LIPID ASSOCIATED BIOMARKERS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND RHEUMATOID ARTHRITIS

A THESIS SUBMITTED TO THE UNIVERSITY OF MANCHESTER FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF MEDICAL AND HUMAN SCIENCES

BY

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Abstract
Abstract

The University of Manchester

Awal Zaki Almohedhusain

PhD

Lipid Associated Biomarkers in Patients with Systemic Lupus Erythematosus and Rheumatoid Arthritis

30th September 2012

Patients with chronic inflammatory conditions such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) experience premature cardiovascular mortality and morbidity compared with the general population. The increased risk of cardiovascular disease (CVD) may in part, result from an interaction between traditional and non-traditional risk factors, modulated by chronic inflammation. The aim of this project was to look at lipid associated biomarkers in patients with SLE/RA and the association between these markers and cardiovascular disease outcomes. We also aimed to study the effect of inflammation reduction on vascular biomarkers.

In the first study we examined 168 SLE patients median (IQR) age was 53 (46-61) years and median disease duration 13 (7, 23) years and 56 healthy controls median age 50 (39-60) years. We demonstrated elevated level of oxidised-LDL in SLE patients compared with healthy controls (76 (57, 99) U/l vs 56 (42, 88) U/l P= 0.02). We further explored the association between oxidant stress and premature atherosclerosis as measured by carotid intima media thickness (cIMT) and plaque. In addition to age and systolic blood pressure, oxidised-LDL and urinary 8-isoprostane were significantly and independently associated with cIMT in SLE patients $\beta$ coefficient 95%CI [0.00007 (5.29 $^{-6}$, 0.0001) and 0.003 (0.0008, 0.004)], respectively. In healthy controls, age was the only independent variable.

In the Norfolk Arthritis Register, 1266 patients with early inflammatory polyarthritist (IP) were studied. A linear regression analysis revealed a significant negative association between CRP and lipid profile namely TC, LDL, TG and ApoA-1. During a median (IQR) follow up=5.5 (3.7-7.7) years 100 (7%) patients died (all causes) of which 33% (33) deaths were attributed to CVD. Forward stepwise regression analysis demonstrated that a low total cholesterol was independently associated with all cause mortality HR (95%CI) 0.75 (0.61, 0.91) and CVD mortality HR (95%CI) 0.49 (0.29, 0.85).

In a small cohort 27 SLE patients and 15 healthy controls. We measured endothelial function using flow mediated dilatation of the brachial artery. At baseline we found a significant increase in TG level [1.36 (0.9, 1.87) mmol/l vs 0.88 (0.64, 1) mmol/l $P= 0.009$] and a significant impaired endothelial function in SLE patients compared to the healthy controls [2.86 (0.6, 5.3) vs 6.81 (3.46, 8.57), $P= 0.03$]. After treatment, there was a trend towards reduce TG level and improved endothelial function. Oxidised-LDL did not change significantly.

In conclusion, oxidant stress is increased in SLE patients and relates to some measures of subclinical atherosclerosis. Control of inflammation may not be sufficient to completely control this in routine practice. In early RA, active inflammation may mask any tendency to hyperlipidemia in this population. Low total cholesterol may be the best biomarker of the overall metabolic and inflammatory status of the patients as well as indicating a group with increased risk of future mortality.
Declaration

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The University of Manchester
Dedication

I dedicate this thesis to my family, especially....

to mum and dad who give me love and support during my life;

to my husband Saleh and my lovely kids Ali and Karrar who are the joy of my life.
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I also extend my thanks to all my family in general; especially my parents for their continuous encouragement and advice and mainly for their prayers without which this work would not be accomplished and to my sisters and brothers, for their love and support. Special thanks to my husband Saleh whose support and loyalty will never be forgotten. Particular thanks to my lovely kids Ali and Karar whose smiles give me a great hope in life. Finally, to all my friends, thank you for your understanding and encouragement in many moments of crisis. Your friendship makes my life a wonderful experience. I cannot list all the names but you are all in my mind.
Preface

I am medically qualified and graduated from King Faisal University in Dammam (Saudi Arabia) with MBChB (2003). I worked as a general practitioner in Dammam Central Hospital (Ministry of health) for one year.

I joined the University of Manchester 2007 and gained my Msc degree in Immunology and Immunogenetics. I registered for a PhD in 2008. My study in UK is supported by a scholarship from the Saudi Ministry of Higher Education. On completion of my study, I plan to return to Saudi and continue my research.
Publication arising from this thesis

Abstracts


Presented at British Society of Rheumatology Annual Meeting. Brighton UK.


Publications


• C. Chew, P. Pemberton, A. Alhusain, S. Haque, I. Bruce. Serum cystatin C is independently associated with renal impairment and inflammation in Systemic Lupus Erythematosus. Clinical Experimental Rheumatology. (Accepted for publication)

Prizes

• 2011 Rheumatology Young Researcher Travel Award.
## List of abbreviation

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR</td>
<td>American College of Rheumatology</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>Anti-phosphlipid antibodies</td>
</tr>
<tr>
<td>Apo</td>
<td>Apolipoprotein</td>
</tr>
<tr>
<td>BILAG</td>
<td>British Isles Lupus Assessment Group</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>cIMT</td>
<td>Carotid intima-medial thickness</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CS</td>
<td>Corticosteroids</td>
</tr>
<tr>
<td>CV</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DMARD</td>
<td>Disease modifying antirheumatic drug</td>
</tr>
<tr>
<td>FMD</td>
<td>Flow mediated dilatation</td>
</tr>
<tr>
<td>GTN</td>
<td>Glyceryl Trinitrate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Meaning</td>
</tr>
<tr>
<td>--------------</td>
<td>---------</td>
</tr>
<tr>
<td>HAQ</td>
<td>Health activity questionnaire</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>HMG Co-A</td>
<td>Hydroxymethyglutaryl coenzyme A</td>
</tr>
<tr>
<td>Hs-CRP</td>
<td>High density C-reactive protein</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IP</td>
<td>Inflammatory polyarthritis</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>Lipoprotein (a)</td>
</tr>
<tr>
<td>Lp-PLA2</td>
<td>Lipoprotein associated phospholipase A2</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PAT</td>
<td>Peripheral arterial tonometry</td>
</tr>
<tr>
<td>pi-HDL</td>
<td>Proinflammatory-HDL</td>
</tr>
<tr>
<td>PON1</td>
<td>Paroxonase 1</td>
</tr>
<tr>
<td>RA</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>RF</td>
<td>Rheumatoid factor</td>
</tr>
<tr>
<td>RHI</td>
<td>Reactive hyperemic index</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operator characteristics</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 activity</td>
</tr>
<tr>
<td>SMR</td>
<td>Standardised mortality ratio</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>TRF</td>
<td>Traditional risk factors</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low density lipoprotein</td>
</tr>
</tbody>
</table>
Chapter 1

Background

1.1 Introduction

Patients with chronic inflammatory conditions such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) experience premature cardiovascular mortality and morbidity compared with the general population. The increased risk of cardiovascular disease (CVD) might result from an interaction between traditional and non-traditional risk factors, both modulated by chronic inflammation. In the following sections we will review the prevalence of CVD in SLE and RA, the risk factors that contribute to increased burden of cardiovascular events in these populations.

1.2 Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease that affects females of all ages. It predominantly affects women of child bearing age, and thus can have great impact on morbidity and mortality. The
female: male ratio is 9-13:1.

1.2.1 Incidence and prevalence

The incidence and prevalence of SLE vary among different racial groups and between geographic locations. This may be due to multiple genetic and environmental factors. The majority of epidemiological studies to date have been performed in US, Europe and fewer studies in Australia, Africa, Asia and the Middle East.

In the United States, the overall incidence has been estimated to be 1.6-7.6 cases per 100,000. In Europe the incidence ranges from 2.15-4.8 per 100,000 in cohorts from England, Sweden, Iceland and Spain [110]. In all previous studies females had a higher incidence rate than males. The highest incidence rate occurs between ages 15-45 years.

The overall prevalence in the United States ranges from 14.6-50.8 per 100,000 persons. In Europe, the prevalence is slightly lower ranging from 12.5 to 39 per 100,000 persons [110]. A study from Birmingham, UK, demonstrates that the prevalence in Afro-Caribbean is five times the prevalence in Caucasians (111.8 vs 20.7 per 100,000 respectively) [124]. Similarly, patients from Asia have twice the prevalence as Caucasians (46.7 per 100,000 persons) [124].

1.2.2 Clinical features

Systemic lupus erythematosus is a multi-system disease with a wide range of clinical and laboratory manifestations. The clinical symptoms can be constitutional or due to inflammation in various organ systems including skin, joints, kidneys, mucus membranes, lungs, heart and less frequently the gastrointestinal tract.
The involvement of organ systems can be individual or multi-organ. Vital organ involvement is the major cause of morbidity and mortality. Immune system derangement which is a major characteristic of the disease results in over-production of autoantibodies. These antibodies can cause cytotoxic damage, reflect disease activity and/or participate in immune-complex formation. The autoantibody profile is considered part of the classification criteria of SLE.

1.2.3 Clinical manifestations

SLE is characterised by a relapsing remitting nature with multi-organ involvement. It ranges from a mild disease to rapidly progressive life-threatening disease. The features of disease also vary in different individuals. The course of disease is milder and survival is higher in patients with isolated skin and musculoskeletal involvement than in patients with central nervous system or renal disease.

Patients with SLE commonly present with constitutional symptoms such as malaise, overwhelming fatigue, and fever and weight loss. However, those manifestations are not specific for SLE, for example, they may represent an ongoing infection. The fever in SLE is a challenging problem for the physician as it can indicate a flare of the disease, infection, or can be drug related.

Patients with SLE can present with a diverse clinical pictures including rash, arthritis, pleurisy, proteinuria, Raynaud’s phenomena, seizure or pyrexia of unknown origin. Cardiac involvement is one of the most common causes for morbidity and mortality in SLE patients and this will be discussed in details later on. Thus, a definite diagnosis of SLE requires a good history, physical examination and appropriate laboratory confirmation.

The overall survival rate for patients with SLE has improved in the last five
decades, from 50% at 4 years in the 1950s [172] to 70% at 10 years in the 1980s [86] and recently has been reported to exceed 90% at 10 years [37]. This improvement in survival has been attributed to: better use of steroids and immunosuppressive agents, earlier diagnosis and general improvements in overall health care. In spite of this, SLE is still considered a potentially fatal disease. The most common cause of death includes; infection, atherosclerosis, and organ failure due to active disease.

1.2.4 Diagnosis

The diagnosis of SLE is based on clinical and laboratory findings. The specific cause of SLE is unknown. Many factors are thought to be associated with its development including genetic, racial, hormonal and environmental factors. Immune disturbances of both innate and adaptive systems occur in SLE. Production of autoantibodies and immune complexes is a major finding in this disease which is thought to be due to a lack of immune tolerance. The presence of some autoantibodies can also aid in the diagnosis of disease.

The American College of Rheumatology (ACR) developed classification criteria for diagnosis of SLE in 1971 and these criteria have been revised subsequently in 1982 [37] with the last revision by Hochberg [111]. To be classified as definite SLE, patients should have at least 4 out of the 11 criteria (Table 1.1).
<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malar rash</td>
<td>A butterfly rash of flat or raised fixed erythema that affects the malar eminence and sparing the nasolabial fold.</td>
</tr>
<tr>
<td>Discoid rash</td>
<td>Erythematous raised patches with adherent keratotic scaling lesions.</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>Skin rash that results from exposure to sunlight.</td>
</tr>
<tr>
<td>Oral ulcers</td>
<td>Often painless oral or nasopharyngeal ulceration.</td>
</tr>
<tr>
<td>Arthritis</td>
<td>Non-erosive arthritis tenderness, swelling, or effusion affecting 2 or more of the peripheral joints.</td>
</tr>
<tr>
<td>Serositis</td>
<td>Pleuritis or pericarditis</td>
</tr>
<tr>
<td>Renal disorder</td>
<td>Persistent proteinuria &gt; 0.5 g/day or &gt; 3+ or cellular cast.</td>
</tr>
<tr>
<td>Neurologic disorder</td>
<td>Seizures or psychosis in the absence of offending drugs or metabolic disorder.</td>
</tr>
</tbody>
</table>
| Immunologic disorder | Positive LE* cell preparation, anti-DNA, anti-Sm, or anti phospholipid antibody (LAC/APL) *.
| Haematological disorder | Haemolytic anemia, leucopenia, lymphopenia or thrombocytopenia. |
| Antinuclear antibody | Abnormal titre of ANA* at any point in time in the absence of drugs known to be associated with drug induced lupus syndrome. |

Table 1.1: Criteria for diagnosis of SLE adapted from 1997 Updated Revised Criteria for Classification of SLE. Four criteria are required for the diagnosis. *LE= lupus erythematosus, *ANA= anti nuclear antibody. *LAC=lupus anticoagulant, *APL= antiphospholipid antibody.
1.2.5 Assessment of disease activity

The assessment of disease activity is complex and there are more than 60 tools developed for this purpose. None of these tools is considered as a gold standard in this area. The most commonly used measures are the British Isles Lupus Assessment Group (BILAG) and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI).

BILAG is a comprehensive scoring system that assesses disease activity over the preceding four weeks. It was first developed in 1988 and refined later to improve its sensitivity. Further revision led to the development of the BILAG 2004 [291]. This revision summarises disease activity in 9 organ systems and retains the "intention-to-treat" principle to reflect disease activity.

The SLEDAI was first developed in Toronto in 1992 [20] and passed through refinement and modifications. In 2002 Gladman et al. published their modification to SLEDAI (SLEDAI-2K) [87]. SLEDAI 2K is a global scoring system that summarises disease severity as a total score. Each of the 24 items is weighted according to potential severity. Disease activity is summarised over the previous 10 days and in the SLEDAI 2K, persistent disease activity as well as new disease is scored.

1.2.6 Management of SLE

The management of SLE patients is individualised according to the degree of organ involvement and the severity of symptoms. Supportive measures are very important such as smoking cessation, and avoidance of sun exposure. Pharmacological treatment includes non-steroidal anti-inflammatory drugs which are first line therapy for the general and constitutional symptoms and musculoskeletal
manifestations and mild serositis.

Anti-malarial agents such as hydroxychloroquine and chloroquine phosphate are very effective for chronic constitutional symptoms, cutaneous and musculoskeletal symptoms.

Corticosteroids are the mainstay therapy for SLE. The dosing of steroid is varied according to the severity of manifestations. Most manifestations respond very well to steroids. Corticosteroids are some times used in combination with immunosuppressant agents in moderate to severe disease activity. Immunosuppressive agents are very useful in life threatening conditions and can be used as steroid sparing agents.

A new modality of treatment has been introduced over the last decade which coexists with the development of biological agents. Understanding the pathophysiology of SLE has led to the use of agents that target specific parts of the immune system (B-cell depleting agents).

1.3 Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory condition that mainly affects joints. The initial manifestations of RA can be fatigue, pain, morning stiffness and symmetric inflammation of small joints of hands and feet.

1.3.1 Incidence and prevalence

The incidence of RA varies in different populations. A Low incidence rate was reported in France 8.8 per 100,000 [102], while Scandinavian countries have a relatively higher incidence rate 24-36 per 100,000. The highest incidence rate
was observed in the United States 42-45 per 100,000 [110]. The incidence in the UK was reported by studies from the Norfolk Arthritis Register (NOAR) which was established in 1989 [257]. The overall incidence was 36 per 100,000 in women and 14 per 100,000 in men.

In most populations, the overall age-specific annual incidence rate increases with age until the eighth decade then it starts to decline. RA is more common in females. However, the variation in incidence between men and women is dependent on age with a four fold higher incidence rate in women in the 35-44-year age group but almost a similar incidence rate in the 75-84 year age group. The peak incidence in women is 55-64 and in men 75-84 year age group [110]. Data from NOAR reported low incidence of RA in men under 45 years and higher incidence in females at age of 65-74 years and after the age 75 years higher incidence in men than women [257].

The reported prevalence of RA according to the American College of Rheumatology (ACR) criteria ranges from 0.5-1% in populations older than 16 years of age [240]. Low prevalence (<0.2%) was reported in Yugoslavia. In some European countries such as Italy and France, the prevalence was also low at around 0.3%. The observed prevalence in UK is slightly higher at around 0.8% and similarly in Finland. In the UK, the reported prevalence in 2001 using the 1987 ACR criteria was 0.8% [257].

The prevalence of RA is known to differ geographically. For example, a low prevalence (0.3%) has been noted in Saudi Arabia and China [4][49]. A higher prevalence in Japan (1.7%) and Argentina (2.0%) was reported. The highest prevalence was reported in Native-American populations including the Inupiat, Chippewa Indians and Pimma Indians. The prevalence in African populations is relatively low. There are also reports on regional variations of the prevalence of
RA within some countries [110].

There is a considerable variation of incidence and prevalence of RA worldwide. These reports provide some evidence for the contribution of both genetic and environmental factors to the aetiology of RA e.g smoking rate and alcohol consumption.

1.3.2 The aetiology of RA

Rheumatoid arthritis is a multi-factorial complex disease. Risk factors for RA are thought to be a combination of genetic and environmental factors. The contribution of environmental factors is difficult to estimate. The contribution of genetics to RA susceptibility was interpreted from twin studies, family studies and genome wide association studies.

It is estimated that the genetic contribution to the risk is 50-60% [270]. Much research has been carried out to identify the genetic loci associated with predisposition to RA. The HLA-DRB1 ”shared epitope” alleles were first identified in the late 1970s, and then PTNPN22 was recognised in 2004. Approximately 30 genes have now been confirmed to be associated with RA [246]. Many of these alleles however have modest effects on the risk of RA and some may also influence the long-term progression in RA [17].

Smoking is the key environmental risk factor for developing RA [258]. Interestingly, smoking is a risk factor known to be associated with the serological markers of RA namely rheumatoid factor (RF) and anti-citrullinated peptide antibody (ACPA). In other words, associated with sero-positive RA and confers no risk or little risk for seronegative RA [240]. It is also reported that smoking exerts its biological effect on predisposition to RA years before diagnosis. There is an
interaction between smoking and genetic susceptibility (HLA-DRB1) alleles [68].

A recent meta-synthesis by Lahiri et al extensively reviewed the contribution of smoking to the risk for RA [139]. They found that smoking was consistently associated with increased risk for RA in particular in males with RF+ve OR (95% CI) $= 3.91$ (2.78, 5.50), and in females 1.29 (0.94, 1.77). The estimated risk contribution of smoking to RA ranges from 18-25% of the population attributable risk. They also concluded that the effect of smoking is dose related, stronger in males and shared epitope carriers for ACPA+ve RA. Upon smoking cessation there is a 20 years latency in risk to be back to the baseline risk.

Other variables such as lower alcohol intake, increased coffee intake, low vitamin D status, non-use of oral contraceptive and socioeconomic status, may be factors although the supporting evidence is not as strong for these as smoking [147]. Lahiri and colleagues also noted a protective role for antioxidants and breast feeding [139]. On the other hand, coffee intake was associated with increased risk, three studies in their review showed an inverse association with alcohol intake and higher education.

### 1.3.3 Clinical features

Patients with RA present with polyarthritis involving the small joints of the hands and feet, although mono-articular presentation can occur initially. Around half of the patients present with rheumatoid factor/anti-citrullinated peptide antibodies (RF/ACPA) even prior to diagnosis of RA. Most patients experience morning stiffness that persists for more than one hour. Patients may present with difficulties performing daily activities and have reduced grip strength. Profound fatigue and anorexia are other common manifestations. Extra-articular manifestation includes subcutaneous nodules, serositis, vasculitis, episcleritis, glumerulonephritis
and rheumatoid lung disease.

1.3.4 Diagnosis of RA

The diagnosis of RA is based on the clinical history and physical examination. Classification criteria for RA have been developed and were designed to identify patients with RA and distinguish them from other inflammatory arthropathies. The American College of Rheumatology (ACR) 1987 criteria have typically been used for classification of patients. According to the ACR criteria, patients must have 4 or more of the following to be diagnosed with RA:

1. Morning stiffness $>2$ hours.
2. Arthritis of three or more joint areas.
3. Arthritis of hand joints.
4. Symmetrical arthritis.
5. Rheumatoid nodules.
6. Positive Rheumatoid Factor (RF).
7. Radiographic changes typical for RA which includes erosions and/or osteopenia.

The first four clinical features should have been present for at least 6 weeks. We will use these criteria for classification of the cohort in this study. Recently, a set of new criteria developed by the ACR and the European League Against Rheumatism (EULAR) has been published [8]. These criteria are meant to identify RA at an early stage and used mainly for risk stratification of patients with high risk
<table>
<thead>
<tr>
<th>Joint involvement</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 medium-large joint</td>
<td>0</td>
</tr>
<tr>
<td>2-10 medium-large joints</td>
<td>1</td>
</tr>
<tr>
<td>1-3 small joints</td>
<td>2</td>
</tr>
<tr>
<td>4-10 small joints</td>
<td>3</td>
</tr>
<tr>
<td>More than 10 small joints</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serology</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>RF(-) and anti-CCP(-)</td>
<td>0</td>
</tr>
<tr>
<td>RF(+) or anti-CCP(+)</td>
<td>2</td>
</tr>
<tr>
<td>High RF(+) or anti-CCP(+)</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Duration of symptoms</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6 weeks</td>
<td>0</td>
</tr>
<tr>
<td>≥ 6 weeks</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Acute phase reactants</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP and ESR within normal</td>
<td>0</td>
</tr>
<tr>
<td>Elevated CRP or ESR</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1.2: The updated ACR/EULAR 2010 criteria for RA diagnosis.

for erosions. They are a point based system with a cut point of 6 or more for RA. The criteria are shown in (Table 1.2). In this study, we used the ACR 1987 criteria as the EULAR criteria were published after the study was established.
### 1.3.5 Disease activity

Assessment of disease activity in RA is based on clinical examination and enumeration of tender and swollen joints as well as global assessments that estimate disease activity and health status. Measurements of acute phase response such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are also included in a number of scales. The most widely used scale is the 28-joint count of swollen and tender joints. Disease activity is estimated by calculating the DAS-28 (disease activity score) which is based on 28-joint counts of tender and swollen joints, markers of inflammation e.g. ESR or CRP at the time of presentation and a patient global health visual analogue scale. The interpretation of this scoring are listed in (Table 1.3).

### 1.3.6 Management of RA

As in SLE, the nature of organ involvement and the severity of manifestation will guide the physician to the treatment. Non-pharmacological treatment includes patient education. Pharmacological treatment includes the use of non-steroidal anti-inflammatory drugs (NSAIDs). These agents provide anti-inflammatory therapy for the patient.

Glucocorticosteroids are potent anti-inflammatory drugs. In RA, short term use of low doses of steroids improves disease activity and reduces the joint damage.

<table>
<thead>
<tr>
<th>DAS-28 score</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAS \leq 3.2</td>
<td>Low disease activity</td>
</tr>
<tr>
<td>3.2 &lt; DAS \leq 5.1</td>
<td>Moderate disease activity</td>
</tr>
<tr>
<td>DAS &gt; 5.1</td>
<td>High disease activity</td>
</tr>
<tr>
<td>DAS &lt; 2.6</td>
<td>Remission</td>
</tr>
</tbody>
</table>

Table 1.3: Interpretation of DAS-28 score.
Different ways of administrating the drug can be used (oral, injection or intra-articular). However, the side effects are common and they are dose-dependent. Methotrexate (MTX), an analogue of folic acid, is currently considered the first line agent for management of RA and the anchor drug for combination therapy. It is widely used and, when monitored carefully, has a very good efficacy and is well tolerated.

For patients who do not respond well to MTX, other disease modifying antirheumatic drugs (DMARDs) are added to the regime of their management. Examples of traditional DMARDs include antimalarials, sulfasalazine, IM gold salts and leflunomide.

In the last decade, biological therapies has been introduced to the management of RA. Agents targeting both B cell and T cell can be used in RA. Inhibition of TNF with monoclonal antibodies is an example of a specific cytokine inhibition. There is an increased interest in using biological agents in patients with active refractory disease. It is thought that these agents would have less side effects than general immune suppression. Randomized controlled trials have shown these agents are efficacious in RA; and their effects on radiological changes also suggests important disease modifying properties. Details about these agents and their effect on the risk of CVD will be discussed in more details in the relevant section.

1.4 Cardiovascular disease in SLE

In 1976, Urowitz et al introduced the concept of the "bimodal mortality" in SLE suggesting that the early deaths in patients with SLE (2-5 years) were due to active lupus or infection and late deaths (>5 years) due to atherosclerotic cardiovascular disease [268]. Other studies confirmed that accelerated atherosclerosis
was a significant cause of morbidity and mortality in SLE [2][155][224].

The overall incidence of CHD in SLE patients is approximately 5-6 times that of the general population. Surprisingly, SLE patients aged between 35-44 year old are at 50-fold increased risk of myocardial infarction compared to the general population [155]. Studying factors that contribute to CHD in SLE are challenging. Prospective clinical studies are the ideal but are, by nature, long-term and require a large number of subjects. Several such studies including these from the Toronto and Baltimore cohorts have been conducted [30][198]. Case control studies have also been undertaken including one in the UK [107] detailed in the following section. Therefore, much research has employed surrogate markers for clinical CHD to investigate the contributing factors to the development and progression of atherosclerosis in SLE.

1.5 Subclinical atherosclerosis in SLE

The prevalence of subclinical atherosclerosis is also increased in SLE patients compared to healthy controls with up to 40% of SLE patients having signs of subclinical disease [156][28]. This has been noted using a range of surrogate markers of subclinical disease including Single Photon Emission Computed Tomography Dual Isotope Myocardial Perfusion Imaging [27], coronary artery calcification score (CAC)[126][210], ankle brachial pressure index [232], carotid intima media thickness (cIMT), pulse wave velocity and flow mediated dilatation (FMD) [63]. Both cIMT and FMD will be used in this study on different cohorts of patients and will be discussed in more detail and the methods will be described fully in the Method section.

Carotid IMT is widely used as a surrogate marker for atherosclerosis. It has
been reported that cIMT improves the risk prediction of CHD when added to traditional risk factors [179]. The advantage of this technique is that it is non-invasive, reproducible, inexpensive, and radiation free. This facilitates its use as a research tool, thus understanding its role in CVD prediction might eventually also lead to its use as a screening tool.

A cross sectional study in Manchester by Ahmad et al including 200 SLE patients and 100 healthy controls reported that SLE patients aged < 55 years have a significant increase in plaque prevalence [3]. There was no significant difference in the cIMT between SLE patients and healthy controls despite the controls in this study being significantly older (deliberately chosen by design of the study) than patients. The predictors of sub-clinical atherosclerosis in SLE patients were slightly different from the healthy controls in this study; this will be further discussed later in more details in the risk factors section.

With regards to atherosclerosis progression, Haque et al has shown that SLE patients had greater change in the cIMT than would be expected in healthy females [107]. Factors associated with cIMT progression were older age, metabolic syndrome and low HDL-cholesterol. This is in agreement with a recent longitudinal study that reported a significant increase of cIMT after two years follow up of SLE patients [223]. Age, baseline cIMT, complement level (C3, C5a) and homocysteine were associated with this increase. Thompson et al from the Pittsburgh Lupus Registry also found SLE patients had increased plaque prevalence and accelerated progression compared with healthy controls after around four years of follow up [261]. Among SLE related factors: high serum C3 levels and use of immunosuppressant were related to plaque progression independently from traditional risk factors (TRFs).
1.6 Mortality in rheumatoid arthritis

Rheumatoid arthritis was considered a disease of great suffering but with little impact on survival until 1953 when Cobb et al noted that patients with RA died earlier than individuals without RA [45]. The causes of mortality in RA patients include infection, renal, gastrointestinal and pulmonary disease. A recent review by Symmons [256] suggested that around 40% of all death is due to cardiovascular causes including IHD and stroke. This is similar to the proportion of death in the general population due to CVD. This finding confirms that RA patients die from the same causes as the general population but at an earlier age (prematurely).

Cardiovascular mortality has been extensively studied over the past years, 50% of premature deaths in RA were attributed to CVD. A recent meta-analysis included 17 studies reporting CV mortality in which a total number of 91,916 patients were analyzed [173]. The overall standardised mortality rate (SMR) was 1.6 (95% CI 1.5, 1.8). This is in agreement with an earlier meta-analysis of 24 studies that also showed increased CV mortality in RA patients (SMR 1.5 95% CI 1.39-1.61) [15]. It can be concluded that there is a 50-60% increase in the risk of cardiovascular mortality in RA patients compared to the general population. Despite major changes in the management of RA over the past few decades, the SMR for CV mortality remained increased and unchanged.

Several explanations for increased CVD mortality in RA exist;

a A higher burden of atherosclerosis

Del Rincon and colleagues studied 204 RA patients and 102 age and sex-matched healthy controls [55]. The age range was 40-83 years and 89% were females. There was a trend towards higher plaque prevalence and cIMT in RA patients but this did not reach statistical significance. On the other
hand, Roman et al studied 98 RA patients aged 20-80 years and 98 age, sex, and ethnicity matched healthy controls [219]. They found that RA patients have significantly higher plaque prevalence (44% vs 15%) compared to their peers. However, there was no significant difference in the cIMT between the two groups.

The contrast between the two studies in plaque prevalence can be explained by the difference in age of the RA patients as it is indicated from the literature that younger individuals are at higher risk of CVD. With regards to cIMT, in Roman’s study they excluded areas of plaque from their measurements of cIMT which made the cIMT lower in RA patients.

b A higher CVD event rate and/or more ”silent event”

Del Rincon et al conducted a study to compare the incidence of cardiovascular events in RA patients compared to the general population [54]. They studied 236 patients with RA and compared them to participants aged 25-65 in an epidemiological study of atherosclerosis and CVD. Outcomes were defined as any cardiovascular related hospitalization including MI, stroke and occlusive arterial events or arterial revascularization. The other outcome measure was CV death. In this study, patients with RA have an almost four-fold increased risk of CV events, the incidence rate ratio (IRR) being 3.96 (95% CI 1.86, 8.43). Adjusting for the cardiovascular risk factors, just slightly reduced the IRR to 3.17. Solomon et al 2003 used myocardial infarction as the outcome measure in the Nurses Health Study (NHS) and they found an increased incidence of CV events in RA patients [244]. This result has been replicated by other studies in different RA cohorts. In patients with rheumatoid arthritis, there is also increased risk of unrecognized coronary heart disease (silent event) as well as increased
sudden death [157].

RA patients often experience silent events i.e ischemic heart disease with no signs or symptoms preceding the sudden cardiac death. Sudden cardiac death in RA patients was reported to be almost twice that of the general population HR (95% CI)= 1.99 (1.06, 3.55) [157].

c More severe outcomes from events (case-fatality)

One of the explanations for increased CV mortality in RA patients is increased death rate after an acute event such as myocardial infarction. Van Doornum et al reported that the 30-day case-fatality rate following myocardial infarction in RA patients is almost twice that of the general population [271]. The cause for this increased fatality was described by the same author in a subsequent study and attributed to the discrepancies of treatment between RA patients and general population. The former received less frequent medical care [272].

In conclusion, RA patients have higher mortality than the general population. The main cause of mortality is CVD. Patients with RA have more frequent cardiovascular events, but also more severe incidents with higher case-fatality rates compared to the general population.

1.7 Do patients with RA have increased CVD risk prior to diagnosis?

Of particular interest also is the timing of this increased risk. The results in the literature are controversial regarding this aspect. Holmqvist et al compared the risk of CVD in two Swedish cohorts [113]. There was no apparent increase
in the odds ratio for CVD prior to the onset of RA. In contrast, a previous study has shown that patients with RA had an increased prevalence of MI and subclinical atherosclerosis at the time of diagnosis [157]. Although in this study fulfilling the ACR criteria was the date of diagnosis, which may have meant that synovitis for some time prior to the diagnosis have accumulated the risk over that period. Holmqvist et al studied 7469 patients from the Swedish National Register and reported an increased relative risk of MI (RR= 1.6 95% CI 1.4, 1.9) in the first five years of disease [112]. Franklin et al reported increased hospital admissions in patients with recent onset inflammatory arthritis compared to the general population after a median follow up period of 7 years, the relative risk for hospitalisation due to CVD being 2.0 (95% CI 1.4, 1.9) [75].

Therefore, it is likely that CVD risk is increased from early in disease especially in younger patients who may already have accumulated the risk even before the diagnosis. Further work is needed to determine whether such risk actually precedes the disease onset in patients with RA preferably at earlier stages of arthritis. Table (1.4) gives a summary of the studies reporting the increased risk of CVD in RA patients.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Outcome definition</th>
<th>Follow up</th>
<th>Absolute risk RA/non RA</th>
<th>Relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Del Rincon, (US) ([54])</td>
<td>Self reported hospitalization CV event, chart verified</td>
<td>1996-1997</td>
<td>0.3/0.06/1,000 py</td>
<td>IRR: 3.96(1.86, 8.43)</td>
</tr>
<tr>
<td>Solomon, (US) ([244])</td>
<td>Self reports of MI, chart verified</td>
<td>Confirmed diagnosis of RA-death/ event of (31 may 1996)</td>
<td>2.7/1.01/1,000 py</td>
<td>IRR 2.0(1.23, 3.29)</td>
</tr>
<tr>
<td>Goodson, (UK) ([98])</td>
<td>Hospitalizations report of MI, IHD</td>
<td>1 April 1994-death or admission (31 March 2002)</td>
<td>NA</td>
<td>IRR for MI: 1.3(0.6, 2.5), IHD 0.8(0.5, 1.3)</td>
</tr>
<tr>
<td>Maradit-Kremers, (US) ([157])</td>
<td>Hospitalization MI, unrecognized MI detected by chart review</td>
<td>Fulfilment of ACR criteria for RA-death, events (January 2001)</td>
<td>Hospitalized: 4.0/4.6/1,000py Unrecognized: 2.7/1.5/1,000 py</td>
<td>HR: hospitalized: 1.09(0.71, 1.68), unrecognized: 2.13(1.13, 4.03)</td>
</tr>
<tr>
<td>Solomon, (Canada) ([243])</td>
<td>Hospitalization MI</td>
<td>3rd admission for RA-event, death (end of 2003)</td>
<td>Incidence rate: 5.3/2.9/1,000 py</td>
<td>IRR: 1.9(1.7, 2.0)</td>
</tr>
</tbody>
</table>

Table 1.4: Studies reporting the risk for cardiovascular morbidity in RA patients. py; Person years of follow up, IRR; incidence rate ratio, HR; hazard ratio, NA; data not available.
1.8 Risk factors for CVD

1.8.1 Traditional risk factors for cardiovascular disease

There are certain factors that are associated with the future development of cardiovascular disease in the general population. Many of these factors were deduced from the large community-based studies such as the Framingham study in the USA. The Framingham Heart Study aimed to identify common risk factors associated with the development of CVD (http://www.framinghamheartstudy.org/).

From this and other pivotal studies several potentially modifiable risk factors have been identified including dyslipidemia, hypertension, obesity, physical inactivity, diabetes and cigarette smoking. A number of non-modifiable risk factors including age, gender (male) and family history in a first degree relative (male under age of 55 and females <65 years) were associated with increased CVD risk (http://www.bhf.org.uk). The precise attributable risk of each factor is controversial. However, according to the Framingham study, a combination of 6 risk factors contributes to 50% of the coronary risk in a population. In the INTERHEART study, which is a standardised case-control study of acute myocardial infarction in 52 countries, a combination of 9 risk factors accounted for 90% of the attributable risk [293]. Other novel factors including genetic markers and additional biomarkers are also being actively explored.

Several studies have been conducted to determine the prevalence of these factors in SLE and RA and their contribution to the CVD burden.
1.8.2 Traditional risk factors in SLE and RA

This topic has been thoroughly researched and lots of evidence is available from the literature. In the following sections, we will give a summary of the evidence available for the prevalence of traditional risk factors in SLE/RA, their association with CVD in these patients and to what extent these factors can explain the risk of CVD and CVD mortality.

1.8.3 Do SLE patients have more risk factors?

Several studies have been conducted to determine the risk factors for cardiovascular disease in SLE and to estimate the contribution of these factors to cardiovascular events.

Svenungsson and colleagues have identified dyslipidemia as a more prevalent traditional risk factor in SLE patients with a history of CVD (and who have increased cIMT) compared to age-matched SLE patients without a previous history of CVD and to healthy controls. There was no significant difference in smoking, BP, diabetes, BMI between cases and controls [255].

Bruce et al reported that hypertension and diabetes were more prevalent in SLE patients than in healthy controls in the Toronto Risk Factor Study [30]. SLE patients also had higher levels of triglycerides and VLDL, premature menopause and a more sedentary life style compared to healthy controls. However, the 10-year risk of CHD related events was similar in both patients and controls. Summary of the studies reporting risk factors is shown in Table (1.5).

In conclusion, patients with SLE have higher prevalence of traditional risk factors, the contribution of these factors in the Framingham risk formula being different from their contribution in the general population.
<table>
<thead>
<tr>
<th>Author</th>
<th>CV outcome</th>
<th>Traditional risk factors</th>
<th>Lupus-related, other factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manzi (1997) ([154])</td>
<td>CHD</td>
<td>↑cholesterol, post-menopausal.</td>
<td>↑age at diagnosis, ↑disease duration, ↑duration of steroid use.</td>
</tr>
<tr>
<td>Petri (1992) ([200])</td>
<td>CHD</td>
<td>↑BP, ↑cholesterol</td>
<td>↑age at diagnosis, ↑disease duration, ↑duration of steroid use.</td>
</tr>
<tr>
<td>Svenungsson (2001) ([255])</td>
<td>CHD, stroke, PVD</td>
<td>↑VLDL, LDL, Lp(a), ↓HDL</td>
<td>ESR, CRP, lupus anticoagulant, homocysteine, osteoporosis, cumulative steroid dose.</td>
</tr>
<tr>
<td>Urowitz (2007) ([267])</td>
<td>CHD, stroke, PVD</td>
<td>↑BP, ↑cholesterol, smoking, number of traditional factors.</td>
<td>Raynaud’s, renal disease, neuropsychiatric disorder, vasculitis, ↑prothrombin time, steroid or immunosuppressive treatment.</td>
</tr>
<tr>
<td>Haque (2010) ([107])</td>
<td>CHD</td>
<td>↑BP, ↑cholesterol, Male gender</td>
<td>SDI, azathioprine, steroid therapy.</td>
</tr>
</tbody>
</table>

Table 1.5: Summary of the studies of risk factors for cardiovascular events in SLE patients. CHD= coronary heart disease, BP= blood pressure, TG= triglycerides, DM= diabetes melitus, CHF= congestive heart failure, PVD= peripheral vascular disease, VLDL= very low density lipoprotein, LDL= low density lipoprotein, Lp(a)= lipoprotein (a), SDI= SLE damage index.
1.8.4 Do traditional risk factors influence cardiovascular risk in SLE?

Several case-control studies have indicated that traditional risk factors are associated with the development of atherosclerotic disease in SLE. The following section will tackle some of these studies in more details.

Doria et al evaluated the association between traditional and non-traditional risk factors and subclinical atherosclerosis in a prospective cohort study including 78 SLE patients [57]. Outcomes for this study were cIMT and plaque. Patients were assessed at baseline and 5 years after follow up. The strongest predictor variables were age and hypertension. Hypercholesterolemia was also a predictor variable in univariable analysis. A UK wide multicenter case control retrospective study has confirmed that SLE patients with clinical CHD were older mean (SD) 53 (10) vs 42 (10) years $P < 0.001$, more likely to be male (20% vs 7%), and had more exposure to all traditional risk factors compared to SLE patients without CHD [107]. Other disease related factors were identified and will be discussed later.

1.8.5 Do traditional risk factors fully explain excess risk in SLE?

A prospective cohort study by Esdaile and colleagues found that patients with SLE are at 7.5-17 fold excess risk of cardiovascular risk even after adjusting for the baseline Framingham risk confounders [66]. Although patients with SLE have a higher prevalence of traditional risk factors, excess CVD morbidity and mortality risk remain after adjusting for the traditional risk factors.
In a case-control study by Ahmad et al, traditional risk factors perform less well in risk prediction of subclinical atherosclerosis in SLE patients than healthy controls [3]. The model including age, smoking, and systolic blood pressure give an area under the curve-receiver operating characteristic (AUC-ROC= 0.90 in controls and 0.75 in SLE patients). Adding SLE related factors significantly improves the model (AUC-ROC= 0.87, P<0.01).

There are other factors that render patients with SLE at high risk of CVD such as early menopause, renal impairment, hyperhomocysteinaemia, systemic inflammation and longer disease duration [30][26][184].

### 1.8.6 Do patients with RA have more traditional risk factors?

There are several reports in the literature reporting the prevalence of traditional factors in patients with RA. A prospective nested case-control study by Goodson et al examined the prevalence of baseline traditional risk factors (TRF) in patients with early inflammatory polyarthritis (IP) compared to age and sex-matched controls [98]. Smoking was significantly higher in patients with IP. There was a trend towards increase diabetes mellitus but this did not reach statistical significance. On the other hand, another study reported lower alcohol intake and higher past smoking in females with RA compared to those without RA [243]. In this study, there was no significant difference in other TRF including current smoking, hypertension, diabetes mellitus, physical inactivity, family history or lipid profile.

Boyer and colleagues conducted a meta-analysis looking at the difference in the prevalence of TRFs between patients with RA and healthy controls [25]. They included 15 case-control studies in their analysis. They found that patients
with RA have a higher prevalence of smoking compared to their counterparts OR (95% CI) 1.56 (1.35, 1.80). There was also a higher prevalence of diabetes mellitus in RA patients OR (95% CI) 1.74 (1.22, 2.5) and a higher prevalence of reduced HDL in RA [24]. No statistical difference in other TRFs was observed. Results from 196 patients with early inflammatory polyarthritis from the Norfolk Arthritis Register has shown that insulin resistance was associated in addition to the classic risk factors (obesity, blood pressure, triglycerides and HDL), as was tender joint count, and HAQ score ($\beta$ coefficient (95% CI); 0.029 (0.002, 0.056), and 0.709 (0.237, 1.182) respectively. It was also significantly and independently associated with the serological status of the patients ($\beta$ coefficient (95% CI)) for RF = 0.867 (0.204, 1.530) and for ACPA = 1.423 (0.701, 2.146)[175]. Summary of the studies reporting risk factors in RA patients is shown in Table (1.6).

In summary, patients with RA have higher prevalence of some TRFs. There are contrasting results between different studies owing to the heterogeneity in study designs and different study populations and possibly also according to the stage of disease being studied.
<table>
<thead>
<tr>
<th>Author</th>
<th>CV outcome</th>
<th>Traditional risk factors</th>
<th>RA-related, other factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chung (2005) ([43])</td>
<td>CAD</td>
<td>Unadjusted model age, male sex, ↑ systolic BP, smoking</td>
<td>ESR.</td>
</tr>
<tr>
<td>Maradit-Kremers (2005) ([159])</td>
<td>CVD mortality</td>
<td>BMI.</td>
<td>NA.</td>
</tr>
<tr>
<td>Myasedova (2010) ([178])</td>
<td>CVD, heart failure</td>
<td>↑ TG, HDL, ↓ TC</td>
<td>ESR, CRP.</td>
</tr>
<tr>
<td>Han (2006) ([104])</td>
<td>IHD, CVD, PVD, CHF, atherosclerosis</td>
<td>Hypertension, diabetes, hyperlipidemia.</td>
<td>NA.</td>
</tr>
<tr>
<td>Roman (2006) ([218])</td>
<td>Subclinical atherosclerosis</td>
<td>Age, hypertension, dyslipidemia.</td>
<td>Age at diagnosis, use of biological therapy.</td>
</tr>
</tbody>
</table>

Table 1.6: Summary of the studies of risk factors for cardiovascular events in RA patients. CAD= coronary artery disease, IHD= ischemic heart disease, BP= blood pressure, TG= triglycerides, CHF= congestive heart failure, PVD= peripheral vascular disease, HDL= high density lipoprotein, TC= total cholesterol
1.8.7 Do traditional risk factors influence CV risk in RA?

The effect of TRFs on the cardiovascular outcome in RA has been investigated. We will discuss some of the studies that reported this influence. It has been reported that age and male gender were predictors of early (within 5 years) and late (10 years) all-cause and cardiovascular mortality in patients with early inflammatory polyarthritis [182]. Another study has reported an interaction between smoking and genetic predictors of RA (shared epitope) and ACPA all of which were associated with the highest risk of cardiovascular death [68].

With regards to the association with cardiovascular morbidity, age and male gender were reported to be associated with all vascular hospitalisation in patients with early onset inflammatory polyarthritis [75].

Some traditional risk factors have an adverse effect on the risk for CVD as compared to the general population. This is described in the literature as a paradoxical effect. Two factors have been reported to have such effect; namely obesity and dyslipidemia. Dyslipidemia will be discussed in more details later.

With regards to obesity, it would be expected that patients, with a chronic disabling disease such as RA, will do less physical activity and hence will gain weight. While being overweight and obesity are known risks for CVD in general population, in RA the opposite has been observed in some studies. This was reported by a study investigating the effect of body mass index (BMI) on the cardiovascular death. They found an inverse association between BMI and mortality [65]. Maradit Kremers et al [161] and Gonzalez 2008 [93] also noted that having a BMI <20kg/m² was associated with increased CVD morbidity and a 3-folds increased risk of mortality.

In RA low BMI might indicate uncontrolled, active systematic inflammation
and thus, support the hypothesis that inflammation plays a major role in the pathogenesis of CVD. Another explanation may be that, in RA patients, there is a profound alteration of body composition and this is not reflected by the BMI threshold. It is apparent that patients with RA have reduced muscle mass and increased fat mass which increases the fat: muscle ratio and this may be reflected as a deleterious effect of low BMI on the outcome measure. There is also some evidence of the variation in the distribution of the visceral fat area and subcutaneous fat area in RA patients compared with healthy controls despite similar BMI and waist circumference [85]. Higher visceral fat area (above 75th percentile) was associated with elevated fasting glucose level, hypertension, other composites of metabolic syndrome. It was also associated with more severe disease in terms of RF positivity, higher steroid dose, and higher CRP.

1.8.8 Do traditional risk factors fully explain excess risk in RA?

Del Rincon and colleagues conducted a study in which they found, after adjusting for classic risk factors (age, sex, diabetes, hypertension, body mass index, smoking and hypercholesterolemia), RA remained associated with a three-fold increase in the incidence rate of CVD IR= 3.17 (95% CI 1.33, 6.36) [54].

It has been reported that the estimation of the absolute risk by age group is more informative than estimating the relative risk in patients with RA [158]. The absolute cardiovascular risk in patients with RA is equivalent to the risk in healthy individuals who are 5-10 years older.

Maradit-Kremers suggested that the relative contribution of different risk factors is less strong in RA than their influence on the general population. The
chronic inflammatory state might have a dilution effect on the TRFs. This make the contribution of each individual risk factor appear less significant or have an adverse effect on the risk of CVD. Thus, a risk score based on TRFs alone might underestimate the cardiovascular risk in patients with RA. There is a recent EU-LAR suggestion of multiplying the risk calculated by Framingham formula by a factor of 1.5 to determine the risk in RA patients [194]. In particular, patients with disease duration > 10 years, RF/ACPA positive and/or patients with extra-articular manifestation.

1.8.9 Overview of SLE, RA traditional risk factors

1. The importance of premature atherosclerosis in SLE and RA is well documented. Both population and cohort based studies have confirmed this and it is well replicated in most publication. Generally speaking, there are fewer studies in the epidemiology of CVD in SLE than in RA. This is due to the fact that SLE is less prevalent than RA. There is also a lack of large cohort studies that study the cause specific mortality in SLE patients. The majority of CV morbidity and mortality data were obtained from a few large cohorts in North America and Toronto. Much of the research in SLE is focused on subclinical atherosclerosis.

2. Atherosclerosis and atherosclerotic consequences are the main cause of late death in SLE. Similarly, in RA, it contributes to the mortality in half of RA patients and appears to begin early in the course of disease. This is similar to the occurrence in the general population but occurs at an earlier age. The incidence rate of MI and angina is up to 50-fold higher in young SLE patients than healthy controls. In RA, the observed incidence of MI is only two times higher than general population.
3. One explanation for this is that RA patients tended to be generally older than SLE patients and hence there is not such a big gap in the risk between RA patients and healthy people. With regards to subclinical atherosclerosis, studies have shown increased prevalence in both SLE and RA compared with age and sex matched healthy individuals. Certain conventional risk factors seem to be increased in both SLE and RA. However, there is a wide range in their prevalence among different studies owing to the different design and study populations. The exact contribution of these factors to increased risk of CVD remains controversial.

4. Traditional risk factors therefore do not explain fully the increased prevalence of cardiovascular morbidity and mortality in patients with SLE/RA (Tables 1.5 and 1.6). There is increasing evidence for the presence of factors other than traditional factors that may contribute to the development of cardiovascular events in SLE and RA in particular inflammation associated factors. The cardiovascular events in patients with SLE/RA may be the result of exposure to a combination of genetic and environmental factors in patients ’set’ to be at high risk due to the inflammatory process responsible for the pathogenesis of these diseases.

In the following sections, I will consider more disease specific factors that contribute to CVD/atherosclerosis risk in SLE/RA.
1.9 Disease related factors

1.10 Chronic inflammation in the general population

In the general population, markers of the acute phase response such as high sensitivity C-reactive protein (hsCRP) were hypothesized to be associated with clinical and subclinical CVD.

CRP was identified as an independent risk marker for CVD by Ridker et al. Elevated levels of CRP were reported to be associated with first, future and fatal CVD events independent of traditional risk factors [211][216]. The precise contribution of CRP to risk prediction in the general population is controversial. A study by Ridker et al found that CRP was the strongest predictor of CVD compared to traditional risk factors [216]. In contrast, Danesh and colleague reported that adding CRP to the model of risk prediction (TRFs) has a modest effect and certainly less than the TRFs [50]. In this study, the odds ratio of CRP for having CVD was around 1.5 which was higher than other inflammatory markers but less than high cholesterol, hypertension and smoking status.

Recently, data published from the Mendelian study by Zacho et al have added to this debate [294]. They found that patients with CRP >3 mg/l have a 1.6 times increased risk of having ischemic heart disease compared to patients having CRP <1 mg/l. On the other hand, patients with a genetic polymorphism associated with elevated CRP (64% higher CRP) did not have an increased risk of ischemic heart disease. This may indicate that a constant, high level of CRP has no causative role in CVD.
The influence of RF on CVD and mortality in the general population has been investigated [60]. They found that RF was associated with increased risk for IHD HR (95% CI) = 3.1 (1.7, 5.4) and the adjusted HR 2.9 (1.6, 5.3). Another population-based study, had noted that RF was associated with increased all-cause mortality (HR 1.47, 95% CI 1.19, 1.80), cardiovascular mortality (HR 1.57, 95% CI 1.15, 2.14) age and gender adjusted [262]. This was modestly attenuated by adjusting for TRFs and ESR. There was increased CHD in participants who were RF positive but this was not statistically significant. The same trend of association was observed in patients with RF who have no signs of joint involvement. Although patients with joint symptoms were excluded from this analysis, this does not mean that all patients with RA were excluded. Of particular note, RF was measured by an old method that requires subjective interpretation and has poor reproducibility. Further studies to determine the association between RF and CVD in the general population are required.

With regards to ACPA association with CVD in the general population, I could not find a report on the association between ACPA and CVD in the general population.

1.10.1 Chronic inflammation in SLE

SLE represents the classic model for chronic immune complex mediated inflammatory disease of the blood vessels. The association between inflammatory mediators and atherosclerosis (both clinical and subclinical) has been reported. A study investigating the risk for CVD in Toronto noted that previous inflammation of the lung was a predictor of cardiovascular events in multivariable analysis [29]. It has been reported that patients with a previous history of pericarditis have impaired myocardial perfusion [117]. Elevated CRP and C3 were also linked to
clinical and subclinical atherosclerosis in patients with SLE [255].

A UK wide multicenter study suggested that SLE patients with a history of clinical CHD had higher SLICC damage index OR (95% CI) 2.2 (1.09, 4.44) and were more likely to be treated with steroid 2.46 (1.03, 5.88) and azathioprine 2.33(1.16, 4.67) [107]. This indicates that patients with active and persistent inflammation who have accumulated more organ damage are at higher risk of developing CVD compared with patients with mild disease or in remission. Those patients need good control of their disease and regular assessment.

Antiphospholipid syndrome is an autoimmune condition characterized by thrombotic events and repeated miscarriages. Antiphospholipid syndrome is prevalent (30-40%) in patients with SLE. This condition is characterized by production of auto-antibodies against phospholipids (aPL) including cardiolipin (anti-cardiolipin) and $\beta$-glycoprotein I. These antibodies have pro-thrombotic properties. Serum lipoproteins contain phospholipids which make them a target for aPL antibodies.

As indicated by the definition of this condition, it would be expected that patients with aPL/anti-cardiolipin will be prone to increased risk of clinical atherosclerosis. This was confirmed by Vaarala et al in a nested case-control study [269]. Patients who developed MI had anticardiolipin antibody (ACLA) at baseline.

1.10.2 Chronic inflammation in RA

Increased erythrocyte sedimentation rate (ESR) a year after diagnosis of RA was associated with an increased risk of CVD. This association between inflammation and CVD in RA has been replicated by many studies. Maradit-Kremers et
al demonstrated the association between ESR (three or more measurements) and cardiovascular death [160]. Similarly, Goodson noted that CRP levels more than 4 mg/l at baseline were associated with an increased risk of cardiovascular death during the follow up period [98]. Gonzalez-Gay et al also demonstrated that high levels of both ESR and CRP were associated with both cardiovascular mortality and morbidity [94]. Although this has been convincingly replicated, a few studies have failed to demonstrate a similar association [127]. The contrast between different results could be explained by different settings and different study groups. However, overall the evidence support an association between inflammation and CVD.

Goodson et al also showed that RF positivity was associated with a 3-fold increased risk of cardiovascular mortality after adjusting for age, sex, disease severity and smoking [99]. The association between genetic risk factors for RA and increased risk for CVD was first published in 2007 by Gonzalez-Gay et al who investigated the role of HLA-DRB1 on both CVD mortality and morbidity and found that having a single or double copies of HLA-DRB1*0404 was associated with the first CV event and cardiovascular mortality [94]. Earlier on, an association between HLA-DRB1*04 and risk of endothelial dysfunction, which is thought to be the earlier stage of CVD, (was reported) in RA [97].

In 2008, Farragher et al demonstrated an association between HLA-DRB1 alleles and cardiovascular mortality [68]. In their study, they found that carrying two copies of HLA-DRB1*01/04 was associated with a two-fold increased risk of cardiovascular death compared with having one or no shared epitope allele. They also reported an interaction of smoking, shared epitope alleles and anti-citrullinated peptide antibodies (ACPA). This interaction was associated with the highest CVD risk of mortality from CVD HR (95% CI)= 7.81 (2.6–23.2).
They also studied the effect of PTPN22 and no association with cardiovascular mortality was noticed. A recent study by Palomino-Morales et al indicated that patients with RA who have a polymorphism in the MTHFR gene have a significantly increased risk of suffering cardiovascular events compared with those who do not have this polymorphism [189]. Data from the Norfolk Arthritis Register (NOAR) suggest that patients with early inflammatory arthritis who were RF+ve have HR (95% CI) = 1.99 (1.3, 3.0) for CVD mortality and similarly patients with nodules 2.28 (1.2, 4.5) [182]. Both factors are indicators of higher disease severity.

1.10.3 Mechanisms by which chronic inflammation, in the general population and in SLE/RA, lead to atherosclerosis and CVD

Several investigators have proposed that CVD, endothelial dysfunction and atherosclerosis are triggered by chronic injury to the endothelium, followed by invasion of inflammatory cells and lipid deposition. In other words, atherosclerosis is the result of an interplay between three factors namely; endothelial injury, inflammation and lipid deposition. Currently, inflammation is thought to be a major factor in the initiation and progression of the atherosclerosis process. Modified lipids such as oxidised-LDL and glycated LDL stimulate the endothelium to secrete chemokines and consequently more inflammatory cells (monocytes, dendritic cells and T lymphocytes) are recruited to the site of injury. Monocytes are then differentiated to macrophages and become lipid laden cells known as foam cells. These cells make the core of the plaque, activation of these macrophages leads to secretion of cytokines, reactive oxygen and nitrogen species and proteases. Chronic release of these factors leads to thinning of the vascular wall and
rupture of the plaque. Loss of endothelial function leads to increased invasion by inflammatory cells, smooth muscle proliferation and neo-intima formation.

In both SLE and RA, there is a chronic inflammatory state during the disease process. The following paragraph will tackle the possible mechanisms by which these conditions can be thought to be a classic model for atherosclerosis. I will consider a general inflammatory condition shared by both diseases.

A chronic release of acute phase reactants such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) can lead to activation of monocyte chemotactic protein (MCP) which is an intracellular signal for recruitment of monocytes during the inflammatory response. Chronic release of inflammatory cytokines and acute phase reactant may also act on adipose tissue, muscles and liver hence producing metabolic changes and consequently resulting in dyslipidemia as will be discussed later.

Inflammation can result in immune complex deposition at different sites and may be associated with systemic complement activation. This may lead to endothelial cell activation and enhance recruitment of inflammatory cells and further increase the release of inflammatory cytokines, chemokines and adhesion molecules. These steps are the same steps proposed for atherosclerosis pathogenesis. In other words, theoretically, SLE and RA may provide a perfect model for atherosclerosis.

In the following section I will focus on the following factors: dyslipidemia, oxidant stress and endothelial dysfunction. I will also consider anti-inflammatory treatments for SLE/RA and how they affect the risk of cardiovascular disease.
1.11 Dyslipidemia

Dyslipidemia is one of the major risk factors for cardiovascular disease in the general population. According to the third report of the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATPIII), atherogenic dyslipidemia comprises a triad of: increased triglycerides and small dense LDL particles, and reduced HDL cholesterol [1].

Lipoproteins are macromolecular structures composed of lipids-cholesterol, cholesteryl ester, triglycerides, phospholipids and proteins. The outer layer of lipoproteins is a monolayer of phospholipid. Apolipoproteins are the protein component and they differ in their function and distribution. Lipoproteins are classified according to the size, density or apolipoprotein content. There are four major types of lipoprotein particles: chylomicrons and very low density lipoprotein (VLDL) (triglyceride-rich), low density lipoprotein and high density lipoprotein (LDL, HDL) are cholesterol rich lipoproteins. Apolipoproteins will be detailed later in Section 1.12.

The relationship between total cholesterol (TC) and the risk of coronary heart disease (CHD) in the general population is "curvilinear". However, there is no threshold that can be referred to for risk determination. Low HDL and high triglycerides (usually in combination) are more prevalent in patients with CHD than high cholesterol. Thus, HDL is considered as a more predictive risk factor than other lipids.

1.11.1 Dyslipidemia in SLE and RA

Dyslipidemia in SLE and RA has been widely studied. The most common abnormality is reduced HDL but there are inconsistent results for TC, LDL and TG.
Bruce et al found that in an inception cohort of SLE patients with persistent hypercholesterolemia who were followed up for a mean of 12 years, 24% developed a new CVD event compared with only 3% of those with normal cholesterol levels [29]. In the UK General Practice Research Database, the combination of SLE and hypercholesterolemia was associated with an 18-fold increase in the risk for MI compared with the general population [72]. There are multiple factors that might lead to dyslipidemia in this cohort such as chronic inflammation, steroids, and disease activity. Chronic inflammation is associated with alteration in lipid metabolism and alteration in lipid functions [103]. Patients with active disease require high dose of steroids and this may be associated with metabolic disturbance.

In RA, Myasoedova et al [177] has noticed a significant reduction of TC and LDL levels five years prior to diagnosis but other markers (HDL and TG) were similar in both RA and controls. A recent study by the same group described the impact of lipids on the risk for CVD by the term "lipid paradox". In this study, they suggested that low levels of TC and LDL were associated with the future CVD. A low lipid level was correlated with a high level of ESR. A similar trend of association with CRP was observed (although this did not reach statistical significance) [178].

1.11.2 Possible mechanisms for dyslipidemia in inflammatory diseases

Gabay and Kushner documented that the acute phase response was associated with altered hepatic synthesis of some coagulation proteins and lipoprotein metabolism [82]. Thus, an inflammatory disease process itself can induce alterations in the
lipid profile which can be considered as a risk factor for cardiovascular disease.

A common finding during infection or inflammation is reduced HDL which is the most consistent finding in SLE/RA. The exact mechanism is unknown but there are possible explanations such as inhibition of reverse cholesterol transport. This will lead to continuous efflux of cholesterol from cells to HDL [131].

Another possibility is reduced ApoA-1 or its displacement by other components such as serum amyloid A or haptoglobin. This could lead to loss of the protective function of HDL, possibly altering it to a pro-inflammatory mediator [131]. Shifting of HDL and ApoA-1 from the circulation to the site of infection or inflammation is another possibility. Ananth et al had reported that joints of RA patients are infiltrated by ApoA-1 [12]. It could be also due to activation of the reticuloendothelial system (RES) which leads to reduction in lipid components. Choy et al suggested a cytokine induced activation of the reticuloendothelial system which would lead to reduction in HDL, and total cholesterol [42].

Lipid abnormalities observed in SLE patients with active or inactive disease could be explained by the accumulation of triglyceride-rich lipoproteins, particularly VLDL and chylomicrons. These molecules are degraded and metabolized by lipoprotein lipase (LPL). The activity of this enzyme has been reported to be reduced in SLE [91][23]. Reduced LPL activity leads to accumulation of these lipoproteins and eventually high TG levels. A high frequency of antibodies to LPL (anti-LPL) has also been noted in SLE which might alter the activity of LPL [125]. These auto-antibodies are associated with higher TG levels, higher disease activity and markers of inflammation supporting a role for the humoral immunity in contributing to dyslipidemia.

The possible role of inflammation in dyslipidemia can also be deduced from the association between a raised TNF-α level and hypertriglyceridemia. Svenungsson
et al studied the correlation between TNF-α and CVD and lipids in patients with SLE. They found that patients with CVD have high TNF-α levels which correlate positively with triglyceride levels [254]. Administration of TNF-α was associated with sustained increase of VLDL and triglycerides in mice [171]. More evidence for the effect of TNF-α on lipid metabolism can be deduced from the effect of blocking TNF-α as will be discussed in the treatment section later.

As a consequence of inflammation, the structure of lipoprotein is also altered in a way that makes it more susceptible to oxidation. Enhanced levels of oxidised-phospholipids would therefore be expected in such a cohort of patients and this might generate neoepitopes that bind macrophages through scavenger receptors. This will contribute to the process of foam cell formation.

1.12 Lipoproteins and apolipoproteins

A lipoprotein is defined as a biochemical assembly composed of a core of cholesterol (in the form of cholesteryl ester) and an outer layer of phospholipids and proteins. Lipoprotein particles transfer hydrophobic molecules from the sites of synthesis to tissues where they are utilized for energy, storage, membrane assembly or hormone synthesis. This transport system is regulated by enzymes and cell surface receptors. The major component of lipoproteins is apolipoproteins. Apolipoproteins are essential for maintaining the structure and integrity of lipoproteins during processing in the circulation and for directing them for metabolism.
1.12.1 The attribution of lipids to CVD risks

Low density lipoprotein (LDL) is established as a primary risk factor for CHD. To have a complete picture of the association between lipids and CVD predisposition, other lipids should be considered for risk determination as indicated by several guidelines (ATP-III, European Guidelines)[1]. Recently, interest has focused on the importance of apolipoproteins as potentially more informative risk markers for cardiovascular diseases than a conventional lipid profile. Thus ApoB, which represents the potentially atherogenic particles, and ApoA-I which reflects the antiatherogenic particles, may indicate the risk more accurately than LDL and other non-HDL. The apoB/apoA-I ratio has been found to be strongly related to the risk of myocardial infarction and stroke in the Apolipoprotein-related Mortality Study (AMORIS) and in the INTERHEART study as will be discussed later [293][280].

1.12.2 Apolipoprotein B

Apolipoprotein B (ApoB) represents the potentially atherogenic lipoprotein particles. It is found in vLDL, LDL, IDL and small dense LDL, one molecule of ApoB being present in each particle of these lipoproteins. Hence, the serum concentration of ApoB yields the number of potentially atherogenic particles. ApoB is produced in the liver and allows the transport of cholesterol rich lipoproteins in the plasma. It serves as ligand for the ApoB receptor by which it enhances the uptake of cholesterol in peripheral tissues and the liver. In the blood, 90% of ApoB is found in LDL. However, in people with normal LDL range, high ApoB may indicate higher sdLDL level which is considered the more atherogenic particle (easily oxidised and promotes an inflammatory response).
In a prospective study of 2155 men aged 45-76 years followed for 5 years, Lamarche et al indicated that ApoB was strongly associated with the onset of ischemic heart disease (IHD) (relative risk (RR) 1.4, 95% CI 1.2-1.7) independent of other variables namely age, smoking, diabetes and systolic blood pressure. Adjusting for triglycerides and HDL-cholesterol did not affect the relation between apoB and incidence of IHD (RR 1.4, P<0.0001). Similarly, the RR of IHD associated with ApoB levels remained significant after controlling for the total/HDL ratio (RR 1.29, 95% CI 1.04, 1.60) [140].

1.12.3 Is ApoB better than other lipids in predicting risk?

In the NCEP-ATPIII guidelines, non-HDL is recommended for risk prediction especially in individuals with hypercholesterolemia who have moderate to normal levels of LDL-C. This is based on the close relationship between non-HDL cholesterol and ApoB. The correlation between the two is strong (R= 0.8-0.85).

In the AMORIS study, ApoB has a stronger association with the risk of fatal myocardial infarction than non-HDL cholesterol [281]. A similar finding was reported from a nested case-control study among 18225 participants in the Health Professional Follow-up Study. In this study, ApoB was more predictive of the development of CHD than other non-HDL particles [202]. On the other hand, Ridker and others found no significant difference between apolipoproteins and other lipid measures in prediction of cardiovascular events in his prospective study [215]. This is to be expected as the study group included relatively low risk individuals.
1.12.4 Apolipoprotein A-1

Apolipoprotein A-1 (ApoA-1) is considered as the active component of HDL particles. ApoA-1 reflects the atheroprotective component of the lipids. ApoA-1 initiates the reverse cholesterol transport and returns excess cholesterol from peripheral tissues to the liver via HDL molecules [162].

ApoA-1 has anti-inflammatory and anti-oxidative properties which might contribute to its cardio-protective role as both inflammation and oxidation are believed to play a key role in the process of atherosclerosis [162]. This could lead to the assumption, that ApoA-1 in particular HDL sub-fractions are important in protection from atherosclerosis. Of particular note, HDL is in a dynamic state, changing its size and lipid contents. Hence, the ApoA-1 concentration is a reflection of this dynamic state.

The Apolipoprotein-related mortality risk study (AMORIS): this is a large prospective study started in 1985. The main aim of the study was to assess the predictive power of apolipoproteins [281]. Individuals with low ApoA-1 values were at risk of developing MI.

In the literature, reports regarding ApoA-1 in SLE are scarce. Only one study reported the level of ApoA-1 in SLE. Zhang et al found that ApoA-1 is reduced in Chinese patients with multiple sclerosis and other related autoimmune diseases [295]. In the same study SLE patients had the lowest level of ApoA-1 (Table 1.7). However, in this report there are no details regarding disease activity and treatment regimes.
Chapter 1  Background

<table>
<thead>
<tr>
<th>Group</th>
<th>Multiple sclerosis</th>
<th>SLE</th>
<th>RA</th>
<th>Healthy control</th>
</tr>
</thead>
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<td>Gender female/male</td>
<td>32/28</td>
<td>22/14</td>
<td>20/15</td>
<td>27/37</td>
</tr>
<tr>
<td>Mean age±SD</td>
<td>35.9±14.8</td>
<td>31.6±10.7</td>
<td>36.3±9.8</td>
<td>35.7±10.2</td>
</tr>
<tr>
<td>Apo A-1</td>
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<td>0.940±0.061</td>
<td>1.03±0.061</td>
<td>1.179±0.047</td>
</tr>
</tbody>
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Table 1.7: Apo A-1 in autoimmune diseases vs healthy controls [295].

1.12.5 The ApoB/ApoA-1 ratio vs lipids ratio

In the LDL/HDL ratio, LDL is calculated using the Friedwald formula [76] from HDL, TC and TGs. As a result, HDL is present on both sides of the ratio. Similarly in TC/HDL, HDL is contained in the TC and hence present in nominator and dominator.

In contrast, (ApoB/ApoA-1) both components of the ratio are measured directly using standardised and validated techniques. The components of this ratio reflect the two sides of the risk and the ratio represent the balance of cholesterol transport. Of particular note, ApoB and ApoA-I also can be measured in samples from non-fasting individuals.

The International Federation of Clinical Chemistry (IFCC) recognized the important role of ApoA-1 and ApoB and hence a standardization committee has been established [163][164]. Based on these documents, a common reference material for measurement of ApoA-1 and ApoB has been accepted by the World Health Organisation (WHO) and IFCC. This reference material improved the agreement of measurements between different laboratories.

In contrast, there is no common reference for the measurement of LDL and HDL, making the accuracy and comparability of their measurements less accurate [162]. This inaccuracy is due to problems with the assays themselves and
not due to inaccuracy in calibration. Laboratory LDL estimated by the Fried-
wald equation [76] has a greater chance of error as it is estimated from three measurements each with its own inherent error (TC, HDL and TGs). Measurement of triglycerides in general clinical laboratories gives higher values usually as it includes monoglycerides and diglycerides. Direct HDL measurement has an inherent method-dependent inaccuracy.

1.12.6 ApoB/ApoA-1 ratio and events

As indicated before, results from AMORIS have revealed a strong association between ApoB and fatal MI in both men and women. ApoA-1 was negatively associated with the risk of MI in keeping with its protective effect. These associations were irrespective of age, gender, TC and TG levels. Later on, the ApoB/ApoA-1 ratio was found to be the most predictive marker [280]. The addition of other lipids or cholesterol ratios to the risk model did not improve the strong predictive value of the apoB/apoA-1 ratio.

The INTERHEART study was a large international (52 countries) standardised case control study. The main aim was to assess the importance of risk factors for CHD in different regions and different ethnic groups [293]. This study included 15152 patients with acute MI as well as 14820 age and sex matched controls. Different markers were analyzed including ApoB, ApoA-1, smoking, hypertension, fasting blood glucose, obesity, psychosocial stress, vitamin intake and exercise. The ApoB/ApoA-1 ratio was the strongest marker in multivariate analysis and found to be independent of age, gender and ethnicity [293][292].

In the AIR study, the primary aim was to investigate the relationship between progression of atherosclerosis (as indicated by carotid IMT) and baseline
risk factors (ApoB, ApoA-1, LDL particle size, blood pressure, fasting blood glucose, insulin and hsCRP) in 58 men with variable degrees of obesity and insulin resistance [229]. Among these factors, only the ApoB/ApoA-1 ratio and insulin were significantly associated with carotid IMT progression rate. A similar finding was reported for the association between ApoB/ApoA-1 ratio and femoral artery plaque [228].

From this review, it can be concluded that ApoB, ApoA-1 are strongly associated with CHD in the general population. It can be also deduced that ApoB and ApoA-1 are more reliable and stable markers. Given the fact that ApoB and ApoA-1 can be measured directly in non-fasting individuals, ApoB and ApoA-1 can be used as risk predictors for CVD.

However, the contribution of these markers to risk prediction in SLE/RA is unknown and the level of agreement between apolipoproteins and lipid concentrations in these conditions has not been studied. What is the best marker in SLE/RA is unclear and how inflammation influences these markers has not so far been determined.

1.13 Oxidative stress

Phospholipids are major components of lipoproteins that undergo radical or enzymatic oxidation in stressful conditions such as infection and in inflammatory conditions such as SLE, RA and atherosclerosis [18][143]. At the atherosclerotic lesion, oxidized phospholipids are taken up by macrophages leading to the transformation of macrophages to lipid-laden foam cells which are one of the major characteristics of atherosclerotic lesion [80][149]. Oxidized lipids are chemotactic, immune-stimulatory and have other inhibitory and toxic properties [90][247].
Moreover, oxidized phospholipids can trigger humoral immune response via formation of auto-antibodies such as anti-oxLDL antibodies [78][77].

Oxidative stress is a normal response to various stimuli and is usually self-limiting. However, in chronic inflammatory conditions, the process is continuous for a longer period and ultimately leads to accumulation of oxidized lipids mainly oxidised-LDL. This particle has been shown to be present in atherosclerotic lesions. Holvoet et al reported a strong correlation between oxidised-LDL and CAD with a significant link to Framingham risk factors [115].

It has been reported that oxidised-LDL levels were higher among SLE patients than healthy controls [84]. Similarly, Lee and colleagues documented, in a case-control study of SLE patients that oxLDL levels were higher among SLE patients than in controls and the level correlated with endothelial dysfunction [142]. El-Magadmi and colleagues reported increased level of oxidised-LDL in SLE patients with the metabolic syndrome [152].

Anti-oxLDL antibodies were also detected in patients with cardiovascular disease [235]. Doria et al demonstrated that the titre of these auto-antibodies was higher in SLE patients and the level was correlated with increased intima-media thickness (IMT) [57]. Cross reactivity between these autoantibodies and anticaldilipin antibodies was reported which might explain the association between the latter and low HDL and apolipoprotein A-1 (ApoA-1) [78].

Anti-oxLDL, however, may be protective. It has been reported that high levels of antibodies against oxidised-LDL were associated with reduced development of carotid atherosclerosis in hypertensive patients [250]. Fukumoto et al also found a negative correlation between anti-oxLDL titre and carotid IMT in a healthy population [81].

Furthermore, it was found in animal models that immunization of LDL−/−
mice (knockout animal model) with anti-oxLDL protected them from inflamma-
tion and plaque formation [264]. The role of these antibodies is still controversial. 
There are several possible explanations for that. One is that there are different 
subclasses of the antibody; IgG antibodies are more likely to be pathogenic while 
IgM seem to be protective [250]. These antibodies might also differ in their affin-
ity and specificity. A case-control study by Frostegard has also shown increased 
IgM-antibodies to phosphoryl-choline (aPC) in a population from non-westernised 
Kitava in New Guinea compared to a Western population from Sweden. These 
'protective' antibodies may explain the lower incidence of CVD among the for-
mer population [79]. It was also documented in SLE patients that this subclass 
of antibodies was associated with lower disease activity [251]. Anti-PL antibodies 
may contribute to atheroprotection in SLE and other populations.

1.14 Endothelial dysfunction

The endothelium is a single layer of cells that lines the interior surface of blood 
vessels and separate blood constituents from the sub-endothelial layers. The 
endothelium is an important endocrine, paracrine and autocrine organ that has 
a crucial role in maintaining vascular smooth muscle tone, vascular growth and 
blood adhesion. Thus, endothelial cells are capable of secreting a number of 
factors such as prostacycline, nitric oxide (NO) and endothelin-1. It also has a 
major role in maintaining homeostasis by acting as a surface with anti-thrombotic 
properties under normal conditions. In the event of tissue damage, it exhibits 
procoagulant activity to minimise blood loss and accelerate the repair process. 
The endothelium has a role in the immune process against infectious agents by 
promoting leukocyte migration to the site of entry of foreign organisms. This role
also can be part of a pathological process in inflammatory autoimmune diseases.

Endothelial dysfunction (ED) is broadly defined as reduced endothelial associated vasodilatation, anti-inflammatory properties and a pro-thrombotic state. This can result from an imbalance between reduced NO bioavailability and excess oxidative stress. ED is thought to be the earliest stage of atherosclerosis and probably a key trigger for plaque formation and progression. It is thought that excess damage to endothelial cells and/or impaired reparative mechanisms can contribute to endothelial dysfunction. Endothelial progenitor cells are thought to be important in the repair of endothelial damage. Hence, reduced numbers of circulating endothelial progenitor cells will contribute to ED and cardiovascular disease [230].

1.14.1 Markers of endothelial function

Endothelial function can be assessed by measuring the ability of the artery to dilate in response to chemical or mechanical stimuli or by measuring markers of endothelial activation/dysfunction. There are several methods, both invasive and non-invasive, for the assessment of endothelial function.

1.14.2 Circulating biomarkers of endothelial function

Endothelial dysfunction can be associated with the release of cellular adhesion molecules (CAMs), namely vascular cell adhesion molecule (VCAM), intra-cellular adhesion molecule (ICAM) and E-selectin. These molecules are involved in cell rolling, margination and extravasation. In addition, other pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumour necrosis factor (TNF-α) are
secreted. These molecules are also shed and can be measured in the circulation. Soluble E-selectin level was documented to be associated with CHD [252]. ICAM-1 and VCAM were expressed in the atherosclerotic plaque and found to be correlated with CHD [217].

Several studies have also shown increased levels of E-selectin, ICAM and VCAM in SLE patients and which were correlated with disease activity (SLEDAI) and subclinical atherosclerosis [226][266][277].

Also in RA, Sodergren et al showed significant increase in VCAM, ICAM and MCP-1 levels. In this study, L-selectin and MCP-1 were significantly associated with IMT while platelet activation inhibitor (PAI-1) and L-selectin were associated with FMD [242].

Of particular note, these markers are not specific for ED (e.g in RA, synovial pannus is highly vascular and it may be a source of CAM and cytokine release independent of endothelial dysfunction) which makes their derangement difficult to interpret [19].

1.14.3 Non-invasive techniques to measure endothelial function

Flow mediated dilatation
Flow Mediated Dilatation is a non-invasive high-resolution ultrasonographic method to measure endothelial dependent vasodilatation of medium sized blood vessels. Brachial artery flow mediated dilatation (FMD) is commonly used. FMD was first described in 1992 by Celermajer et al [35]. Since then, FMD has been proposed as a functional bioassay for endothelium-derived NO bioavailability (endothelial function). Hence, guidelines for its use was developed thereafter [46].
This technique is widely used for research despite its variability between and within individuals. The mechanism behind this technique is the ability of endothelium to dilate in response to nitric oxide release, stimulated by shear stress. There is a good correlation between brachial artery FMD and coronary artery dilatation demonstrated by Takase et al \( (R=0.79, P<0.001) \) [260].

Flow mediated dilatation has been widely used in investigating cardiovascular risk in patients with chronic diseases such as diabetes, hypertension, chronic kidney disease, RA and SLE.

Several studies reported impaired endothelial function in SLE [63][253]. A recent meta-analysis looked at the difference in FMD between SLE patients and healthy controls [153] and included 13 studies (total number of SLE patients 580, 381 age, sex matched controls). Although the authors pointed out the heterogeneity between studies, endothelium dependant FMD was significantly reduced in SLE patients compared to healthy controls. Of particular note, is the effect of increasing age and disease duration on the endothelial reactivity in patients with SLE which may interfere with the use of endothelial function as a predictor of early atherosclerosis in patients with SLE.

Some medication, dietary products and hormones are known to have an effect on FMD. Many medications have direct or indirect vascular effects. If possible, drugs that target the cardiovascular system such as \( \beta \)-blockers, calcium channel blockers and angiotensin converting enzyme inhibitors (ACEI) should be stopped prior to the assessment of FMD. Some times it is not possible to stop these medications but their confounding effect should be taken into account. There is evidence that the use of ACEI (quinapril) was associated with improvement in FMD in patients with impaired cardiac function [135].

The use of vitamin supplement should be noted as these directly affect free
radicals [67]. Simvastatin which is a member of the statins (lipid-lowering agents) was associated with significant improvement of FMD in patients with hypercholesterolemia irrespective of its dose [134].

Smoking is a traditional risk factor associated with impaired endothelial function [34]. Passive smoking as well as active smoking is associated with impaired FMD [129]. Thus smoking and smoking exposure are ideally avoided when assessing participants for FMD. Caffeine is also thought to attenuate FMD as it inhibits soluble guanylate cyclase which is involved in the NO-mediated vasodilatation. Caffeinated coffee drinks were associated with reduced FMD [190].

**Carotid intima media thickness (cIMT)**

Carotid IMT is determined by non-invasive scanning using B-mode image of the carotid artery. It is defined by the lumen-intima and the media-adventitia interfaces. These interfaces are interpreted as the cIMT and also used for plaque detection. There is variability among methods used for obtaining cIMT among the literature. cIMT is now widely used as a surrogate marker for sub-clinical atherosclerosis, some studies reporting that cIMT is an independent predictor of coronary heart disease [38].

In SLE, several cross-sectional studies using cIMT reported increased prevalence of plaque in patients compared to the controls [156][184][3]. The majority of these studies indicated that there was an increased incidence of plaque in SLE patients less than 55 years. In RA, cIMT was found to be associated with CVD events [97]. Accelerated progression of atherosclerosis in RA patients had also been reported [53].

**Peripheral arterial tonometry**

Peripheral Arterial Tonometry (EndoPat) is also a non-invasive technique
used to estimate endothelial function. This technique was developed by Itamar Medical Ltd (Israel). The basic idea of the method is to measure the changes in digital pulse wave amplitude (PWA) using pneumatic finger probes and record the changes in response to hyperemia (RH-PAT). Thus, it measures peripheral micro-vascular endothelial function. A study by Bonetti et al has shown that patients with CAD have reduced digital hyperaemic response as indicated by RH-PAT [21].

Along with other measures of endothelial function, RHI show modest correlation e.g with FMD (R= 0.47) [231] and with cIMT (R= -0.35) [73]. However, most of the studies investigating these correlations were carried out in relatively healthy individuals. Another explanation is that different methods may evaluate different aspects of endothelial function e.g. macrovascular vs microvascular, the physiology of each aspect being quite different and affected by multiple variables which may not necessary be the same.

1.15 Treatment

In sections (1.2.6) and (1.3.6), we discussed broadly the treatment of SLE/RA. We also touched on the recent increased use of biological agents. There is accumulating evidence from observational studies indicating that the use of DMARDs/Biological therapies may reduce the risk of cardiovascular disease in patients with RA/SLE which can be translated as a lower incidence of cardiovascular events and their related mortality. In this section, I will review the evidence about how drugs used in treatment of RA/SLE may influence CVD risk and/or cardiovascular risk factors.
1.16 Effects of traditional DMARDs on cardiovascular risk in RA/SLE

1.16.1 Steroid therapy

Steroids were first introduced (by Hench, Kendal and colleagues) into the Western medical practice over 50 years ago for use in the treatment of RA patients [109]. Steroids are widely used in the treatment of a variety of rheumatic and other diseases due to their potent anti-inflammatory and immunosuppressive effects. The prognosis of some diseases such as SLE and RA has been greatly improved by the use of steroids. However, this improvement has been associated with an increased prevalence of atherosclerotic cardiovascular disease [32].

Steroid therapy has been thoroughly investigated but the results are still controversial. Steroids are assumed to be a key mediator of CHD. As is known in Cushing’s syndrome, steroids can cause many metabolic abnormalities including central obesity, hypertension, glucose intolerance and alterations in lipid profiles. It would be expected that patients on steroid therapy would have a higher risk of cardiovascular events since all these side effects are known risk factors for CVD. In 1975, Bulkeley et al noted that treatment with steroids for more than a year was associated with an increased risk for atherosclerosis and CHD [32].

In patients with SLE, chronic steroid use is associated with increased total cholesterol, LDL, HDL and TGs [199][29]. The effect of steroids is thought to be dose dependent. As demonstrated by Petri et al, each 10 mg increase in prednisolone dose was associated with a 7.5% increase in serum cholesterol [199].

Another study found that a low dose of steroid (<10 mg) daily did not, for example, adversely affect the lipid profile in SLE but doses > 10 mg daily caused...
increased LDL cholesterol and triglycerides [151]. This effect of high dose steroid has been confirmed by other studies [130][29]. Conversely, Okawa-Takatsuji *et al* reported that corticosteroid use in SLE patients with renal impairment led to decreased Lp(a) levels, a potentially atheroprotective effect [187].

Steroids are the mainstay treatment for active and severe SLE and used as background treatment in 20-40% of RA patients. This may, in part, explain the association of the disease severity with CVD vulnerability, so called confounding by indication or "channeling bias". There are other factors that contribute to the association of steroids with cardiovascular risk such as the dose of steroid, duration of use, mean daily dose and cumulative dose. Although a high dose of steroid is associated with a number of metabolic abnormalities, its use leads to improved survival of patients with SLE indicating its beneficial role in controlling inflammatory conditions. To date however, the ideal treatment regime with steroids remains unclear. In SLE, various measures have been associated with cardiovascular or sub-clinical disease including steroid ever use [3] and high steroid dose [199]. All of these are however highly co-related.

### 1.16.2 Anti-malarial drugs

The use of antimalarial drugs as anti-inflammatory agents was considered previously to be of limited value in the treatment of rheumatic disorders. Recently, however, hydroxychloroquine has been shown to protect against cardiovascular and thrombotic events and it facilitates the action of other disease modifying agents in patients with renal problems [195][128]. Several investigators have also shown that antimalarials have lipid lowering properties in SLE patients [195][279]. In particular, in combination therapy with steroids, Rahman *et al* noted a 10% reduction in total cholesterol [207][278].
Sachet et al suggested that the increased lipoprotein synthesis induced by steroids can be counteracted by the lipid lowering effect of antimalarial agents [225]. In addition to lipid lowering, El-Magadmi et al also noted that antimalarial agents have glucose lowering properties [152]. Wasko and colleagues reported that patients with RA treated with hydroxychloroquine have a lower incidence of diabetes than patients who never had this agent. The reduction of diabetes was more apparent (up to 77%) with longer duration (>4 years) of hydroxychloroquine use [282].

In a case-control study of SLE patients from LUMINA (Lupus in Minorities: Nature vs nurture), a multi-ethnic US cohort, it was reported that increased hydroxychloroquine use had a protective effect on the overall survival of SLE patients and prevented disease flares, reduced the damage index and facilitated the effect of other agents in renal impairment [5]. Antimalarial agents have, therefore, been noted to have anti-inflammatory, antithrombotic, lipid-lowering, antiglycaemic and immunomodulatory effects [278]. So it is not surprising that antimalarials have a protective effect in patients with SLE and a combination of steroid and antimalarial seems to have a synergistic effect in countering the metabolic side effects of steroids and reduces chronic inflammation.

1.16.3 Methotrexate

Since the 1980s Methotrexate (MTX) has been the most frequently used disease-modifying agent in the treatment of RA. The current guidelines from the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) for treatment of RA recommend MTX as a first line therapy [241]. MTX is an analogue of folic acid. The anti-inflammatory action of
MTX may occur via adenosine release from cells and this is triggered by polyglutamate metabolites of the drug. However, the exact mechanism of action is not fully understood.

The safety of MTX has been extensively investigated in observational studies and association with both CVD mortality and morbidity has been reported in the literature. Choi et al conducted a prospective study which included 1240 patients with RA with a mean follow-up of 6 years to investigate the effect of MTX on mortality in RA patients. In this study, there was 60% reduction in all-cause mortality and a 70% reduction in CVD mortality in patients treated with MTX [HR (95% CI)= 0.3(0.2, 0.7)]. Adjustment for the confounding effect of clinical indications for MTX did not make any difference [41]. This protective effect was confirmed by Van Halm et al in a case-control study including 613 patients treated with MTX which was associated with a significant CVD risk reduction (OR, 95% CI; 0.16, 0.04-0.66) [273]. On the other hand, a prospective cohort study by Alarcon et al [7] indicated increased all-cause mortality in RA patients treated by MTX and a trend towards increased CVD mortality which was not statistically significant (OR, 95% CI; 1.4, 0.5-2.6).

The effect of MTX on CVD morbidity has been extensively researched. However, there is increased heterogeneity between studies with regards to the definition of CVD outcome, study design and the population included. A large US research database study (n=16752) has shown that prior treatment with MTX was associated with significant reduction of risk for cardiovascular disease [HR (95% CI)] was [0.65 (0.61, 0.82)]. However, the design of this study (retrospective and including patients on insurance claims) limits the generalisation of these results and introduces a selection bias. Naranjo et al studied the effect of years of exposure to MTX on CVD outcome (MI, angina, coronary disease, coronary
bypass and stroke) [180]. The adjusted [HR (95% CI)] for all CVD events was [0.85 (0.81, 0.89)]. This indicates that MTX use is associated with reduced risk of CVD events even after adjusting for potential confounders. A systematic review by Westlake et al [286] has reviewed the effect of MTX on cardiovascular disease in patients with RA. In their review, there were two studies looking at the effect on CVD mortality, one study reported a significant reduction in the mortality and the other showed a trend towards reduction. With regards to the all-cause/CVD morbidity, four studies demonstrated a significant reduction in the CVD morbidity. They also noted that MTX use before developing RA was associated with 3-4 years reduction in the risk of CVD.

The effect of MTX on lipid profiles has been studied but the results are inconclusive due to the small number of patients included in these studies as well as the use of other DMARDs or dietary interventions. However, a recent study has shown an effect of MTX treatment on the gene expression of the atheroprotective protein 27-hydroxylase (HY27) and ATP-binding cassette transporter-A1 (ABCA1) [40]. There was no significant difference in the lipid profile between MTX treated or untreated patients. Both HY27 and ABCA1 are anti-atherogenic reverse cholesterol transporters in humans. MTX, via induction of these proteins may potentially improve dyslipidemia by enhancing the outflow of cholesterol and hence reduce atherogenesis.

In summary, the results of the studies are controversial owing to potential confounding associated with the choice of MTX and the severity of disease. As a general conclusion, the majority of these studies point towards a protective effect of MTX on the overall and CVD morbidity and mortality. This effect may be important early in the disease course. The mechanism for this effect is hard to determine and could possibly be multi-factorial. Adequate control of
inflammation in RA may be an important mechanism that reduces CVD event risk
and MTX specifically may have particular benefits due to its anti-inflammatory
properties and effects on lipids.

1.16.4 Cyclophosphamide

Cyclophosphamide is an immunosuppressive agents used mainly in severe organ
damage or life threatening conditions. There is little evidence that cyclophos-
phamide has an effect on cardiovascular risk. Apras and colleagues have noted
that in patients with scleroderma, treatment with cyclophosphamide was asso-
ciated with a reduction in some adhesion molecules which might indicate an
immune-modulating effect on endothelial function [13]. A study by Roman and
colleagues reported an independent, reduced risk of carotid plaque development
in patients with SLE treated with cyclophosphamide [219], although this has not
been replicated by others.

1.16.5 Azathioprine

Azathioprine is a potent immunosuppressive used for the treatment of moderate
to severe forms of SLE. There is very little data in the literature about the role
of azathioprine as a risk factor for cardiovascular disease. It has been reported
that azathioprine was associated with decreased survival (18.3% died) in patients
who underwent cardiac transplant surgery compared to Mycophenolate mofetil
(MMF) treated patients (11.3% died) [62]. Intravascular ultrasound of patients
treated with azathioprine revealed increased mean ± SD IMT compared to MMF
treated patients (0.13±0.03 mm vs 0.06±0.03 mm). Post mortem examination,
performed on a subset of patients revealed that around 60% of patients treated
with azathioprine had evidence of moderate to severe coronary artery disease compared to 29% in the MMF group. Azathioprine was also associated with increased dyslipidemia and enhanced atherogenic lipid profile when used as immunosuppressant agent after renal transplantation [206]. In this study, patients after renal transplantation were categorized into three groups; a) prednisolone and azathioprine, b) prednisolone and cyclosporine, c) prednisolone, cyclosporine and azathioprine. The authors reported an increase in the serum level of triglycerides, LDL, VLDL in all patients. This was more significant in female patients and more pronounced in patients in group c. It is hard to determine the cause of this hypertriglyceridemia hence patients were treated with drugs and no further studies looking specifically on the effect of azathioprine on lipid profile.

A study by Doria and colleagues documented that azathioprine use was associated with increased intima-media thickening and plaque formation in a univariate analysis [57]. A similar finding was reported by Ahmad *et al* in a cross sectional study [3]. In SLE patients, azathioprine is used for moderate to severe disease, to treat patients with lupus nephritis or as a maintenance in patients with CNS involvement. As a result, this may be due to the severity of the disease confounding by other factors or it could be inadequate disease suppression to control CHD risk. In addition, those patients may already have accumulated risk over time as they have renal impairment which is a known risk for CVD.

1.16.6 **Mycophenolate mofetil (MMF)**

Mycophenolate mofetil (MMF) is an immunomodulatory agent that acts via inhibition of inosine monophosphate dehydrogenase (IMPDH). By inhibiting this enzyme, MMF suppresses the de novo synthesis of purine and hence expresses a selective and reversible anti-proliferative activity in macrophages and lymphocytes.
In addition, MMF can down-regulate the expression of adhesion molecules, and reduce macrophage and monocyte responses [274]. As a result, it could be hypothesized that MMF may have an anti-atherogenic effect. Romero et al noted that diet induced atherosclerosis was reduced in rabbits treated with MMF [220]. In a randomized double-blind active-controlled trial of MMF vs azathioprine (AZA) in cardiac transplanted patients followed up for 36 months, it has been noticed that MMF treated patients have better graft survival and a lower mortality rate than azathioprine treated patients [62].

In a recent lupus nephritis trial (Aspreva Lupus Maintenance Study-ALMS) patients were randomized to either MMF or intravenous cyclophosphamide (IVC). In addition to renal response, adiponectin levels (indicative of vascular protection) were higher in patients treated with MMF than those treated with IVC [44]. MMF may, therefore, provide an additional vascular protective effect. Compared to cyclophosphamide, MMF has fewer side effects, less predisposition to infection, infertility, haemorrhagic cystitis and malignancy [197]. Overall, MMF may be more favourable for use in patients with lupus nephritis. However, further studies are needed to confirm whether MMF affects the vasculature in patients with SLE.

1.17 Conclusions of usual treatment

As discussed previously, patients with SLE/RA have chronic inflammation which is the main characteristic of the disease process. Chronic inflammation is now viewed as a major component for risk of CVD. Thus, it has been suggested that good control of inflammation should reduce the risk of CVD. However, whether this is due to reduced inflammation or due to the drug itself is unknown. Different treatments have different effects and can be categorised as:
a) “Beneficial” such as anti-malarials, MTX, low dose steroids and MMF.

b) Controversial such as high dose steroid.

c) Detrimental or (not good enough) such as azathioprine.

1.18 Effects of the usual preventative agents for traditional cardiovascular risk factors on cardiovascular risk and disease activity in RA/SLE

1.18.1 Statins

The use of 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors (statins) was associated with a reduction of cardiovascular events in a large number of studies in a variety of primary and secondary preventive settings. Statins are known as cholesterol-lowering agents but they have other immune-modulatory effects. Their influence on immune-modulation was first suggested in 1995 when pravastatin was reported to reduce rejection episodes in cardiac transplant patients independent of their cholesterol level [133]. Studies in animal models reported that simvastatin was effective in treating collagen-induced arthritis and was associated with a reduction in serum IL-6, a major pro-inflammatory component [144]. In addition to LDL lowering, statins may act via several anti-inflammatory mechanisms to reduce atherosclerosis. For example there is evidence that statins increase bioavailability of nitric oxide (NO) and decrease levels of CRP, chemokines and
pro-inflammatory cytokines (IL-6, IL-1β, TNF-α). They also reduce platelet activity and enhance fibrinolysis [31].

In patients with CAD, statins therapy is associated with a reduction in CRP and reduced incidence of subsequent myocardial events [185][212]. This could be a possible rationale for the use of statins in patients with chronic inflammatory conditions in order to suppress inflammation, which is now viewed as a major risk factor for CVD. However, other effects of statins (immune-modulation) should be considered since a number of statin induced lupus cases have been documented [16][105].

An open-label trial investigating the effect of atorvastatin on patients with SLE has been carried out [71]. Atorvastatin treated patients had a significant increase in flow mediated arterial dilation (FMD) independent of the lipid profile or other CVD risk factors. Moreover, atorvastatin reduced homocysteine, LDL and total cholesterol as well as the SLEDAI score (a measure of disease activity). A similar finding was documented by Felea et al. who demonstrated a significant improvement in FMD in atorvastatin-treated SLE patients [70].

The effect of Fluvastatin on cardiac outcomes was investigated in SLE patients who underwent renal transplant [186]. Although the size of the study was relatively small, this reduces the event rate, the authors reported a 70% risk reduction in the event rate in patients in the Fluvastatin treated arm compared with placebo.

In RA, a randomized placebo-controlled Trial of Atorvastatin in Rheumatoid Arthritis (TARA) has also shown that atorvastatin improved disease activity and reduced joint swelling and ESR [167]. In another trial of high dose atorvastatin (80mg/day), patients (n=20) were randomized to placebo or atorvastatin in addition to RA therapy for 12 weeks. Atorvastatin-treated patients had reduced high
sensitivity CRP levels, an effect not observed in placebo-treated patients [39].

This demonstrates that statins have other effects apart from the most commonly known lipid-lowering one. As mentioned above, statins are widely used for the treatment of hyperlipidaemia and prevention of cardiovascular disease. Pravastatin is a member of the statin family and its effect on SLE patients was studied by Costenbader and colleagues [47]. 41 SLE patients treated with pravastatin were compared with 22 SLE control patients. In this study, 10 mg pravastatin/day was associated with a 16% reduction in total cholesterol and a 24% reduction in LDL whereas 40 mg a day was associated with a 21% reduction in cholesterol and a 35% reduction in LDL levels. The level of CRP was not affected by pravastatin use in this study. The lipid lowering effect of pravastatin was impaired by the use of glucocorticoids.

1.18.2 Angiotensin converting enzyme inhibitor

Angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) are agents used in the treatment of hypertension, congestive heart failure and renal disease. Studies in animal models showed that ACE inhibitors and ARB can reduce the progression of atherosclerosis [205][249].

A large double-blind randomized study, Heart Outcomes Prevention Evaluation (HOPE), evaluated the effect of Ramipril on high risk patients (aged ≥55 year old, had evidence of vascular disease, diabetes + one traditional risk factor) [121]. The primary outcome of the study was a composite of myocardial infarction, stroke or death from CVD. They found that ramipril use was associated with reduced relative risk for the primary outcome [RR (95%CI)] [0.78 (0.70, 0.86 )]. In this study, patients had a high risk of CVD with more than one traditional risk factors and by definition were on one or more protective treatments such as
lipid-lowering drugs. The results of this study indicated that ramipril had an additional protective effect over and above reducing blood pressure as the majority of patients were not hypertensive and the effect on blood pressure in this group was minimum.

In one trial, studying ACE inhibitors in 15 patients with RA subjects, showed improved symptoms and decreased vascular viscosity and CRP [165]. A recent study also reported that the use of ramipril (10mg/day) significantly increased FMD and hence improved endothelial function in RA patients [74]. The trials of ACE inhibitors and CVD in patients with SLE are surprisingly few in the literature.

1.18.3 Aspirin

It is well documented in the general population that low dose aspirin is effective in the primary and secondary prevention of cardiovascular risk (especially myocardial infarction). Aspirin use was associated with a 32% reduction of the risk for myocardial infarction [61]. However, its overall effect on the risk of cardiovascular mortality and stroke is not conclusive yet. Of particular note, most of the trials on the effect of aspirin included mainly men. The vast majority of SLE patients are women so these results may not be applied to women.

Ridker et al performed a randomized trial on 39,876 apparently healthy women aged ≥45 years [213]. Participants were randomized to aspirin or placebo and were followed for 10 years for the occurrence of first major CVD event. They found that aspirin has no significant effect on the risk for CVD (RR=1.02 95% CI (0.84-1.25)) and for CVD mortality (RR=0.95 95% CI (0.74-1.22)). Therefore, aspirin may not be recommended as a primary prevention in women as indicated by Ridker’s trial.
1.18.4 Fish oils in SLE

Omega-3 (Ω-3) polyunsaturated fatty acids are found in oily fish such as sardine, salmon, herring and kippers. These fatty acids were found to be beneficial in secondary prevention trials in patients with ischaemic heart disease [33]. There is some evidence for improvement of RA symptoms and reduced requirement for NSAIDs, but this is not conclusive.

Wright et al. carried out a randomized double-blind placebo-controlled parallel trial on SLE patients to determine the clinical effect of Ω-3 on disease activity and endothelial function [289]. This study revealed an improvement in disease activity. Moreover, low dose supplementation of Ω-3 was associated with improved endothelial function and reduced oxidative stress. However, the disease activity in this study was estimated by Systemic Lupus Activity Measure (SLAM-R) which gives a similar weight to mild and severe manifestation. The major improvement in this study was in the constitutional symptoms with a minor improvement in joints and other individual scores. There was a worsening in the GI symptoms at week 12 which may indicate intolerance to (3g) of fish oil and 4 patients withdrew from the study because of the GI disturbance.

This suggests that fish oil might have a favorable effect; the issue regarding its effect on disease activity needs further study and the optimum dose needs to be determined.

1.18.5 Do novel interventions affect the CHD risk in RA/SLE?

Recently a number of agents have been introduced in the protocols for the treatment of patients with rheumatic diseases either for the treatment of disease activity or as preventive measures. Of particular interest, is the effect these agents
may have on the CVD risk factors as will be discussed later.

1.18.6 Anti TNF (RA)

Biological agents that target TNF-α such as infliximab, etanercept and adalimumab have been approved recently for use in the treatment of rheumatic conditions. These drugs are of particular interest because they target specific components of the immune system and most likely will have fewer side effects.

Given the fact that TNF-α plays a major role in the development and progression of atherosclerosis by endothelial activation and enhanced endothelial dysfunction, it would be expected that the use of anti-TNF therapy would reduce the risk of CVD.

A number of studies have reported that TNF blockade was associated with reduced levels of markers of inflammation (important CVD risk). Combined treatment of infliximab and methotrexate rapidly reduces matrix metalloproteinase-3, ICAM-1, IL-8 and TNF-α [276]. Hurlimann et al demonstrated improvement in vascular endothelial function after 12 weeks treatment of RA patients with infliximab. This improvement was correlated with decreased disease activity and improved inflammatory markers [118]. A similar finding was confirmed subsequently by Gonzalez-Juanatey although the improvement was transient [96]. Similar findings were reported for the use of adalimumab (fully humanised monoclonal anti TNF-α antibody)[92].

According to Popa et al, anti TNF-α agents may also have a positive effect on the lipid profile in the period of treatment between 6 months and one year; however proatherogenic patterns of lipid were also noted [204]. A study by Kiortsis et al looking at the effect of infliximab on lipid profile in patients with RA
and ankylosing spondylitis has also shown increased total cholesterol level, but no change to other lipid components [132]. The atherogenic index as indicated by TC/HDL or TG/HDL was unaltered. This suggests that infliximab has a neutral effect on lipids. A recent systematic review included 24 studies evaluating the long term effect of anti-TNF therapy on lipid profile in RA patients [203]. The results of these studies were highly variable. The majority of studies (11) showed increased levels of TC and HDL. The interesting finding was that there was no significant change in the ApoB/ApoA-1 ratio. This indicates that ApoB/ApoA-1 might be a more reliable and stable marker for risk determining. It also indicates that the change in lipid might not have a deleterious effect; it could be rather a normalization and restoration to the usual lipid profile.

The effect of TNF inhibition on lipids is controversial. This may be due to the differences; in study design (observational, cohorts), the fact that different controls have been used in different studies (healthy, DMARDs, placebo), and the use of different biological agent (infliximab, adalimumab).

The majority of clinical trials of anti-TNF therapy were not designed to look at CVD events and were not sufficiently powered to detect a modest change in the incidence of rare events such as MI. Thus, we have to rely on results from observational studies. At a population based study, Jacobsson and colleagues compared 531 RA patients treated with etanercept or infliximab with 452 patients who had not been treated with anti-TNF agents. Patients treated with anti-TNF had half the risk of developing a first cardiovascular event compared to those not receiving these agents, the adjusted rate ratio being 0.46 (95% CI 0.25-0.85, p = 0.013) in patients treated with anti-TNF. The use of anti-TNF agents was also associated with less disability and disease severity [123].

In a large prospective analysis of British Registry data, 8,670 patients were
treated with anti-TNF compared to 2,170 patients treated with conventional DMARDs. There was no significant difference in the rate of MI between the two groups after adjusting for baseline risk factors (incidence rate ratio= 1.44, 95% CI: 0.56-3.67). However, after stratifying patients according to their response to treatment there was a significant reduction in the incidence rate in the responders arm compared to the non-responders (incidence rate ratio 0.36, 95% CI: 0.19-0.69) [56].

With regards to mortality events a study by Lunt et al was reported in the British Society for Rheumatology Biologics Register (BSRBR) [150]. They found no significant difference in the cardiovascular mortality in anti-TNF treated patients compared to patients treated with conventional DMARDs (the weighted hazard ratio (95% CI) 0.73 (0.44-1.23)). In association with CVD morbidity, Westlake et al performed a systematic review on this aspect [285]. Although there was no definite association with the risk of individual events such as MI, stroke, or heart failure, they suggest that the use of anti-TNF therapy may be associated with a decreased risk of all CVD morbidity in patients with RA. They highlighted the small number of individual events which reduce the statistical significance.

In summary, the effect of anti-TNF on CVD risk is still inconclusive and further studies are needed. As highlighted by Westalke et al, the response to TNF antagonists may be the key to the risk reduction, with TNF responders having the significant reduction in CVD events compared to the non-responders. This also suggests that the mechanism of effect may be through a reduction in the systemic inflammation. However, a firm conclusion cannot be drawn from the available evidence.
1.18.7 B-Cell depletion

B-cell plays a central role in the pathogenesis of SLE. Hypereactive B-cells produce autoantibodies that cause tissue damage. This can be due to immune complex formation, complement activation or a direct effect on cells. B-cells also can cause immune dys-regulation by producing cytokines, presenting antigen and regulating T cells. Therefore, B-cells are an attractive target for selective treatment in SLE.

B-cells have been targeted in different ways in SLE. B-cell depletion can be achieved by targeting CD20 (Rituximab). Other approaches involve blocking B-lymphocyte stimulating factor (BLYS) or targeting CD22 with (Ezpratuzumab).

Rituximab is a monoclonal antibody that is recently approved to be used for the treatment of patients with RA refractory to other DMARDs. Several trials have been carried out to examine the efficacy and safety of rituximab in patients with RA. Rituximab was proven to be effective in the treatment of long standing active rheumatoid arthritis. The disease activity was reduced significantly and it was effective in the prevention of joint damage.

Gonzalez-Juanatey and colleagues reported a dramatic improvement in the FMD in 6 RA patients (age 55-79 years; with active RA refractory to anti TNF therapy) treated with rituximab two weeks after the start of therapy [95]. This improvement in vascular function was associated with a decline in CRP and disease activity score (DAS-28) but the strength of association was not reported. They also showed a mild, but not significant decrease in TG level and increase in HDL, and a significant increase in TC and LDL levels compared to the baseline level. This may be attributed to the effect of steroid which is co-administered with rituximab, but the persistent improvement observed after 24 weeks indicates
a role for rituximab.

In contrast, Mathieu et al reported no change in the arterial stiffness in 30 RA patients (mean age 61 years) after 6 months or one year of rituximab treatment [166]. Both TC and LDL significantly increased following treatment in these patients, no change in HDL or TGs was found. Disease activity and inflammation improved following therapy. The contrasting results between the two studies may be attributed to the size of study. Both studies examined different outcome FMD vs arterial stiffness. Arterial stiffness may need a longer time to improve.

In SLE patients, studies looking at the effect of biological therapy on CVD risk are scarce. One report indicated that rituximab treatment was associated with improvement in the lipid profile (↓ total cholesterol, ↓ LDL-cholesterol, ↓ atherogenic index and ↓ TG) after one year of treatment [193]. This study included 12 SLE patients who were assessed at baseline and one year after therapy. The change in lipid profile correlated with a reduction in disease activity (BILAG from 11 at baseline to 5 one year later) and the mean steroid dose. This improvement in lipid profile might be a direct reflection of reduced disease activity and inflammation. There was no studies that looked at the effect of treatment on oxidant stress in patients with SLE or RA.

1.19 Overall hypothesis

Dyslipidemia, oxidant stress, and endothelial dysfunction are associated with inflammation in patients with SLE/RA and these factors contribute to cardiovascular risk in these populations.
1.20 Aims

1. To determine the association of oxidant stress, lipid profiles and endothelial dysfunction, markers of subclinical atherosclerosis in SLE.

2. To assess the relation between dyslipidemia and cardiovascular outcome in patients with early inflammatory polyarthritis.

3. To compare oxidant stress, lipids, and endothelial dysfunction markers in SLE/RA patients vs healthy controls.

4. To compare changes over time in oxidant stress, lipids, and endothelial dysfunction markers in active SLE after treatment.

5. To compare changes over time in oxidant stress, lipids, and endothelial dysfunction markers in active RA after treatment.
Chapter 2

Methods

2.1 Introduction

This chapter will describe the general methods used in this thesis. For the purpose of simplicity, there were three cohorts studied in this project. Some of the methods were common to more than one cohort. Thus I will describe in this chapter the general methods that were commonly used. A detailed description of each cohort will be introduced in each chapter. Generally, the study cohorts are:

1. A cross sectional study of SLE patients looking at lipids and oxidant stress in association with sub-clinical atherosclerosis.

2. Patients with early inflammatory polyarthritis recruited from the Norfolk Arthritis Registry (NOAR). In this cohort we looked at the lipid profile and apolipoprotein at baseline and their ability to predict all-cause/CVD mortality.

3. A prospective group of patients with SLE/RA with active disease looking at the effect of treatment on endothelial function and vascular biomarkers.
2.1.1 Contribution of the candidate

The first cohort was assembled over five years as part of another study looking at the novel risk factors for CVD in SLE. Patients who satisfy the inclusion criteria (females, age $>18$ years and have $\geq 4$ ACR modified criteria for SLE) were recruited from the Lupus outpatient clinic in Manchester Royal Infirmary Hospital. The candidate Awal Almohmedhusain (AA) was not involved in patients recruitment or assessment. However, the sample analysis (stored samples) for oxidised-LDL, urinary 8-isoprostane, RAGE, lipoprotein (a) were done by the candidate after a period of training and under the supervision of Mr. Philip Pemberton, Mrs. Eifen Leu and Dr. Valentine Charlton-Menys. Data cleaning and preparation of the dataset and statistical analysis and interpretation of the results were performed by the candidate.

With regards to the NOAR cohort, similarly, it is an ongoing primary care inception cohort. Patients aged $\geq 16$ years, with two or more swollen joints lasting for $\geq 4$ weeks are involved in this study. Data collection take place in the Norfolk and transferred to the main dataset in Manchester. The data collection was performed by the research nurse. The laboratory analysis for lipid profile and ApoA-1 and ApoB were done by the candidate (Cardiovascular Lab, University of Manchester). Lipid profile for around 300 samples were analysed in the Norfolk and Norwich University Hospital (NNUH) as part of the cardiovascular sub-study, thus we repeated the analysis for 30 random samples to examine the correlation between the two measurements (details later). The data cleaning and preparation of the dataset, cause of death and the ICD-code interpretation were performed by the candidate. Planning of the statistical analysis and interpretation of the results were done by AA. Statistical models were discussed with Dr. Mark Lunt and Dr. Suzanne Verstappen.
The third study was a collaborative work with Dr. Benjamin Parker. I was involved in the sample collection, preparation (details in the following subsection), and laboratory analysis for lipid profile and lipid modification. We also performed validation assessment as will be described later for the reliability and repeatability of the vascular scans. Data preparation, statistical analysis and interpretation were performed by AA.

2.1.2 Sample collection

The following samples were collected from the patient:

1. Red top tubes for serum
2. Purple top EDTA tubes for plasma
3. Urine samples

The blood tubes were gently mixed by inversion. Plasma and urine samples were placed immediately on ice. Serum tubes were allowed to clot for 30 min at room temperature. Blood tubes were centrifuged at 4°C at 3000rpm for 15 minutes and the upper layer removed. Samples were aliquoted into clearly marked cryotubes (2ml screw-capped polypropylene tubes, Alpha Laboratories, Eastleigh, UK) as follows :- Plasma: Inhibitors were added to prevent oxidation on storage. 1ml plasma + 5mg reduced glutathione + 2ml butylated hydroxytoluene (10% BHT in methanol). Divide the remaining plasma into aliquots. Serum: Divide into aliquots

Urine: Inhibitor was added to prevent oxidation on storage, 1.5ml urine + 1.5 l indomethacin (1% in methanol). The remaining urine was stored in polypropylene
tubes. All samples were stored at -80°C in a freezer where the temperature was constantly monitored.

2.2 Assays used in this project

Most samples were analysed using ELISA methods, so I will start with a brief description of the general principle of ELISA followed by details of each assay used. Details of the methods used for lipid and apolipoproteins analysis are reported. Finally, the methods for assessment of endothelial function are also described.

2.3 Principles of ELISA technique

Enzyme-linked immunosorbent assay (ELISA) or enzyme immunoassay (EIA) is a biochemical technique used to determine the presence of an antigen or antibody in the examined sample. The principle of these techniques is based on an antibody sandwich procedure. The first step in this assay involves the addition of a capture antibody against the substance of interest to the microtitre plate. This will create the solid phase. A blocking agent is then added and the plate is incubated at room temperature for one hour. After washing the plate, the standards and samples are added to the plate and incubated with the solid phase. The plate is emptied and washed again and the detection antibody is added at this step. The final step involves the addition of a substrate that will generate color change in a rate proportional to the amount of the substance of interest. In this thesis we used ELISA to measure; oxidised-LDL, sRAGE, glycated-LDL and 8-isoprostane. Detailed methods (reagents and steps) for each assay are attached (see Index).
2.4 Oxidised LDL

The oxidative modification of LDL to oxidised-LDL is now considered to be a major step in the biological process of development and progression of atherosclerosis. It has been reported that LDL becomes more atherogenic after it is converted to oxidised-LDL. This assay (Mercodia AB Sweden, reference number 10-1143-01) determines the level of oxidised-LDL in human plasma or serum see Appendix (I).

2.4.1 Principles of the assay

The Mercodia oxidised-LDL is a solid phase two site enzyme immunoassay. This assay is a direct sandwich technique where two monoclonal antibodies are directed against separate antigenic determinants on the oxidised ApoB molecule. The oxidised-LDL in the sample reacts with anti-oxidised LDL antibodies bound to the microtitre wells on the plate during the incubation. Washing the plate removes the non-reactive plasma components. Oxidised-LDL attached to the solid phase is recognised by a peroxidase-conjugated anti-human ApoB antibody. The bound conjugate is detected by tetramethylbenzidine (TMB) and the reaction is stopped by adding acid to give a colorimetric endpoint.

2.4.2 Materials and reagents

The following materials and reagents are supplied with the kit:

1. A 96 well microtitre plate coated with mouse monoclonal anti-oxidised LDL.

2. Lyophilised human oxidised LDL 1-5 calibrators reconstituted with 1000 µl of distilled water.
3. H and L lyophilised human antigen controls reconstituted with 1000 µl of distilled water.

4. Enzyme conjugate and enzyme conjugate buffer.

5. Assay buffer.

6. Sample buffer diluted with 150 ml of distilled water.

7. Wash buffer reconstituted with 1000µl of distilled water.

8. Substrate TMB.

9. Stop solution.

2.4.3 Assay procedure

We used plasma samples for this assay. Anti oxidation reagent was added to the samples at the time of processing and then samples were stored at -80°C. Samples were diluted in two steps to reach a final dilution of 1/6561. All reagents and samples were brought to room temperature before use. The assay procedure was as follows:

1. Enzyme conjugate solution, sample buffer, wash buffer and samples were prepared.

2. 25 µl of each calibrator, control and diluted samples were added to the coated plate in duplicate.

3. 100 µl of the assay buffer was added to each well.

4. The plate was incubated on the plate shaker at room temperature for 2 hours.
5. The plate was washed 6 times using the wash buffer. After the last wash, the plate was blotted onto paper towel.

6. A 100 $\mu$l of the enzyme conjugate was added to each well.

7. The plate was incubated at room temperature on the shaker for one hour.

8. The plate was washed again 6 times.

9. 200 $\mu$l of substrate TMB was added to each well.

10. The plate was incubated at room temperature for 15 minutes. 50 $\mu$l of stop solution was added to each well.

11. Optical density was read at 450 nm using (MRX plate reader from Dynex Technologies (Worthing, UK) using Revelation 4.21 software.) and the results were calculated.

### 2.4.4 Calculation of the results

The results were calculated by plotting a standard curve of oxidised-LDL against the absorbance values obtained for the calibrators as shown in Figure (2.1). Blank and standard values were entered into Fig P (version 2.98) from Biosoft (Ferguson, MO) and the best curve-fit determined. Sample values were averaged and the concentrations determined by extrapolation from the standard curve, final values being adjusted for the dilution factor ($\times 6561$).

### 2.4.5 Assay characteristics

Assay Dynamic Range: the range of the assay is up to 25mU/l and the minimum detection limit was calculated from the mean plus two standard deviations of
Figure 2.1: This graph shows the standard curve of oxidised-LDL. Oxidised-LDL level in the standards is plotted against the absorbance.

8 analyses of reagent blank and was found to be 0.037mU/l. The intra-assay (within run) Variation was calculated from repeated measurement of one sample 10 times.

- **mean** = 78.46U/l
- **SD** = 4.57U/l
- **CV** = 5.83%
2.5 Urinary 8-Isoprostane

This assay determines Isoprostane in urine (ELISA kit from Oxford Biomedical Research (EA 85). It is a competitive ELISA test. Urine samples were analyzed in duplicates.

2.5.1 Reagents and materials

1. A 96-well plate that is coated with anti 15-Isoprostane $F_{2t}$.

2. 15-Isoprostane $F_{2t}$ standard (1µg/ml).

3. Enhanced dilution buffer (to dilute the samples, ready for use).

4. Wash buffer for washing the plate (diluted with deionized water before use).

5. TMB substrate (ready for use).

6. 15-Isoprostane F2t HRP conjugate.

7. Samples are diluted ($\times 4$)

2.5.2 Assay procedure

1. 100 µl of standards and samples were added to each well.

2. 100 µl of diluted 15-Isoprostane $F_{2t}$ HRP conjugate was added to all wells except the reagent blank to which enhanced dilution buffer was added.

3. The plate was incubated for 2 hours at room temperature with shaking.

4. The plate was washed three times with 2-3 minutes standing between the washes.
5. 200 µl TMB was added to each well and the plate was incubated for 20-40 minutes. A blue color developed at this stage.

6. 50 µl 3 M sulphuric acid was added to each well to stop the reaction. The color changed from blue to yellow.

7. The plate was read at 450 nm using (MRX plate reader from Dynex Technologies (Worthing, UK) using Revelation 4.21 software.) and the results were calculated.

8. A standard curve of the concentration vs absorbance was plotted Figure (2.2).
2.6 Glycated LDL

Non-enzymatic glycation of LDL is one of the post-secretory modifications of LDL that affects its atherogenic potential. It has been reported that premature and severe atherosclerosis were associated with diabetes mellitus and increased lipid glycation. This method allows direct determination of glycated-LDL in plasma. It is based on non-radioactive immunoassay (GLYCACOR, Exocell Philadelphia).

2.6.1 Reagents and materials

1. GLYCACOR assay plate is a plate pre-coated with a standardised preparation of glycated-LDL.

2. GLYCACOR wash buffer supplied in a concentrated form and had to be diluted 1:10 (900 ml) of distilled water before use.

3. LDL diluent used to dilute the glycated-LDL in standards and samples.

4. Glycated-LDL standard, a preparation of human glycated-LDL, reconstituted with 1.0 ml of distilled water. Then serial dilutions of this standard were made and this should give values between 0.01-0.1 mg/dl.

5. Positive control (lyophilised glycated-LDL) reconstituted with 0.5 ml of distilled water and meant to give a value >0.2 mg/dl.

6. Assay control, a vial of lyophilised human plasma reconstituted with 0.5 ml of distilled water, targeted to give a value within the assay range.

7. ES12 anti-glycated antibody, a mouse monoclonal antibody that specifically binds to the glycated apoB site within the LDL complex. This was diluted with 4.4 ml of LDL diluent.
8. HRP conjugate anti-mouse IgG-HRP conjugate, goat origin ready to be used.

9. Color developer TMB ready to be used

10. Color stopper 2N sulphuric acid supplied to stop color development.

2.6.2 Principles of the assay

This is a competitive ELISA assay in a micro-plate format. It uses the mouse monoclonal antibody (ES12) to recognize the epitope on glycated-ApoB in the LDL complex. The antibody is added to the plate well that contains glycated-LDL in the solid phase. The glycated-LDL from standards and/or samples is added in the soluble phase. The antibody binds to glycated-LDL in the solid or soluble phase at the initial incubation hence the notion of competitive binding. Subsequent washing, removes all binding in the soluble phase and the bound antibody on the solid phase is detected with HRP-conjugated goat anti-mouse antibody. This assay is competitive hence the reading of color intensity upon addition of TMB is inversely proportional to the concentration of glycated-LDL in the soluble phase samples. The amount of glycated-LDL is calculated from the standard curve.

2.6.3 Dilution of glycated-LDL standards

1. 5 microfuge-tubes are prepared and labeled from 0-4.

2. 0.4ml of freshly made glycated-LDL standard is added to tube 0.

3. 0.2ml of LDL diluent is added to tubes 1-4.

4. 0.2ml of standard from tube 0 is transferred to tube 1 and vortex.
5. 0.2ml of content from tube 1 is transferred to tube 2 and vortex.

6. 0.2ml of content from tube 2 is transferred to tube 3 and vortex.

7. 0.2ml from tube 3 is transferred to tube 4 and vortex.

2.6.4 Dilution of plasma samples

1. 800µl of LDL diluents was added to the microfuge tubes

2. 20µl of the samples were added to the diluent, the inside of the tip is washed by repeated aspiration/discharge.

3. The content was mixed by vortex.

2.6.5 Test procedure

1. All the wells were drained and washed 5 times with diluted glycacor wash buffer, the residual fluid was removed after the last wash by inverting the plate on adsorbent paper and gently tapping.

Diluted Standard and samples application

A. A1-B1 wells 100 µl of LDL diluent was added (Blank wells; No Ag, No Ab)

B. C1-D1 wells 50 µl of LDL diluent was added(Maximum absorbance, No Ag)

C. E1-F1 wells 50 µl of LDL standard from tube 0 was added (Standard 0)

D. G1-H1 wells 50 µl of standard from tube 1 was added (Standard 1)
E. A2-B2 wells 50 µl of standard from tube 2 was added (Standard 2)
F. C2-D2 wells 50 µl of standard from tube 3 was added (Standard 3)
G. E2-F2 wells 50 µl of standard from tube 4 was added (Standard 4)
H. G2-H2 wells 50 µl of positive control was added (+ve control)
I. A3-B3 wells 50 µl (Assay control)
J. From C3-D3 wells onwards 50 µl of diluted sample was added in duplication

2. ES12 anti-glycated-LDL was reconstituted in 4.4 ml LDL diluent.
3. 50 µl of ES12 antiglycated-LDL was added to each well starting from well C1.
4. The plate was covered with the cling film and incubated for 1 hour at room temperature.
5. Put HRP conjugate on the bench to bring it to the room temp.
6. After incubation, the plate was drained and the strip was washed 10 times with buffer. After the final wash the plate was plotted on paper towel.
7. 100 µl of HRP conjugate was added to each well. The plate was covered and incubated for 1 hour at room temperature.
8. The color developer and color stopper were brought to room temperature.
9. After incubation, the plate was drained and washed 10 times with buffer.
10. 100 µl of color developer was added to all the wells.
11. The plate was covered and incubated for 10 minutes at room temperature.
Figure 2.3: This graph represents the standard curve of glycated LDL in which the concentration of glycated-LDL of the standards is plotted against the absorbance.

12. 100 µl of color stopper was added to all the wells.

13. The absorbance was read at 450 nm using A1 as blank using (MRX plate reader from Dynex Technologies (Worthing, UK) using Revelation 4.21 software.) and the results were calculated.

2.6.6 Calculation of results

Standard curve was drawn by plotting the standard concentration against absorption as in Figure (2.3). Then the concentration of glycated-LDL was calculated from the standard curve and multiplied by the dilution factor.
2.7 Assay for Human RAGE (receptor for advanced glycation end products)

2.7.1 Principles of the assay

This assay is a quantitative sandwich enzyme immunoassay (Mercodia AB, Sweden). A monoclonal antibody specific for the extracellular domain of RAGE has been pre-coated onto a microplate. Standards and samples are pipetted into wells and any present RAGE will bound by the immobilised antibody. After washing away any unbound substances, an enzyme linked polyclonal antibody specific for RAGE is added to the wells. A second wash is performed to remove unbound antibody-enzyme reagents, then a substrate solution is added to the wells and color is developed in proportion to the amount of RAGE bound in the initial step. This process is stopped by adding the stop solution and the intensity of the color is measured.

2.7.2 Assay reagents and buffers

1. RAGE microplate: a 96 well polystyrene microplate coated with a mouse monoclonal antibody against RAGE.

2. RAGE conjugate: 21 ml/vial of polyclonal antibody against RAGE conjugated to horseradish peroxidase with preservatives.

3. RAGE standard: recombinant human RAGE/Fc Chimera in a buffer with preservatives, lyophilized.


8. Color reagent B: 12.5 ml/vial of stabilized chromogen.


2.7.3 Reagents preparation

All reagents were brought to room temperature prior to use.

Wash buffer
20 ml of the wash buffer concentrate was diluted with distilled water to get a final volume of 500 ml of wash buffer. If crystals were formed in the concentrate it was warmed to room temperature and mixed gently till the crystals were dissolved.

Substrate solution
Color reagent A and B were mixed together in equal volumes. This was performed within 15 minutes of use and was protected from light.

RAGE standard
The RAGE standard was reconstituted with 1 ml of distilled water to perform the stock solution of 50,000 pg/ml. This solution was mixed and left for around 15 minutes to ensure complete reconstitution.

Seven tubes were prepared and labeled for serial dilutions. 900 µl of the calibrator diluent RD 6-10 was pipetted into the 5000 pg/ml tube and 500 µl to the remaining tubes. A serial dilution was produced using the stock solution.
Each tube was mixed thoroughly before the next transfer. The 5000 pg/ml standard served as the high standard and the calibrator diluent the zero standard (0 pg/ml).

2.7.4 Assay procedure

1. All reagents and standards were prepared as shown previously and brought to room temperature.

2. 100 µl of the assay diluent RD1-60 was added to each well

3. A 50 µl of standard, or sample was added per well, the plate was incubated for two hours at room temperature.

4. The plate was washed four times manually.

5. 200 µl of RAGE conjugate was added to each well and the plate was incubated for another two hours at room temperature.

6. The wash step was repeated.

7. 200 µl of substrate solution was added to each well and incubated for 30 minutes at room temperature, the plate was kept in dark to protect it from light.

8. 50 µl of stop solution was added to each well and optical density was measured at 450 nm.

2.7.5 Calculation of results

The mean absorbance of each standard was plotted against the concentration and draw a best fit curve. The concentration of serum RAGE of the samples was
calculated from the curve.

2.8 Measurements of lipids and apolipoproteins

2.8.1 Lipoprotein and apolipoproteins measures

Serum cholesterol, triglycerides, and HDL cholesterol were all measured by enzymatic-colorimetric determination using a Cobas Mira auto-analyzer (ABX Diagnostics). Apolipoproteins AI and B were determined by turbidimetry analysis on the same device. The measurements took place at the Cardiovascular Research Laboratories, The University of Manchester.

Some samples were analyzed for TC, HDL and TG at the Norfolk and Norwich University Hospital (NNUH). Validation of the two measurements will be discussed later.

2.9 Methods

2.9.1 Serum Cholesterol

The serum cholesterol assay is based on the conversion of cholesterol ester (main form of cholesterol within transporter lipoprotein) to free cholesterol by the enzyme cholesterol esterase (Cat. No. CH200, Randox, UK) according to the following formula:

\[ \text{Cholesterol ester} + \text{H}_2\text{O} \rightarrow \text{cholesterol} + \text{fatty acids}. \]

Then free cholesterol is used to generate hydrogen peroxide by the enzyme cholesterol oxidase.
• Cholesterol +$O_2 \rightarrow$ cholestene-3-one + $H_2O_2$.

The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. This then can be read by the auto-analyzer at 500nm.

• $2H_2O_2 +$ phenol+ 4-aminoantipyrine $\rightarrow$ quinoneimine + 4$H_2O$.

Samples were calibrated using Randox Calibrator (Cat. No. CAL 2351). Randox multi-sera level 2 (Cat No HN 1530) and level 3 (Cat. No. HE 1532) were used as controls.

### 2.9.2 Serum triglycerides

The principle of the procedure is enzymatic hydrolysis of triglycerides with lipases in a multi-step reaction. The indicator is a quinoneimine which is formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic effect of peroxidase.

• Triglycerides +$H_2O \rightarrow$ glycerol + fatty acids.

• Glycerol + ATP $\rightarrow$ glycerol-3-phosphate + ADP.

• Glycerol-3-phosphate + $O_2 \rightarrow$ dihydroxyacetone + phosphate+ $H_2O_2$.

• $H_2O_2 +$ 4-aminophenazone + 4-chlorophenol $\rightarrow$ quinoneimine + HCl + 4$H_2O$.

Samples were calibrated using Randox calibration serum level 3 (Cat. No. CAL 2351), while Randox Assayed multi sera level 2 (Cat No HN 1530) and level 3 (Cat. No. HE 1532) were used as controls.
2.9.3 Serum direct HDL

This assay (Randox, Cat. No. CH 2652) was used and it involves two steps:

1. Elimination of non-HDL lipoproteins by cholesterol estrase, cholesterol oxidase and then catalase;
   - Cholesterol ester $\rightarrow$ cholesterol + fatty acids.
   - Cholesterol + $O_2$ $\rightarrow$ cholestenone + $H_2O$.
   - $2H_2O_2$ $\rightarrow$ $2H_2O + O_2$.

2. Measurement of HDL after its release via detergents in reagent 2;
   - Cholesterol ester $\rightarrow$ cholesterol + fatty acids.
   - Cholesterol + $O_2$ $\rightarrow$ cholestenone + $H_2O_2$.
   - $2H_2O_2 + N-4$-aminoantipyrine + 2-hydroxy-3-sulfopropyl-3,5-dimethoxyaniline $\rightarrow$ Quinone pigment + $4H_2O$.

The intensity of the quinone dye concentration is directly proportional to HDL when measured at (600 nm). The second catalase reaction is inhibited by a detergent in reagent 2 (sodium azide). The precision of the three assays is indicated in (Table 2.1).
<table>
<thead>
<tr>
<th>Assays</th>
<th>Total cholesterol</th>
<th>HDL</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>0.19-3.37 mmol/l</td>
<td>0.13-12.70 mmol/l</td>
<td></td>
</tr>
<tr>
<td>Intra-assay precision (%CV) low level</td>
<td>3.73% at 1.71 mmol/l</td>
<td>1.80% at 0.79 mmol/l</td>
<td>3.29% at 0.31 mmol/l</td>
</tr>
<tr>
<td>Intra-assay precision (%CV) high level</td>
<td>3.84% at 7.7 mmol/l</td>
<td>3.11% at 2.00 mmol/l</td>
<td>1.77% at 5.61 mmol/l</td>
</tr>
<tr>
<td>Inter-assay precision (%CV) low level</td>
<td>1.33% at 1.67 mmol/l</td>
<td>3.81% at 0.82 mmol/l</td>
<td>3.51% at 0.64 mmol/l</td>
</tr>
<tr>
<td>Inter-assay precision (%CV) high level</td>
<td>1.39% at 7.52 mmol/l</td>
<td>2.73% at 2.01 mmol/l</td>
<td>1.33% at 3.03 mmol/l</td>
</tr>
</tbody>
</table>

Table 2.1: This table represents the intra-assay and inter-assay coefficients of variability for low and high levels of total cholesterol, HDL and triglycerides (TG)

2.9.4 The agreement between measurement in Manchester and Norwich laboratories

As mentioned before, some of the samples were analyzed at the Department of Clinical Biochemistry in the Norfolk and Norwich University Hospital (NNUH). To test the agreement between the two measurements, we repeated the measurements of 30 random samples in the Cardiovascular Lab, University of Manchester and we examined the correlation between the two measurements. The correlation between the two measurements was very good as shown in (Table 2.2).

We further tested the agreement by plotting Bland-Altman graphs. Figures (2.4-2.6) show the Bland-Altman plots for total cholesterol, HDL and LDL. The X-axis represents the average of the two measurements and the Y-axis show the difference between them. The line in the middle is the bias line and the two lines above and below represents the upper and lower limits of agreement (bias±1.96(SD). As can be seen from Figure it can be concluded that one of the labs reads slightly higher than the other and this drift increases towards the higher values. Despite this, the limit of agreement (-0.4-1.1) are small enough for us to
Table 2.2: This table shows Spearman’s correlation between results from NNUH and Cardiovascular lab in Manchester.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>0.96</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL</td>
<td>0.96</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TG</td>
<td>0.95</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

be confident to use the two measurements. The mean difference between the two labs is less than 10% which is clinically acceptable. Similarly, for triglycerides measurement the agreement is even better with a narrower limit of agreement (-0.01-0.4). Bland-Altman of HDL, readings are scattered around the bias line evenly which indicates a good agreement between the two measurements.
2.9.5 LDL cholesterol determination by the Friedewald equation

LDL-cholesterol was calculated using an indirect method described by Friedewald et al [76]. The formula uses total cholesterol, triglycerides and HDL to determine LDL as following provided TGs level less than 4.5 mmol/l.

\[ \text{LDL} = \text{serum cholesterol} - [\text{HDL} - (\text{serum triglycerides}/2.2)] \]

2.9.6 Determination of Apo A-I and Apo B

This method (Randox Cat. No. LP 2116) is based on a reaction of samples containing human Apo A-I/Apo B and specific anti-serum to form insoluble complex that can be determined turbidimetrically at 340nm. Table (2.3) shows the precision and minimum detection of the assays.
Figure 2.5: Bland-Altman plot of HDL the x-axis represents the average readings against their difference. Horizontal lines represents the bias (middle line) and upper and lower limit of agreement.

Figure 2.6: Bland-Altman plot of TG the x-axis represents the average readings against their difference. Horizontal lines represents the bias (middle line) and upper and lower limit of agreement.
<table>
<thead>
<tr>
<th>Assays</th>
<th>Apo A-1</th>
<th>Apo B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Range</td>
<td>6.5-239 mg/dl</td>
<td>13.5-193 mg/dl</td>
</tr>
<tr>
<td>Intra-assay precision (%CV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>low level</td>
<td>2.67 mg/dl</td>
<td>3.86 mg/dl</td>
</tr>
<tr>
<td>high level</td>
<td>4.1 mg/dl</td>
<td>4.13 mg/dl</td>
</tr>
<tr>
<td>Inter-assay precision (%CV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>low level</td>
<td>3.19 mg/dl</td>
<td>1.79 mg/dl</td>
</tr>
<tr>
<td>high level</td>
<td>3.22 mg/dl</td>
<td>2.57 mg/dl</td>
</tr>
</tbody>
</table>

Table 2.3: A table illustrates the intra-assay and inter-assay coefficient of variability for low and high level for ApoA-1 and ApoB.

### 2.10 Assay for lipoprotein (a)

Quantitative determination of lipoprotein (a) (Lp(a)) is based on immunolatex enhanced immunoassay (LEIA). In this assay, anti Lp(a) antibody coupled to latex particles forms high molecular weight immuno-complexes with Lp(a) antigens in the sample. This leads to a change in the turbidity of the sample which can be determined photometrically by turbidimetric and nephelometric analyzers. This is usually directly proportional to the antigen concentration. The change in absorbance can be converted to antigen concentration using a reference curve prepared from standards of known concentration.

#### 2.10.1 Assay procedure

Plasma samples were used for this assay. Immuno LEAI antihuman Lp(a) latex reagents and diluent were ready for use. The reference standard and control human were lyophilized and were reconstituted with 0.5 ml of distilled water at least 30 minutes before use. The samples (controls and patients) were pre-incubated with immuno LEAI Lp(a) diluent for 5 minutes. At the same time the intrinsic blank reaction was completed. The immune reaction started when the latex reagent was added.
2.10.2 Calculation of results

A reference standard was provided with the kit and used to construct a reference curve from which the concentration of Lp(a) was calculated.

2.11 Study of endothelial function

2.11.1 Hypothesis:

The endothelium is a single layer of cells that maintains the integrity of blood vessels. It has a number of physiological functions which mainly maintain a balance between vasodilatation and vasoconstriction. Endothelial function is impaired in conditions such as SLE/RA. Endothelial dysfunction is triggered by endothelial injury. There are several factors that may cause endothelial injury in those patients including: chronic inflammation, oxidant stress, metabolic derangement, etc. In patients with active SLE/RA, reduction in inflammatory activity may improve the endothelial function.

2.11.2 Assessment of endothelial function

In this study we used two methods to examine endothelial function namely:

1. Peripheral arterial tonometry was obtained using the EndoPat 2000 device (Itamar Medical Inc., Caesarean, Israel). The EndoPat probe assesses the ability of endothelium to induce dilatation of the vessel wall by detecting a change in the pulse volume after reactive hyperaemia. This is a measurement of the finger arterial pulsatile volume changes. This reflects the peripheral artery tone (PAT).
2. Flow mediated dilatation of the brachial artery (FMD). This is a non-invasive method to assess the endothelial-dependant vasodilatation of the brachial artery in response to ischemic stimulus on the brachial artery. Endothelial independent vasodilatation is also assessed by this method using 300µg sublingual nitre-glycerine (GTN) tablets.

2.12 The settings

Assessment of participants was carried out in the morning around (9:00-11:00am) at the Wellcome Trust Clinical Research Facility (WTCRF) in a temperature controlled room. All subjects were asked to fast overnight, withheld any anti-hypertensive medicines until after the assessment. Participants were advised not to smoke in the morning of the study and abstain from alcohol for 24-48 hours before the assessment. All participants were consented on arrival. Demographic measurements were taken (height, weight, hip and waist circumferences, and bio-impedance). Blood was collected for immunological and other biochemical measurements. The participant was asked to lie on the bed with their head at 30° and told to relax for 5-10 minutes. During this time, the ultra-sound scan setting took place. The FMD protocol was adapted from previous publication [35][46]. The setting of both EndoPat and FMD will be described in more details in the following sections.

2.12.1 EndoPat

The main components of the EndoPat are the specially designed disposable probes shown in Figure (2.7). These are composed of inflatable latex air cuff connected via the pneumatic tubes to the inflating device. The EndoPat system was pre-
Chapter 2 Methods

Figure 2.7: This is a photo of the EndoPat. The EndoPat 2000 device, pneumatic tubes and the probes. The picture on the right side shows the foam rubber attached to the middle finger to fix the probe and prevent any mechanical intervention when the test takes place.

pared for data acquisition and was attached to a PC software where patients details (ID, age, gender, blood pressure) were recorded. For each patient 2 new probes were used and connected to the pneumo-electrical tubes. The probes were inserted into the sockets of the arm-supports. The patient was asked to place one of their fingers (preferably the index) into the probe all the way till they feel the back of the probe by the tip of their finger. Thereafter, the probe was inflated. The probes were kept in a steady position by attaching a foam anchor from the tube to the middle finger and tape a loop around the middle finger gently. The tubes were kept out of contact with any objects including arm-rest, foam ring anchor, the mattress or other fingers. The patient’s arms were repositioned with the forearms supported on the arm-rest; fingers are positioned free at the edge of the arm-rest. The standby mode was applied for 5 minutes and the gain was adjusted for the best PAT signals on both probes. The study measurement included five minutes baseline recording, then five minutes when the blood pressure
Figure 2.8: This photo shows the mechanism of EndoPat measurement of endothelial function. It uses one arm as a control and assesses the endothelial function on the occluded arm as compared with the control arm.

cuff was inflated (a minimum of 200 mm Hg is required for data acquisition by the EndoPat or 50 mm Hg above the systolic blood pressure) and five minutes for the post deflation recordings. An automatic analysis was performed by the software and the results were displayed on the screen. It quantified the endothelium dependent changes in vascular tone triggered by 5 minutes occlusion of the brachial artery. This was expressed as Reactive Hyperemia and this was analyzed by the software as an increase in the PAT signal amplitude. The ratio of post occlusion to pre-occlusion was calculated by the EndoPAT software providing the reactive hyperemia index (RHI) or what is known as the Endoscore. Figure (2.8) shows an example of the EndoPat output.
2.12.2 Flow mediated dilatation

The setting of this procedure as shown in Figures (2.9, 2.10) includes: Philips ultrasound machine connected by (12.5 MHz linear array transducer) to a probe and probe holder to fix the probe after acquisition of the scan. The ultrasound machine was set on the cardiovascular, arterial, peripheral arteries mode.

In the first scan the B-mode ultrasound was used to measure flow mediated endothelium-dependent dilatation of the brachial artery in response to ischemic stimulus. The forearm was rested on an arm-rest with the head of bed tilted at about 30°. The brachial artery was scanned 2-15 cm above the ante-cubital fossa. The best position of the probe was identified when an image with clear medial and lateral wall of the brachial artery was observed on the PC screen. The transducer position then is fixed by the probe holder. Depth and focus were adjusted to obtain the best image. The ultrasound scan was connected to edge detecting and wall tracking PC software (Figure 2.11) that automatically calculates the FMD to minimize the observer error. A blood pressure cuff was wrapped around the forearm and inflated to around 50 mm Hg above systolic pressure (a minimum of 200 mm Hg) and left inflated for 5 minutes. This cuff causes temporary cessation of blood flow. A release of this cuff leads to an increase in the blood flow and hence causes reactive hyperaemic vasodilatation in normal blood vessel. Example of the computer screen output of FMD is shown in Figure 2.12.

We measured the brachial artery’s diameter at baseline and 45-90 seconds after deflation according to the maximum response observed. The percentage FMD then calculated by the software. The principle of this calculation is to measure the percentage change in diameter after the cuff release (caused by reactive hyperaemia) compared to the resting diameter. The formula for this calculation is:
Figure 2.9: This photo shows the room setting of the ultrasound scan for FMD and EndoPat measurements, the participant is lying comfortably on the bed, on the right hand side of the participant is the ultrasound machine, PC, and on the other side the EndoPat device attached to the computer. Participants arms are rested on the arm-rest on each side.

\[
FMD = \frac{\text{Post-deflation diameter} - \text{Baseline diameter}}{\text{Baseline diameter}} \times 100
\]

After 5-10 minutes rest, we repeated the same procedure for the second scan, this time with the use of 300\(\mu\)g sublingual of nitroglycerine tablet as a stimulus. By this we test for endothelium-independent vasodilatation of the brachial artery. After a baseline run of 2 minutes, a sublingual GTN tablet was taken by the participant. Once a full response was noticed on the screen of the monitor, the participant was asked to take the tablet out to avoid any headache or dizziness which are common side effects of this agent.
Figure 2.10: This photo shows the setting of the ultrasound scan for FMD measurement, the ultrasound probe is attached to the participant arm and fixed at position by the probe holder, blood pressure cuff around the forearm. The participant index is attached to the EndoPat probe.

Figure 2.11: This photo shows the ultrasound of the FMD. The far left photo showing the edge tracking of the brachial artery, then a baseline diameter before (photo at the middle) and post deflation diameter after cuff release (photo on the right).
Figure 2.12: A snapshot of the screen reading for the FMD software the reading between yellow and green lines represent the baseline diameter followed by the occlusion period (between green lines), post deflation diameter is between the pink lines.

2.13 Validation of the FMD on healthy volunteers

We aimed to assess the reproducibility of our measurements in healthy volunteers. For this purpose we performed two sets of preliminary assessment:

- Study 8 subjects at two time points.
- Repeat the same measurement in one subject 8 times.

2.13.1 The reliability of measurements

As the requirement of the study is to assess the endothelial function at two occasions, we needed to test the reliability and reproducibility of our measures. For this purpose, we studied 8 healthy volunteers, (3 men, 5 women), age ranges (27-39 years), all of them were free from any CVD or other clinical morbidity. All volunteers were assessed at two time points. We used the same setting mentioned
above. Both FMD and RHI (EndoPat) were assessed at both visits simultaneously. We assessed the reliability of measurement of baseline diameter, FMD and RHI at two different times.

2.13.2 Reliability of baseline resting/post-deflation diameter measurement

The measurement of baseline diameter at visit (1) was very close to the baseline diameter at visit (2) with good correlation between the two measurements (R=0.9524, P=0.0003) [192]. Similarly, the association between the post deflation diameter in visit (1) vs visit (2) was very significant (R=0.9286, P=0.0009).

2.13.3 The reliability of %FMD/RHI measurements

We examined the association between a repeated measurement of %FMD and RHI in the healthy volunteers at two visits for details see [192]. Briefly, the %FMD was similar in visit 1 and visit 2 with a correlation coefficient of (R=0.7857, P=0.02). The correlation coefficient for RHI Figure (2.13) was less significant than that for the FMD (more variation between the two visits) as indicated by the correlation coefficient (R=0.42, P=0.39).

2.13.4 The association between resting diameter and %FMD

We also explored the association between resting diameter and %FMD Figure (2.14) and we found a negative correlation between them (R=-0.3, P=0.29). However, this was not statistically significant.
Figure 2.13: Correlation between RHI measurements at two visits.

Figure 2.14: Correlation between baseline diameter and %FMD.
2.13.5 The correlation between FMD and RHI

We tested the correlation between FMD and RHI in our preliminary assessment on 8 healthy volunteers. There was a reasonable correlation between the two measures in healthy volunteers ($R = 0.73$, $P=0.003$) for visit (1) and $R = 0.6429$, $P=0.1194$ at visit (2) Figure (2.15). However, at visit (2) the association was not statistically significant. This may be due to the variability of the RHI between the two visits.
<table>
<thead>
<tr>
<th></th>
<th>Mean(SD)</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting diameter</td>
<td>4.2(0.148)</td>
<td>3.5%</td>
</tr>
<tr>
<td>Reactive diameter</td>
<td>4.59(0.156)</td>
<td>3.4%</td>
</tr>
<tr>
<td>% FMD</td>
<td>6.27(2.648)</td>
<td>42.2%</td>
</tr>
<tr>
<td>%FMD (no outlier)</td>
<td>7.01 (1.77)</td>
<td>25.3%</td>
</tr>
</tbody>
</table>

Table 2.4: Reproducibility testing by repeated measurements on one subject 8 times.

## 2.13.6 The reproducibility of measurements

To achieve this, we did 8 repeated measurements of the resting diameter and %FMD on one healthy volunteer over a period of time around 2 months. Then we calculated the coefficient of variability from the formula SD/mean*100. Table (2.4) indicates the main results. We got a very good reproducibility for the resting diameter as indicated by a low CV of 3.5%.

As indicated in the table below, there was high variability in the %FMD with a CV of 42.23%. When the outlier was excluded, the variability improved to 25.3%. Reproducibility of measurement of resting diameter and %FMD are illustrated in Figures (2.16 and 2.17).

We further explored the digital measurement variation. The images were digitized to 0.03-0.06 mm/pixel using the automated method. We tested the variation of digital difference on resting diameter measurement. For instance, if we consider visit 1 and visit 3 from the data on (Table 2.5), we found that the resting diameter at visit 1 was 4.33 mm, and at visit 3 was 4.37 mm, i.e. there was 0.04 mm difference between the two visits. However, when it comes to the FMD, there was a 1.29% difference which represents 19% of the mean %FMD.

It can be speculated that the higher the %FMD is the lower the variability. If we exclude %FMD readings with values <7, and recalculate the CV, we get a mean (SD) of 8.2 (0.86) and CV of 9.7% as compared to 42.2%.
<table>
<thead>
<tr>
<th>Visit number</th>
<th>Baseline diameter $mm$</th>
<th>Maximum post deflation $mm$</th>
<th>%FMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.33</td>
<td>4.65</td>
<td>7.39</td>
</tr>
<tr>
<td>2</td>
<td>4.07</td>
<td>4.38</td>
<td>7.62</td>
</tr>
<tr>
<td>3</td>
<td>4.37</td>
<td>4.64</td>
<td>6.10</td>
</tr>
<tr>
<td>4</td>
<td>4.29</td>
<td>4.54</td>
<td>5.84</td>
</tr>
<tr>
<td>5</td>
<td>4.16</td>
<td>4.20</td>
<td>1.12</td>
</tr>
<tr>
<td>6</td>
<td>3.99</td>
<td>4.34</td>
<td>8.98</td>
</tr>
<tr>
<td>7</td>
<td>4.34</td>
<td>4.52</td>
<td>4.13</td>
</tr>
<tr>
<td>8</td>
<td>4.07</td>
<td>4.4</td>
<td>8.98</td>
</tr>
</tbody>
</table>

Table 2.5: This table represents the measurements of baseline diameter, maximum post-deflation diameter and %FMD one subject repeated over time.

Figure 2.16: Scatter plot of resting diameter measurements repeated 8 times in one subject.
2.14 Discussion

Lipids agreement

The estimation of lipoprotein variables was done with the help and supervision of a biochemist and laboratory technician. The performance of the auto-analyzer is regularly checked and assessed. The results showed high accuracy. The method used for lipoprotein estimation was identical in the two laboratories.

We repeated the measurement of lipids in 30 random samples to examine the agreement between the two measures. We found a very strong correlation between measurements in the Cardiovascular Lab, University of Manchester and in the Norfolk and Norwich University Hospital (NNUH) with a correlation coefficient of R=0.95-0.96. Although one of the laboratories seems to read slightly higher than the other lab the bias lines were narrow which indicate that this is accepted and should not affect the results dramatically.
Assessment of FMD

We validated the FMD technique by assessing multiple factors. Initially, we tested for the correlation between the first and second 8 measurements made by one observer. In our study, there was a good correlation between the two measurements (R=0.95). Welsch et al reported a correlation coefficient (R=0.74) after 13 scans repeated two times by one observer [284]. We also assessed the correlation of %FMD at two visits (R=0.78). This is similar to the previously reported correlation coefficient by De Roos et al [51].

With regards to the reproducibility of measurement of the resting diameter and %FMD over time, we demonstrated a %CV of 3.5%. Other investigators reported %CV ranging from 1.5%-6% as cited by De Roos et al [51]. The mean (SD) %FMD was 6.27 (2.648) with a CV=42%. This is improved after we excluded the outlier to be 25.28% as discussed previously.

High variability of %FMD was reported previously [108]. De Roos repeated measurements in 16 subjects 6 times and also reported a high variability of FMD (CV=50%). Although some groups reported a low inter-observer variability of 1.4% [35], this was criticised and attributed to the method of expressing FMD as the percentage of baseline diameter rather than percentage change. Liang et al also reported a CV of around 10% but that was calculated in 30 subjects from two visits only [146].

There are inherent problems with the technique such as the limit of resolution of the ultrasound probe. This can also contribute to the high variability of FMD. We used the automated edge detector and analysis of brachial artery to increase the accuracy and reduce the variability. The automated method was introduced by Sonka et al [245]. This method involves automated detection of the wall properties, border detection in image sequence, quality control of borders over
individual frames and calculation of markers of vascular function.

There is evidence from previous reports that FMD is influenced by a number of factors as discussed in the first chapter such as fasting, alcohol, smoking, exercise, medications and hormonal factors [34]. We tried to control most of these factors to minimise the variability. However, FMD is sensitive to many factors and it is impossible to eliminate them all. There is a residual effect of unknown variables on FMD.

Then we examined the correlation between FMD and EndoPat reactive hyperaemic index. In our preliminary analysis, we found a good correlation between the two measures with a correlation coefficient R=0.7. The correlation between the two measures has been reported previously and ranged between R=0.3-0.55 [138][137].

Both FMD and RHI are surrogate markers for endothelial function. However, they may be influenced by different factors. For example, the cutaneous vascular beds at the finger tips are more sensitive to a change in the room temperature than the larger conduit arteries such as the brachial artery. The magnitude of the blood flow is also different between the two sites. It is possible that FMD and EndoPat may be affected in the same direction but not in the same magnitude.

We managed to show that our validity technique was similar to the majority of the previously reported validation studies. Some sources of variability were ascertained including the digital measurements of resting diameter and the magnitude of %FMD.
Chapter 3

Oxidant Stress in SLE Patients and Subclinical Atherosclerosis

3.1 Introduction

Premature atherosclerosis is a major cause of morbidity and mortality in patients with systemic lupus erythematosus (SLE) [28]. Women with SLE have up to 50-fold increased risk of myocardial infarction [155]. There is also an increased risk of having subclinical atherosclerosis, measured as the presence of plaque on carotid ultrasound [3]. Traditional cardiac risk factors explain to some extent this increased risk [30]. However, cardiovascular events are still common in patients with SLE even after adjustment for traditional risk factors [66]. Thus, although patients with SLE have higher prevalence of traditional risk factors, these do not explain the increased cardiovascular morbidity and mortality and suggests the presence of other novel or disease related factors.
Over the last three decades a large body of evidence has indicated that oxidative stress plays an important role in the initiation and progression of atherosclerosis [288][136]. Navab et al have extensively reviewed the oxidation hypothesis and the role of oxidised-LDL in atherogenesis [181].

Oxidative stress may therefore function as a link between chronic inflammation and lipid deposition in the arterial vasculature. It has also been reported that increased 8-isoprostane (a measure of oxidative stress) is associated with coronary artery calcification in young healthy adults from the CARDIA study which supports the association between oxidative stress and the early stage of atherosclerosis [101]. It has also been noted that the level of oxidised-LDL was associated with the degree and extent of coronary artery disease [265].

Recently, oxidised-LDL has been studied as a predictor of future CVD events and some evidence has been published; Meisinger et al has shown that high oxidised-LDL level is a predictor of CHD in apparently healthy men [170]. The adjusted HR for the upper tertile of oxidised-LDL compared to the lower tertile was 4.25 (95% CI 2.09-8.63; P<0.001). According to their study plasma oxidised-LDL is the strongest predictor of CHD among other lipid profile and traditional risk factors. There is also a report on patients with type 2 diabetes that indicates increased hazard ratio for cardiac events in patients with high oxidised-LDL. After adjusting for other traditional risk factors HR=3.6 (95% CI 1.5-8.8; P=0.005). The cut off point in this study was oxidised-LDL>24.7 U/l [236].

3.1.1 Hypothesis

Patients with SLE have increased levels of oxidant stress and this contributes to premature atherosclerosis.
3.1.2 Aims

- To compare lipids, modified lipids and markers of oxidant stress in SLE patients and healthy controls.

- To examine the correlation between oxidant stress and markers of inflammation and disease activity.

- To determine the association between the above markers and subclinical atherosclerosis (carotid IMT and plaque) in SLE patients and healthy control.

3.2 Study design and setting

3.2.1 Study setting

This is a cross-sectional study of SLE patients and healthy controls. All participants in this study were assessed at the Wellcome Trust Clinical Research Facility (WTCRF), Manchester Royal Infirmary. This cohort was assembled over five years as part of a study examining novel risk factors for atherosclerosis with specific focus on telomere length and endothelial progenitor cells (Chief Investigator: Prof. Ian Bruce). The cohort and its clinical assessment was part of a PhD thesis by Dr. Sahena Haque [106] and recruitment was in its final year when I started my programme of study. Serum and plasma from this cohort was stored for additional studies including lipid sub-analysis and markers of oxidation. All laboratory experiments were performed at the University of Manchester: the Lipid Research Laboratory, Cardiovascular Research Group, Core Technology Facility Building; and the Specialist Assay Laboratory, Central Manchester
Chapter 3  
Oxidant Stress in SLE Patients and Subclinical Atherosclerosis

Foundation Trust.

3.2.2 Study population

A total of 168 patients with SLE were enrolled in the study. Ethical approval was granted by the National Research Ethical Service (Appendix I). The majority of patients were recruited from outpatient clinics at the Central Manchester Foundation Trust. All patients were females, aged >18 years, and fulfilled at least four of the American College of Rheumatology (ACR) 1997 revised classification criteria for SLE [111]. Subjects were excluded if they were pregnant, lactating, or had been diagnosed with malignancy. Fifty-six control subjects, matched by sex and age (within 10 years) were also recruited (using a "best friend" scheme). All patients had a detailed clinical assessment and examination on the day of assessment. Disease activity in SLE patients was assessed by the use of Systemic Lupus Erythematosus Disease Activity Index-2K (SLEDAI-2K)[87] and organ damage was assessed by the ACR/Systemic Lupus International Collaborating Clinics (ACR/SLICC) Damage Index [248]. After an overnight fast, a blood sample was obtained on the morning of assessment. As part of the routine assessment, full blood count, liver function test, total cholesterol, LDL, HDL cholesterol, triglycerides, fasting blood glucose and biochemical profile, including creatinine, were measured for each patient. An autoantibody profile was also performed, which included anti-double stranded DNA (dsDNA) and anticardiolipin antibodies. A plasma sample was used to measure lupus anticoagulant. C-reactive protein (CRP) and levels of C3 and C4 complement factors were also determined.
3.3 Methods

3.3.1 Estimation of subclinical atherosclerosis

Subclinical atherosclerosis was assessed using a non-invasive technique to measure carotid intima media thickness (cIMT). B-mode ultrasound scan of the carotid artery was employed and was performed by one of the vascular technicians as previously described [3]. Briefly, both right and left carotid arteries were examined in longitudinal and cross-sectional planes. The areas examined included common carotid artery (CCA), carotid bulb, and the first 1.5 cm of the internal and external carotid arteries. The carotid artery thickness was measured at the areas of highest thickness on the far wall of the CCA around 1 cm proximal to the carotid bulb. The mean IMT was taken as the average of six readings from both sides (3 readings each side) [238]. In this study, the median IMT of the normal population, estimated using data from the healthy controls in the original study, was used as a cut off point. As previously reported, the Intra-Class Correlation Coefficient (95% CI) for this measure was high in our laboratory (0.92 [0.84, 1.00]) [3]. Carotid plaque was defined if two of the following three conditions were met: (a) a distinct area of protrusion, >50% compared with the surrounding area, into the vessel lumen, (b) increased echogenicity compared to the adjacent boundaries and (c) IMT >0.15 cm [145].

3.3.2 Measurement of oxidant stress and modified lipids

Details of the methods we used to measure oxidised-LDL, Lp(a), and 8-IP are reported in Chapter (2). These were performed by the candidate after a period of training and under the supervision of Dr. Valentine Charlton-Menys and Mr.
3.4 Statistical analysis

Data were analyzed using STATA 10 (STATA Corporation, Texas). Comparison of continuous data was carried out using the Mann-Whitney U test. For categorical variables, chi-squared test was employed to calculate the significant difference between the groups. The correlation between variables was examined by the Spearman’s rank correlation test. Linear regression analysis was used to test the association between lipids and cIMT. Logistic regression was conducted using plaque (yes or no) as the outcome variable. The significance level was set at P values less than 0.05. Forward stepwise linear regression/logistic regression was applied after the univariable analysis to determine which variables were independently associated with cIMT/plaque.

3.5 Results

3.6 Description of the study cohort

3.6.1 Demographic data

A total of 168 patients and 56 healthy controls were included in this analysis. The median (IQR) age of SLE patients was 53 (46-61) years and controls 50 (39-60) years. The majority of participants were Caucasian (81% patients, 81% controls). There were 16 (9%) patients who were current smokers and their median (IQR) number of cigarettes smoked per day being 9 (5, 20). Among controls, there were
Chapter 3

Oxidant Stress in SLE Patients and Subclinical Atherosclerosis

3.6.2 SLE related factors

The median (IQR) disease duration was 13 (7, 23) years. This cohort generally had a low disease activity as most of the patients were recruited from outpatient clinics. The median (IQR) of the SLEDAI-2K was 2 (0, 5). Of the cohort, 76 (45%) had a SLEDAI-2K=0. There were 18 (10%) patients who had a SLEDAI-2K>8 indicating active disease. Figure (3.1) shows the percentage of patients in different SLEDAI categories. The median SLICC damage index (SDI) was 1 (0, 2).

Figure 3.1: This graph shows the percentage of patients in different SLEDAI score categories.

9 (13%) current smokers; the median (IQR) of cigarettes smoked/day being 15 (7, 20).
3.6.3 Treatment

There were 81 (45%) of the patients using steroids at the time of assessment. The current steroid daily dose was low-moderate with a median (IQR) 8mg (5, 10). In total, 139 (76%) patients had used steroids at some stage, with a median (IQR) daily dose of 10mg (5, 15). Almost half of the patients 87 (48%) were using anti-malarial therapy at the day of interview, and 25 (14%) reported past use of anti-malarials. Lipid lowering therapy was taken by 67 (40%) of the patients and the majority of them, 64 (94%), were on statins however the duration for statin use was not recorded. There were also 69 (39%) on anti-hypertensive treatment.

3.6.4 Traditional risk factors in SLE vs healthy control

Table (3.1) summarises the traditional risk factors in both SLE and controls. SLE patients were significantly older than healthy controls. Systolic blood pressure was also significantly higher in SLE patients, as was the prevalence of hypertension see Figure 3.2. Total cholesterol, LDL and ApoB were lower in SLE patients and these lipid parameters remained significantly different after age adjustment. The prevalence of high total cholesterol (>6.2 mmol/l) and LDL (>4.1 mmol/l) was also higher in the control than SLE patients. There were 67 (45%) of patients on statins therapy, yet total cholesterol, LDL and ApoB remained significantly lower in SLE patients after stratifying them according to statin use (Table 3.2). The remaining factors did not differ between the two groups. As can be seen in Figure (3.1) more patients have hypertension, hypercholesterolemia and diabetes than the controls.
### Variables  | Control (n=56) | SLE (n=168) | Age adjusted P value
---|---|---|---
Age (years) | 50 (39-60) | 53 (46-61) | -
Total cholesterol (mmol/l) | 5.3 (4.8, 6.1) | 4.6 (4.0, 5.3) | <0.0001
HDL-cholesterol (mmol/l) | 1.6 (1.3, 2.1) | 1.7 (1.4, 2.0) | 0.9
LDL-cholesterol (mmol/l) | 2.8 (2.2, 3.2) | 2.3 (1.8, 2.9) | <0.0001
Triglycerides (mmol/l) | 1.1 (0.8, 1.5) | 1.0 (0.8, 1.5) | 0.9
Glucose (mmol/l) | 4.7 (4.5, 4.9) | 4.5 (4.2, 5.1) | 0.4
SBP (mmHg) | 120 (110, 130) | 127 (115, 143) | 0.4
DBP (mmHg) | 72 (65, 80) | 71 (64, 76) | 0.2
BMI (kg/m²) | 25 (22, 27) | 26 (22, 31) | 0.9
ApoB (U/l) | 1.02 (0.9, 1.4) | 1 (0.8, 1.2) | <0.0001

Table 3.1: Traditional risk factors in SLE and healthy controls. Data are described as median (IQR). Mann-Whitney test was used to compare between patients and controls, Age adjusted analysis was assessed by linear regression.

### Variables  | Control (n=56) | SLE (n=168) | Age adjusted P value
---|---|---|---
Total cholesterol (mmol/l) | 5.2 (4.5, 5.9) | 4.9 (4.26, 5.4) | <0.0001
HDL (mmol/l) | 1.64 (1.4, 1.9) | 1.6 (1.4, 1.9) | 0.87
LDL (mmol/l) | 2.9 (2.3, 3.7) | 2.5 (2, 3) | 0.001
TG (mmol/l) | 0.85 (0.7, 1.3) | 1.1 (0.7, 1.5) | 0.05
ApoB (U/l) | 1 (0.9, 1.4) | 1 (0.9, 1.2) | 0.02

Table 3.2: Lipid profile in SLE patients not on statins compared to healthy controls. Data are described as median (IQR), differences between the two groups were were analysed using Mann-Whitney test, Age adjusted analysis was assessed by linear regression.
Figure 3.2: This graph shows the prevalence of traditional risk factors in SLE patients and healthy controls. High TC $>$ 6.2 mmol/l, High LDL $>$ 4.1 mol/l, high TG $>$ 2.3 mmol/l, low HDL $<$ 1.03 mmol/l, hypercholesterolemia TC $>$ 6.2 mmol/l or on statins, hypertension BP $>$ 140/90mmHg or on blood pressure medication.

### 3.6.5 Modified lipids and markers of oxidation

Table (3.3) illustrates the main findings of modified lipids in SLE and healthy controls. In an age adjusted analysis the level of oxidised-LDL was significantly higher in SLE patients than healthy controls. There was also a trend towards higher lipoprotein(a) [Lp(a)] in SLE patients than controls. Glycated LDL was measured in a subset of the study cohort (126 patients, 29 controls) and showed no significant difference between them.
Table 3.3: Modified lipids in SLE and healthy control. Data are shown as median (IQR). Age adjusted analysis was assessed by linear regression. * (SLE n=126, controls n=29)

### 3.7 Predictors of oxidant stress in SLE patients and healthy controls

The focus of this section is to study the oxidant stress in patients with SLE and to find out what factors are associated with increased markers of oxidant stress in SLE patients and whether those factors are similar in healthy controls or not. We chose two markers for this purpose: oxidised-LDL and urinary 8-isoprostane (u 8-IP). We studied their association with markers of inflammation, disease activity, renal function and metabolic factors.

#### 3.7.1 Oxidised-LDL

Spearman’s correlation, detailed in (Table 3.4), showed that there was no association between oxidised-LDL and clinical markers of disease activity or damage.
(SLEDAI, SLICC). In contrast, there was a positive association between oxidised-LDL and C3, C4 complements and lupus anticoagulant (LAC), but not with ds-DNA or CRP in SLE patients. There was a positive correlation with steroid dose (R=0.24, P=0.029) and smoking (R=0.18, P=0.017). CRP and increasing age were positively associated with oxidised-LDL only in controls (R=0.32, P=0.007 and R=0.39, P=0.001 respectively).

There was no significant correlation of oxidised-LDL with eGFR or creatinine in both SLE and healthy controls. Obesity markers (BMI, waist circumference and hip circumference) were generally associated with increased oxidised-LDL in both SLE and healthy controls, though their association was stronger in the healthy controls. In SLE: BMI (R=0.19, P=0.01), waist circumference (R=0.15, P=0.049), hip circumference (R=0.15, P=0.05); in healthy controls: BMI (R=0.55, P<0.0001), waist circumference (R=0.47, P=0.0001), hip circumference (R=0.43, P=0.0003).
### Table 3.4: Spearman’s correlation of oxidised-LDL with SLE factors and metabolic factors in SLE patients and healthy controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>SLE R</th>
<th>P value</th>
<th>Controls R</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.08</td>
<td>0.3</td>
<td>0.39</td>
<td>0.001</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>0.12</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SLICC</td>
<td>0.02</td>
<td>0.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ds-DNA</td>
<td>0.08</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IgG aCL</td>
<td>0.06</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IgM aCL</td>
<td>0.14</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LAC</td>
<td>0.17</td>
<td>0.04</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C3</td>
<td>0.24</td>
<td>0.003</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C4</td>
<td>0.23</td>
<td>0.005</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CRP</td>
<td>0.09</td>
<td>0.2</td>
<td>0.32</td>
<td>0.007</td>
</tr>
<tr>
<td>eGFR</td>
<td>-0.12</td>
<td>0.1</td>
<td>-0.2</td>
<td>0.09</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.05</td>
<td>0.5</td>
<td>0.03</td>
<td>0.8</td>
</tr>
<tr>
<td>Steroid dose</td>
<td>0.24</td>
<td>0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Antimalarial</td>
<td>-0.03</td>
<td>0.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FBG</td>
<td>0.12</td>
<td>0.1</td>
<td>0.07</td>
<td>0.6</td>
</tr>
<tr>
<td>TC</td>
<td>0.29</td>
<td>0.0001</td>
<td>0.58</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL</td>
<td>0.36</td>
<td>&lt;0.0001</td>
<td>0.59</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.15</td>
<td>0.04</td>
<td>-0.05</td>
<td>0.6</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>0.21</td>
<td>0.005</td>
<td>0.39</td>
<td>0.001</td>
</tr>
<tr>
<td>TGs</td>
<td>0.17</td>
<td>0.02</td>
<td>0.47</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SBP</td>
<td>0.07</td>
<td>0.3</td>
<td>0.34</td>
<td>0.005</td>
</tr>
<tr>
<td>DBP</td>
<td>0.10</td>
<td>0.2</td>
<td>0.25</td>
<td>0.047</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.18</td>
<td>0.02</td>
<td>0.07</td>
<td>0.6</td>
</tr>
<tr>
<td>BMI</td>
<td>0.19</td>
<td>0.01</td>
<td>0.54</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.15</td>
<td>0.049</td>
<td>0.47</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hip circumference</td>
<td>0.15</td>
<td>0.05</td>
<td>0.43</td>
<td>0.0003</td>
</tr>
</tbody>
</table>
3.7.2 Forward stepwise regression for predictors of oxidised-LDL

We performed a forward stepwise regression analysis (Table 3.5) using all of the variables with $P<0.2$ at the univariable analysis. In SLE patients, we included SLEDAI score, IgM aCL, LAC, C3, CRP, eGFR, steroid dose, glucose level, total cholesterol, LDL, triglycerides, diastolic blood pressure, smoking, BMI, waist circumference and hip circumference. In this model, increased current steroid dose, LAC, current smoking and decreased LDL level were associated with increased oxidised-LDL in SLE patients. The $R^2$ for this model was 0.69, which means that 69% of increased oxidised-LDL can be attributed to these factors.

In healthy controls we included age, CRP, eGFR, systolic BP, BMI, TC, LDL and triglycerides. In the forward stepwise regression model, increased total cholesterol $\beta$ (95% CI) = 17.23 (10.01, 24.45) and triglycerides 33.82 (7.58, 60.05) were independently associated with increased oxidised-LDL in healthy controls. The $R^2$ for this model was 0.42.

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\beta$ (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid dose</td>
<td>4.46 (2.11, 6.81)</td>
<td>0.001</td>
</tr>
<tr>
<td>LAC</td>
<td>40.73 (19.56, 61.91)</td>
<td>0.001</td>
</tr>
<tr>
<td>Current smoking</td>
<td>33.58 (7.73, 59.42)</td>
<td>0.013</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.62 (0.09, 1.14)</td>
<td>0.022</td>
</tr>
<tr>
<td>LDL</td>
<td>-12.39 (-23.21, -1.55)</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Table 3.5: Forward stepwise regression for predictors of oxidised-LDL in SLE patients.
Oxidised-LDL/LDL ratio

We did some further exploratory analysis using a new variable (oxidised-LDL/LDL) which is the ratio of oxidised-LDL to LDL. The median (IQR) of oxidised-LDL/LDL in SLE patients was significantly higher than healthy controls 30.45 (22.5, 37.28) u/mmol vs 19.50 (15.17, 27.01), P<0.0001. Patients who were being treated with lipid lowering therapy had significantly higher oxidised-LDL/LDL than patients who were not on statin 32.07 (26.64, 40.52) compared to 28.22 (20.41, 35.46) with P=0.009. This would be expected as patients treated with statin will have lower LDL level.

We looked at the factors associated with increased oxidised-LDL/LDL ratio. In SLE patients, we found a significant association with ds-DNA, SLICC-Damage Index, waist circumference and a weak association with IgM aCL antibodies, C4 complement and hip circumference. There was a negative association with eGFR. When we exclude patients who were treated with statin, we found a significant association with anti ds-DNA, smoking and a trend towards association with SLEDAI. In healthy controls, metabolic factors and markers of obesity were the predominant associations (BMI and waist circumference). There was a trend towards association with age, CRP, TG, and systolic blood pressure.
<table>
<thead>
<tr>
<th>Variables</th>
<th>SLE R</th>
<th>P value</th>
<th>Controls R</th>
<th>P value</th>
<th>SLE no statin R</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.009</td>
<td>0.9</td>
<td>0.23</td>
<td>0.06</td>
<td>-0.06</td>
<td>0.56</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>0.12</td>
<td>0.1</td>
<td>-</td>
<td>0.06</td>
<td>0.19</td>
<td>0.05</td>
</tr>
<tr>
<td>SLICC-DI</td>
<td>0.18</td>
<td>0.01</td>
<td>-</td>
<td>0.06</td>
<td>0.16</td>
<td>0.1</td>
</tr>
<tr>
<td>Ds-DNA</td>
<td>0.21</td>
<td>0.007</td>
<td>-</td>
<td>0.06</td>
<td>0.20</td>
<td>0.04</td>
</tr>
<tr>
<td>IgG aCL</td>
<td>0.06</td>
<td>0.5</td>
<td>-</td>
<td>0.06</td>
<td>0.03</td>
<td>0.81</td>
</tr>
<tr>
<td>IgM aCL</td>
<td>0.17</td>
<td>0.06</td>
<td>-</td>
<td>0.06</td>
<td>0.15</td>
<td>0.18</td>
</tr>
<tr>
<td>LAC</td>
<td>0.06</td>
<td>0.4</td>
<td>-</td>
<td>0.06</td>
<td>0.03</td>
<td>0.77</td>
</tr>
<tr>
<td>C3</td>
<td>0.07</td>
<td>0.4</td>
<td>-</td>
<td>0.07</td>
<td>0.07</td>
<td>0.51</td>
</tr>
<tr>
<td>C4</td>
<td>0.15</td>
<td>0.07</td>
<td>-</td>
<td>0.05</td>
<td>0.05</td>
<td>0.62</td>
</tr>
<tr>
<td>CRP</td>
<td>0.05</td>
<td>0.5</td>
<td>0.22</td>
<td>0.08</td>
<td>0.06</td>
<td>0.55</td>
</tr>
<tr>
<td>eGFR</td>
<td>-0.20</td>
<td>0.007</td>
<td>0.007</td>
<td>0.95</td>
<td>-0.16</td>
<td>0.11</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.15</td>
<td>0.05</td>
<td>-0.18</td>
<td>0.14</td>
<td>0.03</td>
<td>0.78</td>
</tr>
<tr>
<td>Steroid dose</td>
<td>0.09</td>
<td>0.4</td>
<td>-</td>
<td>0.02</td>
<td>0.02</td>
<td>0.9</td>
</tr>
<tr>
<td>Antimalarial</td>
<td>-0.009</td>
<td>0.9</td>
<td>-</td>
<td>0.03</td>
<td>0.03</td>
<td>0.76</td>
</tr>
<tr>
<td>FBG</td>
<td>0.09</td>
<td>0.3</td>
<td>0.08</td>
<td>0.53</td>
<td>-0.06</td>
<td>0.49</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.08</td>
<td>0.3</td>
<td>0.05</td>
<td>0.6</td>
<td>-0.11</td>
<td>0.24</td>
</tr>
<tr>
<td>TGs</td>
<td>0.03</td>
<td>0.7</td>
<td>0.22</td>
<td>0.07</td>
<td>0.01</td>
<td>0.89</td>
</tr>
<tr>
<td>SBP</td>
<td>0.02</td>
<td>0.8</td>
<td>0.22</td>
<td>0.07</td>
<td>-0.09</td>
<td>0.38</td>
</tr>
<tr>
<td>DBP</td>
<td>0.01</td>
<td>0.8</td>
<td>0.21</td>
<td>0.10</td>
<td>-0.08</td>
<td>0.39</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.1</td>
<td>0.1</td>
<td>0.03</td>
<td>0.81</td>
<td>0.2</td>
<td>0.03</td>
</tr>
<tr>
<td>BMI</td>
<td>0.14</td>
<td>0.05</td>
<td>0.32</td>
<td>0.009</td>
<td>0.06</td>
<td>0.5</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.15</td>
<td>0.04</td>
<td>0.31</td>
<td>0.01</td>
<td>0.03</td>
<td>0.72</td>
</tr>
<tr>
<td>Hip circumference</td>
<td>0.09</td>
<td>0.7</td>
<td>0.17</td>
<td>0.18</td>
<td>0.05</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Table 3.6: Spearman’s correlation for oxidised-LDL/LDL in SLE patients and controls.
3.7.3 Urinary 8-isoprostane

While urinary 8-IP did not differ between patients and controls, we wished to explore factors that determine its level. Table (3.7) summarises the main predictors. These are eGFR and creatinine, markers of renal function, especially in SLE patients. In SLE patients, higher eGFR was associated with increased level of urinary 8-IP (R=0.22, P=0.003), while creatinine was negatively associated with urinary 8-IP (R=-0.18, P=0.02). There was also a negative correlation with IgG and IgM anticardiolipin antibodies (R=-0.23, -0.21; P=0.01, 0.03 respectively).

In controls, we found a negative correlation with age (R=-0.38, P=0.002) but there was no association with other metabolic or CVD markers.
### Table 3.7: Spearman’s correlation for the association of u8-IP and other factors in SLE patients and healthy controls. *Lupus anticoagulant.

<table>
<thead>
<tr>
<th>Variables</th>
<th>SLE R</th>
<th>P value</th>
<th>Controls R</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.003</td>
<td>0.9</td>
<td>-0.38</td>
<td>0.002</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>0.01</td>
<td>0.88</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ds-DNA</td>
<td>-0.06</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IgG aCL</td>
<td>-0.23</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IgM aCL</td>
<td>-0.21</td>
<td>0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LAC*</td>
<td>-0.03</td>
<td>0.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C3</td>
<td>0.07</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C4</td>
<td>0.06</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CRP</td>
<td>-0.04</td>
<td>0.6</td>
<td>-0.03</td>
<td>0.8</td>
</tr>
<tr>
<td>eGFR</td>
<td>0.25</td>
<td>0.001</td>
<td>0.21</td>
<td>0.09</td>
</tr>
<tr>
<td>Creatinine</td>
<td>-0.19</td>
<td>0.01</td>
<td>-0.09</td>
<td>0.5</td>
</tr>
<tr>
<td>Steroid dose</td>
<td>0.17</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Antimalarial</td>
<td>0.08</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.12</td>
<td>0.1</td>
<td>-0.16</td>
<td>0.2</td>
</tr>
<tr>
<td>TC</td>
<td>0.09</td>
<td>0.3</td>
<td>-0.12</td>
<td>0.3</td>
</tr>
<tr>
<td>LDL</td>
<td>0.03</td>
<td>0.7</td>
<td>-0.06</td>
<td>0.6</td>
</tr>
<tr>
<td>HDL</td>
<td>0.09</td>
<td>0.2</td>
<td>-0.15</td>
<td>0.2</td>
</tr>
<tr>
<td>TGs</td>
<td>-0.14</td>
<td>0.05</td>
<td>-0.18</td>
<td>0.1</td>
</tr>
<tr>
<td>SBP</td>
<td>-0.03</td>
<td>0.7</td>
<td>-0.18</td>
<td>0.2</td>
</tr>
<tr>
<td>DBP</td>
<td>-0.05</td>
<td>0.5</td>
<td>0.01</td>
<td>0.9</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.11</td>
<td>0.1</td>
<td>0.05</td>
<td>0.7</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.02</td>
<td>0.8</td>
<td>0.007</td>
<td>0.9</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.006</td>
<td>0.9</td>
<td>-0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Hip circumference</td>
<td>0.007</td>
<td>0.9</td>
<td>0.01</td>
<td>0.9</td>
</tr>
</tbody>
</table>
Urinary 8-isoprostane stratified by renal function (eGFR)

Because of the strong association between urinary 8-isoprostane and markers of renal function and 8-IP is an excreted product, we repeated the analysis considering renal function as the stratification variable with results in (Table 3.8). We divided SLE into two groups according to their eGFR: patients with unequivocally normal renal function (eGFR >90), and patients with potentially impaired renal function (eGFR <90). We found a statistically significant difference in the level of urinary 8-isoprostane between the two groups; it was significantly higher in SLE patients with normal eGFR compared to patients with impaired eGFR. The median (IQR) for each group were 1.59 (1.07-2.01) and 1.23 (0.78-1.79), P=0.029.

However, within the group of patients with normal eGFR there remained no clear association between urinary 8-IP and any measures of inflammation or metabolic factors such as obesity or lipid level.
### Table 3.8: Spearman’s correlation for u8-IP stratified by renal function (eGFR).

<table>
<thead>
<tr>
<th>Variables</th>
<th>SLE with eGFR $\geq$ 90 (n=72)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.09</td>
<td>0.41</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>0.05</td>
<td>0.6</td>
</tr>
<tr>
<td>Ds-DNA</td>
<td>-0.08</td>
<td>0.5</td>
</tr>
<tr>
<td>IgG aCL</td>
<td>-0.13</td>
<td>0.3</td>
</tr>
<tr>
<td>IgM aCL</td>
<td>-0.17</td>
<td>0.2</td>
</tr>
<tr>
<td>LAC</td>
<td>-0.13</td>
<td>0.3</td>
</tr>
<tr>
<td>C3</td>
<td>-0.02</td>
<td>0.9</td>
</tr>
<tr>
<td>C4</td>
<td>0.04</td>
<td>0.8</td>
</tr>
<tr>
<td>CRP</td>
<td>-0.07</td>
<td>0.6</td>
</tr>
<tr>
<td>eGFR</td>
<td>0.32</td>
<td>0.006</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.004</td>
<td>0.9</td>
</tr>
<tr>
<td>Steroid dose</td>
<td>0.19</td>
<td>0.3</td>
</tr>
<tr>
<td>Antimalarial</td>
<td>0.09</td>
<td>0.5</td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.08</td>
<td>0.5</td>
</tr>
<tr>
<td>TC</td>
<td>-0.05</td>
<td>0.7</td>
</tr>
<tr>
<td>LDL</td>
<td>-0.12</td>
<td>0.3</td>
</tr>
<tr>
<td>HDL</td>
<td>0.09</td>
<td>0.5</td>
</tr>
<tr>
<td>TGs</td>
<td>-0.02</td>
<td>0.9</td>
</tr>
<tr>
<td>SBP</td>
<td>-0.18</td>
<td>0.1</td>
</tr>
<tr>
<td>DBP</td>
<td>-0.04</td>
<td>0.8</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.04</td>
<td>0.7</td>
</tr>
<tr>
<td>BMI</td>
<td>0.001</td>
<td>0.9</td>
</tr>
</tbody>
</table>
3.8 Subclinical atherosclerosis

The mean cIMT was slightly higher, but not statistically significant, in healthy controls compared to SLE patients. Mean (SD) IMT: 0.07 (0.1) in controls vs 0.06 (0.010) in SLE patients, age adjusted P= 0.2. SLE patients also had higher prevalence of carotid thickening, or plaque 45% compared to 24% of the controls; P=0.002. The overall prevalence of plaque in SLE patients was 45 (24%) compared to 10 (14%) in controls; (P=0.07). However, the plaque prevalence was significantly higher in SLE patients aged <55 years 14/101 (14%) compared to 1/43 (2%) in healthy controls; (P=0.04) see Figure (3.3).
### Table 3.9: Linear regression analysis for the association between TRF and cIMT in SLE patients and healthy controls. * P<0.05

<table>
<thead>
<tr>
<th>Variables</th>
<th>β (95% CI) controls</th>
<th>β (95% CI) SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age years</td>
<td>-0.001 (-0.004, 0.0006)</td>
<td>0.0006 (0.0004, 0.0007)*</td>
</tr>
<tr>
<td>Systolic BP mmHg</td>
<td>0.0003 (-0.001, 0.002)</td>
<td>0.0002 (0.0001, 0.0003)*</td>
</tr>
<tr>
<td>TC mmol/l</td>
<td>-0.006 (-0.037, 0.024)</td>
<td>0.003 (0.0003, 0.005)*</td>
</tr>
<tr>
<td>LDL mmol/l</td>
<td>0.0003 (-0.03, 0.03)</td>
<td>0.003 (0.0006, 0.005)*</td>
</tr>
<tr>
<td>HDL mmol/l</td>
<td>-0.04 (-0.13, 0.036)</td>
<td>-0.001 (-0.006, 0.004)</td>
</tr>
<tr>
<td>TG mmol/l</td>
<td>0.03 (-0.09, 0.16)</td>
<td>0.001 (-0.006, 0.009)</td>
</tr>
<tr>
<td>BMI</td>
<td>0.00008 (-0.005, 0.005)</td>
<td>0.0002 (-0.0002, 0.0005)</td>
</tr>
<tr>
<td>Smoking</td>
<td>-0.01 (-0.1, 0.08)</td>
<td>0.002 (-0.005, 0.0099)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.003 (-0.03, 0.03)</td>
<td>-0.003 (-0.01, 0.007)</td>
</tr>
<tr>
<td>Oxidised-LDL</td>
<td>0.006 (-0.02, 0.03)</td>
<td>0.00006 (−9.0⁻⁶, 0.0001)</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>0.008 (-0.009, 0.025)</td>
<td>0.00007 (0.00002, 0.0001)*</td>
</tr>
<tr>
<td>sRAGE</td>
<td>0.002(0.0004, 0.0003)*</td>
<td>2.75⁻⁶ (−2.66⁻⁶, 5.77⁻⁶)</td>
</tr>
<tr>
<td>Glycated-LDL</td>
<td>0.005 (-0.002, 0.011)</td>
<td>0.001 (-0.003, 0.005)</td>
</tr>
<tr>
<td>Urinary 8-IP</td>
<td>-0.0003 (-0.017, 0.016)</td>
<td>0.001 (-0.0006, 0.003)</td>
</tr>
</tbody>
</table>

### 3.8.1 Association between TRF, lipids, modified lipids and cIMT

Among traditional risk factors, increased age, systolic BP, total cholesterol and LDL were significantly associated with cIMT in SLE patients by univariable analysis in (Table 3.9). After adjusting for age, only LDL remained significantly associated with cIMT. In healthy controls, none of these factors have a direct association with cIMT. There was increased cIMT in patients with high Lp(a) level and in healthy controls with high sRAGE.
Table 3.10: Forward stepwise regression of association with cIMT in SLE patients (age, BMI, DM, smoking, hypercholesterolemia, sRAGE, oxidised-LDL, Lp(a) and urinary 8-IP).

### 3.8.2 Forward stepwise regression analysis for predictors of cIMT in SLE

We further tested for factors which were independently associated with cIMT by a multivariable forward stepwise regression modeling. In this model, we included TRF+ lipids+ modified lipids and all variables with \( P < 0.2 \) at univariable regression. Factors included were: age, systolic BP, TC, LDL, BMI, smoker-current, oxidised-LDL, Lp(a), RAGE, glycated-LDL and and urinary 8-IP, see (Table 3.10). In this model increased age, systolic BP, oxidised-LDL and urinary 8-IP were independently associated with increased cIMT in SLE patients.
3.8.3 Forward stepwise regression analysis in controls

We repeated the same model described above for healthy controls and found that age was the only independent variable associated with higher cIMT, $\beta (95\% \text{ CI})$ was 0.00004 (0.0002, 0.0006); $P=0.001$.

3.9 Association between TRF, lipids, modified lipids and plaque

In a univariable logistic regression, see (Table 3.11): increased age, systolic blood pressure and hypercholesterolemia were significantly associated with plaque development in both SLE patients and healthy controls. Smoking was more significantly associated in SLE patients. Lp(a) was associated with increased odds for plaque only in SLE patients.
### Table 3.11: Univariable logistic regression of predictors of plaque in healthy controls and SLE patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls</th>
<th>SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>CI</td>
</tr>
<tr>
<td>Age</td>
<td>1.2</td>
<td>1.07-1.40</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.28</td>
<td>0.69-2.30</td>
</tr>
<tr>
<td>BMI</td>
<td>1.01</td>
<td>0.9-1.10</td>
</tr>
<tr>
<td>Smoking-ever</td>
<td>1.72</td>
<td>0.44-6.76</td>
</tr>
<tr>
<td>TC</td>
<td>1.01</td>
<td>0.9-1.10</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.22</td>
<td>0.60-2.45</td>
</tr>
<tr>
<td>BMI</td>
<td>0.9</td>
<td>0.70-1.10</td>
</tr>
<tr>
<td>LDL</td>
<td>1.13</td>
<td>0.53-2.39</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>3.7</td>
<td>1.81-7.56</td>
</tr>
<tr>
<td>SBP</td>
<td>1.04</td>
<td>1.01-1.08</td>
</tr>
<tr>
<td>BMI</td>
<td>1.01</td>
<td>0.9-1.10</td>
</tr>
<tr>
<td>sRAGE</td>
<td>1.00</td>
<td>1.00-1.002</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.99</td>
<td>0.99-1.00</td>
</tr>
<tr>
<td>Oxidised-LDL</td>
<td>1.00</td>
<td>0.98-1.02</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>1.00</td>
<td>0.99-1.02</td>
</tr>
<tr>
<td>Urinary 8-IP</td>
<td>0.36</td>
<td>0.11-1.13</td>
</tr>
</tbody>
</table>

Multivariable logistic regression in SLE

A forward stepwise logistic regression for the assessment of independent variables associated with carotid plaque was performed (Table 3.12). Variables with P<0.2 were included: age, smoking, hypercholesterolemia, sBP, diabetes, BMI, oxidised-LDL and Lp(a). Increased age, smoking and hypercholesterolemia were independently associated with increased odds for plaque in SLE patients. Oxidised-LDL was not significantly associated with carotid plaque in this model.

In healthy controls, the same model was employed. Age was the main independent predictor of plaque.
<table>
<thead>
<tr>
<th>Variables</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.09 (1.03-1.14)</td>
<td>0.001</td>
</tr>
<tr>
<td>Smoker ever</td>
<td>3.43 (1.45-8.11)</td>
<td>0.005</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>3.15 (1.32-7.52)</td>
<td>0.01</td>
</tr>
<tr>
<td>Oxidised-LDL</td>
<td>1.01 (0.99-1.02)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Table 3.12: Multivariable stepwise logistic regression for plaque in SLE patients.

### 3.10 Summary of the main findings

This section summarises the main findings.

- 168 Female SLE patients with moderate-low disease activity and 56 healthy controls were assessed in this study.

- Around half of the patients were treated with steroids at the time of assessment with a median dose 8 (5, 10)mg.

- Less than half the patients were treated with lipid lowering agents mainly with statins.

- SLE patients have higher prevalence of hypercholesterolemia, hypertension and diabetes but significantly lower total cholesterol and LDL level.

- Oxidised-LDL was significantly higher in SLE patients compared to the healthy controls and there was a trend towards higher Lp(a) level in SLE patients.

- Higher oxidised-LDL levels were related mainly to metabolic markers and markers of obesity in both SLE patients and healthy controls while urinary 8-IP was influenced by markers of renal function.

- Plaque prevalence was significantly higher in SLE patients aged < 55 years.
Oxidised-LDL and urinary 8-IP were independently associated with cIMT in SLE patients.

3.11 Discussion

For this cohort, patients were recruited from the outpatient clinics in Manchester. Most of the patients in this study had participated in previous studies investigating the risk for carotid atherosclerosis in SLE patients. This may explain the relative older age of study participants compared to other younger SLE cohorts. This may also explain the low disease activity score as these participants were considered as high risk patients. Most of the patients had established disease with a median disease duration of 13 years and a median SDI 1 (0, 2). This is similar to the reported median damage index of SLE patients with relatively low disease activity at 10 and 15 years [89].

At the time of assessment 43% of the patients were on corticosteroids with an average daily dose of 8mg (5, 10) which is a relatively low dose. A similar proportion of patients had been treated with anti-malarial drugs. This cohort benefited from low disease activity which minimised the confounding effect of disease severity and high inflammatory burden.

We assessed the prevalence of traditional risk factors in SLE patients compared to controls. SLE patients had higher prevalence of hypertension than the healthy controls, although the median level 127 (115, 143) was within the normal range. This comes in agreement with previous studies [30] in which SLE patients had higher prevalence of hypertension and diabetes. Total cholesterol and LDL were significantly lower in SLE patients compared to the healthy controls. Of particular note, is the fact that around 40% of SLE patients were on statins...
which may explain the lower level of lipids found. However, after adjusting for statins use, SLE patients still had low total cholesterol and LDL. This may in part be explained by the chronic low inflammatory state of the disease itself.

The level of oxidised-LDL was significantly higher in SLE patients compared to the healthy controls. Lp(a) also tended to be higher. Higher oxidised-LDL and Lp(a) were reported previously in SLE patients [255][77]. El-Magadmi et al also reported high oxidised-LDL in SLE patients with metabolic syndrome [64]. There was no significant difference in urinary 8-isoprostane levels between patients and controls. Increased oxidised-LDL in SLE patients and in particular in patients with CVD or metabolic syndrome support the role of oxidised-LDL in the development of cardiovascular disease.

We looked at predictors of oxidant stress in patients and controls. In SLE patients, we found that complement and levels of lupus anticoagulant antibodies were significantly associated with increased oxidised-LDL level. This may reflect increased stress itself or cross reactivity between oxidised-LDL antibodies and other autoantibodies as reported in the literature [77]. Wu et al reported cross-reactivity between oxidised-LDL and $\beta_2$ glycoprotein which is a plasma protein involved in the coagulation cascade and cofactor for anticardiolipin antibodies [290]. They also reported a close correlation between antibodies to endothelial cells and anti oxidised-LDL antibodies. This report suggests a shared antigenic epitopes between endothelial cells and oxidised-LDL.

Higher steroid dose, is indicative of more severe disease leading possibly to higher oxidant stress. As would be expected, oxidised-LDL was significantly associated with high total cholesterol, LDL and triglycerides. High total cholesterol and LDL may lead to more lipids being oxidised. Although in the forward stepwise modeling, we found a negative correlation with LDL. This may be due to the
effect of inflammation on LDL. LDL in this cohort was significantly reduced yet there was higher oxidised-LDL. Inflammation can alter the quantity and quality of lipids [103]. High triglycerides are also associated with smaller dense particle more prone to oxidation [131]. Smoking, high BMI, waist and hip circumference were also associated with increased oxidised-LDL level in SLE patients, all these factors are expected to be associated with higher oxidised-LDL as all of them are risk factors of CVD.

In healthy controls, increased age and CRP were significantly associated with increased lipid oxidation. A stronger association of lipids with metabolic factors and oxidised-LDL was noted in healthy controls which may be due to higher levels of lipids in controls than SLE. Weinbrenner et al reported a direct association between oxidative stress and markers of obesity [283]. They reported an independent association between oxidised-LDL and abdominal fat as indicated by waist circumference. This association may be driven by low grade systemic inflammation in association with obesity or SLE which induces the production of free radicals and enhances lipid peroxidation. Another association between oxidised-LDL and the incidence of metabolic syndrome (in particular with the obesity component) was reported in a population based study [114]. This association may be due to increased levels of small dense LDL which are more prone to oxidation or could be due to increased production of oxidised-LDL by adipose tissue which induces the enzymes involved in LDL oxidation. Interestingly, in our exploratory analysis, we found the ratio of oxidised-LDL/LDL was associated with some inflammatory markers, obesity markers and markers of renal impairment. Therefore, the proportion of oxidised-LDL may reflect better the inflammatory milieu.

With regards to the excretion of urinary 8-isoprostane, we found no significant
difference between patients and controls. Other studies have reported conflicting results [122][11]. The discrepancy between our result and others could be explained by the differences between the two published studies and the current study with regards to the sample size and degree of disease severity. In one study the sample size was 36 patients with BILAG score $>6$ indicating moderately active disease while the median (IQR) SLEDAI score in our study was 2 (0, 5), which reflects mild disease activity. The other study also included a different spectrum of SLE patients and disease activity. In agreement with our study, Avalos et al found no significant difference in the excretion of urinary 8-isoprostane between patients and controls [14]. In their study, they found a significant correlation between urinary 8-isoprostane and disease activity but that was only in patient-reported symptoms namely fatigue and visual analogue scale (VAS). Similar to our finding, there was no association between urinary 8-isoprostane level and the clinical measures of disease activity (SLEDAI) and inflammation. We found that 8-isoprostane was significantly related to measures of renal function and anti-cardiolipin antibodies. A correlation with these antibodies has been reported by Iuliano et al [122].

There was no significant difference in the cIMT between SLE patients and healthy controls in this study. The mean cIMT in our SLE patients is similar to the mean reported in another study [219]. However, compared with previous reports, there was no significant difference between patients and controls. A similar finding was reported by Svenungsson and colleagues [255]. This could be due to the age of the study cohort. The average age in previous reports was 44 while in our study it was 53 years. The majority of reports indicate premature atherosclerosis in SLE which occurs at a young age. This is confirmed in the present study. When we stratify the prevalence of plaque by age, we found that
plaque was significantly higher in SLE patients < 55 years, which is the cut off age below which there is an increased atherosclerosis burden in SLE.

Using univariable analysis, we found that increased age, hypertension, total cholesterol, LDL and Lp(a) were significantly associated with increased cIMT in SLE patients only. Age, high systolic blood pressure, sRAGE urinary 8-isoprostane and oxidised-LDL were independently associated with increased cIMT in SLE patients in the multivariable analysis. This indicate that lipid modification in particular oxidation may indeed promote early and premature atherosclerosis.

With regards to the association with carotid plaque, increased age, hypercholesterolemia and systolic blood pressure were associated with increased odds of carotid plaque in controls when tested univariately. In addition to these factors, smoking and Lp(a) were associated with plaque in SLE patients.

In a multivariable model, only age was independently associated with a 30% increase in the odds for plaque in controls, while in SLE patients age, smoking, and hypercholesterolemia were all significantly and independently associated with increased risk of plaque.

Predictors of subclinical atherosclerosis varied in previous reports [219][3]. The fact that more influence of the traditional risk factors found in our analysis may be due to the age of this cohort and the mild disease severity. Oxidised-LDL was associated with an increase in the odds of plaque although not after adjustment for other factors. This may be attributed to a ”colinear” association between oxidised-LDL and increased cholesterol or increased LDL. Several factors might contribute to lipids alteration in patients with inflammatory conditions, their contribution to atherosclerosis being influenced by the degree of inflammation, disease severity and other factors yet to be discovered.
In conclusion, there is evidence of an increased level of oxidant stress in patients with SLE regardless the severity of the disease. We were able to demonstrate an association between level of oxidant stress and subclinical atherosclerosis in SLE patients.

3.12 Possible model for the influence of inflammation on oxidant stress

From this analysis we proposed a possible mechanism for the association between inflammation and oxidant stress in patients with systemic lupus erythematosus. There are several pathways that can be involved both triggered by the chronic inflammatory state of the disease Figure 3.4. The first pathway is, chronic inflammation is associated with increased levels of cytokines and in particular TNF-α. Increased TNF-α is associated with dyslipidemia and mainly increased levels of triglycerides and VLDL. Triglycerides and VLDL are more prone to oxidation thus lead to increased lipid oxidation in SLE. The other mechanism, is the use of steroid to control the inflammation in patients with SLE. One of the side effects of steroids is central obesity. Central obesity is associated with increased adipocytes and further increased the TNF-α production and hence increased oxidation. Steroid use may also be associated with hypercholesterolemia and other metabolic derangements that may lead to increased oxidant stress. One of the main characteristics of the disease is autoantibodies production.
3.13 Limitations of the study

As the design of the study was cross-sectional, any finding could be representative of the time of assessment and may not reflect a long-term association. No causal relation can be concluded as the design of the study was cross-sectional. The study cohort was relatively older than that of published SLE studies and the majority of our patients had mild disease whereas younger patients/with severe disease could have a different pattern of association. The variable nature of lipids and oxidant stress over time make the interpretation of this study only representative of a single time point.
Figure 3.4: Schematic representation of the possible pathways for the influence of inflammation on oxidant stress in SLE.
Chapter 4

Lipids and Apolipoproteins and Mortality in Patients with Inflammatory Polyarthritis

4.1 Introduction

Patients with RA have increased risk of premature mortality/CVD mortality [45][256]. In the general population, dyslipidemia was found to be associated with increased risk of CVD. Less is known about their role in IP/RA. The association between lipids and CVD in patients with RA is uncertain and appears to be more complicated compared to the general population.

Studies investigating lipid profiles in RA have shown controversial results which could be explained by the effect of systemic inflammation on lipid derangement. Reports on patients with active untreated RA have shown reduced levels of TC, LDL and HDL. Reduced HDL was the most consistent finding in the literature. A recent finding from the Mayo clinic indicated a paradoxical
association between lipids and risk of CVD in patients with RA [178].

In this study, we aimed to assess the relationship between inflammation and lipids and apolipoproteins. We also set out to examine the impact of lipids on all cause/CVD mortality in patients with early inflammatory polyarthritis. The study cohort is part of a primary care-based inception cohort recruited to the Norfolk Arthritis Register (NOAR). A number of studies have been published in this cohort looking at the predictors of CVD morbidity and mortality including different aspects such as environmental factors and serological markers [182][75]. However, none of these studies have looked at the association between lipids and all-cause or CVD mortality.

4.2 Aims and hypothesis

We hypothesized that lipids and lipoprotein levels at baseline predict future mortality in an early IP cohort. The aims of this study were;

1. To assess baseline lipid and lipoprotein levels in patients with early IP.
2. To examine how inflammation, serological status and other classic risk factors associated with lipid and lipoprotein levels in early IP.
3. To examine how baseline lipid and lipoprotein levels predict future mortality with a special focus on CVD mortality.

4.3 The Norfolk Arthritis register

The Norfolk Arthritis Register (NOAR) is a primary care-based inception cohort of patients with recent onset inflammatory polyarthritis (IP). It was established
in 1989, several publication were published from this cohort, summary of the establishment and key results are found in [259].

The initial setting up of the register was to capture new cases of IP presenting to primary care and study the natural course and history of the disease in a prospective cohort of these patients. The region of the former Norwich Health Authority, which has a population of almost 800,000, was chosen as the area of interest.

The reasons for choosing this area were:

1. It provided a good sample of both rural and urban populations.

2. The population is relatively stable with little inward or outward migration.

3. There is a central pattern of referral to Norwich and Ayleshan hospitals, now formalized to the Norfolk and Norwich University Hospital (NNUM)

The local base for NOAR is the Norfolk and Norwich University hospital. Data are collected in Norfolk and then the paper records are transported to the Arthritis Research UK Epidemiology Unit in Manchester where the data are stored in the main NOAR database and blood samples are analysed or stored for later analyses. The Chief Investigator in Manchester is Prof. Deborah Symmonds and Prof. Ian Bruce is the Chief Investigator of the NOAR Cardiovascular Sub-study.

4.3.1 NOAR criteria

For patients to be eligible for entry into the register they need to be aged 16 years or over, be registered with the participating centers, have two or more swollen
joints that started after January 1989. The swelling should persist for at least four weeks.

Patients who fulfilled criteria are enrolled in the register. Any patients who satisfy a diagnosis other than RA, psoriatic arthritis or undifferentiated polyarthritis are discharged from the registry. Patients who met criteria for RA are continued to be followed up regardless of other diagnoses. Patients are followed up annually for the first three years (0-3) then assessed at years 5, 7, 10 and 15, with a 20 year follow-up planned. For this study we used patients entered into the cohort since January 2000.

4.3.2 Baseline assessment

When patients are referred to the register, they are interviewed by the research nurse who performs a detailed interview. During the interview, the patients have a 51-joint examination (swollen, tender), questionnaires are given and blood samples are taken from each participant.

The questionnaire includes a detailed history of joint symptoms at baseline. Areas of social history including marital status, smoking history, and employment status are covered. Details about the current medication at baseline assessment are gathered. A Health Assessment Questionnaire (HAQ) was also completed by patients.

Non-fasting blood samples are collected (serum and plasma) and aliquots are stored at -80 C for later analyses. These samples are tested for rheumatoid factor and ACPA. An aliquote was taken to measure a detailed lipid profile (TC, HDL, TG, ApoA-1 and ApoB) for this study, detailed methods for lipid profiling are described in Chapter 2.
For RF measurement, a tube latex dilution test was used. Titers of RF were measured and a titre of 1/40 or above was considered positive result. CRP was measured using end point immunoturbidimetric agglutination method and this is used to calculate the disease activity score DAS-28_{CRP}.

### 4.4 Ascertainment of deaths

Around 98% of UK residents are registered with a National Health Service (NHS) general practitioner. The NHS Central Register (NHSCR) is a computerized registry for all NHS patients’ records. Access to the NHSCR for residents of UK and Wales can be obtained via the NHS Information Service (NHS-IS) formerly the Office for National Statistics (ONS). The patients are flagged with the NHS-IS which records details about date and cause of death. The NHS-IS send regular reports to the Arthritis Research UK (ARUK) Epidemiology Unit with details about any event of death or embarkations regarding NOAR participants. The death notifications are send in the form of death certificate or death draft.

The cause of death is coded according to the International Statistical Classification of Disease and Related Health Problems, Tenth Revision (ICD-10). In this analysis, CVD deaths were defined as the main cause of death and coded by ICD-10 (I00-I99). Patients were followed from time of enrolment until death or until 31\textsuperscript{st} of December 2010 which ever came first. Patients who moved from the UK, or were no longer registered with the general practitioner were given a date of embarkation. The vital status of these patients cannot be obtained therefore they were censored at the time of embarkation.
4.5 Statistical analysis

Data were described by mean (SD) or median (IQR) according to their distribution. Linear regression analysis was used to ascertain the association between lipid sub-types and markers of inflammation/disease activity. For predictors of mortality, survival analysis (Cox proportional hazard modeling) was used to identify predictors of mortality in the study cohort. Patients that had embarked were censored at the date of embarkation to enable to capture their contribution to years of follow up.

Multivariable model forward stepwise regression analysis was used to test for independent predictors of mortality. In this model the variables are added according to their significance level. Variables that are significantly associated (P<0.05) were retained in the final model.

4.6 Results

4.6.1 Demographic data and anthropometric measures at baseline assessment

The total number of patients who were analyzed for the purpose of this study was 1266. The baseline characteristics of patients with IP are summarized in (Table 4.1). The median (IQR) age of the study cohort at the time of baseline assessment was 58 (47, 68) years. As is typical in an early IP cohort, around two thirds of the patients were females.

The mean (SD) BMI of participants was 27.3 (4.5) kg/m². According to the WHO classification, 468 (37%) of patients were overweight (25 ≤ BMI ≤ 30)
Chapter 4
Lipids and Apolipoproteins and Mortality in Patients with Inflammatory Polyarthritis

### Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>IP patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (n=1266) years median (IQR)</td>
<td>58 (47, 68)</td>
</tr>
<tr>
<td>BMI (n=1237 kg/m² m(SD))</td>
<td>27.3 (4.5)</td>
</tr>
<tr>
<td>Females</td>
<td>826 (65.21%)</td>
</tr>
<tr>
<td>Smoker (current)</td>
<td>287 (23.7%)</td>
</tr>
<tr>
<td>Smoker (previous)</td>
<td>493 (39%)</td>
</tr>
<tr>
<td>Hypertension (self reported)</td>
<td>291 (22.26%)</td>
</tr>
<tr>
<td>Diabetes (self reported)</td>
<td>102 (7.8%)</td>
</tr>
</tbody>
</table>

Table 4.1: Demographic data for IP patients at baseline assessment. Data are expressed as n (%) except when otherwise indicated.

<table>
<thead>
<tr>
<th>Variables</th>
<th>IP patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (m)</td>
<td>1.67 (0.09)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76 (17)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>93 (14)</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>106 (11)</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.87 (0.09)</td>
</tr>
</tbody>
</table>

Table 4.2: Anthropometric measures in IP subjects at the time of assessment. Data are shown as mean m(SD) or N(%).

...and 349 (28%) were obese individuals (BMI > 30) (Table 4.2).

Blood pressure, was only systematically measured from 2004 onwards. The median (IQR) for systolic BP (n=453) was 138 (124, 149) mmHg and diastolic BP 81 (75, 88) mmHg. Hypertension was defined as self reported or from co-morbidity data provided by the patients (ICD-codes) or assumed if the patient was on anti-hypertensive medication. Based on this definition, 291 (22.3%) of patients were hypertensive. Similarly, diabetes was defined according to history, co-morbid data or diabetic treatment. According to this definition, 102 (7.8%) of the patients had diabetes.
Table 4.3: Arthritis associated features in the NOAR cohort at baseline. All data are expressed as median (IQR) except were otherwise indicated.

### 4.6.2 Arthritis related factors

At baseline, the median (IQR) symptom duration was 8 (4.5, 15) months. In this cohort, 515 (41.5%) patients were RF-positive, 332 (26.2%) ACPA-positive, and 243 (19%) patients were positive for both RF and ACPA. Therefore overall, 486 (38.4%) of the patients were sero-negative and 61.6% of the patients were “sero-positive”. In total, 568 (41.25%) patients fulfilled the 1987 ACR criteria for RA at the time of first assessment (Table 4.3).

The median (IQR) DAS-28 was 3.68 (2.82, 4.62) and CRP 11 (5.4, 20.8) mg/l which indicated moderate disease activity at baseline. Around half of the patients had started treatment with DMARDs. There were around 25% of patients taking steroids before or at the time of presentation. The median (IQR) for duration of treatment for patients treated with DMARD was 45 (18, 217) days.
4.6.3 Lipid profile at baseline assessment

The distributions of lipids are shown in Figures (4.1-4.3). The lipid profile of the participants is also summarized in (Table 4.4). Of note, the median (IQR) of total cholesterol was 5.0 (4.1, 5.8) mmol/l and HDL was 1.13 (0.85, 1.44) mmol/l.

According to the National Cholesterol Education Panel Adult Treatment Program III (NCEP ATP III) definition of dyslipidemia [1], 237 (17.2%) of patients had high total cholesterol (TC > 6.2) mmol/l. 229 (17.7%) of patients had LDL > 4.1 mmol/l, 182 (13.36%) had TG > 2.3 mmol/l and 465 (39%) of patients had HDL < 1.0 mmol/l (Table 4.4).

Therefore, while the average levels of lipid sub-fraction appeared to be within the normal level, almost half of patients had at least one form of dyslipidemic profile. The most prevalent ”dyslipidemia” was reduced HDL level with 40% of the patients having low HDL levels.

We stratified the analysis of lipid profile according to gender as the majority of this cohort was female (Table 4.5). Females tended to have higher levels of all lipid sub-fractions with the exception of TG which was significantly higher in male participants.

4.7 Association between lipid profile and markers of inflammation in patients with inflammatory arthritis

The aim of this section was to study the interplay between inflammation and lipid sub-fractions. For this analysis we performed a linear regression analysis
### Chapter 4

**Lipids and Apolipoproteins and Mortality in Patients with Inflammatory Polyarthritis**

<table>
<thead>
<tr>
<th>Variables</th>
<th>IP patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol mmol/l</td>
<td>5.0 (4.06, 5.8)</td>
</tr>
<tr>
<td>HDL mmol/l</td>
<td>1.13 (0.85, 1.44)</td>
</tr>
<tr>
<td>TG mmol/l</td>
<td>1.45 (1.1, 1.97)</td>
</tr>
<tr>
<td>LDL mmol/l</td>
<td>3.03 (2.32, 3.80)</td>
</tr>
<tr>
<td>ApoA-1 g/l</td>
<td>1.62 (1.29, 2.0)</td>
</tr>
<tr>
<td>ApoB g/l</td>
<td>0.89 (0.7, 1.09)</td>
</tr>
<tr>
<td>High TC (&gt;6.2) mmol/l n (%)</td>
<td>237 (17.22%)</td>
</tr>
<tr>
<td>High LDL (&gt;4.1) mmol/l n (%)</td>
<td>229 (17.7%)</td>
</tr>
<tr>
<td>High TG (&gt;2.3) mmol/l n (%)</td>
<td>182 (13.36%)</td>
</tr>
<tr>
<td>Low HDL (&lt;1.0) mmol/l n (%)</td>
<td>465 (38.98%)</td>
</tr>
<tr>
<td>Any dyslipidemia n (%)</td>
<td>722 (57.03%)</td>
</tr>
</tbody>
</table>

**Table 4.4:** Lipid profile in patients with IP. Data are shown as median (IQR) and N(%).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Males (440)</th>
<th>Females (826)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol mmol/l</td>
<td>4.65(3.76, 5.5)</td>
<td>5.16(4.3, 5.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL mmol/l</td>
<td>1.03(0.79, 1.29)</td>
<td>1.24(0.94, 1.59)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TG mmol/l</td>
<td>1.56(1.18, 2.02)</td>
<td>1.39(1.02, 1.88)</td>
<td>0.0006</td>
</tr>
<tr>
<td>LDL mmol/l</td>
<td>2.89(2.12, 3.77)</td>
<td>3.14(2.47, 3.85)</td>
<td>0.0003</td>
</tr>
<tr>
<td>ApoA-1 g/l</td>
<td>1.54(1.22, 1.85)</td>
<td>1.69(1.32, 2.09)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ApoB g/l</td>
<td>0.87(0.65, 1.1)</td>
<td>0.9(0.72, 1.09)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

**Table 4.5:** Lipid profile in patients with IP stratified by gender. The comparison between the two groups was analysed using Mann-Whitney test.
Figure 4.1: Distribution graph of TC, the Y axis shows the number of patients with different cholesterol level.
Figure 4.2: Distribution graph of HDL, the Y axis shows the number of patients with different HDL level.
Figure 4.3: Distribution graph of TG, the Y axis shows the number of patients with different TG level.
adjusted for age and gender using lipids as the dependent variable and markers of inflammation and disease activity (CRP, DAS-28, number of swollen joints and number of tender joints) as the independent variables.

4.7.1 The correlation between CRP, DAS-28 score and lipids

A linear regression analysis revealed a significant negative association between CRP and TC, LDL, TG, and ApoA-1. There was no significant association between CRP and HDL or ApoB or lipid ratios (Table 4.6).

With regards to the association with DAS-28 score, only ApoA-1 and the TC/HDL ratio had a significant negative association. There was no significant association with the remaining lipids as detailed in (Table 4.7).

4.7.2 Correlation between 51-swollen/tender joints and lipids

A linear regression analysis was performed to test the association between lipids and swollen/tender joints using the 51-swollen/tender joints count. A higher number of tender joints was associated with higher TG levels (Table 4.8). There was a trend towards association with ApoA-1. A higher number of swollen joints was associated with lower HDL levels. There was also a trend towards an association with lower cholesterol level and lower ApoA-1 level (Table 4.9).

In the previous analysis there might be an issue of multiple testing. We performed a bonferroni adjustment although this might be too conservative due to the fact that these variables are very closely related. We multiplied the P
Table 4.6: Linear regression analysis for the association between lipids and CRP adjusting for age and gender.

<table>
<thead>
<tr>
<th>Variables</th>
<th>β coefficient</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-cholesterol</td>
<td>-0.006</td>
<td>(-0.011, -0.002)</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.0008</td>
<td>(-0.002, 0.005)</td>
<td>0.235</td>
</tr>
<tr>
<td>TG</td>
<td>-0.004</td>
<td>(-0.006, -0.001)</td>
<td>0.003</td>
</tr>
<tr>
<td>LDL</td>
<td>-0.004</td>
<td>(-0.007, -0.005)</td>
<td>0.025</td>
</tr>
<tr>
<td>ApoA-1</td>
<td>-0.001</td>
<td>(-0.004, -0.002)</td>
<td>0.031</td>
</tr>
<tr>
<td>ApoB</td>
<td>-0.0009</td>
<td>(-0.0001, 0.0001)</td>
<td>0.11</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>-0.007</td>
<td>(-0.014, 0.001)</td>
<td>0.092</td>
</tr>
<tr>
<td>ApoB/apoA-1</td>
<td>0.0009</td>
<td>(-0.0006, 0.002)</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Table 4.7: Linear regression analysis for the association between lipids and DAS-28\textsubscript{CRP} adjusting for age and gender.

<table>
<thead>
<tr>
<th>Variables</th>
<th>β coefficient</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-cholesterol</td>
<td>-0.036</td>
<td>(-0.099, 0.026)</td>
<td>0.25</td>
</tr>
<tr>
<td>HDL</td>
<td>0.008</td>
<td>(-0.014, 0.031)</td>
<td>0.47</td>
</tr>
<tr>
<td>TG</td>
<td>-0.03</td>
<td>(-0.069, 0.001)</td>
<td>0.06</td>
</tr>
<tr>
<td>LDL</td>
<td>-0.037</td>
<td>(-0.092, 0.018)</td>
<td>0.18</td>
</tr>
<tr>
<td>ApoA-1</td>
<td>-0.04</td>
<td>(-0.069, -0.014)</td>
<td>0.003</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.002</td>
<td>(-0.014, 0.019)</td>
<td>0.77</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>-0.13</td>
<td>(-0.25, -0.006)</td>
<td>0.04</td>
</tr>
<tr>
<td>ApoB/apoA-1</td>
<td>0.017</td>
<td>(-0.0004, 0.034)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

value by 10 (the number of variables) to adjust for multiple testing. In summary, CRP was significantly associated with (TC and TG) after adjustment. With regards to the other markers of disease severity i.e. tender joint, swollen joint and DAS-28\textsubscript{CRP}, they were less consistently associated with lipids (one marker per measure). Of particular note, the trends were generally in the same direction with the exception of TG vs 51-tender/swollen joints which were positively and only significantly associated with tender joint count.
LIPIDS AND APOLIPROTEINS AND MORTALITY IN PATIENTS WITH INFLAMMATORY POLYARTHITIS

CHAPTER 4

Table 4.8: Linear regression analysis for the association between lipids and 51-tender joints, age and gender adjusted.

<table>
<thead>
<tr>
<th>Variables</th>
<th>β coefficient</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-cholesterol</td>
<td>-0.0001</td>
<td>(-0.006, 0.006)</td>
<td>0.97</td>
</tr>
<tr>
<td>HDL</td>
<td>0.0001</td>
<td>(-0.002, 0.002)</td>
<td>0.89</td>
</tr>
<tr>
<td>TG</td>
<td>0.005</td>
<td>(0.001, 0.007)</td>
<td>0.006</td>
</tr>
<tr>
<td>LDL</td>
<td>-0.003</td>
<td>(-0.008, 0.002)</td>
<td>0.32</td>
</tr>
<tr>
<td>ApoA-1</td>
<td>-0.003</td>
<td>(-0.005, 0.0006)</td>
<td>0.05</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.0006</td>
<td>(-0.0009, 0.002)</td>
<td>0.45</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>-0.004</td>
<td>(-0.018, 0.0098)</td>
<td>0.58</td>
</tr>
<tr>
<td>ApoB/ApoA-1</td>
<td>0.001</td>
<td>(-0.0004, 0.003)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Table 4.9: Linear regression for the association between lipids and 51-swollen joints, age and gender adjusted.

<table>
<thead>
<tr>
<th>Variables</th>
<th>β coefficient</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-cholesterol</td>
<td>-0.009</td>
<td>(-0.02, 0.001)</td>
<td>0.09</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.004</td>
<td>(-0.009, -0.0008)</td>
<td>0.02</td>
</tr>
<tr>
<td>TG</td>
<td>0.003</td>
<td>(-0.002, 0.009)</td>
<td>0.22</td>
</tr>
<tr>
<td>LDL</td>
<td>-0.007</td>
<td>(-0.012, 0.002)</td>
<td>0.17</td>
</tr>
<tr>
<td>ApoA-1</td>
<td>-0.006</td>
<td>(-0.01, 0.0003)</td>
<td>0.06</td>
</tr>
<tr>
<td>ApoB</td>
<td>-0.0005</td>
<td>(-0.003, 0.003)</td>
<td>0.9</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>0.005</td>
<td>(-0.020, 0.031)</td>
<td>0.67</td>
</tr>
<tr>
<td>ApoB/ApoA-1</td>
<td>0.002</td>
<td>(-0.001, 0.005)</td>
<td>0.29</td>
</tr>
</tbody>
</table>
4.8 Predictors of all-cause mortality in patients with early inflammatory polyarthritis

This section aimed to determine the factors that were associated with mortality in patients with inflammatory polyarthritis with a focus on lipid profile. Initially, we performed a univariable Cox-proportional hazard analysis using each predictor variable individually. Then, we adjusted, for age and gender as those significantly affected other predictor variables. Finally, a multivariable analysis was performed including all predictors with \( p < 0.2 \) from the univariable analysis being included in the initial model. Factors included as independent (predictors) were: age, gender, TC, TG, LDL, HDL, ApoA-1, ApoB, BMI, HTN, DM, CRP, RF, ACPA and DAS-28\(_{CRP}\). The outcome variable was death from any cause.

The median (IQR) follow up period was 5.5 (3.7-7.7) years. There was 100 (7%) deaths 33 (33%) of which had CVD as a primary cause of death (ICD10 codes I00-I99). Three patients were lost follow up (embarkation date was available for each patient). The total number of person year follow up was 7607 PY.

4.8.1 Univariable Cox-regression analysis for all-cause mortality

The data are presented in (Table 4.10). Increasing age and self reported hypertension were associated with increased mortality. Markers of disease severity including CRP, DAS-28\(_{CRP}\), HAQ score and steroid use were all associated with increased hazard of mortality. High levels of TC, LDL, HDL, female gender and increased BMI were apparently associated with reduced risk of mortality.

Due to the effect of age and gender on the predictor variables we repeated the
Table 4.10: Univariable Cox regression for all-cause mortality.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.087</td>
<td>1.067, 1.106</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>0.522</td>
<td>0.353, 0.773</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI</td>
<td>0.929</td>
<td>0.889, 0.971</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Self reported hypertension/medication</td>
<td>2.207</td>
<td>1.470, 3.31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Self reported diabetes</td>
<td>1.729</td>
<td>0.946, 3.162</td>
<td>0.075</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.30</td>
<td>0.83, 2.02</td>
<td>0.25</td>
</tr>
<tr>
<td>TC</td>
<td>0.791</td>
<td>0.676, 0.926</td>
<td>0.004</td>
</tr>
<tr>
<td>LDL</td>
<td>0.756</td>
<td>0.621, 0.921</td>
<td>0.006</td>
</tr>
<tr>
<td>HDL</td>
<td>0.467</td>
<td>0.273, 0.805</td>
<td>0.003</td>
</tr>
<tr>
<td>TG</td>
<td>1.219</td>
<td>0.918, 1.619</td>
<td>0.171</td>
</tr>
<tr>
<td>ApoA-1</td>
<td>1.279</td>
<td>0.912, 1.796</td>
<td>0.153</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.526</td>
<td>0.268, 1.029</td>
<td>0.061</td>
</tr>
<tr>
<td>ApoB/ApoA-1</td>
<td>0.599</td>
<td>0.294, 1.224</td>
<td>0.160</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>1.02</td>
<td>0.977, 1.073</td>
<td>0.312</td>
</tr>
<tr>
<td>CRP</td>
<td>1.008</td>
<td>1.0009, 1.02</td>
<td>0.027</td>
</tr>
<tr>
<td>DAS28CRP</td>
<td>1.171</td>
<td>1.001, 1.375</td>
<td>0.049</td>
</tr>
<tr>
<td>HAQ-score</td>
<td>1.532</td>
<td>1.217, 1.929</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RF</td>
<td>1.638</td>
<td>1.11, 2.402</td>
<td>0.011</td>
</tr>
<tr>
<td>ACPA</td>
<td>1.98</td>
<td>1.147, 2.857</td>
<td>0.002</td>
</tr>
<tr>
<td>Sero-positive</td>
<td>1.905</td>
<td>1.207, 3.006</td>
<td>0.006</td>
</tr>
<tr>
<td>Current DMARDs</td>
<td>0.723</td>
<td>0.496, 1.054</td>
<td>0.092</td>
</tr>
<tr>
<td>Steroid ever</td>
<td>2.085</td>
<td>1.429, 3.043</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Analysis adjusting for both of these variables (Table 4.11). However, adjusting for age and gender did not significantly change the results. The trend of the major findings remained the same although less significant for some variables.

### 4.8.2 Forward stepwise Cox regression analysis

We performed a forward stepwise regression analysis to find which variables are independently predictors of all-cause mortality. We included in this model lipid profile (TC, LDL, HDL, TG, ApoA-1 and ApoB). We adjusted for traditional risk factors (age, gender, BMI, hypertension, diabetes and smoking). We also added
<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.06</td>
<td>1.04, 1.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>0.599</td>
<td>0.41, 0.88</td>
<td>0.01</td>
</tr>
<tr>
<td>BMI</td>
<td>0.93</td>
<td>0.88, 0.97</td>
<td>0.003</td>
</tr>
<tr>
<td>Self reported hypertension</td>
<td>1.02</td>
<td>0.67, 1.56</td>
<td>0.9</td>
</tr>
<tr>
<td>Self reported diabetes</td>
<td>1.18</td>
<td>0.65, 2.18</td>
<td>0.58</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.94</td>
<td>1.22, 3.06</td>
<td>0.005</td>
</tr>
<tr>
<td>TC</td>
<td>0.82</td>
<td>0.70, 0.90</td>
<td>0.01</td>
</tr>
<tr>
<td>LDL</td>
<td>0.75</td>
<td>0.61, 0.92</td>
<td>0.006</td>
</tr>
<tr>
<td>HDL</td>
<td>0.51</td>
<td>0.29, 0.87</td>
<td>0.014</td>
</tr>
<tr>
<td>TG</td>
<td>1.12</td>
<td>0.82, 1.5</td>
<td>0.45</td>
</tr>
<tr>
<td>ApoA-1</td>
<td>0.97</td>
<td>0.68, 1.37</td>
<td>0.84</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.57</td>
<td>0.28, 1.17</td>
<td>0.12</td>
</tr>
<tr>
<td>ApoB/ApoA-1</td>
<td>0.72</td>
<td>0.36, 1.42</td>
<td>0.34</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>1.03</td>
<td>0.97, 1.08</td>
<td>0.34</td>
</tr>
<tr>
<td>CRP</td>
<td>1.007</td>
<td>0.99, 1.01</td>
<td>0.058</td>
</tr>
<tr>
<td>DAS28</td>
<td>1.16</td>
<td>0.99, 1.01</td>
<td>0.07</td>
</tr>
<tr>
<td>HAQ-score</td>
<td>1.39</td>
<td>1.09, 1.78</td>
<td>0.006</td>
</tr>
<tr>
<td>RF</td>
<td>1.53</td>
<td>1.03, 2.26</td>
<td>0.04</td>
</tr>
<tr>
<td>ACPA</td>
<td>1.786</td>
<td>1.13, 2.83</td>
<td>0.01</td>
</tr>
<tr>
<td>Sero-positive</td>
<td>1.654</td>
<td>1.05, 2.61</td>
<td>0.03</td>
</tr>
<tr>
<td>On DMARDs current</td>
<td>0.79</td>
<td>0.54, 1.16</td>
<td>0.23</td>
</tr>
<tr>
<td>Steroids ever</td>
<td>1.28</td>
<td>0.87, 1.89</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Table 4.11: Predictors of all-cause mortality age and gender adjusted.
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CHAPTER 4

Table 4.12: Forward stepwise regression analysis: all variables with $P < 0.2$ at univariable analysis were included.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.06</td>
<td>1.04, 1.09</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.78</td>
<td>0.62, 0.99</td>
<td>0.04</td>
</tr>
<tr>
<td>HAQ</td>
<td>1.62</td>
<td>1.16, 2.29</td>
<td>0.005</td>
</tr>
<tr>
<td>Female gender</td>
<td>0.54</td>
<td>0.29, 0.99</td>
<td>0.04</td>
</tr>
<tr>
<td>DMARD</td>
<td>0.69</td>
<td>0.39, 1.21</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Table 4.12: Forward stepwise regression analysis: all variables with $P < 0.2$ at univariable analysis were included.

the markers of disease activity (DAS-28$_{CRP}$, HAQ score, ACPA and RF) and the use of DMARDs and steroids see (Table 4.12). In this model, increased age and HAQ score were associate with increased odds ratio of all-cause mortality. On the other hand, female gender was associated with improved survival. Interestingly, total cholesterol level was associated with reduced all-cause mortality.

To reduce the effect of co-linearity we repeated the test swapping between different variables with possible close relation. For example, DAS-28$_{CRP}$ was calculated from CRP we used only CRP in this analysis. Due to the co-linearity between ACPA and RF we did not include it. This model included age, gender, hypertension, current smoking, diabetes mellitus, BMI, total cholesterol and ACPA. In this model age, ACPA and total cholesterol were independently predictors of mortality in patients with IP (Table 4.13). When we add CRP to this model ACPA become no longer independent and similarly when replace ACPA by sero-positive.

We also repeated the analysis testing for other lipid sub-fractions individually. HDL, LDL and ApoB were independently associated with reduced risk of overall mortality. Results are shown in (Tables 4.16-4.17).
<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>P-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.08</td>
<td>&lt;0.0001</td>
<td>1.06, 1.11</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>0.63</td>
<td>0.06</td>
<td>0.38, 1.03</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.75</td>
<td>0.004</td>
<td>0.61, 0.91</td>
</tr>
<tr>
<td>ACPA</td>
<td>1.75</td>
<td>0.02</td>
<td>1.08, 2.84</td>
</tr>
</tbody>
</table>

Table 4.13: Stepwise forward Cox regression for predictors of all-cause mortality with total cholesterol.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.08</td>
<td>1.05, 1.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL</td>
<td>0.67</td>
<td>0.51, 0.88</td>
<td>0.005</td>
</tr>
<tr>
<td>Female gender</td>
<td>0.65</td>
<td>0.37, 1.12</td>
<td>0.13</td>
</tr>
<tr>
<td>ACPA</td>
<td>1.46</td>
<td>0.84, 2.53</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Table 4.14: Stepwise forward Cox regression for predictors of all-cause mortality with LDL replacing TC.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.07</td>
<td>1.05, 1.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CRP</td>
<td>1.01</td>
<td>0.99, 1.02</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI</td>
<td>0.95</td>
<td>0.89, 1.01</td>
<td>0.09</td>
</tr>
<tr>
<td>Female gender</td>
<td>0.66</td>
<td>0.39, 1.09</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Table 4.15: Stepwise forward Cox regression for predictors of all-cause mortality with HDL replacing TC.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.07</td>
<td>1.05, 1.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CRP</td>
<td>1.01</td>
<td>1.0, 1.02</td>
<td>0.041</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.32</td>
<td>0.11, 0.93</td>
<td>0.04</td>
</tr>
<tr>
<td>Gender</td>
<td>0.57</td>
<td>0.32, 1.01</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Table 4.16: Stepwise forward Cox regression for predictors of all-cause mortality with TG replacing TC.

Table 4.17: Stepwise forward Cox regression for predictors of all-cause mortality model 5 with ApoA-1, ApoB.
4.9 Predictors of CVD mortality

This section studied the predictors for cardiovascular mortality. Cardiovascular mortality was defined as the primary cause of death due to cardiovascular reasons.

There were 33 (33% of the total deaths) deaths due to CV causes. Similar to the previous analysis we performed a univariable Cox-regression then multivariable regression to determine the association between the traditional risk factors, and cardiovascular mortality with a focus on lipid profile.

4.9.1 Univariable Cox-regression analysis for predictors of CVD mortality

As in the overall mortality, increasing age, and self reported hypertension were associated with increased risk of mortality due to CVD. Female gender and increased total cholesterol were associated with reduced risk of CVD mortality. There was no significant association between the remaining lipid sub-fractions and CVD mortality but the trend is similar to that observed in the all-cause mortality (Table 4.18). Because of the reduced number of events this did not reach statistical significance. Adjusting for age and gender attenuates the influence of TC and hypertension on CVD mortality (Table 4.19).

4.9.2 Forward stepwise Cox regression of predictors of CVD mortality

We performed the initial model similar to the model used in all-cause mortality. The following variables were included in the model age, gender, hypertension,
<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.09</td>
<td>1.06, 1.14</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>0.39</td>
<td>0.19, 0.77</td>
<td>0.007</td>
</tr>
<tr>
<td>BMI</td>
<td>0.99</td>
<td>0.92, 1.05</td>
<td>0.676</td>
</tr>
<tr>
<td>Self reported hypertension</td>
<td>4.45</td>
<td>2.24, 8.84</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Self reported diabetes</td>
<td>1.76</td>
<td>0.62, 5.02</td>
<td>0.29</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.05</td>
<td>0.47, 2.36</td>
<td>0.89</td>
</tr>
<tr>
<td>TC</td>
<td>0.71</td>
<td>0.54, 0.94</td>
<td>0.02</td>
</tr>
<tr>
<td>LDL</td>
<td>0.67</td>
<td>0.46, 0.97</td>
<td>0.03</td>
</tr>
<tr>
<td>HDL</td>
<td>0.38</td>
<td>0.15, 0.96</td>
<td>0.04</td>
</tr>
<tr>
<td>TG</td>
<td>1.23</td>
<td>0.75, 1.99</td>
<td>0.41</td>
</tr>
<tr>
<td>Apoa-1</td>
<td>1.32</td>
<td>0.72, 2.39</td>
<td>0.37</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.19</td>
<td>0.05, 0.77</td>
<td>0.02</td>
</tr>
<tr>
<td>ApoB/Apoa-1</td>
<td>0.21</td>
<td>0.04, 1.019</td>
<td>0.05</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>0.99</td>
<td>0.89, 1.12</td>
<td>0.98</td>
</tr>
<tr>
<td>CRP</td>
<td>1.001</td>
<td>0.99, 1.02</td>
<td>0.53</td>
</tr>
<tr>
<td>DAS28</td>
<td>1.19</td>
<td>0.90, 1.58</td>
<td>0.22</td>
</tr>
<tr>
<td>RF</td>
<td>1.08</td>
<td>0.52, 2.24</td>
<td>0.84</td>
</tr>
<tr>
<td>ACPA</td>
<td>1.22</td>
<td>0.49, 2.98</td>
<td>0.66</td>
</tr>
<tr>
<td>Seropositive</td>
<td>1.69</td>
<td>0.72, 3.96</td>
<td>0.23</td>
</tr>
<tr>
<td>HAQ-score</td>
<td>1.99</td>
<td>1.35, 2.96</td>
<td>0.001</td>
</tr>
<tr>
<td>Steroids ever</td>
<td>3.23</td>
<td>1.69, 6.16</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>DMARDs current</td>
<td>0.69</td>
<td>0.36, 1.34</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Table 4.18: Univariable Cox regression for CVD mortality.
## Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.10</td>
<td>1.06, 1.13</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>0.50</td>
<td>0.25, 1.00</td>
<td>0.05</td>
</tr>
<tr>
<td>BMI</td>
<td>1.02</td>
<td>0.94, 1.10</td>
<td>0.57</td>
</tr>
<tr>
<td>Self reported hypertension</td>
<td>1.98</td>
<td>0.97, 4.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Self reported diabetes</td>
<td>1.08</td>
<td>0.37, 3.09</td>
<td>0.89</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.61</td>
<td>0.70, 3.69</td>
<td>0.26</td>
</tr>
<tr>
<td>TC</td>
<td>0.77</td>
<td>0.59, 1.02</td>
<td>0.07</td>
</tr>
<tr>
<td>LDL</td>
<td>0.73</td>
<td>0.51, 1.05</td>
<td>0.09</td>
</tr>
<tr>
<td>HDL</td>
<td>0.44</td>
<td>0.17, 1.16</td>
<td>0.097</td>
</tr>
<tr>
<td>TG</td>
<td>1.09</td>
<td>0.65, 1.82</td>
<td>0.75</td>
</tr>
<tr>
<td>ApoA-1</td>
<td>0.96</td>
<td>0.52, 1.77</td>
<td>0.89</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.30</td>
<td>0.08, 1.11</td>
<td>0.07</td>
</tr>
<tr>
<td>ApoB/apoA-1</td>
<td>0.45</td>
<td>0.09, 2.28</td>
<td>0.33</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>1.00</td>
<td>0.88, 1.14</td>
<td>0.95</td>
</tr>
<tr>
<td>CRP</td>
<td>1.00</td>
<td>0.99, 1.02</td>
<td>0.55</td>
</tr>
<tr>
<td>DAS28</td>
<td>1.19</td>
<td>0.91, 1.55</td>
<td>0.19</td>
</tr>
<tr>
<td>RF</td>
<td>1.01</td>
<td>0.49, 2.09</td>
<td>0.98</td>
</tr>
<tr>
<td>ACPA</td>
<td>1.33</td>
<td>0.54, 3.28</td>
<td>0.54</td>
</tr>
<tr>
<td>Seropositive</td>
<td>1.54</td>
<td>0.66, 3.61</td>
<td>0.32</td>
</tr>
<tr>
<td>HAQ score</td>
<td>1.90</td>
<td>1.27, 2.84</td>
<td>0.002</td>
</tr>
<tr>
<td>Steroids</td>
<td>1.96</td>
<td>1.02, 3.79</td>
<td>0.044</td>
</tr>
<tr>
<td>DMARDs current</td>
<td>0.79</td>
<td>0.41, 1.54</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Table 4.19: Univariable Cox regression of CVD mortality age and gender adjusted.
diabetes, BMI, smoking, total cholesterol, LDL, HDL, ApoB, ACPA, CRP, DAS-28, HAQ score, use of DMARDs and steroids. We found that increased age, HAQ score and hypertension were associated with increased odds of CVD mortality. Similar to all-cause mortality, high total cholesterol was independently associated with reduced risk of CVD mortality.

We tested other lipids: replacing total cholesterol by TG and HDL revealed only age and hypertension were associated with CVD mortality (Tables 4.22 and 4.23). Reduced LDL level was marginally associated with increased risk (Table 4.21). When ApoA-1 and ApoB were included in the model ApoB was associated with reduced risk of cardiovascular mortality and age with increased risk (Table 4.24).

### Table 4.20: Forward stepwise regression analysis for CVD mortality all TRF+variables P<0.2 at univariable analysis were included.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.11</td>
<td>1.03, 1.21</td>
<td>0.009</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.08</td>
<td>0.007, 0.92</td>
<td>0.04</td>
</tr>
<tr>
<td>Steroid</td>
<td>7.49</td>
<td>1.61, 34.89</td>
<td>0.01</td>
</tr>
<tr>
<td>HAQ-score</td>
<td>2.22</td>
<td>1.03, 4.78</td>
<td>0.003</td>
</tr>
<tr>
<td>Hypertension</td>
<td>4.42</td>
<td>0.57, 124.81</td>
<td>0.09</td>
</tr>
</tbody>
</table>

### Table 4.21: Forward stepwise Cox-regression of predictors of CVD mortality with LDL replacing TC.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.16</td>
<td>1.06, 1.27</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL</td>
<td>0.56</td>
<td>0.29, 1.06</td>
<td>0.08</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3.36</td>
<td>0.88, 12.90</td>
<td>0.08</td>
</tr>
</tbody>
</table>
### Table 4.22: Forward stepwise Cox-regression of predictors of CVD mortality with HDL replacing TC.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.13</td>
<td>1.05, 1.21</td>
<td>0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>4.08</td>
<td>1.08, 15.34</td>
<td>0.04</td>
</tr>
<tr>
<td>Gender</td>
<td>0.39</td>
<td>0.12, 1.33</td>
<td>0.14</td>
</tr>
</tbody>
</table>

### Table 4.23: Forward stepwise Cox-regression of predictors of CVD mortality with TG replacing TC.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.12</td>
<td>1.06, 1.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3.45</td>
<td>1.10, 9.94</td>
<td>0.02</td>
</tr>
<tr>
<td>Gender</td>
<td>0.44</td>
<td>0.16, 1.24</td>
<td>0.12</td>
</tr>
</tbody>
</table>

### Table 4.24: Forward stepwise Cox regression of predictors of CVD mortality with ApoA-1, ApoB replacing TC.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.11</td>
<td>1.04, 1.18</td>
<td>0.003</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.05</td>
<td>0.003, 0.66</td>
<td>0.02</td>
</tr>
<tr>
<td>Gender</td>
<td>0.27</td>
<td>0.07, 1.03</td>
<td>0.06</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2.61</td>
<td>0.64, 10.55</td>
<td>0.17</td>
</tr>
</tbody>
</table>
4.10 Summary of the main result

The aim of this chapter is to examine lipid profile and determine the association between inflammation and lipids and to examine the predictors of mortality in patients with early IP. This section provides a summary of the main findings of this chapter.

- 1266 patients were assessed of which (20%) had hypertension and (7%) had diabetes.
- The majority of patients (66%) were obese or overweight.
- There was a moderate disease severity in this cohort.
- Around half of the patients were treated with DMARDs and (20%) were exposed to steroids, both the median duration of therapy was 45 days.
- Females had significantly higher levels of all lipid sub-fractions except TG.
- Around (57%) of patients had a dyslipidemic profile and the most prevalent dyslipidemia was reduced HDL (40% patients).
- CRP was the most consistent marker of disease activity associated with alterations in lipid subfractions.
- Independent predictors of all-cause mortality were age, HAQ-score, low total cholesterol and female gender.
- Independent predictors of CVD mortality were age, low total cholesterol, steroid, HAQ score and hypertension.
4.11 Discussion

This is the first study to report lipid profile and risk of mortality in a large inception cohort of patients with IP.

This study involved patients recruited to the Norfolk Arthritis Register (NOAR). The setting and design of this study allowed involving a large number of patients from the community prospectively and following them over time. The inclusion criteria gave a chance to include a larger sample size which makes it "representative" of the whole RA cohort in that community. One of the major concerns about NOAR is that not all patients fulfilled the 1987 ACR criteria for RA however previous studies indicated that around 70% of the patients satisfied RA diagnosis by their fifth anniversary [287].

In this cohort two thirds of the patients were females. 42% of the patients were RF positive and a similar number fulfilled the 1987 ACR criteria for RA both of which naturally increase during the disease course. Around a quarter of the patients were ACPA positive. The median level of CRP was 11 and DAS-28 \(_{CRP} \) (3.68). It would be expected that patients with chronic inflammatory condition such as RA would have a high level of CRP and DAS-28\(_{CRP} \). However, in this cohort this may be attributed to the low level of inflammatory, disease burden in the early stage of disease. A few patients also have been established on treatment and may already have been responding although there was actually a short disease duration.

With regards to lipid profile, the median values of lipids were within the normal range. However, more than half of the patients had one or more kind of dyslipidemic reading at the time of assessment. The main dyslipidemia found in this cohort was reduced HDL which was present in 40% of the study subjects.
Reduced HDL in patients with RA has also been the most consistent finding in studies looking at dyslipidemia in patients with RA [227].

We examined the association between markers of inflammation (CRP) and disease activity (DAS-28\(_{CRP}\), swollen/tender joints) and lipids adjusting for age and gender. Generally the trend was towards negative association between these markers and lipids. CRP was the most consistent marker associated with levels of most lipids namely; TC, TG, LDL, and ApoA-1. Although there was no significant association between HDL and CRP, a high level of CRP was significantly associated with reduced ApoA-1. ApoA-1 is the protein content of HDL. This may indicate that although the level of HDL was not significantly altered by inflammation, its protein content is altered. A similar pattern was also observed in patients with RA in a recent study by Toms et al [263]. This suggests that function of HDL may also be impaired and this may explain the conversion of HDL from protective, anti-oxidant to pro-inflammatory [103]. Alterations of HDL content have also been described in other conditions [168][169].

In association with DAS-28 (calculated from CRP, swollen/tender joints, and Visual Analogue Scale VAS) both ApoA-1 and the TC/HDL ratio were negatively associated with the disease activity. There was also a negative trend towards association between DAS-28\(_{CRP}\) and ApoB/ApoA-1 ratio and TGs. The effect of DAS-28\(_{CRP}\) on the lipids seems to be less than the effect of CRP. This may be due to the difference in the sensitivity of both markers. DAS-28\(_{CRP}\) may also detect signs of chronic disease, disease progression, synovial thickening and signs of inflammation. It may also be prone to subjective variability as it contains the VAS and tender joints both are subjective measurements.

The number of tender joints was significantly associated with increased TG level and swollen joints were negatively associated with HDL. Looking at the
trend towards an association between tender joints and HDL there is a reverse in the trend although it was not significant but this might be explained as an acute phase response where there is an increased need for anti-inflammatory and protective response. Some studies reported increased levels of HDL at the site of inflamed joints [188]. The swollen/tender joints are similar to DAS-28_{CRP} and may also contain a subjective elements in their assessment and may not be such a precise estimate of the inflammatory state.

Previous work in the general population indicated that the TC/HDL and ApoB/ApoA-1 ratios are more stable and more predictive for risk prediction [280]. We looked at the association between these ratios and markers of inflammation and disease severity. We found that there is a trend towards a negative association between CRP and TC/HDL ratio and a significant association with DAS-28_{CRP}. The association between markers of inflammation and the lipid ratios may be dependant on the effect of these markers on individual lipids which subsequently influence the effect of inflammation on the ratios.

The main aim of this study was to determine the baseline predictors of all-cause/CVD mortality. We found in a univariable analysis that increased age, hypertension (self reported), RF, ACPA were associated with increased risk of all cause mortality and CVD mortality. In contrast and somewhat surprisingly perhaps, female gender, higher total cholesterol, HDL and LDL level were significantly associated with reduced risk of overall mortality/CVD mortality. Surprisingly, low BMI was also associated with increased risk of mortality. Age and gender adjustment did not affect these findings apart from RF and hypertension where the crude estimate remained increased.

Substituting other lipid fractions also demonstrated similar association with all-cause mortality. Therefore, in the context of inflammation there is a shift in
the lipid profile generally towards reduced lipid levels. In other words inflammatory activation leads to a reduction in the circulating lipid profile. It has been confirmed that patients with early active untreated RA have normal or reduced TC, TG in association with high grade of inflammation [222]. Sattar et al also reported that TC, HDL, ApoB, and ApoA-1 are inversely related to markers of inflammation and disease activity in patients with RA and related conditions [227]. In this cohort we confirmed a negative correlation with CRP.

Others have also noted that CRP is inversely related to HDL in patients with RA [58]. Intriguingly, one report has also found that reduced level of lipids may indeed predate the diagnosis of RA by up to 5 years prior to the diagnosis [177].

Increased mortality in patients with low level of lipids does not necessarily mean that low level of lipid itself is deleterious. It does however suggest that patients with chronic inflammation in which lipid levels are reduced have an excess mortality. This has been confirmed by several other studies investigating the effect of treatment on lipids in patients with RA. Lee et al found that treatment in RA patients was associated with increased HDL and TC in association with a reduction in ESR and this was noted more in the DMARD responders compared to the non-responders [141]. A recent metanalysis of the effect of TNF-α blocking agents on lipid level in RA showed increased TC, HDL level after treatment [275]. A sub-group of patients from this cohort were followed up for two years and their lipids were found to increase in association with an improvement in inflammatory disease [174]. Therefore, this increase in lipid profile might not be a "deterioration" of their lipids but rather may represent a normalization of previously low lipid induced by inflammatory burden.

Inflammation mediates many of the changes on lipid levels through multiple mechanisms. This is mainly via changes in the acute phase proteins. This alters
the metabolism, contents and structure of lipids. Some of the changes in the metabolism of LDL are associated with increased risk of atherogenesis and hence CVD. These changes include reduced a level of LDL which is associated with a reduction in the particle size. This leads to accumulation of small dense LDL which is more pro-atherogenic [69]. Small dense LDL particles have low affinity to the LDL receptors which leads to reduced clearance and its accumulation in the circulation [183]. These small lipid particles can easily cross the endothelial barrier and bind the proteoglycans in the vascular wall intima which causes LDL retention [120][119]. Small dense LDL particles are also more prone to oxidative modification compared with LDL and this leads to increased accumulation of lipids and uptake by macrophages.

Many changes in HDL metabolism also occur during inflammation which impairs the HDL functions. Some of these changes include a reduction in the components of HDL (ApoA-1, LCAT, CETP), these proteins are involved in the reverse cholesterol transport process. Reduction in the LCAT leads to reduced clearance of cholesterol from the cells [131]. If this continued for a long time, as in chronic inflammation, it will potentially lead to cholesterol deposition in macrophages, foam cell formation and eventually atherosclerosis. Alterations in protein content (ApoA-1, PON-1) also impairs a major function of HDL (anti-oxidation) and this increases the oxidation of lipids and accumulate oxidant-stress.

We also found a paradoxical association between survival and BMI where reduced BMI was associated with decreased survival. This finding was reported previously in patients with RA by Escalante and colleagues [65]. In the general population a low BMI is not associated with increased risk of mortality or cardiovascular death. However, in RA patients, low BMI might reflect uncontrolled active systematic inflammation and "cachexia”. Thus low BMI is found to be
associated with a three fold increased risk of CVD death even after adjustment for other risk factors in patients with RA [161]. RA patients are at risk of developing "rheumatoid cachexia" in which there is a redistribution of the body composition with a differential loss of body lean muscles and increased fat mass. Adipose tissues are metabolically active and may further influence the inflammatory burden through adipocytokines. There is new evidence [85] that abdominal fat in patients with RA is distributed differently between the visceral and subcutaneous tissues. The visceral fat is strongly associated with increased risk of cardiovascular disease.

These findings may support the concept of "reverse causation" which described low levels of traditional risk factors (lipids, BMI, BP) as deleterious [208][116][83].

Female gender is associated with reduced risk of mortality in this study. This would be expected as males have more severe symptoms and progressive disease nature which reduce the survival. Current smokers at baseline had increased risk of overall mortality; the risk for CVD mortality was high but not statistically significant. Increased all-cause mortality with smoking was reported previously in NOAR patients [68]. The effect of smoking on mortality may be due to its effect on RF/ACPA status. RF/ACPA status may account for some of the effects of smoking in our model i.e. RF/ACPA becomes a "path variable".

The finding of this study enhances the importance of systemic inflammation as a major player in lipid alterations in patients with IP. While high lipid levels in the general population are associated with increased risk of CVD and mortality, low total cholesterol and LDL were independently associated with overall and CVD mortality in this study. The results from the AMORIS (Apolipoprotein-related MORtality RISk) study found the association between TC and MI in patients
with RA was weaker than general population [233]. Similarly, Myasedova et al found an increased risk of cardiovascular events in patients with low TC, LDL and high CRP [177].

Our findings and previous reports suggest a pivotal role of the impact of inflammation on lipids and alteration of their effects. However, the exact mechanism by which inflammation confounds the association between lipids and outcome remains to be elucidated. Further prospective studies are needed with serial measurements of both lipids and inflammatory markers are needed to determine the exact interplay between the two measures and their effects on outcome.

4.12 Strengths and limitations

The design of the study allows us to identifying patients with early disease, prospectively from the community and follow them over a period of time. This design provides a good sample representative of all RA patients and is generalizable to a UK population of early RA. This design also gives us the chance of getting more information about the progress of the disease and the development of any co-morbidity. The design of this study minimises selection bias where any patient with two swollen joints for more than four weeks is eligible to participate in the study. There is also complete follow-up information via the NHS-IS of a large primary-care based inception cohort for mortality over the follow-up period.

One of the major criticisms on this cohort is that not all patients fulfilled the criteria for RA; however this number is increasing with the progress of disease course. Another limitation of the study is the opportunity of recall bias during filling the questionnaires. There might also be an issue of misclassification of the cause of death on the death certificate in the absence of a post-mortem report
but the degree of misclassification is likely to be similar to that in the general population.

### 4.13 Clinical impact

- The paradoxical effect of lipids on the survival highlight the importance of monitoring the risk for CVD in patients with chronic inflammatory conditions especially in patients with chronic active disease flares.

- A serial measurement of lipid profile in patients with active inflammatory conditions is advisable in risk assessment and risk estimate.

- Lipid profile is affected by the inflammatory activity thus risk assessment in patients with chronic inflammatory conditions is different from individuals without inflammatory condition.

- These results suggest the importance of primary CVD prevention and the need for aggressive control of inflammation from the time of presentation of IP, as part of this strategy.
Chapter 5

Lipids profile and modified lipids in patients with active SLE/RA.

5.1 Introduction

Mortality in systemic lupus erythematosus (SLE) follows a bimodal rhythm [268]. Active disease and infection is the main cause for the early mortality and myocardial infarction and stroke is the cause for second peak mortality. In particular young patients age 35-44 have up to 52 times increased risk of CVD compared to the general population [155]. SLE patients were found to have increased risk of both clinical and subclinical atherosclerosis with 30-40% of women with SLE having carotid plaque or perfusion abnormalities in some studies [156][27]. Similarly patients with rheumatoid arthritis (RA) have an increased risk of cardiovascular disease mortality which accounts for up to 50% of excess mortality.

However, the exact mechanism underlying this is unclear, several studies have shown that the traditional cardiovascular risk factors do not explain the excess rate of atherosclerosis in SLE/RA patients [66][54]. Thus, disease related factors,
systemic inflammation and immune dysregulation are thought to contribute to increased risk of atherosclerosis [221].

Vascular endothelial dysfunction has been widely regarded as the earliest stage of atherosclerosis. It has been widely described in association with CHD, diabetes mellitus, smoking and hypercholesterolemia [35]. Endothelial dysfunction can be assessed by a validated non-invasive technique using the ultrasound scan of the brachial artery in response to hyperemic stimuli known as flow mediated dilatation (FMD) [35]. As described by Celermajer et al, endothelial dysfunction is associated with a number of traditional risk factors such as smoking [34]. Patients who have FMD <4.5% are likely to have myocardial perfusion defect and may be at risk of developing subclinical atherosclerosis [237].

In those patients there are a number of factors that may contribute to increased endothelial dysfunction such as chronic inflammation, Raynaud’s phenomenon, increased risk of vascular thrombosis in patients with antiphospholipid syndrome and metabolic derangement. Endothelial dysfunction has been described in patients with SLE/RA by a number of investigators [148][63]. Raza and colleague described endothelial dysfunction in patients with systemic vasculitis and treatment with immunosuppressant was associated with improvement in endothelial function [209]. Several reports on the effect of treatment on endothelial dysfunction in RA with contrasting results ranges from active transient improvement to long-term sustained improvement [118][96].

5.2 Aims

- Assess endothelial function in patients with SLE/RA
- Examine the predictors of endothelial function in SLE/RA patients
• Ascertain the level of oxidant stress as indicated by oxidised-LDL, lipids and apolipoproteins in patients with active SLE/RA.

• Determine the effect of treatment on endothelial function, oxidant stress and lipid profile in patients with active SLE/RA.

5.3 Methods

This section is based on analysis of a cohort of SLE/RA patients recruited from the Lupus clinics at the Manchester Royal Infirmary Hospital. These patients are seen regularly at the Rheumatology department. Patients fulfilled the 1997 revised ACR criteria for SLE and 1987 criteria for RA, and have active flare of the disease and needing change of therapy are nominated for the study. Patients are contacted directly in the clinics or by post with a request to participate in the study. Patients who agree to participate are invited to the Wellcome Trust Clinical Research Facility Center (WTCRF). The study was approved by the North West Ethic Committee (NRES), (Chief Investigators: Prof. Ian Bruce and Dr. Benjamin Parker) see appendix IV.

All participants were asked to sign a written consent form. The first part of the study was interview, clinical assessment, and collection of fasting blood samples. The second part was the vascular scans to assess the endothelial function using two methods: brachial artery scan for flow mediated dilatation (FMD), and EndoPAT which assesses peripheral artery tone. The patients were assessed at two time points before the start and four months after their therapy. Fifteen healthy controls, age and sex matched, were recruited from the University and hospital staff.
5.4 Clinical assessment

The clinical assessment included a complete history including age, ethnicity, marital status, education, and occupation. Data on SLE/RA criteria, date of diagnosis, current, past and treatment to be commenced were recorded. Any history of Raynaud’s, previous thrombosis, co-morbidity was recorded. Patients’ menstrual status and any hormonal replacement therapy were reported. Information about any history of CVD disease (CHD, MI, angina, etc) and classic risk factors were gathered. Patients were also asked about their smoking and alcohol consumption.

Disease activity in patients with SLE was assessed by Systemic Lupus Disease Activity Index (SLEDAI), and organ damage by ACR/Systemic Lupus International Collaborating Clinics (SLICC/ACR). For RA patients: swollen, tender joint count was performed. Disease activity was assessed by the disease activity score DAS-28crp and Health Assessment Questionnaire score (HAQ).

5.5 Processing of blood samples

Blood samples were obtained on the day of interview after overnight fasting, and 48-hour abstain from alcohol. As part of the routine assessment biochemical profile was performed and included (creatinine, glucose, liver function, lipid profile and full blood count). In addition autoimmune and autoantibody profile were assessed including C3, C4, anti ds-DNA, and anti cardiolipin antibodies. In addition, blood samples (serum and plasma) were collected for later analysis. These samples were centrifuged and separated. Separated serum and plasma samples were divided into aliquots and stored at $-80^\circ$ for later analysis. The experimental methods for this chapter are outlined in Chapter (2).
5.6 Statistical analysis

Values are presented as median (IQR) unless indicated otherwise. Differences in parameters between patients and controls or in patients at baseline and after therapy are compared using Mann-Whitney test. Correlation between the variables was ascertained using Spearman’s correlation coefficient. P values <0.05 were accepted as statistically significant.

5.7 Results

5.8 SLE patients

5.8.1 Demographics

We studied a total of 27 SLE patients and 15 healthy controls. The median (IQR) age of SLE patients was 43 (28-53) years and for controls 36 (33, 44) years. There was no statistical difference in the age between patients and controls P=0.6.

5.8.2 Clinical features of SLE patients

All patients satisfied at least 4/11 ACR criteria for SLE, Table (5.1) illustrates the prevalence of ACR criteria in SLE patients. The most common manifestation was positive ANA (96%) followed by arthritis which present in 80% of the patients. With regards to the clinical features of SLE, 9 patients had current nephritis, 15 had Raynaud’s phenomenon. One patient gave a history of previous stroke, and another gave a history of MI.
## ACR criteria

<table>
<thead>
<tr>
<th>ACR criteria</th>
<th>SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malar rash</td>
<td>16 (59%)</td>
</tr>
<tr>
<td>Discoid rash</td>
<td>13 (48%)</td>
</tr>
<tr>
<td>Oral ulcers</td>
<td>17 (62%)</td>
</tr>
<tr>
<td>Serositis</td>
<td>8 (29.6%)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>22 (81.5%)</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>19 (70%)</td>
</tr>
<tr>
<td>Renal</td>
<td>11 (40.7%)</td>
</tr>
<tr>
<td>Neurologic</td>
<td>6 (22%)</td>
</tr>
<tr>
<td>Haematological</td>
<td>19 (70%)</td>
</tr>
<tr>
<td>Immunological</td>
<td>14 (51.8%)</td>
</tr>
<tr>
<td>ANA</td>
<td>26 (96%)</td>
</tr>
</tbody>
</table>

Table 5.1: Table summarises the prevalence of ACR criteria in SLE patients.

## BILAG item

<table>
<thead>
<tr>
<th>BILAG item</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Mucocutaneous</td>
<td>3</td>
<td>5</td>
<td>9</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Neurological</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>7</td>
<td>4</td>
<td>8</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Cardiorespiratory</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>Renal</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Haematological</td>
<td>-</td>
<td>2</td>
<td>12</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 5.2: Distribution of BILAG scores and components in SLE patients (n=27) at baseline.

### 5.8.3 Disease activity and damage score

The median (IQR) disease duration from the diagnosis date was 7 (3.5, 12) years and SLEDAI score was 6 (5, 13) which indicates active disease. The key BILAG item involvement detailed in (Table 5.2) suggests disease activity mostly involving renal and musculoskeletal systems. The median (IQR) SLICC-Damage Index (SLICC-DI) score was 1 (1, 2). The most prevalent item of the SLICC-DI was deforming arthritis, present in 8 (30%) of the patients.
5.8.4 Therapy

At the time of assessment the majority of patients 24 (89%) were treated with steroids with a median (IQR) dose of 12.5 (10, 17.5)mg/day, 10 patients were treated with steroid at some point during their disease course.

20 patients were on anti-malarials, 12 patients on immunosuppressants (2 patients were on azathioprine, 6 patients MMF and 2 MTX). Three patients were on lipid lowering treatment and two patients on hormonal replacement therapy. 13 (48%) of SLE patients were assigned to start rituximab, 6 (22%) to start azathioprine, 6 (22%) to start MMF and one patient to start IV cyclophosphamide infusions.

5.8.5 Lifestyle factors

Three patients were current smokers and the median number of cigarettes smoked per day was 20 (10, 20), none of the controls were smoker. Twelve patients (44%) admitted to alcohol consumption as did 10 (70%) of the controls. Around one quarter (7) of the patients were post menopausal. The average age for menopause in SLE patients was 45 (40, 49) years. In healthy controls 4 participants were post menopausal with a median age at menopause of 53 (50, 53) years. Summary of the life style and hormonal factors in SLE patients and healthy controls is in table (5.4).
<table>
<thead>
<tr>
<th>SLE features</th>
<th>Median (IQR)/ N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease duration years</td>
<td>7 (3.5, 12)</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>6 (5, 13)</td>
</tr>
<tr>
<td>SLICC</td>
<td>1 (1, 2)</td>
</tr>
<tr>
<td>BILAG</td>
<td>17 (12, 22)</td>
</tr>
<tr>
<td>ESR mm/h</td>
<td>28 (9, 46)</td>
</tr>
<tr>
<td>hsCRP mg/dl</td>
<td>2.4 (0.5, 5.1)</td>
</tr>
<tr>
<td>Nephritis current</td>
<td>9 (33.33%)</td>
</tr>
<tr>
<td>Nephritis past</td>
<td>6 (22.22%)</td>
</tr>
<tr>
<td>Nephritic syndrome current</td>
<td>4 (14.81%)</td>
</tr>
<tr>
<td>Nephritic syndrome past</td>
<td>3 (11.11%)</td>
</tr>
<tr>
<td>On Steroids current</td>
<td>24 (89%)</td>
</tr>
<tr>
<td>Past</td>
<td>10 (38%)</td>
</tr>
<tr>
<td>Current steroid dose</td>
<td>12.5 (10, 17.5)</td>
</tr>
<tr>
<td>Anti malarial current</td>
<td>20 (74%)</td>
</tr>
<tr>
<td>Anti malarial past</td>
<td>10 (38%)</td>
</tr>
<tr>
<td>Current immunosuppresssion</td>
<td>12 (44)</td>
</tr>
<tr>
<td>Antihypertensive current</td>
<td>12 (44%)</td>
</tr>
<tr>
<td>Antihypertensive past</td>
<td>3 (11.11%)</td>
</tr>
<tr>
<td>Lipid lowering</td>
<td>3 (11.11)</td>
</tr>
<tr>
<td>HRT</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2 (7.41%)</td>
</tr>
<tr>
<td>Raynaud’s</td>
<td>15 (55.5%)</td>
</tr>
<tr>
<td>Anti ds-DNA</td>
<td>7 (26%)</td>
</tr>
<tr>
<td>ANA</td>
<td>23 (88%)</td>
</tr>
<tr>
<td>ACL</td>
<td>8 (32%)</td>
</tr>
</tbody>
</table>

Table 5.3: SLE features in patients at the time of baseline assessment.

<table>
<thead>
<tr>
<th>Social factors</th>
<th>SLE patients (n=27)</th>
<th>Controls (n=15)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoker</td>
<td>3 (11%)</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>12 (44%)</td>
<td>10 (70%)</td>
<td>0.04</td>
</tr>
<tr>
<td>College/university education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hormone contraception</td>
<td>4 (15%)</td>
<td>1 (8%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Post menopausal</td>
<td>7 (27%)</td>
<td>4 (27%)</td>
<td>0.9</td>
</tr>
<tr>
<td>Hysterectomy</td>
<td>3 (11%)</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>HRT use</td>
<td>2</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>15 (55%)</td>
<td>9 (59%)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Table 5.4: Life style and hormonal factors in SLE patients and healthy controls.
5.8.6 Traditional and novel risk factors in SLE and healthy controls

Table (5.5) shows the traditional risk factors in SLE patients and healthy controls. There was no significant difference in the level of total cholesterol, LDL between patients and controls although their level was slightly lower in patients compared to controls. Triglyceride level was significantly higher in SLE patients compared with controls. There was a trend towards lower HDL level in patients than controls. The level of oxidised-LDL and glycated-LDL was similar in patients and controls. There were two patients with diabetes and none of the controls. The family history of CVD was more prevalent in SLE patients than healthy controls.

Generally, SLE patients had more prevalent traditional risk factors than healthy controls. When we stratify patients according to the presence of traditional risk factors we found that the majority of SLE patients have one (7 patients) or more risk factors.
<table>
<thead>
<tr>
<th>Variables</th>
<th>SLE n= 27</th>
<th>Control n=15</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>43 (28, 53)</td>
<td>36 (33, 44)</td>
<td>0.6</td>
</tr>
<tr>
<td>Female</td>
<td>26</td>
<td>14</td>
<td>0.2</td>
</tr>
<tr>
<td>BMI</td>
<td>25.7 (21.5, 28.5)</td>
<td>25.1 (23.1, 30.8)</td>
<td>0.4</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>80 (74, 91.6)</td>
<td>80 (72, 93.5)</td>
<td>0.8</td>
</tr>
<tr>
<td>Hip circumference</td>
<td>96 (84.8, 107)</td>
<td>98 (82.5, 101.3)</td>
<td>0.5</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.82 (0.79, 0.91)</td>
<td>0.84 (0.79, 0.88)</td>
<td>0.7</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>131 (103, 144)</td>
<td>119 (114, 127)</td>
<td>0.8</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>76 (66, 86)</td>
<td>77 (69, 80)</td>
<td>0.8</td>
</tr>
<tr>
<td>% Body fat</td>
<td>33.25 (24.3, 37.8)</td>
<td>32.6 (27.3, 39.4)</td>
<td>0.7</td>
</tr>
<tr>
<td>Bioimpedence</td>
<td>665 (596, 728)</td>
<td>654 (573, 700)</td>
<td>0.4</td>
</tr>
<tr>
<td>Smoker current</td>
<td>3</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>Smoker ex</td>
<td>6</td>
<td>4</td>
<td>0.7</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2 (4%)</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.4 (4.1, 5)</td>
<td>4.9 (4.8, 5.1)</td>
<td>0.05</td>
</tr>
<tr>
<td>Family history CVD</td>
<td>10 (43%)</td>
<td>2 (15%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.2 (4.6, 6.71)</td>
<td>5.54 (4.83, 6.87)</td>
<td>0.5</td>
</tr>
<tr>
<td>TG</td>
<td>1.36 (0.9, 1.87)</td>
<td>0.88 (0.64, 1)</td>
<td>0.009</td>
</tr>
<tr>
<td>HDL</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</td>
<td>2.71 (1.92, 3.6)</td>
<td>3.01 (2.69, 3.58)</td>
<td>0.3</td>
</tr>
<tr>
<td>ApoA-1</td>
<td>1.57 (1.34, 1.81)</td>
<td>1.71 (1.56, 1.9)</td>
<td>0.3</td>
</tr>
<tr>
<td>ApoB</td>
<td>1.3 (1.05, 1.61)</td>
<td>1.16 (0.99, 1.62)</td>
<td>0.9</td>
</tr>
<tr>
<td>Oxidised-LDL</td>
<td>52.15 (37.14, 59.72)</td>
<td>42.29 (36.07, 52.06)</td>
<td>0.5</td>
</tr>
<tr>
<td>Oxidised-LDL/LDL</td>
<td>16.23 (12.44, 23.29)</td>
<td>13.82 (13.22, 16.57)</td>
<td>0.43</td>
</tr>
<tr>
<td>Glycated LDL</td>
<td>2.98(1.89, 3.73)</td>
<td>3.13 (2.73, 3.67)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 5.5: Baseline traditional risk factors and lipids and modified lipids of SLE patients and healthy controls.

### 5.8.7 Endothelial function in SLE vs controls

We studied the vascular function in SLE patients and healthy controls using flow mediated dilatation of the brachial artery (FMD) and reactive hyperaemic index (RHI). Detailed methods and validations are described in Chapter (2).

Endothelial function in SLE patients in this population is the primary focus of another PhD thesis [192]. With regards to the brachial artery diameter, the resting diameter was similar in SLE patients and controls. The absolute %FMD was significantly lower in SLE patients than healthy controls 2.86 (0.6, 5.3) vs
The endothelial independent dilation (GTN dilation) was similar in SLE and healthy controls 15.32 (11.87-19.06) vs 11.99 (10.3-20.45). The median reactive hyperaemic index (RHI) was 1.97 (1.68, 2.54) in SLE and 2.02 (1.79, 2.45) in healthy controls, no statistical difference between the two groups in the RHI.

5.8.8 Comparison of disease activity, lipid profile in SLE patients before and after therapy

There was a significant reduction in the disease activity after therapy as indicated by a drop in the median SLEDAI from 6 to 4 and BILAG from 17 to 3. The number of patients on category A or B on the BILAG has generally reduced. With regards to lipid profile, there was a reduction in the triglycerides level from 1.36 to 0.98 but was not statistically significant. There was no significant change in other lipids. There was a trend towards reduction in oxidised-LDL although was not significant (Table 5.6). As indicated in (Table 5.6), the median change in SLEDAI, SLICC, BILAG and ESR was towards a general reduction in their level. Similarly, there was a trend towards reduction in lipids except HDL and ApoA-1 which tended to increase. This trend indicated a favorable improvement.

With regards to endothelial function, there is improvement in endothelial function after a period of four months therapy although this was statistically not significant. Over time the median %FMD increased from 2.86 (0.6, 5.3) to 4.56 (1.71, 5.87) P=0.62, as did the median RHI 1.97 (1.68, 2.54) vs 2.17 (1.91, 2.51) P=0.39.
Table 5.6: Disease activity, lipid profile and endothelial function in SLE patients at baseline and after therapy.

5.8.9 Correlation between lipids and apolipoproteins and endothelial function

We explored the correlation between lipids and surrogates of endothelial function in both SLE patients and healthy controls. In SLE patients, there was no significant association between lipids and %FMD or resting diameter. In contrast, we found a significant correlation between reactive hyperemic index (RHI) and ApoB, oxidised-LDL and a trend towards association with higher triglycerides level (Table 5.7). There was no significant association with other traditional risk factors, disease activity or immunosuppression use.

In healthy controls, higher HDL level was positively associated with %FMD and negatively with the resting diameter. A similar trend for ApoA-1 was found.
which was only significant in association with the resting diameter (Table 5.8). It can be concluded that the influence of lipids on endothelial function is different in SLE patients from healthy controls.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>%FMD</th>
<th>Resting diameter</th>
<th>RHI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>-0.04 (0.9)</td>
<td>0.10 (0.7)</td>
<td>0.27 (0.2)</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.10 (0.7)</td>
<td>-0.09 (0.7)</td>
<td>-0.11 (0.6)</td>
</tr>
<tr>
<td>LDL</td>
<td>0.005 (0.9)</td>
<td>-0.11 (0.6)</td>
<td>0.27 (0.2)</td>
</tr>
<tr>
<td>TG</td>
<td>-0.07 (0.7)</td>
<td>-0.06 (0.8)</td>
<td>0.38 (0.08)</td>
</tr>
<tr>
<td>ApoA-1</td>
<td>-0.32 (0.3)</td>
<td>-0.08 (0.8)</td>
<td>-0.10 (0.7)</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.11 (0.7)</td>
<td>0.14 (0.6)</td>
<td>0.54 (0.02)</td>
</tr>
<tr>
<td>Oxidised-LDL</td>
<td>0.40 (0.1)</td>
<td>-0.06 (0.8)</td>
<td>0.46 (0.04)</td>
</tr>
<tr>
<td>Glycated-LDL</td>
<td>0.17 (0.5)</td>
<td>-0.16 (0.5)</td>
<td>0.33 (0.16)</td>
</tr>
</tbody>
</table>

Table 5.7: Correlation between lipids and apolipoproteins and endothelial function in SLE patients. Data are shown as R(P).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>%FMD</th>
<th>Resting diameter</th>
<th>RHI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>0.25 (0.4)</td>
<td>-0.22 (0.5)</td>
<td>-0.34 (0.3)</td>
</tr>
<tr>
<td>HDL</td>
<td>0.71 (0.009)</td>
<td>-0.69 (0.008)</td>
<td>0.02 (0.9)</td>
</tr>
<tr>
<td>LDL</td>
<td>0.009 (0.9)</td>
<td>-0.01 (0.9)</td>
<td>0.06 (0.9)</td>
</tr>
<tr>
<td>TG</td>
<td>-0.14 (0.7)</td>
<td>0.35 (0.2)</td>
<td>-0.26 (0.4)</td>
</tr>
<tr>
<td>ApoA-1</td>
<td>0.53 (0.14)</td>
<td>-0.69 (0.03)</td>
<td>-0.02 (0.9)</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.17 (0.7)</td>
<td>0.29 (0.4)</td>
<td>-0.34 (0.3)</td>
</tr>
<tr>
<td>Oxidised-LDL</td>
<td>0.13 (0.7)</td>
<td>0.21 (0.5)</td>
<td>-0.45 (0.2)</td>
</tr>
<tr>
<td>Glycated-LDL</td>
<td>0.29 (0.4)</td>
<td>-0.12 (0.7)</td>
<td>0.06 (0.9)</td>
</tr>
</tbody>
</table>

Table 5.8: Correlation between lipids and apolipoproteins and endothelial function in healthy controls. Data are shown as R(P).

5.9 Correlation between lipid profile and parameters of disease activity in SLE patients

We examined the correlation between lipid profile and the following parameters of SLE (steroid dose, SLEDAI and SLICC-DI) see (Table 5.9). We found higher steroid dose was significantly associated with increased triglycerides levels. There was a trend towards negative correlation between SLEDAI and lipid profile except triglycerides which was positively correlated. However this association was not statistically significant.

Renal impairment was present in almost half of the SLE patients. Thus we
wanted to examine the effect of renal impairment on lipids and modified lipids. We compared the lipid profile in SLE patients according to the presence or absence of lupus nephritis. As indicated in (Table 5.10), SLE patients with lupus nephritis have significantly higher levels of triglycerides and a trend towards higher total cholesterol. Generally, patients with lupus nephritis tended to have higher levels of atherogenic lipids and lower levels of atheroprotective lipids.
5.9.1 Correlation between change in lipid profile and change in SLE parameters

A spearman’s correlation between change in lipids and change in SLEDAI shown a negative correlation between SLEDAI and lipids. This correlation was statistically significant for total cholesterol, HDL and oxidised-LDL and approaching significance for LDL and glycated LDL. Higher steroid dose was significantly associated with higher triglyceride, oxidised-LDL and HDL levels. With regards to the association with endothelial function we found a negative association between high triglycerides and %FMD. Similarly, high ApoB level was associated with reduced %FMD.

5.9.2 The effects of biological agents on lipids.

Almost half of the patients were treated with biological therapy (Rituximab). We wanted to explore the effect of biological therapy compared to the traditional immunosuppression on vascular biomarkers and endothelial function. Of particular note, patients who underwent rituximab treatment usually have more severe or refractory disease. Patients who started rituximab in this study [13 (48%)] had

<table>
<thead>
<tr>
<th>Lipid</th>
<th>SLEDAI</th>
<th>Steroid dose</th>
<th>%FMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>-0.53 (0.0002)</td>
<td>0.28 (0.11)</td>
<td>-0.20 (0.29)</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.35 (0.02)</td>
<td>0.43 (0.01)</td>
<td>0.22 (0.22)</td>
</tr>
<tr>
<td>LDL</td>
<td>-0.31 (0.05)</td>
<td>0.24 (0.21)</td>
<td>0.26 (0.18)</td>
</tr>
<tr>
<td>TG</td>
<td>-0.03 (0.81)</td>
<td>0.46 (0.008)</td>
<td>-0.37 (0.04)</td>
</tr>
<tr>
<td>ApoA-1</td>
<td>-0.32 (0.11)</td>
<td>0.18 (0.42)</td>
<td>-0.19 (0.47)</td>
</tr>
<tr>
<td>ApoB</td>
<td>-0.15 (0.42)</td>
<td>-0.12 (0.54)</td>
<td>-0.5 (0.02)</td>
</tr>
<tr>
<td>Oxidized-LDL</td>
<td>-0.56 (0.001)</td>
<td>0.42 (0.01)</td>
<td>0.27 (0.14)</td>
</tr>
<tr>
<td>OxidisedLDL/LDL</td>
<td>-0.10 (0.67)</td>
<td>0.21 (0.44)</td>
<td>0.06 (0.84)</td>
</tr>
<tr>
<td>Glycated-LDL</td>
<td>-0.36 (0.05)</td>
<td>0.27 (0.17)</td>
<td>-0.18 (0.47)</td>
</tr>
</tbody>
</table>

Table 5.11: Spearman’s correlation between change in lipid profile and SLE parameters. Data are presented as R(P)
longer disease duration at assessment with a median (IQR) 11.7 (7.6, 15.03) years compared with 3.2 (0.6, 4.8) years and P=0.0005. They also have higher global BILAG score at baseline 21 (12, 29) vs 14 (6.5, 21.5) P=0.4. The SLEDAI score was similar with a median of 7 (6, 16) for rituximab and 6 (6, 13.5) for other immunosuppressant candidates, P=0.6.

After treatment the global BILAG score reduced to 3 (2, 9) in the rituximab group and 8.5 (2.5, 10) in the other group, P=0.5. The SLEDAI score improved to the same level in both patients groups 4 (2, 4) with rituximab vs 4 (2.6) in the other treatments, P=0.4.

With regards to the endothelial function, patients who were assigned to biological therapy had lower %FMD at baseline 0.34 (-0.7, 4.7) vs 1.01 (-2.9, 4.1), P=0.6. Patients who had rituximab have a significant better improvement in their endothelial function compared to patients who were on traditional immunosuppression. The median (IQR) %FMD after treatment with biological therapy was 5.3 (4.7, 5.5) compared to 2.7 (0.38, 5.5), P=0.03. No difference in the RHI between the two groups neither before nor after treatment.

In terms of lipid profile, there was no significant difference in the lipid profile between the two treatment groups at baseline assessment (Table 5.12). Apart from ApoB which was slightly higher but not statistically significant in the rituximab group. Following a four months course of treatment we noticed slight increase in the HDL, ApoA-1 in both groups (Table 5.13). Triglycerides level dropped only in the immunosuppressant group and slightly increased in the rituximab arm.

We examined the change in lipids between the two groups, we found that rituximab treatment was significantly associated with increased total cholesterol and ApoA-1. There was also a trend towards increased LDL and HDL level.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Rituximab</th>
<th>Other treatment</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>5.5 (4.1, 6.9)</td>
<td>4.9 (4.7, 5.9)</td>
<td>0.5</td>
</tr>
<tr>
<td>LDL</td>
<td>3.1 (1.9, 3.7)</td>
<td>2.5 (2.1, 3.4)</td>
<td>0.5</td>
</tr>
<tr>
<td>HDL</td>
<td>1.3 (1.2, 1.6)</td>
<td>1.3 (1.2, 1.9)</td>
<td>0.9</td>
</tr>
<tr>
<td>TG</td>
<td>1.3 (0.9, 1.7)</td>
<td>1.5 (1.1, 2.0)</td>
<td>0.3</td>
</tr>
<tr>
<td>ApoA-1</td>
<td>1.4 (1.3, 1.6)</td>
<td>1.6 (1.4, 1.9)</td>
<td>0.2</td>
</tr>
<tr>
<td>ApoB</td>
<td>1.5 (1.2, 1.7)</td>
<td>1.1 (0.8, 1.3)</td>
<td>0.1</td>
</tr>
<tr>
<td>Oxidised LDL</td>
<td>52.2 (37.1, 59.7)</td>
<td>40.9 (32.8, 55.2)</td>
<td>0.4</td>
</tr>
<tr>
<td>Glycated LDL</td>
<td>3.1 (2.7, 3.7)</td>
<td>3.2 (1.9, 4.0)</td>
<td>0.4</td>
</tr>
<tr>
<td>△TC</td>
<td>0.59 (0.56, 1.11)</td>
<td>-0.37 (-0.62, -0.02)</td>
<td>0.002</td>
</tr>
<tr>
<td>△LDL</td>
<td>0.27 (-0.41, 0.76)</td>
<td>-0.09 (-0.67, 0.21)</td>
<td>0.25</td>
</tr>
<tr>
<td>△HDL</td>
<td>0.19 (0.02, 0.29)</td>
<td>0.11 (-0.18, 0.30)</td>
<td>0.48</td>
</tr>
<tr>
<td>△TG</td>
<td>-0.1 (-0.31, 0.6)</td>
<td>-0.49 (-0.81, -0.02)</td>
<td>0.008</td>
</tr>
<tr>
<td>△ApoA-1</td>
<td>0.25 (0, 0.36)</td>
<td>-0.05 (-0.34, 0.21)</td>
<td>0.01</td>
</tr>
<tr>
<td>△ApoB</td>
<td>-0.04 (-0.28, 0.02)</td>
<td>-0.03 (-0.11, 0.06)</td>
<td>0.32</td>
</tr>
<tr>
<td>△Oxidised-LDL</td>
<td>-0.76 (-5.71, 13.53)</td>
<td>-5.99 (-14.47, 2.38)</td>
<td>0.13</td>
</tr>
<tr>
<td>△Glycated LDL</td>
<td>-0.36 (-0.70, 0.76)</td>
<td>-0.21 (-0.63, 0.005)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Table 5.12: Lipid profile in SLE patients at baseline categorised by treatment. Data are presented as median (IQR) and Mann Whitney test was used to compare variables between groups.

and decreased oxidised-LDL and glycated LDL. Both treatment was associated with decreased triglycerides levels but this was more significant in the traditional treatment group.

5.10 RA patients

5.10.1 Demographics

RA patients were recruited from the Rheumatology clinics at the Manchester Royal Infirmary Hospital. All patients fulfilled the 1987 ACR criteria for RA. The setting and design for this cohort were similar to the ones described in SLE section. However, due to the presence of competing studies and shortage of research nurses there was poor recruitment in the time frame of this study.
### Table 5.13: Lipid profile in SLE patients after therapy categorised by treatment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rituximab</th>
<th>Other treatment</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>5.6 (4.7, 6.9)</td>
<td>4.9 (4.2, 5.9)</td>
<td>0.2</td>
</tr>
<tr>
<td>LDL</td>
<td>2.7 (2.5, 3.9)</td>
<td>2.6 (2.2, 2.7)</td>
<td>0.3</td>
</tr>
<tr>
<td>HDL</td>
<td>1.5 (1.4, 1.6)</td>
<td>1.4 (1.1, 1.8)</td>
<td>0.6</td>
</tr>
<tr>
<td>TG</td>
<td>1.5 (1.1, 1.9)</td>
<td>0.9 (0.8, 1.3)</td>
<td>0.2</td>
</tr>
<tr>
<td>ApoA-1</td>
<td>1.6 (1.5, 1.7)</td>
<td>1.7 (1.6, 1.8)</td>
<td>0.6</td>
</tr>
<tr>
<td>ApoB</td>
<td>1.2 (0.8, 1.6)</td>
<td>1.1 (0.9, 1.2)</td>
<td>0.6</td>
</tr>
<tr>
<td>Oxidised LDL</td>
<td>54 (34, 61.1)</td>
<td>43.5 (37.3, 48.0)</td>
<td>0.4</td>
</tr>
<tr>
<td>Glycated LDL</td>
<td>2.6 (2.2, 3.8)</td>
<td>2.5 (2.1, 2.7)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

### Table 5.14: Baseline demographics of RA patients and healthy controls. Data are presented as median (IQR)

<table>
<thead>
<tr>
<th>Variables</th>
<th>RA (n=14)</th>
<th>Control (n=15)</th>
<th>Age-adjusted P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>59.5 (52, 62)</td>
<td>36 (33, 46)</td>
<td>0.002</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>14</td>
<td>na</td>
</tr>
<tr>
<td>Smoking</td>
<td>4</td>
<td>0</td>
<td>na</td>
</tr>
<tr>
<td>BMI</td>
<td>27.6 (24.3, 32.7)</td>
<td>28.1 (24.1, 32.8)</td>
<td>0.3</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>91 (82.5, 101.5)</td>
<td>80 (75.5, 95.9)</td>
<td>0.6</td>
</tr>
<tr>
<td>Hip circumference</td>
<td>102 (95.1, 111)</td>
<td>98.5 (85, 108.3)</td>
<td>0.3</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>123 (113, 140)</td>
<td>123.5 (118, 131)</td>
<td>0.8</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>76.5 (70, 78)</td>
<td>79 (69, 82)</td>
<td>0.3</td>
</tr>
<tr>
<td>% Body fat</td>
<td>32.7 (30.1, 39.1)</td>
<td>34.3 (27.3, 40.1)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

We recruited 14 patients (11 females) with rheumatoid arthritis and 15 healthy controls. The median (IQR) ages of the patients was 59.5 (52, 62) years and controls was 37 (33, 46) years, (P=0.002). In an age adjusted analysis, there was no significant difference between patients and controls with regards BMI, blood pressure, waist and hip circumference. Table (5.14) summarises the demographics for patients and controls.
RA features & Median (IQR)/ N \\
--- & --- \\
Disease duration (years) & 6.05 (0.08, 11.57) \\
DAS-28 & 4.97 (4.32, 5.78) \\
CRP mg/dl & 18.5 (2.9, 34) \\
ESR mm/h & 21 (8, 32) \\
ACPA & 10 (71\%) \\
RF & 10 (71\%) \\
Steroids current & 2 (14\%) \\
Steroids past & 5 (36 \%) \\
Current DMARD & 10 (71\%) \\
Current antihypertensive & 7 (50\%) \\
Past antihypertensive & 1 (10\%) \\
Current statin & 4 (28.5\%) \\

Table 5.15: Disease related features in RA patients at the time of baseline assessment. Data are presented as median (IQR) or percentage of patients.

### 5.10.2 RA related factors

RA patients had active disease with a median (IQR) DAS-28\textsubscript{CRP}; 5.0 (4.3, 5.8). The median (IQR) of disease duration at the time of assessment was 6 (0.08, 11.6) years. Around two thirds (10) of the patients were RF positive and 10 patients were ACPA positive. Two patients were on steroids at the time of assessment and 10 patients were on DMARDs. The median (IQR) of CRP and ESR were 18.5 (2.9, 34) mg/l, 21 (8, 32) respectively (Table 5.15). Five (35\%) patients were due to start methotrexate, 4 (28\%) etanercept and 4 (28\%) were due to start adalimumab.

### 5.10.3 Lipid profile at baseline assessment

Lipid profile for RA patients and healthy controls are shown in (Table 5.16). In an age-adjusted analysis there was no statistical difference in the lipids between patients and controls. However, the total cholesterol and triglycerides level tended to be higher in RA patients than healthy controls. RA patients had a median
<table>
<thead>
<tr>
<th>Variables</th>
<th>RA (n=14)</th>
<th>Control (n=15)</th>
<th>Age-adjusted P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC mmol/l</td>
<td>5.9 (5.5, 6.7)</td>
<td>5.54 (4.8, 6.9)</td>
<td>0.7</td>
</tr>
<tr>
<td>TG mmol/l</td>
<td>1.1 (0.7, 1.5)</td>
<td>0.9 (0.6, 1.0)</td>
<td>0.1</td>
</tr>
<tr>
<td>HDL mmol/l</td>
<td>1.6 (1.3, 2.1)</td>
<td>1.6 (1.3, 1.8)</td>
<td>0.3</td>
</tr>
<tr>
<td>LDL mmol/l</td>
<td>3.03 (2.01, 3.52)</td>
<td>3.01 (2.69, 3.58)</td>
<td>0.7</td>
</tr>
<tr>
<td>ApoA-1 g/l</td>
<td>1.7 (1.4, 2.2)</td>
<td>1.7 (1.6, 1.9)</td>
<td>0.3</td>
</tr>
<tr>
<td>ApoB g/l</td>
<td>1.3 (1.1, 1.7)</td>
<td>1.2 (0.9, 1.6)</td>
<td>0.8</td>
</tr>
<tr>
<td>Ox-LDL U/l</td>
<td>42.67 (35.62, 55.38)</td>
<td>50.67 (42.03, 55.02)</td>
<td>0.7</td>
</tr>
<tr>
<td>oxidised LDL/LDL</td>
<td>15.88 (7.4, 18.18)</td>
<td>14.23 (12.32, 16.66)</td>
<td>0.8</td>
</tr>
<tr>
<td>Glycated-LDL mg/dl</td>
<td>2.58 (2.01, 3.52)</td>
<td>3.24 (2.77, 3.77)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 5.16: Baseline lipid profile and lipids and modified lipids of RA patients and controls.

(IQR) of ApoA-1 was 1.7 (1.4, 2.2) and ApoB was 1.3 (1.1, 1.7) g/l, which was similar to the levels in healthy controls. Oxidised-LDL was also lower in RA patients 42.67 (35.62, 55.38)U/l compared with 50.67 (42.03, 55.02) in healthy controls but this was not statistically significant. Glycated-LDL also tended to be low in RA patients.

5.10.4 Endothelial function at baseline assessment

In RA patients the baseline brachial artery was 3.65 mm (3.33, 4.44) and the median (IQR) of maximum endothelial dependant vasodilation of brachial artery was 5.21% (0.65, 6.60) compared to brachial artery in healthy controls 3.39 (3.04, 4.08) and %FMD 6.81 (3.46, 8.57), age-adjusted P=0.31. Although there was no significant difference in the %FMD between RA patients and healthy controls in this cohort, the %FMD in RA patients tended to be lower than the controls. The endothelium independent vasodilation was normal with a median (IQR) 11.79 (10.35, 18.57) in RA and 11.35 (10.26, 20.45) in the controls, P=0.9. The reactive hyperaemic index measured by EndoPAT was 2.36 (1.77, 3.02) in RA patients.
and 2.21 (1.92, 2.72) in healthy controls (P=0.9).

5.10.5 Inflammatory markers, markers of disease activity, and lipid profile after therapy

In patients with RA four months following treatment there was a reduction of the median DAS-28\textsubscript{CRP} from 4.97 to 3.8. CRP has dropped from a median of 18.5 to 6.5 and ESR from 21 to 19. Generally speaking there was a general reduction in the disease activity as indicated from DAS-28\textsubscript{CRP}, CRP, and ESR although this was not statistically significant. The mean change between baseline measurement and measurement after treatment indicate a reduction in disease activity and inflammatory markers.

We examined the lipid profile before and after treatment in RA patients. Generally, there was no significant difference in the median (IQR) of lipid profile. However, the trend was towards increased TC, HDL, LDL, ox-LDL, ApoA-1 and ApoB. The level of TG, and glycated-LDL tended to decrease following treatment results are shown in (Table 5.17).

We compared endothelial function in RA patients before and after treatment. We found a trend towards reduced %FMD in RA patients after therapy 5.21 (0.65, 7.13) vs 2.14 (-0.83, 3.89), P=0.30. There was no significant difference in the reactive hyperemic index before and after treatment 2.36 (1.75, 3.03) vs 2.53 (2.03, 2.95), P=0.94. Because the sample size was small we could not stratify the patients according to the commenced treatment or according to their age.
### Table 5.17: Comparison of markers of disease activity and lipid profile in RA patients at baseline and after therapy.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Visit 1 (n=14)</th>
<th>Visit 2 (n=12)</th>
<th>P value</th>
<th>Δ in variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAS-28&lt;sub&gt;CRP&lt;/sub&gt;</td>
<td>4.9 (4.32, 5.78)</td>
<td>3.8 (3.8, 4.77)</td>
<td>0.29</td>
<td>-0.61 (-1.7, 0.13)</td>
</tr>
<tr>
<td>CRP</td>
<td>18.5 (2.9, 34)</td>
<td>6.5 (3, 13)</td>
<td>0.11</td>
<td>-11.55 (-27, 0.95)</td>
</tr>
<tr>
<td>ESR</td>
<td>21.0 (8, 32)</td>
<td>19 (8, 27)</td>
<td>0.53</td>
<td>-5.50 (-8.50, 3.50)</td>
</tr>
<tr>
<td>TC</td>
<td>5.9 (5.5, 6.7)</td>
<td>5.9 (5.5, 6.9)</td>
<td>0.67</td>
<td>0.21 (-0.49, 0.58)</td>
</tr>
<tr>
<td>TG</td>
<td>1.1 (0.7, 1.5)</td>
<td>1.09 (0.8, 1.3)</td>
<td>0.87</td>
<td>-0.005 (-0.23, 0.13)</td>
</tr>
<tr>
<td>HDL</td>
<td>1.6 (1.3, 2.1)</td>
<td>1.5 (1.3, 2.01)</td>
<td>0.92</td>
<td>0 (-0.08, 0.06)</td>
</tr>
<tr>
<td>LDL</td>
<td>3.9 (3.06, 4.8)</td>
<td>3.9 (3.3, 4.5)</td>
<td>0.66</td>
<td>0.01 (-0.11, 0.49)</td>
</tr>
<tr>
<td>ApoA-1</td>
<td>1.7 (1.4, 2.2)</td>
<td>1.6 (1.5, 2.05)</td>
<td>0.65</td>
<td>0.05 (-0.13, 0.25)</td>
</tr>
<tr>
<td>ApoB</td>
<td>1.3 (1.1, 1.7)</td>
<td>1.4 (1.1, 1.5)</td>
<td>0.70</td>
<td>0.03 (-0.21, 0.14)</td>
</tr>
<tr>
<td>Oxidised-LDL</td>
<td>44.9 (36.09, 55.38)</td>
<td>46.1 (34.42, 54.62)</td>
<td>0.85</td>
<td>0.82 (-8.38, 7.18)</td>
</tr>
<tr>
<td>Glycated-LDL</td>
<td>3.5 (3.15, 3.52)</td>
<td>2.2 (1.95, 2.83)</td>
<td>0.8</td>
<td>0.37 (-0.25, 1.50)</td>
</tr>
</tbody>
</table>

5.10.6 Correlation between lipid profile and RA parameters

In a spearman’s correlation analysis we found a significant negative correlation between CRP and ApoA-1. A similar trend was observed for other lipids except for ApoB and LDL, and the trend approaching significance with HDL and oxidised-LDL. With regards to ESR, there was a trend towards negative correlation with triglycerides level. High DAS-28<sub>CRP</sub> was significantly associated with reduced triglycerides level see (Table 5.18).

5.10.7 Correlation between change in lipids and change in RA parameters

We explored the correlation between changes in lipids and changes in CRP, DAS-28<sub>CRP</sub> and FMD. Generally, there was a negative correlation between lipids and
Parameters | CRP | ESR | DAS-28  
--- | --- | --- | ---  
TC | -0.08 (0.70) | -0.44 (0.10) | -0.26 (0.40)  
HDL | -0.48 (0.08) | -0.30 (0.30) | -0.34 (0.20)  
LDL | 0.20 (0.50) | -0.29 (0.37) | 0.08 (0.82)  
TG | -0.41 (0.10) | -0.48 (0.08) | -0.64 (0.01)  
ApoA-1 | -0.57 (0.03) | -0.32 (0.26) | -0.33 (0.25)  
ApoB | 0.13 (0.65) | -0.16 (0.58) | 0.12 (0.68)  
Oxidized-LDL | -0.49 (0.07) | 0.15 (0.59) | -0.03 (0.91)  
OxidisedLDL/LDL | -0.29 (0.38) | 0.06 (0.85) | -0.35 (0.29)  
Glycated-LDL | -0.05 (0.83) | -0.04 (0.87) | -0.07 (0.79)  

Table 5.18: Spearman’s correlation between lipid profile and RA parameters. Data are described as R(P)

| Lipid | CRP | DAS-28_{CRP} | %FMD  
--- | --- | --- | ---  
TC | -0.53 (0.07) | -0.71 (0.009) | -0.06 (0.84)  
HDL | -0.36 (0.24) | -0.52 (0.07) | -0.54 (0.08)  
LDL | -0.5 (0.06) | -0.5 (0.06) | -0.27 (0.3)  
TG | -0.10 (0.75) | -0.08 (0.79) | 0.35 (0.29)  
ApoA-1 | -0.14 (0.68) | -0.24 (0.47) | -0.67 (0.04)  
ApoB | -0.16 (0.62) | -0.44 (0.15) | 0.12 (0.71)  
Oxidized-LDL | -0.43 (0.17) | -0.25 (0.41) | 0.11 (0.75)  
OxidisedLDL/LDL | 0.50 (0.31) | 0.50 (0.31) | -0.09 (0.79)  
Glycated-LDL | 0.32 (0.30) | 0.30 (0.33) | 0.10 (0.77)  

Table 5.19: Spearman’s correlation between change in lipid profile and change in RA parameters. Data are described as R(P)

CRP and DAS-28_{CRP}. The correlation was strongest for total cholesterol, HDL and LDL. A stronger correlation between CRP and oxidised-LDL although was not statistically significant. There was a significant negative correlation between FMD and ApoA-1 and a trend with HDL. Table (5.19) summarises the main findings.

5.11 Discussion

SLE  SLE patients were recruited from the Lupus clinics at the Manchester Royal Infirmary Hospital. The median age for this cohort was 43 years and this
is similar to what has been reported in other reports [219]. In keeping with previous studies all patients met the 1997 ACR criteria for SLE. With respect to the clinical features, the prevalence of clinical features is comparable to what has been reported in Europe, Asia, and United States. The prevalence of malar rash in 59% was higher than the reported prevalence in the European lupus [36] but very similar to the prevalence reported by Petri and Alarcon et al in the United states [196] [6]. Similarly, the arthritis in (81.5%) and the previous reports ranged (48-88.1%).

We found impaired endothelial function in SLE patients compared with healthy controls. The same finding was reported previously. In a study by Lima et al they reported a mean (SD) %FMD in SLE patients 5.0 (5.0) compared to 12 (6) in healthy controls [148]. Similarly, Piper et al found that SLE patients had a median (IQR) %FMD of 5.6 (3.1, 7.2) compared with 8.0 (6.3, 9.3) in controls [201]. In this study the median (IQR) of FMD in SLE patients was 2.86 (0.6, 5.3). Similar to our finding, El-Magadmi et al had reported impaired endothelial function in SLE patients in Manchester %FMD 3.6 (-6.3, 13.7) vs 6.9 (6.6, 17.8) in healthy controls [63]. This difference could be attributed to the differences in subject selection, and variation in the technique used to determine FMD. Also in Lima study they excluded postmenopausal women and patients with cardiovascular risk factors.

The correlation between endothelial function and traditional risk factors was not clear from previous studies. While Piper et al found a significant negative correlation between FMD and total cholesterol (R=-0.44) [201], Lima found no association with cholesterol [148]. Of particular note, is that patients with hyperlipidemia and hypertension were excluded from Lima’s study which may obscure or limit the ability to find any correlation with them. In El-Magadmi study, they
found in addition to systolic blood pressure, systemic lupus erythematosus was an independent risk factor for impaired FMD [63]. In this study, we found no association between FMD and traditional risk factors or lipids in SLE patients. In healthy controls, there was a negative correlation with systolic blood pressure (r=-0.6, P=0.04) and a positive significant association with HDL (R=0.7, P=0.009). The association with HDL would be expected as HDL has anti-inflammatory and atheroprotective properties and this function was reported to be impaired in SLE [168][169].

Previous studies have reported that SLE patients have a high prevalence of traditional risk factors. In particular, SLE patients are more likely to have hypertension, post-menopausal at earlier age when compared to controls [30]. We found a similar trend with regards to high prevalence of some traditional risk factors in SLE patients compared with controls. In this study, SLE patients tended to have lower HDL-C level than control which may reflect the effect of inflammation on lipid level[22][23]. A similar finding was also reported in other inflammatory conditions such as rheumatoid arthritis [25]. The triglycerides level was significantly higher in SLE patients than control which may be due to accumulation of triglycerides rich lipids as a result of high inflammatory burden.

Recent evidence suggests that inflammation plays an important role in the initiation and progression of atherosclerosis in the general population. We hypothesised that in the context of SLE, chronic inflammation may be an additional key factor for endothelial dysfunction and alterations in lipids subfractions and a reduction in the inflammation may be associated with improved endothelial function and lipid subfractions. In this study we found that higher steroid dose was associated with significant increase in triglycerides level and a trend to decrease HDL and ApoA-1 and increase in other lipids. This can be explained by
the metabolic effects of steroid and possibly due to its side effect. In contrast, SLEDAI, was generally associated with reduced lipid levels. As discussed in the NOAR study, this can reflect the effect of inflammation on the lipid metabolism which resulted in a shift towards lower lipid profile [131]. In patients with lupus nephritis, we found a trend towards higher oxidised-LDL and other atherogenic lipid subfractions and reduced HDL and ApoA-1 levels. This finding agrees with previous findings [59]. Proteinuria in patients with lupus nephritis alters the apolipoprotein content of lipoproteins. There is also impairment in the concentration of oxidised lipids and an increase in the total amount of oxidised lipids within the lipid subfractions. Patients with lupus nephritis may also be on high dose immunosuppression which may aggravate the accumulation of lipids.

There are several reports on the effect of treatment on endothelial function in RA as will be discussed in the following section. In contrast, the majority of report in SLE looked at the effect of prevention therapy on endothelial function [289]. Gonzalez-Juanatey noted a dramatic improvement in the FMD in RA patients after rituximab treatment although this was transient [93]. This was associated with a reduction in inflammation and disease activity. We found a similar finding in our patients, FMD has improved in association with a significant reduction in the disease activity (SLEDAI, and BILAG). This improvement was more evident in patients who were treated with biological agents than patients treated with conventional treatment. However, the patients on the biological therapy arm had more severe disease to start with and worse FMD. In general, there was a reduction in the triglycerides level. Patients on the biological therapy had significant increased in total cholesterol and a trend towards increase other lipid subfractions and reduction in the oxidised-LDL. A similar finding was observed in patients with RA after suppression of inflammation with DMARDs.
and biological agents [275]. As discussed previously, this may represent a normalisation of blood lipids.

Overall, these findings support the hypothesis that chronic inflammation is associated with impaired endothelial function and lipid alterations which may leads to increased atherosclerosis. Reduction in the inflammation may reduce the risk of cardiovascular disease by several pathways. Details about these pathways mandate further research.

**RA** In this exploratory prospective study we aimed to examine the endothelial function and lipid biomarkers in RA patients and compare it to the healthy controls. We also aimed to ascertain the effect of treatment on endothelial function and lipid profile in RA patients. This cohort represents a small sample of RA patients with active flare as indicated by a median DAS-28 of 5 (4.32, 5.78). The median (IQR) of CRP and ESR also indicate active disease. The majority of patients were seropositive (71%). Patients who are seropositive usually have active disease and at burden of other comorbidity such as cardiovascular disease [182] and metabolic syndrome [175].

Hurliman *et al* reported that TNF-α inhibition was associated with reduced disease activity and significantly improved the endothelial function in RA patients [118]. On the other hand, Gonzalez-Juanatey *et al* reported an active rapid improvement in endothelial function in RA patients which was noticed two days following the anti-TNF therapy. However, this response was transient and 4 weeks later the FMD returned to the baseline level [96]. A study by Sidiropoulos and colleagues measured flow mediated dilatation in 12 RA patients treated with anti-TNF at baseline, 3 months and 18 months and compared them to 5 patients treated with disease modifying therapy [239]. There was no significant difference in the FMD between anti-TNF treated patients and DMARDs treated
patients, despite differences in the age, disease severity, and disease duration between the two groups. In their study, they found no significant difference in the FMD at baseline and 3 months after therapy which agrees with our finding but there was a significant improvement at 18 months. The rapid positive effect of the drug in some reports may indicate the importance of this drug in improving the atherosclerotic complication mediated by endothelial dysfunction in RA patients. However, the small sample size in these studies and selection bias are major limitation in interpreting their results. There is also differences in the frequency and time of infusion and the use of different agents or a mixture of different treatment regime which makes it difficult to confer the effect to which therapy. The age and disease duration are other factors that should be accounted for. As would be expected the gap in endothelial function between patients and controls is narrowed as the age advances.

With regards to the lipid profile, we found no statistical difference in the lipids between patients and controls. Total cholesterol was slightly higher in RA patients but statistically not significant. In this population we also looked at oxidised-LDL and glycated-LDL with no difference. Previous studies reported reduced level of total cholesterol, LDL and HDL level in patients with active RA compared with healthy controls [177]. However, the timing of dyslipidemia in RA patients is an important factor. Myasedova report suggested that dyslipidemia predate the diagnosis of RA.

Our data from NOAR suggests a strong negative association between lipids (in particular; TC, HDL and LDL) and inflammation, thus it would be speculated that a reduction in the inflammation would be associated with improvement or alteration in lipid level and context and improvement in biomarkers of vascular function. There is increasing body of evidence on the effect of treatment on
lipid profile in patients with RA. Munro et al reported increased HDL level in association with hydroxychloroquine treatment and a decreased level with gold therapy [176]. The use of methotrexate was also associated with improvement in HDL level in RA patients [191].

There are several reports on the literature looking at the effect of biological treatment on lipid profile in RA with contrasting results [234][203]. The cause of this contrast is due to differences in study designs or study population. For example, in some studies the controls were either healthy control, RA patients treated with placebo or with other disease modifying therapy or different class of biological agents. The period of follow up was also different and ranging from short term follow up of 14 weeks to 12-24 months. In Pollono’s review, they reported a significant increase in total cholesterol in eleven out of 24 studies. The increase in total cholesterol was associated with increase in HDL level.

In this study there was no change in lipids after treatment. This is in agreement with Sidiropoulos study [239] in which they found slight change in lipids 3 months after treatment and the levels returned back to the baseline level at the second time assessment after 18 months. This could be due to several factors. We measured lipids at baseline and four months after therapy. The change in lipids was more evident in short term studies which lasted up to 14 weeks [9][48] which indicates that those patients may or may not have altered lipids at some point and then returned to baseline level. Another explanation is that RA patients may have a normal lipid level at baseline assessment and may not has been altered after treatment.

When we look to the patients at individual level we found that patients who respond to treatment and have a reduction in their CRP, ESR and DAS-28_{CRP}, their total cholesterol, LDL, and HDL tended to increase. This also was confirmed
when we did a correlation between the change in markers of disease activity and change in lipid profile there was a general trend towards a negative correlation between them. However, the sample size of this study is small with limited power to find clinical relevant or statistical significance. There might be a change in the content of lipids rather than the quantity which is beyond the scoop of this research.

5.12 Conclusions

In this section we studied patients with active systemic lupus erythematosus and rheumatoid arthritis. We used the same study design of a prospective observational study. The aim was to examine endothelial function and the effect of treatment on inflammation reduction, endothelial function and lipid associated biomarkers. As it can be seen from the results sections there are some differences in the findings in SLE and RA. These differences can be attributed to several factors. It can be due to differences in the pathophysiology in both conditions. The age of the study groups was different, age is an important biological factor that affects a number of the outcome variables. Some studies suggested that the difference in the endothelial function between patients and controls narrowed by time. When we look to the studies reporting the prevalence of CVD in patients with SLE and RA, we found that the prevalence reduce by increased age. The effect of sample size should be noted also, the sample size of SLE patients was almost double the size of RA patients.
Chapter 6

General Discussion

6.1 Final conclusions

One of the main objectives of this thesis was to investigate the prevalence and determinants of oxidant stress in SLE patients and the contribution of oxidative stress to sub-clinical atherosclerosis. In the general population, oxidised-LDL was associated with increased CVD. In some studies oxidised-LDL was an independent risk for CVD. The studies regarding oxidant stress in SLE are scarce. Our group has shown previously that oxidised-LDL was elevated in SLE patients with metabolic syndrome [64]. In our cross-sectional study we managed to confirm increased oxidative stress (oxidised-LDL) in SLE patients compared to healthy controls. This is in spite of reduced levels of total cholesterol and LDL. This finding suggest that although the lipid levels were low but the oxidised fraction of lipids is increased which may be due to accumulated oxidation of lipids as a result of inflammation. This may also be explained by a conversion of some lipids to pro-inflammatory (e.g. pi-HDL) and dysfunctional [103]. In relation to subclinical atherosclerosis, oxidised-LDL and urinary 8-IP were independently associated
with increased cIMT. This suggest a role of oxidative stress in the process of atherosclerosis. As this study was cross-sectional and patients in this cohort had relatively low disease activity, it is difficult to generalise this finding. Thus we further explored this on our prospective SLE patients.

In the prospective study, we investigated the effect of treatment on lipid profile and modified lipids and the correlation between lipids and endothelial function. SLE had significantly impaired endothelial function which was improved following treatment. The study was not powered to discover a significant difference in lipid profile and modified lipids. However, this can give an estimate of the sample size required for future research. We found that oxidised-LDL was positively associated with FMD and also with steroid dose. This area needs further research to determine whether high dose steroids prescribed for active disease are associated with improved disease activity, inflammation reduction and improved endothelial function. Alternatively, metabolic disturbance leading to accumulation of oxidant stress may be detrimental to vascular health. This fit with our initial observation that although patients have low disease activity, they also have increased oxidised-LDL which was related to subclinical atherosclerosis. Where the balance lies for a patient may vary according to their disease activity and genetic susceptibility.

With regards to the association between lipids, inflammation and mortality. We noted evidence to support a negative association between lipid profile and inflammation especially with CRP. We also found a paradoxical association between lipids and all cause mortality and CVD mortality. Myasedova et al [178] suggested a non-linear association with mortality, however, they studied RA patients with established disease, almost half of their patients had high total cholesterol and LDL levels. Further studies are needed to find what is the optimal level of lipid
below which there is increased risk. It is also important to determine the level of inflammation that is associated with lipid alterations.

6.2 Remarks from the study

The cause of increased CVD disease and mortality in SLE/RA is likely to be multifactorial. There is increased evidence to support the association between the inflammatory disease process and its treatment, with increased risk. This study highlighted the effect of inflammation on lipid alterations. Further understanding of the precise mechanisms that drive this may suggest targeted interventions to reduce cardiovascular risk in these conditions.

An important role for oxidant stress in the development of atherosclerosis was suggested in SLE patients. Life-style and dietary modification is highly recommended for SLE/RA patients although this advice has shown a modest effect in modifying risk factors [100].

In addition, regular traditional risk factor monitoring and screening is recommended to modify risk. The risk assessment used in the general population is not well validated in patients with active inflammatory conditions, as inflammation can clearly affects plasma lipid levels. Further research is therefore needed to determine how to modify standard risk assessment, for use in RA and SLE.

Results from the Jupiter study suggests the statin use reduces the CVD events in patients with elevated CRP [214]. Besides the lipid lowering effect, statins have anti-inflammatory effects. Thus the use of statins in patients with chronic inflammatory conditions and normal or low lipid level may help reduce inflammation and subsequently reduce the CVD burden. Statins have also shown improved endothelial function and RA disease activity [71][167].
Our study suggests a role for active suppression of the inflammatory disease in improving endothelial function in a small cohort of SLE patients. Other pharmaceutical agents that improve the endothelial function such as angiotensin converting enzyme inhibitors (ACE), folic acid, fish oils and anti-oxidants may also have benefits in these patients [135][289]. The use of these agents may reverse endothelial dysfunction and subsequently may results in a favourable CVD outcome. There is a need for large randomised interventional trials to further investigate whether these therapeutic strategies will also reduce CVD events.

There is increased interest in the use of biological agents in treating patients with active refractory disease. Follow up of these patients for a longer period with a regular assessment of their risk factors may also supply further details in improving survival and reducing risk for CVD.

### 6.3 Clinical implications

There are a number of possible implications arise from this research:

- Classic lipid levels are influenced by inflammation. CRP is negatively correlated with total cholesterol. This indicates that during active inflammation a "normal" lipid profile may be misleading and repeat screening when disease is quiet may be indicated.

- Lower total cholesterol predicts all cause mortality. This may reflect either inflammatory burden or alterations in lipid function, or both.

- Statins are still indicated if hyperlipidemia is identified and their proposed anti-inflammatory effects may also give added benefits in RA.
• There is increasing evidence of higher oxidant stress in SLE. Oxidant stress may reflect in part, both obesity and inflammation.

• Oxidant stress was associated with increased cIMT and may influence aspects of atherogenesis.

• We also found evidence of decreased endothelial function which improved after inflammation suppression.

• There also was a trend to improved lipids after treatment of inflammation. Suppression of inflammation may reduced CVD risk, improve vascular function and improve lipid profiles.

• Separating the effect of inflammation suppression, from a specific beneficial effect of any drug, will require clinical trials and larger studies.

### 6.4 Future directions

There are several directions that arises from this thesis:

• Given our observations, we would seek to explore the influence and degree of CVD risk improvement to be gained by complete suppression of inflammation.

• We also would seek to assess any differential influence of the choice of agents that achieve this goal. In particular, we would wish to explore how ”steroid-base” regime compared to approaches that minimise steroid exposure.

• It remains unknown what effect additional approaches may have in modifying cardiovascular risk in inflammatory diseases. In addition to statins,
future work should explore other potential pathways such as anti-oxidants, ACE inhibitors, and anti-malarial agents.
Appendix I: Ethical Approval
(study 1)
15 November 2005

Dr Ian Bruce
Senior Lecturer and Consultant Rheumatologist
ARC Epidemiology Unit
The University of Manchester
Stopford Building
Oxford Road
MANCHESTER M13 9PT

Dear Dr Bruce

**Full title of study:** Accelerated atherosclerosis in SLE: Lupus factors, telomere shortening and progression of atherosclerosis

**REC reference number:** 05/MRE08/82

Thank you for your letter of 10 November 2005, responding to the Committee’s request for further information on the above research (and for submitting revised documentation).

The further information has been considered on behalf of the Committee by the Chair (Dr P R Kelsey).

**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).

**Ethical review of research sites**

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the research site(s) taking part in this study. The favourable opinion does not therefore apply to any site at present. I will write to you again as soon as one Local Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at sites requiring SSA.

**Conditions of approval**

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

**Approved documents**

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

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The Central Office for Research Ethics Committees is responsible for the operational management of Multi-Centre Research Ethics Committees.
Appendix I

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Research governance approval

The study should not commence at any NHS site until the local Principal Investigator has obtained final research governance approval from the R&D Department for the relevant NHS care organisation.

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.

05/MRE08/62 Please quote this number on all correspondence

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

Yours sincerely,

Dr P R Kelsey
Chair

E-mail: - northwest.mrec@gmsha.nhs.uk

Enclosures: - Standard approval conditions (SL-AC2)

Copy to: - Dr John Rodgers
           Head of the Research Office
           University Research Office
           Christie Building
           The University of Manchester
           Oxford Road
           MANCHESTER M13 9PL

SF1 list of approved sites
Appendix II: Patient Information sheet (study 2)
Appendix II

ARC NORFOLK ARTHRITIS REGISTER (NOAR)

INFORMATION SHEET

The Norfolk Arthritis Register (NOAR) is the largest community-based study in the world investigating the cause and outcome of inflammatory polyarthritis (inflammation and swelling of the joints). The Register is funded by the Arthritis Research Campaign through a grant to its Epidemiology Unit based at the University of Manchester. NOAR is a satellite of the Epidemiology Unit and is based in the Department of Rheumatology at the Norfolk and Norwich University Hospital. The project is run in collaboration with the University of East Anglia.

What Is The Purpose Of The Study?
NOAR started in 1989 and is a long-term study of inflammatory arthritis in the community. The purpose of the Register is to study the natural history of arthritis and to identify genetic and non-genetic factors which may be related to the onset of arthritis, response to treatment, and to long-term outcome. Previous work involving patients participating in the Norfolk Arthritis Register suggests that people with inflammatory arthritis may have an increased risk of developing coronary heart disease in the long-term. One of the current purposes of NOAR, therefore, is to try and identify which patients with arthritis may be at increased risk of heart disease and whether treatment of the arthritis reduces this risk.

Why Have I Been Chosen?
You have been asked to take part in this study because you have recently developed some inflammation in your joints. Your doctor will have asked your permission to forward your name to us. Over 3,700 people are already taking part in this study.

Do I Have To Take Part?
It is up to you to decide whether or not to take part in any or all of this study. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you will still be free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care that you receive.

What Will Happen To Me If I Take Part?
The study consists of a number of parts detailed below. You may choose to take part in any or all of these sections:

1. You will be asked to complete a short questionnaire about the amount of pain you have, whether you have any chest pain, your general well-being and your ability to carry out some everyday activities of daily living.
2. One of our research nurses will interview you about your past medical history, family history of arthritis and heart disease, your smoking history, your current medication, the symptoms related to your arthritis and any possible factors which may have triggered it.

3. Our research nurse will measure your height, weight and blood pressure, check your hip and waist circumference, and examine your joints. You will not be asked to get undressed for these examinations.

4. You will be asked to give a blood sample from an arm vein. (For people taking part in the NOAR Cardiovascular Study, this will be a fasting blood sample taken before 10am – see next page).

The tests on the blood sample will include a measurement of rheumatoid factor (an antibody often found in people with rheumatoid arthritis) and tests for the level of inflammation. In addition, tests may be done looking at, for example, evidence of recent infection that may have triggered your arthritis or at hormonal levels that may influence whether arthritis resolves or persists.

From the same blood sample, DNA (genetic material) will be extracted and stored. This genetic material will be examined for genes which may influence the susceptibility and the outcome of arthritis and response to treatment. Your stored blood samples and DNA will be confidentially coded to allow the results to be linked anonymously to your other results from the study.

5. You will be asked to show all the medication which you are currently taking to our research nurse.

6. Some people are asked to have X-rays taken of their hands and feet. We will try and make the appointments at a time convenient to you and we offer to pay your travel expenses incurred in having the X-rays taken. If you have only recently had X-rays of your hands and feet as part of your hospital appointment, with your permission, we will look at these instead of asking you to have further X-rays.

7. You will be asked if your records can be “flagged” at the NHS Central Office in Southport. This means that NOAR would automatically be notified if you develop a cancer in future and when you die.

**What Happens After The First Visit?**

We will contact you once a year, around the anniversary of your first assessment by the NOAR team. Most of the anniversary assessments involve a further visit either to see a research nurse in a clinic or the research nurse will visit you at home. On each occasion, she will give you a similar self-completed questionnaire to the one you filled in at baseline, ask you some questions about the progress of your arthritis and any treatment you have received, and examine your joints. Some people will be asked to give further blood samples and to have further X-rays of their hands and feet at some of these anniversary visits.
What Is The NOAR Cardiovascular Study?
As mentioned above, previous research from the Norfolk Arthritis Register has shown that people with inflammatory arthritis seem to be at increased risk of developing coronary heart disease. People who have developed inflammatory arthritis in the last 12 months, and who are aged between 18-64, are being asked to take part in the NOAR cardiovascular study. In addition to the standard baseline assessment described above, these people will be asked to give a fasting blood sample (a fasting blood sample is a blood sample taken in the morning after a 12-hour period during which you may drink water but have nothing else to eat or drink) and to attend the hospital for a Doppler ultrasound scan of their carotid arteries. Ultrasound scans do not involve any radiation, needles or injections. We examine the neck arteries to gain information about the general health of your arteries. (Information about exactly how these scans are performed and what will happen to the results is given in a separate leaflet.) In addition to the blood tests outlined above, tests on the fasting sample will include measurement of cholesterol (and other fat) levels, sugar levels and other biochemical factors known to be associated with the development of heart disease.

Will I Get The Results Of My Blood and X-Rays?
The blood specimens are frozen and sent to Manchester University where they are analysed at a later date. The results from the blood tests are used only in the research and are not returned either to you or your GP. However, for those of you taking part in the Cardiovascular Study, part of your blood sample will be analysed for cholesterol and sugar and the results can be sent back to your GP and yourself if you wish.

The results of the Doppler ultrasound scan of the carotid arteries will be sent to you, your GP and filed in your hospital notes, if you agree.

The X-rays are not reported in the same way that X-rays normally are. The X-rays will be looked at by 2 doctors who assess and score them by a special research method. We will send these results to your doctor if he, or you, request it, but it won’t be a report in the normal way. However, following the 5th anniversary assessment, we will, with your permission, write to your doctor with any results which are known to date.

Will The NOAR Staff Answer My Questions
The NOAR staff will answer general questions about arthritis, but it would not be right or appropriate for them to discuss your particular problems or treatment. Your GP or hospital consultant is the person to discuss these with.

If I Am Referred to NOAR, Do I Still Need to See a Consultant or a Nurse Practitioner?
Being notified to NOAR is quite separate from any referral to hospital. It will not mean that you are seen in the hospital clinic any sooner as it will not in any way affect your treatment.
Many Medical Research Studies Involve Taking Drugs – Does NOAR?
NOAR does not involve taking any additional drugs. Your treatment will continue as normal. We simply monitor what is happening to you and record what medication you are taking. Taking part in NOAR does not prevent you from taking part in other studies of arthritis which may be organised by the Department of Rheumatology at the Norfolk and Norwich University Hospital. Whether you take part in any other studies is entirely up to you.

Is The Information Confidential?
Yes. All the information you give will be treated in the strictest confidence. You will be allocated a unique register number and information from the questionnaire, interview, tests and blood samples and any follow-up will only be stored linked with this identification number. The one file that will link this study identification number with any personal information about yourself will be kept under strict security with access to authorised NOAR personnel only. The ARC Epidemiology Unit at Manchester University does co-ordinate other national studies of arthritis patients – in particular related to new treatments. We may seek your permission to link information you have provided us for different studies.

What If Something Goes Wrong?
If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone’s negligence, then you may have grounds for legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, then you may register your complaints via the ARC Epidemiology Unit at the University of Manchester.

What Will Happen to the Results of the Research Study?
There have already been many publications from the Norfolk Arthritis Register Study. These have helped doctors who specialise in the treatment of inflammatory arthritis to understand more about the long term history of this condition. We plan to continue to publish results but you will not be identified in any report or publication, nor will we tell you whether or not you have been included in any particular analysis which is published.

Who Has Reviewed This Study?
This study has been reviewed by the Local Research Ethics Committee of the Norfolk and Norwich University Hospital.

Contacts For Further Information
If you have any further queries about NOAR, please telephone Diane Bunn or any of the NOAR staff on 01603 287974. If the office is not manned, an answerphone will take your message. Alternatively, you can fax us on 01603 452876 or e-mail us on arcnorar@manchester.ac.uk.

Version 7 - 18 December 2003
Appendix III: Patient Information Sheet (study 3)
Appendix III

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.

_Sample_
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 anery 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 Shemardine, Specialist Lupus Nurse
Or
Dr Ben Parker, Clinical Research Fellow

The Keligren 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.
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 Kelklyn Centre for Rheumatology
Manchester Royal Infirmary
Oxford Road, Manchester
M13 9WL
Tel: 0161 275 4626

Study ID

1. 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.

2. 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.

3. 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.

4. 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.

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

6. I know how to contact the research team if I need to, and how to get information about the results of the research.

Continued over...
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 (continued)

Name of researcher: Dr Ian Bruce

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

Study ID

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

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

9. I agree to take part in this study. [ ]

OPTIONAL

10. 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 (If different from researcher) ____________________________ Date ____________________________ Signature ____________________________

Researcher ____________________________ Date ____________________________ Signature ____________________________
Appendix IV: Ethical Approval
(study 3)
NRES Committee North West - Greater Manchester North
3rd Floor, Barlow House
4 Minshull Street
Manchester
M1 3DZ
Tel: 0161 625 7617
Email: cynthia.carter@northwest.nhs.uk

Dr Ben Parker
Clinical Research Fellow
University of Manchester
Arthritis Research UK Epidemiology Unit
Stopford Building
M13 9PT

29 September 2011

Dear Dr Parker:

Study title: 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: 03/H1011/3
Amendment number: 2
Amendment date: 13 September 2011

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

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<th>Document</th>
<th>Version</th>
<th>Date</th>
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<td>3.0</td>
<td>09 September 2011</td>
</tr>
<tr>
<td>Participant Consent Form: Controls</td>
<td>3.0</td>
<td>09 September 2011</td>
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<td>Participant Information Sheet: Patients</td>
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<td>09 September 2011</td>
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<td>2</td>
<td>13 September 2011</td>
</tr>
<tr>
<td>Covering Letter</td>
<td>Email</td>
<td>13 September 2011</td>
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</table>

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.
R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

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.

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

Yours sincerely

Dr Peter Kilmiuk
Chair

Enclosures: List of names and professions of members who took part in the review

Copy to: Research Office, University of Manchester
R&D office for CMUH NHS Foundation Trust

NRES Committee North West - Greater Manchester North
Attendance at Sub-Committee of the REC meeting on 29 September 2011

<table>
<thead>
<tr>
<th>Name</th>
<th>Profession</th>
<th>Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mr Ken Cook</td>
<td>Acute Care Manager - Later Life</td>
<td>Expert</td>
</tr>
<tr>
<td>Dr Peter Kilmiuk</td>
<td>Consultant Rheumatologist</td>
<td>Expert</td>
</tr>
</tbody>
</table>
Appendix V: SLE History
### DEMOGRAPHIC DATA

- **Date of birth:**
  - **_d_** / _m_ / _y_

- **Date of diagnosis of SLE:**
  - **_d_** / _m_ / _y_

- **Date of 1st symptom:**
  - **_d_** / _m_ / _y_

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

- [ ] Malar rash
- [ ] Discoid rash
- [ ] Oral ulcers
- [ ] Serositis
- [ ] Arthritis
- [ ] Photosensitivity
- [ ] Renal disorder
- [ ] Neurologic disorder
- [ ] Haematologic 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:**
  - Number of units per week: ________
  - Number of ml per week: ________

- **Cigarette smoking (delete as appropriate):**
  - Current: Yes / No
  - Ex-smoker: Yes / No
  - If yes, number per day: ________
  - If no, number of years smoking: ________
  - And date stopped: **_d_** / _m_ / _y_
### CLINICAL DATA

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<th>Value</th>
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<td>cm</td>
</tr>
<tr>
<td>Weight (shoes and coat off):</td>
<td>kg</td>
</tr>
<tr>
<td>BMI:</td>
<td></td>
</tr>
<tr>
<td>Waist/hip ratio:</td>
<td>cm / cm</td>
</tr>
<tr>
<td>Blood pressure (systolic/diastolic):</td>
<td>/</td>
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</table>

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

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

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

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<th>If yes, specify date(s):</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>d / m / y</td>
</tr>
</tbody>
</table>

#### Angina (circle as appropriate):

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

#### Angioplasty (circle as appropriate):
- **Ever**: Yes / No
  - If yes, specify date(s):
    - \( d / m / y \)
    - \( d / m / y \)

#### Bypass surgery (circle as appropriate):
- **Ever**: Yes / No
  - If yes, specify date:
    - \( d / m / y \)

#### Previous SLE Cardiac Manifestations

1. **Pericarditis**:
   - **Yes / No**: 
   - If yes, specify date(s):
     - \( d / m / y \)
     - \( d / m / y \)

2. **Myocarditis**:
   - **Yes / No**: 
   - If yes, specify date(s):
     - \( d / m / y \)
     - \( d / m / y \)

3. **Endocarditis**:
   - **Yes / No**: 
   - If yes, specify date(s):
     - \( d / m / y \)
     - \( d / m / y \)

#### Peripheral Vascular

#### Intermittent Claudication (circle as appropriate):

1. **Current**:
   - **Yes / No**: 
   - If yes, specify date(s):
     - \( d / m / y \)
     - \( d / m / y \)

2. **In the past**:
   - **Yes / No**: 
   - If yes, specify date(s):
     - \( d / m / y \)
     - \( d / m / y \)
## Appendix V

### Cerebrovascular

**Transient ischemic attack (circle as appropriate):** Yes / No

If yes, specify date(s):

<table>
<thead>
<tr>
<th>Date 1</th>
<th>Date 2</th>
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<tbody>
<tr>
<td><em><strong>/</strong></em>/___</td>
<td><em><strong>/</strong></em>/___</td>
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</table>

**Stroke (circle as appropriate):** Yes / No

If yes, specify date(s):

<table>
<thead>
<tr>
<th>Date 1</th>
<th>Date 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em><strong>/</strong></em>/___</td>
<td><em><strong>/</strong></em>/___</td>
</tr>
</tbody>
</table>

**Type (if known):**
1 = Hemorrhagic  
2 = Thrombotic

### Hyperlipidemia Therapy

**Current:** Yes / No

If current, specify type (circle as appropriate):

- 0 = None
- 1 = Statins
- 2 = Sequestrants
- 3 = Nicotinic acid
- 4 = Fibrates
- 5 = Combinations
- 6 = Other

**In the past:** Yes / No

If in the past, specify type (circle as appropriate):

- 0 = None
- 1 = Statins
- 2 = Sequestrants
- 3 = Nicotinic acid
- 4 = Fibrates
- 5 = Combinations
- 6 = Other
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<th><strong>HORMONAL FACTORS</strong></th>
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<td><strong>Ovarian function (circle all which apply):</strong></td>
</tr>
<tr>
<td>1 = Menstruating</td>
</tr>
<tr>
<td>2 = Premenarche</td>
</tr>
<tr>
<td>3 = Postmenopausal</td>
</tr>
<tr>
<td>4 = Amenorrhoe</td>
</tr>
<tr>
<td><strong>Age at menopause:</strong></td>
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<tr>
<td><strong>Oral contraceptive</strong></td>
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<tr>
<td>In the past: Yes / No</td>
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<td><strong>Hormone replacement therapy:</strong></td>
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<tr>
<td>2 = Estrogen + progesterone</td>
</tr>
<tr>
<td>3 = Progesterone only</td>
</tr>
<tr>
<td>4 = Other (specify):</td>
</tr>
<tr>
<td>Current course start date: d / m / y</td>
</tr>
<tr>
<td>In the past: Yes / No</td>
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<tr>
<td>1 = Estrogen (specify):</td>
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<tr>
<td>2 = Estrogen + progesterone</td>
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<tr>
<td>3 = Progesterone only</td>
</tr>
<tr>
<td>4 = Other (specify):</td>
</tr>
<tr>
<td><strong>Are you currently pregnant? (circle as appropriate):</strong></td>
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<tr>
<td><strong>Gravida / Para:</strong></td>
</tr>
<tr>
<td><strong>Miscarriage (No):</strong></td>
</tr>
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</table>
# Appendix V

## 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:

**Current:**
- Yes / No
- If yes, specify type (circle as appropriate):
  1. Azathioprine
  2. Cyclophosphamide PO
  3. Cyclophosphamide IV
  4. Cyclosporin
  5. Mycophenolate mofetil
  6. Methotrexate
  7. Other (specify): ___________

**Date initiated:**
- ___________
- Length (weeks): ___________
- To stop? Yes/No

**In the past:**
- Yes / No
- If yes, specify type (circle as appropriate):
  1. Azathioprine
  2. Cyclophosphamide PO
  3. Cyclophosphamide IV
  4. Cyclosporin
  5. Mycophenolate mofetil
  6. Methotrexate
  7. Other (specify): ___________

**Immunosuppressive agent due to start:** (circle as appropriate)
- 1. azathioprine
dose: ___________
- 2. cyclophosphamide IV
dose: ___________
- 3. cyclophosphamide PO
dose: ___________
- 4. rituximab
dose: ___________

**Steroids:**

**Current:**
- Yes / No
- If yes, specify course start date: __/____/____
- and average daily dose: __________ mg

**In the past:**
- Yes / No
- If yes, number of previous courses: __________
- And average daily dose: __________ mg

**Number of previous courses of IV steroids (and details):**

**Number of courses in last 6 months:**

**Antimalarials:**

**Current:**
- Yes / No
- If yes, specify type (circle as appropriate):
  1. Chloroquine
  2. Atabrine
  3. Hydroxychloroquine
  4. Other (specify): ___________

  If yes, specify course start date: __/____/____
  and average daily dose: __________ mg

**In the past:**
- Yes / No
- If yes, specify type (circle as appropriate):
  1. Chloroquine
  2. Atabrine
  3. Hydroxychloroquine
  4. Other (specify): ___________
Current medication list:

SLE DISEASE ACTIVITY:
SLEDAI score: ___________ Date: __ ___ / __ ___ / __ ___
d m y

BILAG-2004 score: ___________ Date: __ ___ / __ ___ / __ ___
d m y

DAMAGE:
SLICC Damage Index score: ___________

QUALITY OF LIFE:

Lupus QOL

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<th>Bodily</th>
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SF36

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Appendix VI: RA History
## CRF Index

**Study number:**

**Principal Investigator:** Dr Ian Bruce

**Participant number:**

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<td>- Examination</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- History</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- DAS 28</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Visit 1: Investigations</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Visit 1: FMD and EndoPAT results</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Visit 1: Questionnaires – HAQ-DI, SF-36, lifestyle</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Visit 1: Visit Activity Chart</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Visit 2: Clinical Examination Data</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Anthropomorphic Measurements</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Examination</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- History</td>
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</tr>
<tr>
<td></td>
<td>- DAS 28</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Visit 2: Investigations</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Visit 2: FMD and EndoPAT results</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Visit 2: Visit Activity Chart</td>
<td></td>
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<tr>
<td>12</td>
<td>Visit 2: Questionnaires – HAQ-DI, SF-36</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Participant Communication</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Discharge Criteria</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Adverse Events</td>
<td></td>
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### Visit 1 Anthropomorphic Measurements

<table>
<thead>
<tr>
<th>Activity</th>
<th>Reading</th>
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<tr>
<td>BP (mmHg)</td>
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</tr>
<tr>
<td>Pulse</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td></td>
</tr>
<tr>
<td>Waist (cm)</td>
<td></td>
</tr>
<tr>
<td>Hips (cm)</td>
<td></td>
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<td>BMI</td>
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### Visit 1 Examination

<table>
<thead>
<tr>
<th>Name:</th>
<th>Study ID:</th>
<th>Date:</th>
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<tbody>
<tr>
<td>Urine</td>
<td>Blood Protein</td>
<td>Temperature</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td>Vascular</td>
</tr>
<tr>
<td>Nodules</td>
<td>Yes/No</td>
<td>Vasculitis</td>
</tr>
<tr>
<td>Rash</td>
<td>Yes/No</td>
<td>Raynaud’s</td>
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<tr>
<td></td>
<td></td>
<td>Nailfold Infarct</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Joints Tenderness (T) Swollen (S) Deformity (D)</td>
</tr>
<tr>
<td>PIPJ</td>
<td>Hips</td>
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<td>Knees</td>
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<td>Wrists</td>
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<td>Elbows</td>
<td>MTPJs</td>
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<td>Shoulders</td>
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<td>Power</td>
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<td>Reflexes</td>
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<td>DP pulse</td>
<td>Sensation</td>
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<tr>
<td>Chest</td>
<td>Abdomen</td>
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</table>
**VISIT 1 HISTORY**

**PATIENT INITIALS:**

**PATIENT ID NO:**

**ASSESSMENT DATE:**

---

### DEMOGRAPHIC DATA

Date of birth: ____ / ____ / _____

Date of diagnosis of RA: ____ / ____ / _____

Date of 1st symptom: ____ / ____ / _____

ACR criteria for diagnosis (tick as appropriate):

- Morning Stiffness > 1 hour
- Arthritis in 3 or more joint areas (synovitis > 6 weeks)
- Arthritis of hands (PIP, MCP, wrists, > 6 weeks)
- Symmetric Arthritis (> 6 weeks)
- Rheumatoid nodules
- Rheumatoid Factor
- Radiographic changes

Extra-articular features:

- Nail fold infarcts
- Systemic rheumatoid vasculitis
- Sjogren’s
- Pulmonary Fibrosis
- Neuropathy
- Leg ulcers

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):

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<thead>
<tr>
<th>Current</th>
<th>Ex-smoker</th>
<th>Never</th>
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</thead>
<tbody>
<tr>
<td>Yes/No</td>
<td>Yes/No</td>
<td>Yes/No</td>
</tr>
</tbody>
</table>

If yes, number per day

If no, number of years smoking

and date stopped

Lifestyle Questionnaire Yes/No
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<th><strong>CLINICAL DATA</strong></th>
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<tr>
<td><strong>Height:</strong> cm</td>
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<tr>
<td><strong>Waist/hip ratio:</strong> cm / cm</td>
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**Antihypertensive therapy (delete as appropriate):**

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<th>If yes, specify type (circle as appropriate):</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td><strong>0 = Diuretics</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>1 = Adrenergic inhibitors</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>2 = Central and agonists</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>3 = Beta blockers</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>4 = Direct vasodilators</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>5 = Calcium antagonists</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>6 = Angiotensin-converting enzyme inhibitors</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>7 = Other</strong></td>
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<td></td>
<td></td>
<td><strong>8 = Combination</strong></td>
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<th>If yes, specify type (circle as appropriate):</th>
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<td></td>
<td><strong>0 = Diuretics</strong></td>
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<tr>
<td></td>
<td></td>
<td><strong>7 = Other</strong></td>
</tr>
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<td></td>
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<td><strong>8 = Combination</strong></td>
</tr>
</tbody>
</table>

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

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<th>If yes, specify date(s):</th>
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</tr>
<tr>
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<td></td>
<td><strong>d / m / y</strong></td>
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</table>

**Angina (circle as appropriate):**

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<th>If yes, specify date of diagnosis:</th>
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<td><strong>d / m / y</strong></td>
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<td><strong>d / m / y</strong></td>
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<tr>
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<td><strong>d / m / y</strong></td>
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**Congestive heart failure (circle as appropriate):**

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

#### Angioplasty (circle as appropriate):  
**Ever**  
Yes / No  
If yes, specify date(s):  
\[ \frac{d}{m}/\frac{y}{y} \]  
\[ \frac{d}{m}/\frac{y}{y} \]

#### Bypass surgery (circle as appropriate):  
**Ever**  
Yes / No  
If yes, specify date:  
\[ \frac{d}{m}/\frac{y}{y} \]

### PERIPHERAL VASCULAR

#### Intermittent claudication (circle as appropriate):  
**Current**  
Yes / No  
If yes, specify date(s):  
\[ \frac{d}{m}/\frac{y}{y} \]  
\[ \frac{d}{m}/\frac{y}{y} \]

**In the past**  
Yes / No  
If yes, specify date(s):  
\[ \frac{d}{m}/\frac{y}{y} \]  
\[ \frac{d}{m}/\frac{y}{y} \]

### CEREBROVASCULAR

#### Transient ischemic attack (circle as appropriate):  
Yes / No  
If yes, specify date(s):  
\[ \frac{d}{m}/\frac{y}{y} \]  
\[ \frac{d}{m}/\frac{y}{y} \]

#### Stroke (circle as appropriate):  
Yes / No  
If yes, specify date(s):  
\[ \frac{d}{m}/\frac{y}{y} \]  
\[ \frac{d}{m}/\frac{y}{y} \]

**Type (if known):**  
1 = Hemorrhagic  
2 = Thrombotic
### HYPERLIPIDEMIA THERAPY

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<th>Yes / No</th>
<th>If current, specify type (circle as appropriate):</th>
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<tr>
<td></td>
<td></td>
<td>0 = None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 = Statins</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = Sequestrants</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 = Nicotinic acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 = Fibrates</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 = Combinations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 = Other</td>
</tr>
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</table>

<table>
<thead>
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<th>In the past</th>
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<tr>
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<td>1 = Statins</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = Sequestrants</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 = Nicotinic acid</td>
</tr>
</tbody>
</table>

### HORMONAL FACTORS

- **Male**
- **Female**

**Menstrual function (circle all which apply):**

- 1 = Menstruating
- 2 = Premenarche
- 3 = Postmenopausal
- 4 = Amenorrhea
- 5 = Pre-menopausal hysterectomy
- 6 = Post-menopausal hysterectomy
- 7 = Pre-menopausal hysterophorectomy

**Age at menopause:**

**Oral contraceptive**

<table>
<thead>
<tr>
<th>Current</th>
<th>Yes / No</th>
<th>If yes, specify number of years:</th>
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<table>
<thead>
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<th>If yes, specify number of years:</th>
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**Hormone replacement therapy:**

<table>
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<tr>
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<th>If current, specify type (circle as appropriate):</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 = Estrogen (specify):</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = Estrogen + progesterone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 = Progesterone only</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 = Other (specify):</td>
</tr>
</tbody>
</table>

**Current course start date:**

\[
\text{d} / \text{m} / \text{y}
\]

<table>
<thead>
<tr>
<th>In the past</th>
<th>Yes / No</th>
<th>If in the past, specify type (circle as appropriate):</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1 = Estrogen (specify):</td>
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<tr>
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<td></td>
<td>2 = Estrogen + progesterone</td>
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<tr>
<td></td>
<td></td>
<td>3 = Progesterone only</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 = Other (specify):</td>
</tr>
</tbody>
</table>

**Are you currently pregnant? (circle as appropriate):**

- Yes / No

**Gravida / Para:**

\[
\text{ } / \text{ }
\]

**Miscarriage (No):**

\[
\text{ } / \text{ }
\]
Appendix VI

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

RENAI.

Renal Impairment:
Current: Yes / No
In the past: Yes / No

Past medical history:

---

Therapy

Disease-Modifying Treatment:
Current: Yes / No
If yes, specify type (circle as appropriate):
1 = Methotrexate  4 = Hydroxychloroquine
2 = Sulphasalazine  5 = Gold IM
3 = Leflunomide  6 = Other (specify):

Date initiated:
length: weeks/months to stop? Yes/No

In the past: Yes / No
If yes, specify type (circle as appropriate):
1 = Methotrexate  4 = Hydroxychloroquine
2 = Sulphasalazine  5 = Gold IM
3 = Leflunomide  6 = Other (specify):
Disease-Modifying treatment due to start: (circle as appropriate)

1 = Methotrexate  
2 = infliximab IV  
3 = etanercept  
4 = adalimumab  
5 = other  
(specify___________)

Steroids:
Current Yes / No  
If yes, specify course start date: ___/___/___
and average daily dose:___________ mg

In the past Yes / No  
If yes, number of previous courses:___________
And average daily dose:______ mg

Number of previous IM steroids (and details):______________________________

Date of last IM/IA steroid:  
___/___/___  
d m y  
dose___________mg

Number of IM steroids in last 6 months:____________________________________

Current medication list:

DISEASE ACTIVITY SCORE (DAS 28):

Tender Joint Count  ___/28
Swollen Joint Count  ___/28
Visual Activity Score  ___/100
ESR  ___
CRP  ___
Physician Global Assessment  ___/100
### QUALITY OF LIFE:

**HAQ-DI**

<table>
<thead>
<tr>
<th>Domain</th>
<th>Dressing</th>
<th>Arising</th>
<th>Eating</th>
<th>Walking</th>
<th>Hygiene</th>
<th>Reach</th>
<th>Grip</th>
<th>Common Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score (0-3)</td>
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<td></td>
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</tbody>
</table>

**SF36**

<table>
<thead>
<tr>
<th>Domain</th>
<th>Physical function</th>
<th>Role physical</th>
<th>Bodily pain</th>
<th>General health</th>
<th>Vitality</th>
<th>Social function</th>
<th>Role emotional</th>
<th>Mental health</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
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<tr>
<td>Investigation</td>
<td>Study No</td>
<td>Date</td>
<td>Participant ID</td>
<td></td>
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# Vascular Ultrasound

## a. Flow-mediated dilatation

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<th>Visit 1</th>
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<td>Baseline Brachial Artery Diameter</td>
<td>mm</td>
</tr>
<tr>
<td>Maximal post-reactive hyperaemia diameter</td>
<td>mm</td>
</tr>
<tr>
<td>% FMD</td>
<td>%</td>
</tr>
<tr>
<td>Pre-GTN diameter</td>
<td>mm</td>
</tr>
<tr>
<td>Maximal post-GTN Diameter</td>
<td>mm</td>
</tr>
<tr>
<td>% endothelium-independent dilatation</td>
<td>%</td>
</tr>
</tbody>
</table>

## b. EndoPAT

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reactive Hyperaemic Index</th>
</tr>
</thead>
</table>
Appendix VII: SLEDAI
<table>
<thead>
<tr>
<th>Weight</th>
<th>SLEDAI score</th>
<th>Descriptor</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td></td>
<td>Seizure</td>
<td>Recent onset. Exclude metabolic, infectious, or drug causes.</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>Psychosis</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></td>
<td>Organic brain syndrome</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, insomnial or daytime drowsiness, or increased or decreased psychomotor activity. Exclude metabolic, infectious, or drug causes.</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>Visual disturbance</td>
<td>Retinal changes of SLE. Include cytoid 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></td>
<td>Cranial nerve disorder</td>
<td>New onset of sensory or motor neuropathy involving cranial nerves.</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>Lupus headache</td>
<td>Severe, persistent headache; may be migrainous, but must be nonresponsive to narcotic analgesia.</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>CVA</td>
<td>New onset of cerebrovascular accident(s). Exclude arteriosclerosis.</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>Vasculitis</td>
<td>Ulceration, gangrene, tender finger nodules, periungual infarction, splinter hemorrhages, or biopsy or angiogram proof of vasculitis.</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Arthritis</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></td>
<td>Myositis</td>
<td>Proximal muscle aching/weakness, associated with elevated creatine phosphokinase/aldolase or</td>
</tr>
</tbody>
</table>
### Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)

<table>
<thead>
<tr>
<th>Weight</th>
<th>SLEDAI score</th>
<th>Descriptor</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<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 celsius. 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>
Appendix VIII: SLICC
### SLICC/ACR Damage Index for Systemic Lupus Erythematosus

#### Overview:

The Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index for Systemic Lupus Erythematosus (SLE) records damage occurring in patients with SLE regardless of causation. This can be used to monitor patients over time especially for comparing periods of disease activity and inactivity.

#### Glossary of Terms

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damage</td>
<td>Nonreversible change not related to active inflammation occurring since diagnosis of lupus ascertained by clinical assessment and present for at least 6 months unless otherwise stated. Repeat episodes must occur at least 6 months apart to score 2. The same lesion cannot be scored twice.</td>
</tr>
<tr>
<td>Cataract</td>
<td>A lens opacity (cataract) in either eye even whether primary or secondary to steroid therapy documented by ophthalmoscopy.</td>
</tr>
<tr>
<td>Retinal damage</td>
<td>Documented by ophthalmoscopic examination may result in field defect legal blindness.</td>
</tr>
<tr>
<td>Optic atrophy</td>
<td>Documented by ophthalmoscopic examination.</td>
</tr>
<tr>
<td>Cognitive impairment</td>
<td>Memory deficit difficulty with calculation poor concentration difficulty in spoken or written language impaired performance level documented on clinical examination or by formal neurocognitive testing.</td>
</tr>
<tr>
<td>Major psychosis</td>
<td>Altered ability to function in normal activity due to psychiatric reasons. Severe disturbance in the perception of reality characterized by the following features: delusions hallucinations (auditory visual) incoherence marked loose associations impoverished thought content marked illogical thinking bizarre disorganized or catatonic behavior.</td>
</tr>
<tr>
<td>Seizures</td>
<td>Paroxysmal electrical discharge occurring in the brain and producing characteristic physical changes including tonic and clonic movements and certain behavioral disorders. Only seizures requiring therapy for 6 months are counted as damage.</td>
</tr>
<tr>
<td>Cerebrovascular accident (CVA)</td>
<td>Cerebrovascular accident resulting in focal findings such as paresis weakness etc. or surgical resection for causes other than malignancy.</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>Damage to either cranial or peripheral nerve excluding optic nerve resulting in either motor or sensory dysfunction.</td>
</tr>
<tr>
<td>Transverse myelitis</td>
<td>Lower extremity weakness or sensory loss with loss of rectal and urinary bladder sphincter control.</td>
</tr>
<tr>
<td>Renal</td>
<td>Estimated or measured glomerular filtration rate &lt;50% proteinuria ≥ 3.5 gm per 24 hours or end-stage renal disease (regardless of dialysis or transplantation)</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>Pulmonary hypertension (right ventricular prominence or loud P2); pulmonary fibrosis (physical or radiograph); shrinking lung (radiograph); pleural fibrosis (radiograph); pulmonary infarction (radiograph); resection for cause other than malignancy.</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Angina or coronary artery bypass; myocardial infarction (documented by electrocardiograph and enzyme studies) ever; cardiomyopathy (ventricular dysfunction documented clinically); valvular disease (diastolic murmur or systolic murmur &gt;3/6);</td>
</tr>
</tbody>
</table>
## Appendix VIII

### Term | Definition
--- | ---
Peripheral vascular | Claudication persistent for 6 months by history; minor tissue loss such as pulp space ever; significant tissue loss such as loss of digit or limb or resection ever; venous thrombosis with swelling ulceration or clinical evidence of venous stasis
Gastrointestinal | Infarction or resection of bowel below duodenum by history resection of liver spleen or gallbladder ever for whatever cause; mesentric insufficiency with diffuse abdominal pain on clinical examination; chronic peritonitis with persistent abdominal pain and peritoneal irritations on clinical examination; esophageal stricture on endoscopy upper gastrointestinal tract surgery such as correction of stricture ulcer surgery etc. ever by history; pancreatic insufficiency requiring enzyme replacement or with a pseudocyst
Musculoskeletal | Muscle atrophy or weakness demonstrated on clinical examination; deformings or erosive arthritis including reducible deformities (excluding avascular necrosis) on clinical examination; osteoporosis with fracture or vertebral collapse (excluding avascular necrosis) demonstrated radiographically; avascular necrosis demonstrated by any imaging technique; osteomyelitis documented clinically and supported by culture evidence; tendon ruptures
Skin | Scarring chronic alopecia documented clinically; extensive scarring or panniculom other than scalp and pulp space documented clinically; skin ulceration (excluding thrombosis) for more than 6 months
Premature gonadal failure | Secondary amenorheria prior to age of 40
Diabetes | Diabetes requiring therapy but regardless of treatment.
Malignancy | Documented by pathologic examination excluding dysplasia

### Scoring

<table>
<thead>
<tr>
<th>Organ</th>
<th>Item</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocular (either eye by clinical assessment)</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 &lt;50%</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Proteinuria ≥ 3.5 g per 24 hours</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>End-stage renal disease (regardless of dialysis or transplantation)</td>
<td>3</td>
</tr>
</tbody>
</table>

SLICC/ACR Damage Index for Systemic Lupus Erythematosus continued.
<table>
<thead>
<tr>
<th>Organ</th>
<th>Item</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<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 &gt;3/6)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Pericarditis for 6 months or pericardiectomy</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) (score 2 if more than one site)</td>
<td>1 or 2</td>
</tr>
<tr>
<td>Gastrointestinal</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</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 panniculium 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 (exclude dysplasia) (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.
Uncertainties in scoring

- Cerebrovascular accident in glossary but not in original table includes resection for cause other than malignancy as an "or" clause.
- Renal damage: assumed that only one of the 3 disease entities mentioned (reduction GFR proteinuria end-stage renal disease) would be scored giving maximum score of 3 rather than 5.
- In valvular disease assumed that the 3 limit applied only to systolic murmur; assumed that any diastolic murmur would apply.
- Tendon ruptures is listed in the glossary but not in the table.
- Primary gonadal failure in the glossary applies only to women; I added testicular failure for males for no substantiated reason.

Interpretation:

- Minimum score: 0.
- Maximum score is unclear. According to Stoll (1996) the maximum total is 46 points but I get 47 on adding up his data (he scores a maximum of 2 for skin when it appears to be 3). A breakdown by sections: 47 points (ocular 2 neuropsychiatric 6 renal 3 pulmonary 5 cardiovascular 6 peripheral vascular 5 gastrointestinal 6 musculoskeletal 6 skin 3 other 4). If tendon rupture is added the maximum would be 48.
- The higher the score the more extensive the damage.

References


SLICC/ACR Damage Index for Systemic Lupus Erythematosus.
Appendix IX: BILAG
### BILAG2004 INDEX

<table>
<thead>
<tr>
<th>Centre</th>
<th>Date</th>
<th>Initials/Hosp No</th>
</tr>
</thead>
</table>

Only record items due to SLE Disease Activity & assessment refers to manifestations occurring in the last 4 weeks compared with the previous 4 weeks.

#### Scoring
- **ND**: Not Done
- **1**: Improving
- **2**: Same
- **3**: Worse
- **4**: New
- **Yes/No OR Value** (where indicated)
- _Q_ indicate if not due to SLE activity (default is 0 = not present)

### CONSTITUTIONAL
- 1. Pyrexia: documented ≤ 37.5°C
- 2. Weight loss: unexplained ≤ 5%
- 3. Lymphadenopathy/splenomegaly
- 4. Anorexia

### MUCOCUTANEOUS
- 5. Skin eruption: severe
- 6. Photosensitivity: mild
- 7. Angio-oedema: severe
- 8. Angio-oedema: mild
- 9. Mucosal ulceration: severe
- 10. Mucosal ulceration: mild
- 11. Panniculitis: Bullous lumps: severe
- 12. Panniculitis: Bullous lumps: mild
- 13. Major cutaneous vascularitis/thrombophlebitis
- 14. Digital infarcts or nodular vasculitis
- 15. Alopecia: severe
- 16. Alopecia: mild
- 17. Peri-ungual erythema/clubbing
- 18. Splinter haemorrhages

### NEUROPSYCHIATRIC
- 19. Acute encephalitis
- 20. Central vasculitis
- 21. Demyelinating syndrome
- 22. Nerve pathology
- 23. Acute confusional state
- 24. Psychosis
- 25. Acute inflammatory demyelinating polyradiculoneuropathy
- 26. Mononeuropathy (single/multiplex)
- 27. Cranial neuropathy
- 28. Mononeuropathy
- 29. Polyneuropathy
- 30. Cerebral vasculitis
- 31. Status epilepticus
- 32. Cerebrovascular disease (not due to vasculitis)
- 33. Cerebral vascular dysfunction
- 34. Movement disorder
- 35. Autonomic disorder
- 36. Central aphasia (isolated)
- 37. Lapse headache: severe unconsenting
- 38. Paraplegic from IC Hypertension

### MUSCULOSKELETAL
- 40. Myositis: mild
- 41. Arthritis (severe)
- 42. Arthritis (moderate/Mild)/Tenosynovitis/Enthesitis
- 43. Arthritis (mild)/Arthralgia/Myalgia

### CARDIORESPIRATORY
- 44. Myocarditis: mild
- 45. Myocarditis/Endocarditis / Cardiac failure
- 46. Arrhythmias
- 47. New valvular dysfunction
- 48. Pulmonary Embolism
- 49. Pericarditis
- 50. Pericardial effusion with dyspnoea
- 51. Pulmonary hypertension
- 52. Intraocular alveolitis/pneumonitis
- 53. Shrinking lung syndrome
- 54. Aortitis
- 55. Coronary vasculitis

### GASTROINTESTINAL
- 56. Lumps peritoneum
- 57. Abdominal sepsis or abscess
- 58. Lumps enteritis/diverticulitis
- 59. Malignant tumour
- 60. Protein losing enteropathy
- 61. Jejunal pseudo-obstruction
- 62. Lumps hepatitis
- 63. Acute hepatitis cholecystitis
- 64. Acute hepatitis pancreatitis

### OPHTHALMIC
- 65. Orbital inflammation/retinopathy/proptosis
- 66. Keratitis: severe
- 67. Keratitis: mild
- 68. Anterior uveitis
- 69. Posterior uveitis/retinal vasculitis: severe
- 70. Posterior uveitis/retinal vasculitis: mild
- 71. Episcleritis
- 72. Scleritis: severe
- 73. Scleritis: mild
- 74. Retinal choroidal vasculitis/occlusive disease
- 75. Isolated cotton-wool spots/cystoid macula
degeneration
- 76. Optic neuritis
- 77. Anterior ischaemic optic neuropathy

### RENAL
- 78. Systolic blood pressure (mm Hg) value
- 79. Diastolic blood pressure (mm Hg) value
- 80. Glomerulonephritis
- 81. Acute glomerulonephritis
- 82. Lupus nephritis
- 83. Lupus nephritis
- 84. 24-hour urine protein
- 85. Lupus nephritis
- 86. Glomerulonephritis
- 87. GFR (calculated) ml/min/1.73 m²
- 88. Glomerulonephritis
- 89. Active urinalysis

### HEMATOLOGICAL
- 90. Anaemia (mild)
- 91. Leucocytosis (> 10³/µl)
- 92. Neutrophilia (x 10³/µl)
- 93. Lymphocytosis (x 10³/µl)
- 94. Platelets (x 10³/µl)
- 95. ANA
- 96. Evidence of active haemolysis
- 97. Coombs test positive

---

**Revision**: 12/Jan/2007
Appendix X: HAQ
# HEALTH ASSESSMENT QUESTIONNAIRE
(FAQ) DISABILITY AND DISCOMFORT SCALES

<table>
<thead>
<tr>
<th>Name</th>
<th>Date</th>
</tr>
</thead>
</table>

In this section we are interested in learning how your illness affects your ability to function in daily life. Please feel free to add any comments on the back of this page.

Please check the response which best describes your usual abilities over the past week:

**Dressing & Grooming**
Are you able to:
- Dress yourself, including tying shoelaces and doing buttons?  

```plaintext
<table>
<thead>
<tr>
<th>Without any difficulty</th>
<th>With some difficulty</th>
<th>With much difficulty</th>
<th>Unable to do</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
```
- Shampoo your hair:

```plaintext
<table>
<thead>
<tr>
<th>Without any difficulty</th>
<th>With some difficulty</th>
<th>With much difficulty</th>
<th>Unable to do</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
```

**Arising**
Are you able to:
- Stand up from a straight chair?
- Get in and out of bed?

```plaintext
<table>
<thead>
<tr>
<th>Without any difficulty</th>
<th>With some difficulty</th>
<th>With much difficulty</th>
<th>Unable to do</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
```

**Eating**
Are you able to:
- Cut your meat?
- Lift a full cup or glass to your mouth?
- Open a new milk carton?

```plaintext
<table>
<thead>
<tr>
<th>Without any difficulty</th>
<th>With some difficulty</th>
<th>With much difficulty</th>
<th>Unable to do</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
```

**Walking**
Are you able to:
- Walk outdoors on flat ground?
- Climb up five steps?

```plaintext
<table>
<thead>
<tr>
<th>Without any difficulty</th>
<th>With some difficulty</th>
<th>With much difficulty</th>
<th>Unable to do</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
```

Please check any aids or devices that you usually use for any of these activities:

- Cane
- Walker
- Crutches
- Wheelchair
- Devices used for dressing (button hook, zipper pull, long-handled shoe horn, etc.)
- Built up or special utensils
- Special or built up chair
- Other [specify ....................................................]
Please check (x) the response which best describes your usual abilities.

**Over the past week:**

<table>
<thead>
<tr>
<th>Hygiene</th>
<th>Without any difficulty</th>
<th>With some difficulty</th>
<th>With much difficulty</th>
<th>Unable to do</th>
</tr>
</thead>
<tbody>
<tr>
<td>Are you able to:</td>
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<td></td>
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<tr>
<td>Wash and dry your body?</td>
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<tr>
<td>Take a tub bath?</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Get on and off the toilet?</td>
<td></td>
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<table>
<thead>
<tr>
<th>Reach</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Are you able to:</td>
<td></td>
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<tr>
<td>Reach and get down a 5 pound object (such as bag of sugar) from just above your head?</td>
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<tr>
<td>Bend down to pick up clothing from the floor?</td>
<td></td>
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<table>
<thead>
<tr>
<th>Grip</th>
<th></th>
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<tbody>
<tr>
<td>Are you able to:</td>
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<td></td>
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<td></td>
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<tr>
<td>Open car doors?</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Open jars which have been previously opened?</td>
<td></td>
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<td></td>
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<tr>
<td>Turn faucets on and off?</td>
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<table>
<thead>
<tr>
<th>Activities</th>
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</thead>
<tbody>
<tr>
<td>Are you able to:</td>
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<td></td>
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<tr>
<td>Run errands and shop?</td>
<td></td>
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<tr>
<td>Get in and out of a car?</td>
<td></td>
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<td></td>
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<tr>
<td>Do chores such as vacuuming or yardwork?</td>
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</tbody>
</table>

Health Assessment Questionnaire (HAQ) Disability and Discomfort continued.
Please check (x) any aids or devices that you usually use for any of these activities:

- [ ] Raised toilet seat
- [ ] Bathtub bar
- [ ] Bathtub seat
- [ ] Long-handled appliances for reach
- [ ] Jar opener
- [ ] Long-handled appliances in bathroom
- [ ] (for jars previously opened)
- [ ] Other [specify .........................]

Please check (x) any categories for which you usually need help from another person:

- [ ] Hygiene
- [ ] Gripping and opening things
- [ ] Reach
- [ ] Errands and chores

We are also interested in learning whether or not you are affected by pain because of your illness. How much pain have you had because of your illness in the past week:

Place a vertical (|) mark on the line to indicate the severity of the pain

No pain [_____________________] Severe pain [_____________________]
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