. Aizer et al reported no correlation between FMD and PAT in their lupus cohort (r² 0.03), but significant moderate correlation in patients with Raynaud’s phenomenon (r² 0.50, p = 0.04) (243). Whilst the endoPAT® system has many potential advantages over FMD in multicentre studies, such as automated protocols and minimal training requirements, it does not eliminate intra-observer variability and is not interchangeable with FMD in SLE.

The correlation between measures of endothelial function (FMD(%) ) and endothelial damage (EMPs) was also examined. A moderate but significant
correlation between paired EMP and FMD measures was observed, both in the whole cohort ($r^2 0.40 \ p = 0.006$) and in SLE patients alone ($r^2 = 0.42; \ p = 0.008$). Several studies have found a similar correlation between EMP levels and endothelial function in other disease states, such as obesity (296), renal failure (274) and heart failure (295) and elevated EMPs predict future cardiovascular outcomes in stable coronary artery disease (278). This is the first time the association between FMD and EMPs has been assessed in an inflammatory condition such as lupus, and suggests that EMPs may be utilised as potential surrogate biomarkers for endothelial dysfunction in SLE. Although FMD remains the gold-standard measure of endothelial function, it is highly observer-dependent and difficult to standardise across sites. EMPs have many potential advantages over FMD in this regard, such as central laboratory processing, and may be a useful adjunctive measure of cardiovascular risk in multi-centre ad large-scale studies and clinical trials.

Finally, the association between disease activity and EMP levels was explored. Brogan et al hypothesised that EMP levels could act as a biomarker of disease activity in childhood vasculitis, a condition in which is difficult to objectively assess inflammatory activity (298). However, despite a significant improvement over time in EMP levels with improved disease control, EMP levels did not correlate well with either change in global BILAG-2004 score or change in SLEDAI-2K score. This may be due to sub-optimally measured disease activity using the composite indices and the correlation may improve with better estimations of disease activity. EMP levels may also correlate better with disease activity in a cohort of patients with a range of disease activities and manifestations.

6.7.4 Study strengths and limitations

To date, most studies investigating endothelial dysfunction in SLE have been cross-sectional and have frequently examined stable, older cohorts of SLE patients. The few longitudinal studies have only examined the effects of interventions aimed at treating traditional cardiovascular risk factors on endothelial function. This limits their ability to explore the interplay between inflammation and vascular risk in SLE. A key strength of this study is the prospective study design and recruitment of patients with active disease that permits an exploration of change over time in disease activity and its influence on endothelial dysfunction. Secondly, several measures of endothelial dysfunction were utilised that have been shown to change over short periods of time. This allowed comparison of techniques and provided internal validation for
novel markers such as EMPs. Finally, the cohort was younger than comparable studies, minimising the influence of age on the outcomes of interest. However, the study also had several limitations. Firstly, although the sample size was sufficient to examine the primary outcome, it did not allow full exploration of secondary outcomes, such as whether the approach to treatment had a differential effect on endothelial dysfunction. Secondly, there was an inherent variability in the methods used to assess endothelial function and damage, despite optimal test conditions and techniques. Whilst this variation does not invalidate any individual technique, it does hamper the analysis of the results in a small study, particularly when assessing correlation and agreement. Finally, not all patients returned for follow-up and this may introduce bias in the results.

The results of this study confirm that endothelial function is significantly impaired and EMPs are significantly elevated in patients with active lupus. Improved control of inflammatory disease activity results in improved indices of endothelial function and damage, in the absence of any changes in traditional CHD risk factors. Endothelial dysfunction is therefore modifiable in patients with active SLE, and may therefore provide useful additional information when planning management of active disease individually. EMP levels also correlate with FMD, and EMPs may therefore serve as a useful biomarker of endothelial dysfunction and cardiovascular risk in SLE.
Chapter 7

Final conclusions and future research directions
7 Final conclusions and future research directions

The main objective of this thesis was to investigate the relative contribution of inflammatory disease activity and its management to the increased cardiovascular risk seen in SLE. Evidence for the impact these factors have on accelerating the atherosclerotic process in SLE is conflicting, and is generally from cross-sectional or retrospective studies. Such uncertainty hampers long-term cardiovascular risk management of patients with SLE, a situation made worse by the poor performance of conventional risk stratification tools in SLE. Prospective studies are needed to delineate this relationship more clearly, and the two studies described in this thesis were designed specifically to address this issue.

Two distinct but complementary approaches were employed in this thesis to explore the effect of inflammation and therapeutic exposures on cardiovascular risk in SLE. Firstly, a large, multicentre, international cohort of patients with SLE recruited within 15 months of diagnosis was utilised to explore the association between inflammatory disease activity, corticosteroid exposure and metabolic syndrome (MetS) over time in a prospective observational study. MetS is associated with adverse cardiovascular outcomes in the general population and was employed as a surrogate of cardiovascular risk in this study. Secondly, a smaller cohort of patients with very active SLE, recruited from a single UK region, was used to prospectively examine change over time in endothelial function and damage following a change in therapy. Endothelial dysfunction is the earliest clinically detectable stage of atherosclerosis and predicts adverse cardiovascular events in the general population, and was therefore also used as a surrogate marker of cardiovascular risk in this study.

Data from an international inception cohort of patients with SLE suggested that MetS was relatively common in young patients with recently diagnosed SLE over the first 2 years of follow-up (12.6-16%), and was persistent in a substantial proportion of patients (4.3%). Although there was no substantial variation in MetS prevalence over the first 2 years, substantial variation in the MetS status was observed within individuals over time, with almost a quarter of the cohort meeting the criteria on at least 1 occasion. Although SLICC-RAS has no control population against which to compare, it would also appear that the MetS phenotype may be different in early SLE. Much of the variation in prevalence over time was due to changes in lipid and glucose parameters, with stable obesity measures over the first 2 years of follow-up. Future studies need to
validate the use of MetS as surrogate for cardiovascular risk in SLE, and the SLICC group will investigate whether prevalent MetS in early SLE, which may represent a different phenotype to that seen in the general population, is associated with adverse cardiovascular outcomes over time.

Subsequent analysis explored the associations of MetS in SLE, both at enrolment and over the initial 2 years of follow-up, and revealed that the risk factors for the development of MetS differed over time. At entry into SLICC, MetS was associated with increasing age, Hispanic and Korean ethnicity, renal lupus and exposure to both higher daily corticosteroid dose and immunosuppressants. Over the first 2 years of follow-up however, MetS was associated with higher baseline peak oral corticosteroid dose, elevated anti-dsDNA antibodies and Hispanic ethnicity, and prevalent MetS strongly predicted future MetS. The observation that Korean and Hispanic ethnicities have high baseline MetS prevalence, which diverges over time, with contrasting MetS and lupus phenotypes is fascinating. The observed ethnic variation in MetS prevalence may reflect an increased susceptibility to developing MetS in some populations (particularly Hispanics), that is aggravated by exposure to inflammatory disease activity and/or corticosteroids. Further studies are therefore required to investigate possible genetic factors that predispose these populations to develop MetS in early SLE and work is currently underway within SLICC to explore potential variation within genes that influence steroid-sensitivity.

Baseline exposure to high disease activity and higher doses of corticosteroids appear to have a persistent influence on MetS phenotype, even after improved disease control and steroid reduction have been achieved. Together with the persistence of MetS in Hispanics but not Koreans, this suggests that the relationship between genetic and non-genetic factors changes over time. SLE itself, and/or exposure to inflammation and corticosteroids may therefore result in epigenetic modifications that imprint a MetS phenotype in SLE patients and explain the persistent effect of the non-genetic factors noted after inflammation is controlled and steroid doses are reduced. Further studies are therefore planned to explore potential epigenetic modifications of relevant genes that influence the development and persistence of MetS in SLE patients.

Data from a longitudinal study found that patients with active SLE had significantly reduced endothelial function (FMD) and elevated measures of endothelial damage and activation (EMPs, VCAM-1) compared to healthy, age-matched controls. Overall, CD31+/AnnexinV+ EMPs correlated with FMD and may therefore serve as a useful biomarker of endothelial dysfunction and cardiovascular risk in SLE. The observed relationship between endothelial
damage (EMPs) and dysfunction (FMD) requires further investigation and replication in larger cohorts with a diverse range of disease activities. If the observed correlation with FMD is replicated and the assay improved, CD31+/AnnexinV+ EMPs could be utilised as a biomarker of cardiovascular risk in SLE in large, multicentre studies.

Both endothelial function and damage improved over time in patients with active disease, following significant improvements in disease activity and in the absence of improvements in traditional CHD risk factors. There was a suggestion that biological therapies had a greater effect on these indices than was observed in patients treated with standard therapies, although the sample size was too small to detect statistically significant results. Nevertheless, this observation adds weight to the theory that judicious use of biological therapies in patients with highly active and refractory disease may reduce the burden of cardiovascular disease over time. The improvement in FMD(%) over time correlated moderately with change in SLEDAI-2K, although this just failed to reach statistical significance, with similar results noted between change in EMP count and change in global BILAG-2004 score over time. Although the association between improved disease control and improvements in endothelial function were inconsistent and not statistically significant, that any correlation was seen at all in a small cohort is a positive finding. Further studies in larger cohorts are required to validate these findings, and may improve the significance of the correlations observed.

Whether CD31+/AnnexinV+ EMPs are markers or mediators of vascular damage in SLE remains unclear, and was not the subject of this thesis. However, the observation that EMPs are elevated in active SLE, improve over time and correlate moderately with FMD will form the basis of further research. As a potential biomarker, EMPs perform well although a lack of consensus on their identification hampers comparison between studies. Locally, studies are underway to explore the functional properties of cell culture-derived EMPs in experiments utilising in vitro markers of endothelial dysfunction and proteomic analysis. Further studies are also planned to assess CD31+/AnnexinV+ EMP levels in a larger cohort of SLE patients.

Together, these prospective studies demonstrate that lupus-related inflammatory disease activity, even in young women with very early disease, can lead to an adverse cardiovascular risk profile characterised by MetS and endothelial dysfunction. This can be further aggravated by high doses of corticosteroids in early disease, the effects of which may be prolonged. Improved disease control can result in improvements in this adverse phenotype over time,
and CD31+/AnnexinV+ EMPs may act as a novel biomarker of cardiovascular risk in SLE. Therefore, even from disease onset, therapeutic regimes should permit corticosteroid doses to be individually tailored, especially in high-risk populations and clinical phenotypes, to limit their adverse and pro-atherogenic metabolic derangements. Ultimately however, controlled clinical trials are required to investigate the effects of different therapeutic regimens on long-term cardiovascular outcomes in early and active SLE. The results described in this thesis therefore support the need for a more personalised approach to the management of active SLE in order to minimise cardiovascular risk.
8 Appendices

Appendix 1: SLE Disease Activity Index (SLEDAI-2K)

<table>
<thead>
<tr>
<th>Weight</th>
<th>SLEDAI score</th>
<th>Descriptor</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Seizure</td>
<td></td>
<td>Recent onset. Exclude metabolic, infectious, or drug causes.</td>
</tr>
<tr>
<td>8</td>
<td>Psychosis</td>
<td></td>
<td>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. Exclude uremia and drug causes.</td>
</tr>
<tr>
<td>8</td>
<td>Organic brain syndrome</td>
<td></td>
<td>Altered mental function with impaired orientation, memory, or other intellectual function, with rapid onset and fluctuating clinical features. Include clouding of consciousness with reduced capacity to focus, and inability to sustain attention to environment, plus at least 2 of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, or increased or decreased psychomotor activity. Exclude metabolic, infectious, or drug causes.</td>
</tr>
<tr>
<td>8</td>
<td>Visual disturbance</td>
<td></td>
<td>Retinal changes of SLE. Include cedematous bodies, retinal hemorrhages, serous exudate or hemorrhages in the choroid, or optic neuritis. Exclude hypertension, infection or drug causes.</td>
</tr>
<tr>
<td>8</td>
<td>Cranial nerve disorder</td>
<td></td>
<td>New onset of sensory or motor neuropathy involving cranial nerves.</td>
</tr>
<tr>
<td>8</td>
<td>Lupus headache</td>
<td></td>
<td>Severe, persistent headache; may be migrainous, but must be nonresponsive to narcotic analgesia.</td>
</tr>
<tr>
<td>8</td>
<td>CVA</td>
<td></td>
<td>New onset of cerebrovascular accident(s). Exclude arteriosclerosis.</td>
</tr>
<tr>
<td>8</td>
<td>Vasculitis</td>
<td></td>
<td>Ulceration, gangrene, tender finger nodules, perungual infarction, splinter hemorrhages, or biopsy or angiogram proof of vasculitis.</td>
</tr>
<tr>
<td>4</td>
<td>Arthritis</td>
<td></td>
<td>More than 2 joints with pain and signs of inflammation (i.e., tenderness, swelling or effusion).</td>
</tr>
<tr>
<td>4</td>
<td>Myositis</td>
<td></td>
<td>Proximal muscle aching/weakness, associated with elevated creatine phosphokinase/aldolase or</td>
</tr>
<tr>
<td>Weight</td>
<td>SLEDAI score</td>
<td>Descriptor</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>--------------</td>
<td>-------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Urinary casts</td>
<td>Heme-granular or red blood cell casts.</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Hematuria</td>
<td>&gt;5 red blood cells/high power field. Exclude stone, infection, or other cause.</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Proteinuria</td>
<td>&gt;0.5 gm/24 hours. New onset of recent increase of more than 0.5 gm/24 hours.</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Pyuria</td>
<td>&gt;5 white blood cells/high power field. Exclude infection.</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>New rash</td>
<td>New onset or recurrence of inflammatory type rash.</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Alopecia</td>
<td>New onset or recurrence of abnormal, patchy or diffuse loss of hair.</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Mucosal ulcers</td>
<td>New onset or recurrence of oral or nasal ulcerations</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Pleurisy</td>
<td>Pleuritic chest pain with pleural rub or effusion, or pleural thickening.</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Pericarditis</td>
<td>Pericardial pain with at least 1 of the following: rub, effusion, or electrocardiogram or echocardiogram confirmation.</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Low complement</td>
<td>Decrease in CH50, C3, or C4 below the lower limit of normal for testing laboratory.</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Increased DNA</td>
<td>&gt;25% binding by Farr assay or above normal range for testing laboratory.</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>Thrombocytopenia</td>
<td>&gt;38 degrees celius. Exclude infectious cause.</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>Leukopenia</td>
<td>&lt;100,000 platelets/mm³.</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>Fever</td>
<td>&lt;3,000 white blood cells/mm³. Exclude drug causes.</td>
</tr>
</tbody>
</table>

**Total SLEDAI score**

*Systemic Lupus Erythematosus Disease Activity Index (SLEDAI).*
Appendix 2: British Isles Lupus Assessment Group 2004

Index

BILAG2004 INDEX  Centre:  Date:  Initials/Hosp No:

Only record items due to SLE Disease Activity & assessment refers to manifestations occurring in the last 4 weeks (compared with the previous 4 weeks).  *** TO BE USED WITH THE GLOSSARY ***

**CONSTITUTIONAL**
1. Pyrexia - documented > 37.5°C
2. Weight loss - unintentional > 5%
3. Lympadenopathy/splenomegaly
4. Anorexia

**MUCOCUTANEOUS**
5. Skin eruption - severe
6. Skin eruption - mild
7. Angular cheilitis
8. Angio-oedema - severe
9. Angio-oedema - mild
10. Nasal septal perforation
11. Nasal polyps
12. Periungual pustules
13. Oral ulcers
14. Oral ulceration
15. Oral ulcers
16. Oral ulcers
17. Oral ulcers
18. Splinter haemorrhages

**NEUROPSYCHIATRIC**
19. Aseptic meningitis
20. Cerebral vasculitis
21. Demyelinating syndrome
22. Myelopathy
23. Acute confusional state
24. Psychosis
25. Aseptic meningitis - demyelinating polyradiculoneuropathy
26. Mononeuropathy (single/multiplex)
27. Caudal neuritis
28. Plexopathy
29. Polynoepy (multiple)
30. Seizure disorder
31. Status epilepticus
32. Cerebrovascular disease (not due to vasculitis)
33. Cognitive dysfunction
34. Movement disorder
35. Autonomic disorder
36. Cerebellar ataxia
37. Lupus headache - severe unremitting
38. Headache from I.C. hypertensive

**MUSCULOSKELETAL**
39. Myositis - severe
40. Myositis - mild
41. Arthritis - severe
42. Arthritis (mild)-Tendinitis/Tenesynovitis
43. Arthritis (mild)-Achilles/Plantar fasciitis

**CARDIOPULMONARY**
44. Myocarditis - mild
45. Myocarditis/Endocarditis - Cardiac failure
46. Aortic dissection
47. New valvular dysfunction
48. Pleurisy/Pneumonitis
49. Cardiac tamponade
50. Pericardial effusion - dyspnoea
51. Pulmonary haemorrhage/vascularitis
52. Interstitial alveolitis/pneumonitis
53. Scleroderma lung syndrome
54. Acute interstitial fibrosis
55. Coroary vasculitis

**GASTROINTESTINAL**
56. Lupus peritonitis
57. Abdominal serositis or ascites
58. Lupus enteritis/coliitis
59. Malabsorption
60. Protein losing enteropathy
61. Intestinal pseudo-obstruction
62. Lupus hepatitis
63. Acute lupus cholecystitis
64. Acute lupus pancreatitis

**OPHTHALMIC**
65. Orbital inflammation/measitis/ proptosis
66. Keratitis - severe
67. Keratitis - mild
68. Anterior uveitis
69. Posterior uveitis/posterior vitreitis - severe
70. Posterior uveitis/posterior vitreitis - mild
71. Episcleritis
72. Scleritis - severe
73. Scleritis - mild
74. Retinal/choroidal vascular occlusion disease
75. Isolated cotton-wool spots (cystoid maculae)
76. Optic neuritis
77. Anterior ischemic optic neuropathy

**RENAL**
78. Systolic blood pressure (mm Hg) value
79. Diastolic blood pressure (mm Hg) value
80. Accelerated hypertension Yes/No
81. Urine dipstick - (+++) value
82. Urine albumin/creatinine ratio mg/mmol
83. Urine protein/creatinine ratio mg/mmol
84. 24 hour urine protein (g) value
85. Nephrotic syndrome
86. Creatinine (plasma/serum) mmol/L
87. GFR (calculated) mL/min/1.73 m²
88. Active urinary sediment
89. Active leucocyturia
90. Active haematuria
91. Nephritis

**HEMATOLOGICAL**
92. Anaemia (g/dl) value
93. Haematocrit (HCT) value
94. Platelets (x 10⁹/L) value
95. TTP
96. Evidence of active haemolysis
97. Coombs’ test positive
98. Serum albumin (g/L)
99. Serum urica (mmol/L)

<table>
<thead>
<tr>
<th>Weight (kg)</th>
<th>Serum urica (mmol/L)</th>
<th>African ancestry: Yes/No</th>
<th>Serum albumin (g/L)</th>
<th>Yes/No</th>
</tr>
</thead>
</table>
## Appendix 3: SLICC/ACR-Damage Index

<table>
<thead>
<tr>
<th>Organ (either eye by clinical assessment)</th>
<th>Item</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocular</td>
<td>Any cataract ever</td>
<td>1</td>
</tr>
<tr>
<td>Neuropsychiatric</td>
<td>Retinal damage or optic atrophy</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cognitive impairment (e.g. memory deficit difficulty with calculation poor concentration difficulty in spoken or written language impaired performance level) or major psychosis</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Seizures requiring therapy for 6 months</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cerebrovascular accident ever (score 2 if more than 1) or surgical resection for causes other than malignancy</td>
<td>1 or 2</td>
</tr>
<tr>
<td></td>
<td>Cranial or peripheral neuropathy (excluding optic)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Transverse myelitis</td>
<td>1</td>
</tr>
<tr>
<td>Renal</td>
<td>Estimated or measured glomerular filtration rate (\leq 50%) or Proteinuria (\leq 3.5) g per 24 hours or</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>End-stage renal disease (regardless of dialysis or transplantation)</td>
<td>3</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>Pulmonary hypertension (right ventricular prominence or loud (P2))</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Pulmonary fibrosis (physical and radiograph)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Shrinking lung (on radiograph)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Pleural fibrosis (on radiograph)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Pulmonary infarction (on radiograph) OR pulmonary resection for cause other than malignancy</td>
<td>1</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Angina or coronary artery bypass</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Myocardial infarction ever (score 2 if more than 1)</td>
<td>1 or 2</td>
</tr>
<tr>
<td></td>
<td>Cardiomyopathy (ventricular dysfunction)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Valvular disease (diastolic murmur or systolic murmur (\geq 3/6))</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Pericarditis for 6 months or pericardectomy</td>
<td>1</td>
</tr>
<tr>
<td>Peripheral vascular</td>
<td>Claudication for 6 months</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Minor tissue loss (pulp space)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Significant tissue loss ever (e.g. loss of digit or limb resection)</td>
<td>1 or 2</td>
</tr>
<tr>
<td></td>
<td>(score 2 if more than one site)</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Venous thrombosis with swelling ulceration or venous stasis</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Infarction or resection of bowel below duodenum spleen liver or gallbladder for any cause (score 2 if more than 1 site)</td>
<td>1 or 2</td>
</tr>
<tr>
<td></td>
<td>Mesenteric insufficiency</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Chronic peritonitis</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Esophageal stricture or upper gastrointestinal tract surgery ever</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Pancreatic insufficiency requiring enzyme replacement or pseudocyst</td>
<td>1</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>Muscle atrophy or weakness</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Deforming or erosive arthritis (including reducible deformities excluding avascular necrosis)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Osteoporosis with fracture or vertebral collapse (excluding avascular necrosis)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Avascular necrosis (score 2 if more than 1)</td>
<td>1 or 2</td>
</tr>
<tr>
<td></td>
<td>Osteomyelitis</td>
<td>1</td>
</tr>
<tr>
<td>Skin</td>
<td>Scarring chronic alopecia</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Extensive scarring or panniculitis other than scalp and pulp space</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>Skin ulceration (excluding thrombosis) for more than 6 months</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Premature gonadal failure</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Diabetes (regardless of treatment)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Malignancy (regardless of treatment) (score 2 if more than 1 site)</td>
<td>1 or 2</td>
</tr>
</tbody>
</table>

SLICC/ACR Damage Index for Systemic Lupus Erythematosus continued.
Appendix 4: SLICC ethical approval

NORTH MANCHESTER
LOCAL RESEARCH ETHICS COMMITTEE
Telephone: 0161 237 2392 / 2385
Fax: 0161 237 2383
E-Mail: danielle.mchugh@gmsha.nhs.uk
sharon.melbourne@gmsha.nhs.uk

Dr IN Bruce
Rheumatism Research Centre
Manchester Royal Infirmary
Oxford Road
Manchester
M13 9WL

Our ref: 03/NM/288
Your ref:
17 July 2003

03/NM/288 - Please quote this number on all correspondence

Dear Dr Bruce

Systemic Lupus International Collaborating Clinics (SLICC). Prospective study of long-term outcomes in SLE.

The Chairman of the North Manchester Local Research Ethics Committee (on behalf of Central Manchester LREC) has considered the amendments submitted in response to the Committee’s earlier review of your application on 26th June 2003 as set out in our letter dated 2nd July 2003. The documents considered were as follows:

Application Form 9/5/03
CV
Protocol
Case record form and glossary for NP-SLE SLICC study Nov.15/02: Version 2:02
Consent Form version 2: July 2003
Patient Information Sheet Version 2: July 2003

The Chairman, acting under delegated authority, is satisfied that these accord with the decision of the Committee and has agreed that there is no objection on ethical grounds to the proposed study. I am, therefore happy to give you the favourable opinion of the committee on the understanding that you will follow the conditions of approval set out below.

Conditions of Approval

- You do not recruit any research subjects within a research site unless favourable opinion has been obtained from the relevant local research ethics committees.

- You do not undertake this research in an NHS organisation until the relevant NHS management approval has been gained as set out in the Framework for Research Governance in Health and Social Care.

Chair: Philip Smith
Chief Executive: Neil Goodwin
• You do not deviate from, or make change to, the protocol without prior written approval of the LREC, except where this is necessary to eliminate immediate hazards to research participants or when the change involves only logistical or administrative aspects of the research. In such cases the LREC should be informed within seven days of the implementation of the change.

• You must complete and return the standard progress report form to the LREC one year from the date on this letter and thereafter on an annual basis. This form should also be used to notify the LREC when your research is completed and in this case should be sent to this LREC within three months of completion.

• If you decide to terminate this research prematurely you send a report to this LREC within 15 days, indicating the reason for the early termination.

• You advise the LREC of any unusual or unexpected results that raise questions about the safety of the research.

Any comments the LREC wished to make are contained in the attached LREC Response Form. The project must be started within three years of the date on which LREC approval is given.

Submissions to other LRECs in Greater Manchester

If you are conducting research at other sites in Greater Manchester it is your responsibility to ensure that you seek approval for locality issues from the relevant LREC before starting their research. To do this you should submit the appropriate number of copies of the following to the relevant LRECs:

• this letter
• the Application Form
• the Health Authority Locality Form (available from www.corec.org.uk)
• a copy of the local investigator’s CV

and one copy of

• the protocol, incorporating any amendments and including the final approved version of the Patient Information Sheet and Consent Form

Yours sincerely

Mrs G Rimington
Chairman, North Manchester Local Research Ethics Committee

Enclosures Annual Report Form
Membership List
Appendix 5: SLICC consent form

Central Manchester University Hospitals
NHS Foundation Trust

Centre Number: Study Number: Version 3, 04/07/2011

CONSENT FORM

THE SYSTEMIC LUPUS INTERNATIONAL COLLABORATING CLINICS

PROSPECTIVE STUDY OF LONG-TERM OUTCOMES IN SLE

Name of Researcher: Professor Ian Bruce, Kellgren Centre for Rheumatology, Manchester Royal Infirmary, Oxford Road, Manchester M13 9WL

Please initial box

1. I confirm that I have read and understand the information sheet dated 04/07/2011 (Version 3) for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I agree to a minimum of 5 years follow up.

4. I agree that my GP will be informed about my involvement in this study.

1. I understand that I will be informed if any of the results of the medical tests done as part of the research are important for my health, and with my permission, my GP and/or consultant will also be informed.

6. I understand that sections of any of my medical notes may be looked at by responsible individuals from Manchester Royal Infirmary, Department of Rheumatology or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.

7. I agree for a blood sample to be sent to the central co-ordinating laboratories in the University of Toronto and Dalhousie University, Halifax, Canada for use in this study.

8. I agree for a genetic sample to be stored in the central co-ordinating laboratory in The University of Toronto, Canada. I understand that it will be used for studies relating to this Registry and that only researchers approved by the SLICC group will have access to this sample. I also understand that this genetic sample will be stored for the duration of the study. If at any time I withdraw consent for its use, the sample will be destroyed.

9. I agree to take part in the study.

10. I offer my blood sample as a gift that may be used for future research and that further ethics committee approval will be sought for any additional use of it in future research.

Please turn over...
CONSENT FORM

THE SYSTEMIC LUPUS INTERNATIONAL COLLABORATING CLINICS
PROSPECTIVE STUDY OF LONG-TERM OUTCOMES IN SLE

Name of Researcher: Professor Ian Bruce, Kellgren Centre for Rheumatology,
Manchester Royal Infirmary, Oxford Road, Manchester M13 9WL

Please sign below:

______________________  _______________  _______________
Name of Patient          Date                        Signature

______________________  _______________
Name of Person taking consent
(if different from researcher)  Date                        Signature

______________________  _______________
Researcher             Date                        Signature
Appendix 6: SLICC data collection (therapies section)

SLICC - Atherosclerosis in SLE Data Collection Protocol – Enrolment (Version #15, 4-Nov-2009)

CENTRE NAME:  

PATIENT INITIALS:  

CENTRE NO: (Assigned by co-ordinating centre) 

PATIENT NO: (Assigned by submitting centre using consecutive numbers i.e., 001, 002, etc.)  

DATE OF ENROLMENT: (yy/mm/dd)  

---

1. DEMOGRAPHIC DATA

<table>
<thead>
<tr>
<th>Gender: (Circle)</th>
<th>M / F</th>
<th>Date of Birth: (yy/mm/dd)</th>
<th>Date of Diagnosis of SLE: (yy/mm/dd)</th>
</tr>
</thead>
</table>

ACR Criteria for Diagnosis: (✓)
- Malar rash
- Discoid Rash
- Oral ulcers
- Serositis
- Arthritis
- Photosensitivity
- Renal disorder
- Neurologic disorder
- Hematologic disorder
- Immunologic disorder
- Antinuclear antibody

New Manifestations since Diagnosis: (✓)
- Malar rash
- Discoid Rash
- Oral ulcers
- Serositis
- Arthritis
- Photosensitivity
- Renal disorder
- Neurologic disorder
- Hematologic disorder
- Immunologic disorder
- Antinuclear antibody

Race: (circle)
- 1 = Caucasian (North American)
- 2 = Caucasian (Indian – sub-continent)
- 3 = Caucasian (Other) – Specify
- 4 = Native North American
- 5 = Black (African)
- 6 = Black (Caribbean)
- 7 = Asian (Chinese)
- 8 = Asian (Filipino)
- 9 = Asian (Japanese)
- 10 = Asian (Korean)
- 11 = Asian (other) – Specify:
- 12 = Hispanic*
- 13 = Mixed - Specify:
- 14 = Native Hawaiian or other Pacific Islander
- 15 = Others – Specify:

* For Hispanic, please indicate which racial group the patient would fit into (as per NIH requirements):
- 1 = American Indian or Alaska Native
- 2 = Asian
- 3 = Black or African American
- 4 = Native Hawaiian or Other Pacific Islander
- 5 = White
- 6 = Other – Specify:
- 7 = unknown

Marital Status:
- 1 = single
- 2 = married
- 3 = widowed
- 4 = divorced
- 5 = separated
- 6 = living with partner (common law)

Education: Number of years prior to college or university

---

2. FAMILY HISTORY AND LIFESTYLE

Physical Activity Index and Family History Questionnaires to be completed by patient interview (Appendices 1 and 2).

Alcohol Consumption units / week
### Cigarette Smoking:
- Current: N Y
- Ex-smoker: N Y Date stopped: (yy/mm/dd)

# cigarettes/day # years smoking

### THERAPY

#### Steroids:
- Current oral steroid: N Y
  - If yes: Current Steroid Type:
  - Current course start date: (yy/mm/dd)
  - Average daily dose over course interval: mg/day
  - Highest dose received in current course: mg/day
- Current pulse steroid: N Y
  - If yes, no. of pulses:
  - Average dose per pulse: mg

#### Previous courses of oral steroid – since diagnosis of SLE: N Y
- If yes, no. of previous courses:
  - Average daily dose previous course(s): mg/day
  - Highest dose received in previous course(s): mg/day

#### Previous pulse steroid – since diagnosis of SLE: N Y
- If yes, no. of pulses:
  - Average dose per pulse: mg

#### Steroids taken prior to diagnosis of SLE (oral or pulse): N Y
  - Indication (specify):

#### Antimalarials:
- Current: N Y
  - If yes, current course start date: (yy/mm/dd)
  - Current daily dose: mg/day
  - Current type (circle):
    - 1 = chloroquine
    - 2 = hydroxychloroquine
    - 3 = atabrine
    - 4 = other (Specify)

#### Previous courses of antimalarials since diagnosis of SLE: N Y
- If yes, previous type (circle):
  - 1 = chloroquine
  - 2 = hydroxychloroquine
  - 3 = atabrine
  - 4 = other (Specify)

#### Antimalarials taken prior to diagnosis of SLE: N Y
  - Indication (specify):

#### Immunosuppressives:
- Current (1): N Y
  - If yes, current course start date: (yy/mm/dd)
  - Current type: (Circle) & indicate current daily dose
    - 1 = imuran
    - 2 = oral cyclophosphamide
    - 3 = i.v. cyclophosphamide
    - 4 = methotrexate
    - 5 = cyclosporin
    - 6 = mycophenolic acid
    - 7 = Other (Specify): Dose (specify unit):

- Current (2) N Y
  - If yes, current course start date: (yy/mm/dd)
Current type: (Circle) & indicate current daily dose

1 = imuran Dose mg/day
2 = oral cyclophosphamide Dose mg/day
3 = i.v. cyclophosphamide Dose mg/month
4 = methotrexate Dose mg/week
5 = cyclosporin Dose mg/day
6 = mycophenolic acid Dose mg/day
7 = Other (Specify): Dose (specify unit):

Previous courses of immunosuppressive since diagnosis of SLE: N Y

If yes, duration of previous course(s) (years) (months)

Previous type: (circle all that apply) & indicate average daily dose

1 = imuran Dose mg/day
2 = oral cyclophosphamide Dose /day
3 = i.v. cyclophosphamide Dose mg/month
4 = methotrexate Dose mg/week
5 = cyclosporin Dose mg/day
6 = mycophenolic acid Dose mg/day
7 = Other (Specify): Dose (specify unit):

Immunosuppressives taken prior to diagnosis of SLE: N Y Indication (specify):

Other Current Medications
(Circle all that apply) (1 = yes)

1 = NSAID’s Specify Type: 10 = iron
2 = anticoagulants 11 = thyroid replacement
3 = aspirin 12 = DHEA
4 = folic acid 13 = ranitidine (zantac)
5 = multivitamins 14 = omeprazole (losec)
6 = vitamin E 15 = bisphosphonates Specify Type
7 = vitamin C 16 = Other (Specify)
8 = vitamin D 17 = None
9 = calcium
Appendix 7: Fellowship approvals

Ref: MP/18845

22nd June 2009

Dr B Parker
arc Epidemiology Unit
University of Manchester
School of Translational Medicine
Stopford Building
Oxford Road
Manchester
M13 9PT

Dear Dr Parker

LETTER OF AWARD

Further to our previous letter, I am pleased to inform you that arc has awarded you a Clinical Research Fellowship at a total cost of £201,991 for 36 months entitled "Suppression of disease activity and improved vascular function in SLE: the role of disease control and immunosuppressive drug effects.". The start date for this award is 01/10/2009 and the expiry date is 30/09/2012. Please inform us as soon as possible if there has been any alteration to these dates.

I enclose full details of the financial support and the conditions of award relating to this grant together with guidelines for the tenure and process of review of arc fellowships; a separate copy has been sent to your administrative authority. The grant holder’s and the host institution’s agreement of these conditions should be signed and returned as soon as possible, since payment of invoices can be authorised only when we have received these documents.

Please ensure you read the conditions of award. In particular, please note clause 13(vi) regarding depositing publications in an open access archive.

Current or recent holders of arc project grants, programme grants, fellowships and PhD studentships are eligible to apply for discretionary travel awards to assist their attendance (or that of research staff employed on the grant or working in their laboratory) at prestigious or relevant national or international scientific meetings in order to present arc-supported work. The maximum total amount allotted for travel is a pro rata sum of £750 per year (project grants and all categories of fellowship), £1,500 per year (programme grants), or £500 per year (PhD studentships). Applications for travel awards may be made at any time on the appropriate form but this must be submitted at least 1 month before the date of the meeting and must include an abstract of the work to be presented. Applications will be accepted for meetings held up to 24 months after the termination of a grant or fellowship.

As arc is a national charity, depending on public donations to ensure the continuance of research into arthritis and rheumatic disease, it is essential that our Press Office be kept informed of potential breakthroughs or work that has a direct patient benefit. Keeping the regional and national media up-to-date with any developments in arthritis research will ensure our profile is raised.
It also helps our fundraising effort to have arc-funded researchers speak at branch meetings and talk to the press about their work if requested. Grant-holders will be required to assist our Area Appeals Managers and Press Office in this vital work.

A condition of an arc Fellowship is attendance at the annual arc Fellows Meeting which takes place over 2 days each year, the next fellows meeting will take place in March 2010.

Any failure to comply with the details outlined in this letter and accompanying grant conditions may lead to sanctions being imposed on either the institution or grant holder.

**Please ensure that the grant reference 18845 is quoted on all future correspondence to avoid delay.**

Please contact Claire Fantom (c.fantom@arc.org.uk) in our Research and Education Department if you have any queries concerning this award, and may I take this opportunity to wish you every success with your Fellowship.

Yours sincerely

[Signature]

Mr Michael Patnick
Head of Research & Education Funding

cc  Dr Yvonne Alexander
    Dr Ian Bruce
    Professor Anthony Heagerty
    Miss Sharon Thompson

Enc  Conditions of award (version 7b)
Dear Ben,

Re: Manchester Biomedical Research Centre Fellowship

Many thanks for attending the interview for a BRC Fellowship on Friday 25th July. I am writing to confirm that we are pleased to be able to offer you a Fellowship for the following project supervised by Dr Ian Bruce:

Effects of biological agents on inflammation and vascular function in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE): inflammation reduction or drug effect?

This offer is **conditional** and will be confirmed following the receipt of two satisfactory references (which have already been requested from your named referees) and successful registration for a MPhil programme with the University of Manchester. This is a formality and will allow the Medical School Postgraduate Admissions Office to issue you with formal written confirmation of your forthcoming postgraduate training. It will also ensure you are sent full registration details.

Please note that you will **not** need to provide copies of references (or the reference cover-sheets) - the versions we have requested will be passed to the Medical School Office on your behalf.

Once you have submitted your application you will receive an automated email confirming receipt. I would anticipate it may take up to a month to process your offer. If you have any questions relating to the application process (or status of your offer) please contact Tasleem Hanif at tasleem.hanif@manchester.ac.uk.

The award is for the period 01/10/2008 to 30/09/2009 in the first instance. We will be able to offer you the equivalent of your current ST3 salary and the fellowship will also cover the UK/EU tuition fee and up to £10,000 consumables. Your tuition payment will be organised on your behalf ahead of registration, and this should be evident when you arrange your online registration.

The **continuation of funding** for this fellowship into a PhD will be subject to a rigorous review of your progress during the MPhil year. If you have any further queries, please do not hesitate to contact me through Sarah Lines (contact details above). May I take this opportunity to wish you every success with your forthcoming research fellowship.

Yours sincerely

Peter E Clayton
Professor of Child Health & Paediatric Endocrinology
Associate Director for Training
Manchester Biomedical Research Centre

cc. Dr Ian Bruce
Appendix 8: Endothelial function ethical approval

Oldham Local Research Ethics Committee
Room 181
Gateway House
Piccadilly South
Manchester
M60 7LP

Telephone: 0161 237 2336
Facsimile: 0161 237 2393

28 January 2009

Dr Ben Parker
Specialist Registrar in Rheumatology
NHSS/ University of Manchester
Kellgren Centre for Rheumatology
Central Manchester and Manchester Childrens Hospital NHS Trust
Stopford Building
Oxford Road
Manchester
M13 9WL

Dear Dr Parker

Full title of study: Effects of Biological Agents on Inflammation and Vascular Function in Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE): Inflammation Reduction or Drug Effect?

REC reference number: 09/H1011/3

Thank you for your letter of 20 January 2009, responding to the Committee’s request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

The Committee has designated this study as exempt from site-specific assessment (SSA). The favourable opinion for the study applies to all sites involved in the research. There is no requirement for other Local Research Ethics Committees to be informed or SSA to be carried out at each site.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.
Management permission at NHS sites (“R&D approval”) should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covering Letter</td>
<td></td>
<td>25 November 2008</td>
</tr>
<tr>
<td>Protocol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Investigator CV</td>
<td>Parker</td>
<td></td>
</tr>
<tr>
<td>Application</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Investigator CV</td>
<td>Bruce</td>
<td></td>
</tr>
<tr>
<td>wound care info</td>
<td>1</td>
<td>25 November 2008</td>
</tr>
<tr>
<td>Compensation Arrangements</td>
<td></td>
<td>27 November 2008</td>
</tr>
<tr>
<td>letter from cmmc</td>
<td></td>
<td>28 July 2008</td>
</tr>
<tr>
<td>Peer Review</td>
<td></td>
<td>08 October 2008</td>
</tr>
<tr>
<td>Questionnaire: HAQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Questionnaire: SF36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Questionnaire: LupusQol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response to Request for Further Information</td>
<td></td>
<td>20 January 2009</td>
</tr>
<tr>
<td>Participant Consent Form: Best Friend Control</td>
<td>1</td>
<td>20 January 2009</td>
</tr>
<tr>
<td>Participant Consent Form: Patient</td>
<td>2</td>
<td>20 January 2009</td>
</tr>
<tr>
<td>Participant Consent Form</td>
<td></td>
<td>pilot</td>
</tr>
<tr>
<td>Participant Information Sheet</td>
<td>1</td>
<td>20 January 2009</td>
</tr>
<tr>
<td>Participant Information Sheet: Patient</td>
<td>2</td>
<td>20 January 2009</td>
</tr>
<tr>
<td>Participant Information Sheet</td>
<td></td>
<td>pilot</td>
</tr>
<tr>
<td>GP/Consultant Information Sheets</td>
<td>2</td>
<td>20 January 2009</td>
</tr>
</tbody>
</table>

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document “After ethical review – guidance for researchers” gives detailed guidance on reporting requirements for studies with a favourable opinion, including:
• Notifying substantial amendments
• Progress and safety reports
• Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

09/H1011/3 Please quote this number on all correspondence

With the Committee’s best wishes for the success of this project

Yours sincerely

Dr Peter Stanley Klimiuk
Chair

Email: carol.ebenezer@northwest.nhs.uk

Enclosures: “After ethical review – guidance for researchers”

Copy to: Dr Karen Shaw
Alison Robinson
Appendix 9: Endothelial function consent form

Effects of Biological Agents on Inflammation and Vascular Function in Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE):
Inflammation Reduction or Drug Effect?

PATIENT CONSENT

Name of researcher Dr Ian Bruce

Contact details The Kellgren Centre for Rheumatology
Manchester Royal Infirmary
Oxford Road, Manchester
M13 9WL
Tel: 0161 276 4626

Study ID

2. I have read and understand the information sheet on this project dated 20/01/09 and have been given a copy to keep. I have been able to ask questions about the project and I understand why the research is being done and any risks involved.

3. I agree to give a sample of blood and urine for use in this research. I understand how the sample will be collected, that giving a sample for this is voluntary and that I am free to withdraw my approval for use of the sample at any time without giving a reason and without my medical treatment or legal rights being affected.

4. I understand that sections of my medical notes may be looked at by responsible individuals from The University of Manchester or from regulatory authorities where it is relevant to my taking part in research. I give my permission for these individuals to have access to my records.

5. I understand that I will be informed if any of the results of the medical tests done as part of the research are important for my health, and with my permission, my GP and/or consultant will also be informed.

6. I understand that I will not benefit financially if this research leads to the development of a new treatment or medical test.

7. I know how to contact the research team if I need to, and how to get information about the results of the research.
PATIENT CONSENT (continued)

PATIENT CONSENT

Name of researcher Dr Ian Bruce

Contact details The Kellgren Centre for Rheumatology
The Royal Infirmary
Oxford Road, Manchester
M13 9WL
Tel: 0161 276 4626

Study ID ____________________________

8. I understand that my blood sample is being gifted to medical research and will be stored in a coded fashion. ☐

9. I understand that further ethics committee approval will be sought for any additional use of my blood sample in future research. ☐

10. I agree to take part in this study. ☐

OPTIONAL

11. I agree to have two gluteal fat biopsies. ☐

11. I have been given a copy of “How to Care for Your Biopsy Wound” ☐

Name of patient ____________________________ Date _____________ Signature ________________

Name of person taking consent ____________________________ Date _____________ Signature ________________ (if different from researcher)

Researcher ____________________________ Date _____________ Signature ________________
Appendix 10: Endothelial function information sheet

Central Manchester University Hospitals
NHS Foundation Trust

Effects of Biological Agents on Inflammation and Vascular Function in Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE): Inflammation Reduction or Drug Effect?

PATIENT INFORMATION

Introduction
Rheumatoid Arthritis (RA) is the most common form of inflammatory arthritis affecting about 1% of the adult UK population. Research has shown that people with RA are more likely to suffer from diseases of the heart and blood vessels (mainly heart attack and stroke). This may be because the high levels of inflammation in RA cause damage to blood vessels.

Systemic Lupus Erythematosus (SLE) is a chronic illness which affects the immune system. SLE can affect any part of the body. It causes inflammation and can lead to organ damage. Research has shown that people with SLE are more likely to suffer from diseases of the heart and blood vessels (arteries). This is partly because the high levels of inflammation in SLE cause damage to arteries by hardening them. This hardening is known as atherosclerosis.

There are several different drugs which can be used to treat both RA and SLE. For this research we are particularly interested in studying the drugs called Methotrexate, Azathioprine, MMF (Mycophenolate Mofetil) and the treatments known as “biological therapies.”

What is the purpose of the study?
We would like to find out whether or not the drugs named above can help reduce the likelihood of future heart disease in people with RA or SLE. We will do this by looking at how the cells lining the arteries (endothelial cells) change over time and compare these changes with the type of drug a patient is using to treat their RA or SLE.

Why have I been asked to take part?
You have been asked to take part in this study because you have RA or SLE and are about to start using one of the medications named in the introduction.

What will happen to me if I take part?
If you agree to take part you will be asked to visit the Wellcome Trust Clinical Research Facility and the Rheumatology Department at Manchester Royal Infirmary. We will ask you to fast for 12 hours, miss any blood pressure medication for 24 hours and avoid alcohol for 48 hours before this visit. Ideally, you should not smoke on the day of the study.

We will take your medical history and examine you so that we can assess the current level of activity of your illness. We will also ask you to complete several short questionnaires.

Samples
A 50ml blood sample (approximately 10 teaspoons) will be taken to measure the level of inflammation in your system, as well as measuring cholesterol, blood glucose and other factors that may influence the risk of heart disease. Some of the blood sample will be used to measure the type and age of your endothelial cells.

A sample of blood will be taken and stored to allow us to measure different aspects of your lipid and cholesterol profile, and other factors that may affect the risk of heart disease. You will also be asked to provide a small urine sample (10ml), which will be frozen and stored to measure signs of inflammation.

Scans
You will also undergo a series of ultrasound scans, which are quick, painless and do not involve needles.

We will use different types of scanning, as we would like to know which of the methods is the most reliable:

The main scanning method we will use is called EndoPAT. This will involve a probe which slips onto your finger. The probe “listens” to your blood vessels to check how they are functioning.
We may also perform a “carotid” scan. This will be done by placing a small probe onto the surface of your neck. The probe will examine the main arteries in your neck (carotid arteries) to see if there is any evidence of atherosclerosis.

Finally, a third scan will be used to measure the artery in the crook of your elbow (brachial artery).

All ultrasound scans are performed at the same time whilst inflating and deflating a blood pressure cuff around the forearm for up to 5 minutes, allowing the resulting change in the artery to be measured. A second measurement will occur after applying a small amount of GTN (Glyceryl Trinitrate) under the tongue. GTN is a spray (commonly used by people with angina) which causes blood vessels to dilate.

Your visit should last approximately 1 – 1 ½ hours and can be arranged to coincide with your routine clinic appointment if necessary.

We will ask you to return 3 months after your initial visit so that we can see how you are responding to your medication and whether there has been any change in the way your endothelial cells are working.

Gluteal Biopsy
We will ask a small number of participants in the study to have two gluteal fat biopsies in addition to the samples and scans named above. The second biopsy will occur 3-4 months after the first.

The biopsy is taken from the gluteal (buttock) area under local anaesthetic. The injection of local anaesthetic can sometimes be uncomfortable. Following this, a small sample of tissue is removed and the small wound (less than 3 cm) will be stitched. The stitches will stay in place for 7 – 10 days and can be removed either by your GP or by the nurses at the Wellcome Trust Facility, depending on what is most convenient to you.

You will be contacted at home after 2 weeks to ensure that the wound has healed satisfactorily, and if necessary, you will be re-assessed.

If you agree to the gluteal fat biopsy, every effort will be made to perform this on the same day as the scans and samples are taken. However, this may not always be possible and you may be asked to return on a different day.

Do I have to take part?
No. It is up to you whether or not you take part.

If you decide not to take part, you do not have to give a reason for this. If you agree to take part, you will be asked to sign a consent form and you are free to withdraw at any time without giving a reason. A decision not to take part, or to withdraw, will not affect the standard of care you receive.

What are the possible risks of taking part?
You may experience some discomfort or bruising from the blood tests. Very occasionally, people can experience light-headedness after using GTN spray, although you will be sitting down when this is given. There is also a small possibility that the spray will give you a sudden, short-lived headache, which may be severe. This is unlikely however as the dose administered is relatively small.

Some patients have experienced discomfort after the gluteal biopsy, which on the whole is mild, short-lived and relieved by simple pain killers such as paracetamol. There is a small chance of infection, as with any other procedure which breaks the skin, so all usual precautions will be in place to prevent this.

There is a chance that the result from a blood test or scan will be clinically relevant. If we find any unexpected abnormalities in the blood tests or scans, a member of the study team will advise you of these and arrange any further test which may be appropriate.

What are the possible benefits of taking part?
You may not receive any direct personal benefit from taking part in this study.

As a result of this study we will have detailed information about patients’ chances of getting heart disease. A member of the research team will discuss your results with you when they are available. You will be told about any significant clinical findings and, with your permission, these will be passed on to either your GP or your consultant, or both.

Will I be paid for taking part?
No, you will not be paid for helping us with this study.

What will happen to the blood samples?
The samples will be gifted to medical research and will be stored in a secure laboratory. Only authorised personnel will have access to the samples.
We would like to retain the samples at the end of the study. They may be valuable in future research. However, we would only use your sample in future research after we have been granted further ethical approval.

What if there is a problem?

If you have a complaint about the way you may have been dealt with during the study, you can talk to patient advice and liaison service (PALS) staff or complaints manager at the Central Manchester and Manchester Children’s University Hospitals NHS Trust. They may be able to resolve your concerns on the spot or can provide you with details of how to make an official complaint.

Will my taking part in this study be kept confidential?

Information collected from you will be sent to The University of Manchester where it will be stored securely under conditions in keeping with the Data Protection Act 1998. Your name and any other personal information from which you could be identified will be kept separately from your clinical data. Only individuals directly involved with the study will have access to this information.

Some parts of your medical records will be looked at by responsible individuals from The University of Manchester and the Central Manchester and Manchester Children’s University Hospitals Trust. This is necessary to make sure that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and will never reveal your identity to anyone not directly involved with the study.

What will happen to the results of the study?

The results of this study will be published in a professional medical journal. Your name, or any details that could be used to identify you, will not be used in any such publications.

You have the right to request information about any personal data that we hold on you, or to request that any inaccuracies be corrected. To make such a request you should contact your rheumatologist or research nurse.

Who has reviewed and approved the study?

This research has been approved by the Oldham Research Ethics Committee.

What do I do now?

The doctor or study nurse organising this study will contact you to discuss whether you wish to take part and make the necessary arrangements for you.

Contact details

For further advice regarding this study you can contact:

Dr Ian Bruce, Senior Lecturer and Consultant Rheumatologist
Or
Sr Joanna Shelmerdine, Specialist Lupus Nurse
Or
Dr Ben Parker, Clinical Research Fellow

The Kellgren Centre for Rheumatology
Manchester Royal Infirmary
Central Manchester and Manchester Children’s University Hospitals NHS Trust
Oxford Road
Manchester M13 9WL
Telephone: 0161 276 4626

Thank you very much for taking the time to read this information.
Appendix 11: GP information sheet

Central Manchester University Hospitals
NHS Foundation Trust

Effects of Biological Agents on Inflammation and Vascular Function in Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE): Inflammation Reduction or Drug Effect?

GP INFORMATION LETTER (v 2.0 20/01/2009)

Dear <Dr Name>

I am writing to inform you that your patient indicated above has agreed to take part in the above study.

The study aims to examine whether part of this increased risk of cardiovascular disease is related to the level of inflammation present, and whether certain drugs improve this risk by controlling inflammation. We aim to assess patients with both RA and SLE who are to start either usual therapy for the first time or who have severe enough disease to warrant newer biologic therapies. We will assess their cardiovascular function before and after using these therapies to see whether any changes can be detected, and whether this is due simply to better control of their disease (and hence inflammation) or possibly related to the type of drug used.

Your patient has agreed to take part in this study (either as a patient or healthy control) and we therefore plan to review their medical notes, take a medical history and examine them.

We will take a 50ml blood sample to look at your patient’s level of inflammation as well as to measure their cholesterol, blood glucose and other factors that may influence the risk of heart disease. A 10ml urine sample will be frozen and stored to measure inflammation markers. Finally, your patient will undergo an ultrasound scan of the carotid artery and brachial artery.

Should the blood tests or scan identify any unexpected clinically relevant abnormalities, your patient will be advised of any additional tests or referrals that may be relevant.

If you require any further information regarding this study please do not hesitate to contact me.

Yours Sincerely

Dr I N Bruce
Senior Lecturer and Consultant Rheumatologist
The Kellgren Centre for Rheumatology
Manchester Royal Infirmary
**VISIT 1 HISTORY**

**PATIENT INITIALS:**

**PATIENT ID NO.:**

**ASSESSMENT DATE:** __/__/__

### DEMOGRAPHIC DATA

<table>
<thead>
<tr>
<th>Date of birth: d/m/y</th>
<th>Date of diagnosis of SLE: d/m/y</th>
<th>Date of 1st symptom: d/m/y</th>
</tr>
</thead>
</table>

**ACR criteria for diagnosis (tick as appropriate):**

- [ ] Malar rash
- [ ] Discoid rash
- [ ] Oral ulcers
- [ ] Serositis
- [ ] Arthritis
- [ ] Photosensitivity
- [ ] Renal disorder
- [ ] Neurologic disorder
- [ ] Haematalogic disorder
- [ ] Immunologic disorder
- [ ] ANA

**Other features:**

- [ ] Raynauds
- [ ] Fatigue
- [ ] Polymyositis
- [ ] Last DXA scan
- [ ] Fractures

**Marital Status (circle as appropriate):**

1. Single
2. Married
3. Widowed
4. Divorced
5. Separated
6. Common law

**Education:**

Number of years prior to college/university: ___

Number of years at college/university: ___

**Occupation (specify):**

---

### FAMILY HISTORY AND LIFESTYLE

**Alcohol consumption:** ___ units per week

**Cigarette smoking (delete as appropriate):**

<table>
<thead>
<tr>
<th>Current</th>
<th>Yes / No</th>
<th>If yes, number per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex-smoker</td>
<td>Yes / No</td>
<td>If no, number of years smoking and date stopped</td>
</tr>
</tbody>
</table>

Lifestyle Questionnaire Yes/No
## CLINICAL DATA

**Height:** _____ cm  
**Weight (shoes and coat off):** _____ kg  
**BMI:** _____

**Waist/hip ratio:** _____ cm / _____ cm  
**Blood pressure (systolic/diastolic):** _____ / _____

**Antihypertensive therapy (delete as appropriate):**

<table>
<thead>
<tr>
<th>Current</th>
<th>Yes / No</th>
<th>If yes, specify type (circle as appropriate):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 = Diuretics</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 = Adrenergic inhibitors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = Central and agonists</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 = Beta blockers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 = Direct vasodilators</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 = Calcium antagonists</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 = Angiotensin-converting enzyme inhibitors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 = Other</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 = Combination</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>In the past</th>
<th>Yes / No</th>
<th>If yes, specify type (circle as appropriate):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 = Diuretics</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 = Adrenergic inhibitors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = Central and agonists</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 = Beta blockers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 = Direct vasodilators</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 = Calcium antagonists</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 = Angiotensin-converting enzyme inhibitors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 = Other</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 = Combination</td>
</tr>
</tbody>
</table>

**Myocardial infarction (circle as appropriate):**

<table>
<thead>
<tr>
<th>In the past</th>
<th>Yes / No</th>
<th>If yes, specify date(s):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>d / m / y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d / m / y</td>
</tr>
</tbody>
</table>

**Angina (circle as appropriate):**

<table>
<thead>
<tr>
<th>Current</th>
<th>Yes / No</th>
<th>If yes, specify date of diagnosis:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>d / m / y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d / m / y</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>In the past</th>
<th>Yes / No</th>
<th>If yes, specify date of diagnosis:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>d / m / y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d / m / y</td>
</tr>
</tbody>
</table>

**Congestive heart failure (circle as appropriate):**

<table>
<thead>
<tr>
<th>Current</th>
<th>Yes / No</th>
<th>If yes, specify date(s):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>d / m / y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d / m / y</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>In the past</th>
<th>Yes / No</th>
<th>If yes, specify date(s):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>d / m / y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d / m / y</td>
</tr>
</tbody>
</table>
**Angioplasty (circle as appropriate):**

<table>
<thead>
<tr>
<th>Ever</th>
<th>Yes / No</th>
<th>If yes, specify date(s):</th>
<th>/   /</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>/   /</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>/   /</td>
</tr>
</tbody>
</table>

**Bypass surgery (circle as appropriate):**

<table>
<thead>
<tr>
<th>Ever</th>
<th>Yes / No</th>
<th>If yes, specify date:</th>
<th>/   /</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>/   /</td>
</tr>
</tbody>
</table>

**PREVIOUS SLE CARDIAC MANIFESTATIONS**

**Pericarditis:**

<table>
<thead>
<tr>
<th>Ever</th>
<th>Yes / No</th>
<th>If yes, specify date(s):</th>
<th>/   /</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>/   /</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>/   /</td>
</tr>
</tbody>
</table>

**Myocarditis:**

<table>
<thead>
<tr>
<th>Ever</th>
<th>Yes / No</th>
<th>If yes, specify date(s):</th>
<th>/   /</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>/   /</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>/   /</td>
</tr>
</tbody>
</table>

**Endocarditis:**

<table>
<thead>
<tr>
<th>Ever</th>
<th>Yes / No</th>
<th>If yes, specify date(s):</th>
<th>/   /</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>/   /</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>/   /</td>
</tr>
</tbody>
</table>

**PERIPHERAL VASCULAR**

**Intermittent claudication (circle as appropriate):**

<table>
<thead>
<tr>
<th>Current</th>
<th>Yes / No</th>
<th>If yes, specify date(s):</th>
<th>/   /</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>/   /</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>/   /</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>In the past</th>
<th>Yes / No</th>
<th>If yes, specify date(s):</th>
<th>/   /</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>/   /</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>/   /</td>
</tr>
</tbody>
</table>
CEREBROVASCULAR

Transient ischemic attack (circle as appropriate): Yes / No

If yes, specify date(s):

\[
\frac{d}{m}/\frac{y}{d}/\frac{m}{y}
\]

Stroke (circle as appropriate): Yes / No

If yes, specify date(s):

\[
\frac{d}{m}/\frac{y}{d}/\frac{m}{y}
\]

Type (if known): 1 = Hemorrhagic 2 = Thrombotic

HYPERLIPIDEMIA THERAPY

Current Yes / No If current, specify type (circle as appropriate):

0 = None 4 = Fibrates
1 = Statins 5 = Combinations
2 = Sequestrants 6 = Other
3 = Nicotinic acid

In the past Yes / No If in the past, specify type (circle as appropriate):

0 = None 4 = Fibrates
1 = Statins 5 = Combinations
2 = Sequestrants 6 = Other
3 = Nicotinic acid

HORMONAL FACTORS

Ovarian function (circle all which apply):

1 = Menstruating 5 = Pre-menopausal hysterectomy
2 = Premenarche 6 = Post-menopausal hysterectomy
3 = Postmenopausal 7 = Pre-menopausal hysteroophorectomy
4 = Amenorrhea

Age at menopause

Oral contraceptive

Current Yes / No If yes, specify number of years:

In the past Yes / No If yes, specify number of years:

Hormone replacement therapy:

Current Yes / No If current, specify type (circle as appropriate):

1 = Estrogen (specify):
2 = Estrogen + progesterone
3 = Progesterone only
4 = Other (specify):

Current course start date:

\[
\frac{d}{m}/\frac{y}{d}/\frac{m}{y}
\]

In the past Yes / No If in the past, specify type (circle as appropriate):

1 = Estrogen (specify):
2 = Estrogen + progesterone
3 = Progesterone only
4 = Other (specify):

Are you currently pregnant? (circle as appropriate): Yes / No

Gravida / Para: \[
\frac{i}{/}\frac{m}{/}\frac{a}{/}\frac{n}{/}\frac{a}{/}\frac{r}{/}\frac{i}{/}\frac{t}{/}\frac{a}{/}\frac{s}{/}\frac{i}{/}\frac{a}{/}\frac{t}{/}\frac{a}{/}\frac{i}{/}\frac{t}{}
\]

Miscarriage (No):
**ENDOCRINE**

*Hypothyroidism:*
- Current: Yes / No
- Ever: Yes / No

*Diabetes:*
- Yes / No
- If yes, specify date of diagnosis: ___ / ___ / ___
- Specify type (delete as appropriate): Type 1 / Type 2

**RENAL**

*Active nephritis:*
- Current: Yes / No
- In the past: Yes / No

*Nephrotic syndrome:*
- Current: Yes / No
- In the past: Yes / No

Past medical history: ____________________________________________
___________________________________________
# Therapy

**Immunosuppressive Agents:**

<table>
<thead>
<tr>
<th>Current</th>
<th>Yes / No</th>
<th>If yes, specify type (circle as appropriate):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 = Azathioprine 4 = Cyclosporin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = Cyclophosphamide PO 5 = Mycophenolate mofetil</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 = Cyclophosphamide IV 6 = Methotrexate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 = Other (specify):</td>
</tr>
</tbody>
</table>

Date initiated: length (weeks): to stop? Yes/No

**In the past**

<table>
<thead>
<tr>
<th>Yes / No</th>
<th>If yes, specify type (circle as appropriate):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 = Azathioprine 4 = Cyclosporin</td>
</tr>
<tr>
<td></td>
<td>2 = Cyclophosphamide PO 5 = Mycophenolate mofetil</td>
</tr>
<tr>
<td></td>
<td>3 = Cyclophosphamide IV 6 = Methotrexate</td>
</tr>
<tr>
<td></td>
<td>7 = Other (specify):</td>
</tr>
</tbody>
</table>

**Immunosuppressive agent due to start:** (circle as appropriate)

| 1 | azathioprine | dose: |
| 2 | cyclophosphamide IV | dose: |
| 3 | cyclophosphamide PO | dose: |
| 4 | rituximab | dose: |

**Steroids:**

**Current**

<table>
<thead>
<tr>
<th>Yes / No</th>
<th>If yes, specify course start date: ____ / ____ / ____ and average daily dose: ________ mg</th>
</tr>
</thead>
</table>

**In the past**

<table>
<thead>
<tr>
<th>Yes / No</th>
<th>If yes, number of previous courses: ________ And average daily dose: ____ mg</th>
</tr>
</thead>
</table>

Number of previous courses of IV steroids (and details):

Number of courses in last 6 months:

**Antimalarials:**

**Current**

<table>
<thead>
<tr>
<th>Yes / No</th>
<th>If yes, specify type (circle as appropriate):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 = Chloroquine 3 = Hydroxychloroquine</td>
</tr>
<tr>
<td></td>
<td>2 = Atabrine 4 = Other (specify):</td>
</tr>
</tbody>
</table>

If yes, specify course start date: ____ / ____ / ____ and average daily dose: ____ mg

**In the past**

<table>
<thead>
<tr>
<th>Yes / No</th>
<th>If yes, specify type (circle as appropriate):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 = Chloroquine 3 = Hydroxychloroquine</td>
</tr>
<tr>
<td></td>
<td>2 = Atabrine 4 = Other (specify):</td>
</tr>
</tbody>
</table>


Isenberg DA. Rituximab—it was the best of times, it was the worst of times. Autoimmun Rev 2012 Feb 12.


Bruce IN. 'Not only...but also': factors that contribute to accelerated atherosclerosis and premature coronary heart disease in systemic lupus erythematosus. Rheumatology 2005 Dec;44(12):1492-502.


(139) Hasunuma Y, Matsuura E, Makita Z, Katahira T, Nishi S, Koike T. Involvement of beta 2-glycoprotein I and anticardiolipin antibodies in oxidatively modified low-


Wright SA, O’Prey FM, McHenry MT, Leahey WJ, Devine AB, Duffy EM, et al. A randomised interventional trial of omega-3-polyunsaturated fatty acids on


(198) Kiani AN, Magder LS, Petri M. Mycophenolate mofetil (MMF) does not slow the progression of subclinical atherosclerosis in SLE over 2 years. Rheumatol Int 2011 Jul 27.


(212) Kahn R, Buse J, Ferrannini E, Stern M. The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the


Devaraj S, Kumaresan PR, Jialal I. C-reactive protein induces release of both endothelial microparticles and circulating endothelial cells in vitro and in vivo: further evidence of endothelial dysfunction. Clinical Chemistry 2011 Dec;57(12):1757-61.


Skeoch S, Haque S, Pemberton P, Shelmerdine J, Bruce IN. E-selectin and VCAM-1 as biomarkers of disease in patients with SLE. Lupus 19[(1 Suppl)], 52. 2010. Ref Type: Abstract


(334) Black MA, Cable NT, Thijssen DH, Green DJ. Importance of measuring the time course of flow-mediated dilatation in humans. Hypertension 2008 Feb;51(2):203-10.


