Associations between inflammation, cardiovascular risk factors, and atherogenic dyslipidemia in patients with systemic lupus erythematosus

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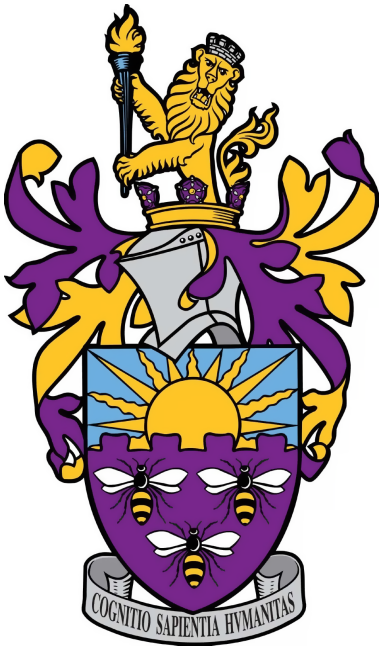

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List of Abbreviations

ANA ..... Anti-nuclear antibodies

AUC ..... Area under curve

CHD ..... Coronary heart disease

CI ..... Confidence interval

CRP ..... C-reactive protein

FA ..... Fatty acid

FFA ..... Free fatty acid

HDL ..... High-density lipoprotein

hsCRP ..... High sensitivity C-reactive protein

IL-6 ..... Interleukin 6

IL-18 ..... Interleukin 18

IL-18BP ..... Interleukin 18 binding protein

IQR ..... Interquartile Range

LDL ..... Low-density lipoprotein

LDLR ..... Low-density lipoprotein receptor

Mdn ..... Median

MetS ..... Metabolic Syndrome

NMR ..... Nuclear Magnetic Resonance

OR ..... Odds ratio

oxLDL ..... Oxidised LDL

PiHDL ..... Pro-inflammatory high-density lipoprotein

ROC ..... Receiver operator characteristic

SD ..... Standard deviation

SLE ..... Systemic Lupus Erythematosus

SLEDAI ..... Systemic Lupus Erythematosus Disease Active Index

SLICC ..... Systemic Lupus International Collaborating Clinics

SLICC-RAS .... SLICC Registry for Atherosclerosis cohort

SMC ..... Smooth muscle cell

TG ..... Triglyceride

TNFα ..... Tumor necrosis factor alpha

VLDL ..... Very-low-density lipoprotein

Abstract

Introduction. Premature atherosclerosis is a long-term complication that affects patients with systemic lupus erythematosus (SLE). Atherogenic dyslipidemia is a product of the metabolic derangement in SLE, which may be linked to the underlying disease activity and cycle of inflammation that is associated with lupus. Improved understanding of the mechanisms involved in lipid transport, and SLE's signature immunological and inflammatory disease components, may help to further our understanding of the disease processes involved in atherogenesis

Methods. Cross-sectional data of newly enrolled lupus patients from an international inception cohort was used to investigate the association of atherogenic dyslipidemia and metabolic syndrome with levels of disease activity, inflammation, and exposure to drug therapies. Statistical interpretation of the data were used to examine the relationship between SLE disease factors and lipid subfractions. Inflammatory cytokines were examined to identify specific biomarkers that may be associated with metabolic syndrome.

Results. Increased total In a multivariate analysis, total cholesterol, triglyceride, and HDL-C levels were independently associated primarily with antimalarial treatments, active renal disease and measures of disease activity. Low HDL-C was significantly more likely in Korean (odds ratio (95% CI)) (4.414 (1916 - 10.169)) and Hispanic (3.503 (1.499 - 8.185)) ethnicities. Increased Leptin was strongly associated with risk of metabolic syndrome (1.026 (1.012 - 1.040)), as were cumulative corticosteroid dose (1.071 (1.014 - 1.128)) and current antimalarial therapy (0.475 (0.311 - 0.726)).

Conclusion. Dyslipidemia is more likely in SLE patients with active disease, whom have increased SLE activity and is associated with more severe disease phenotypes such and renal involvement. Ethnic and geographical factors had an affect on the likelihood of dyslipidemia, but did not influence metabolic syndrome status in cohort subgroup analysis, where raised leptin and cumulative steroid exposure were positively correlated with metabolic syndrome outcome, and antimalarial therapy was protective against metabolic derangement.

Declaration

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

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1 Introduction and Aims

Systemic lupus erythematosus (SLE) is an autoimmune disease with poorly understood pathogenesis. The syndrome associated with SLE is primarily one of tissue inflammation, however, the systemic manifestations that result in physiological dysfunction display a wide degree of heterogeneity (1). It is a syndrome that challenges our understanding of how otherwise protective homeostatic mechanisms can break down and promote disease. In addition to the classic inflammatory manifestations of SLE such as arthritis, serositis and glomerulonephritis, a number of long-term complications are also recognised, highlighting the complexity of the condition. Evidence of organ-specific dysfunction is noted in the increased incidence of coronary heart disease (CHD), which cannot be entirely accounted for with traditional risk factors alone (2). However, the mechanisms underlying this increased risk of atherosclerosis are as poorly understood as those underlying SLE itself. This research will investigate how mechanisms of lipid transport interact with SLE's signature immunological and inflammatory disease components, and how this may contribute to the development of atherosclerosis.

1.1 Background to SLE

1.1.1 Clinical Presentation of SLE

SLE is the most diverse autoimmune connective tissue disease, which may affect multiple organ systems, manifesting in a broad range of clinical and immunological presentations (3). Underlying this diversity is a complex autoimmune pathophysiology. More than 100 autoantibodies have been discovered in SLE, and almost all patients will show signs of anti-nuclear antibodies (ANA) (1). Autoantibodies accumulate over several years before symptoms emerge (4), with onset and diagnosis typically before the age of 30 (3).

Classic features of SLE are: symmetrical small joint arthritis; renal impairment, presenting with proteinuria, haematuria, or renal failure; inflammation and ulceration of mucous membranes, and; photosensitivity, malar and discoid rashes (5). There is a high concordance (25%) among mono-zygotic twins (6), however, the genetic contribution to disease risk only accounts for 5 - 15 % of this association

(1). This suggests that there are significant environmental factors that influence the disease aetiology, and add further to SLE's complexity.

1.1.2 Epidemiology of SLE

SLE is a disease that predominately affects women, with a female:male ratio of 9:1 (3). Recent studies suggest that hormonal abnormalities are a factor in disease aetiopathogenesis, and might help to explain the gender disparity of disease burden (7). There is also a broad age spectrum, despite the tendency for the disease to present earlier in life (8). Studies of racial disparities showed a higher prevalence of specific clinical features in people with African ancestry (9), whilst in the USA SLE is more frequent among all ethnic minorities compared with Caucasians (8).

A comparison of worldwide disease burden found remarkable differences between countries, with the lowest overall incidence in Japan and Iceland, and the highest in the USA and France (8). Multiple environmental factors may explain some of the disparity, with a wide variance of climatic and infectious conditions across countries studied. Influences as diverse as latitude, frequency of exposure to certain pathogens, or psychosocial factors may play a role in the development of SLE.

1.1.3 A Brief History of SLE

Presentations of SLE were recorded as early as 1828, where Biett distinguished the three principle types of 'dartre rongeante' [scaly plaque] that denote SLE's characteristic dermal lesions (10). However, it was not until 1872 that Kaposi gave a description of a single disease that brought together fever, sepsis, and cutaneous lesions resembling erysipelas, into a unified group of clinical manifestations (11, 12).

In 1885 Osler remarked on a series of eleven cases that highlighted the systemic symptoms and the relationship between lesions of the skin and viscera, in what was then termed 'erythema exudativum' (12). Osler noted that "visceral symptoms alone may be present, and to the outward view the patient may have no indication whatever of erythema exudativum" (13).

It was Jadassohn, in 1904, who observed the involvement of the joint, mucous membranes and kidneys (12) stating, “Lupus is directly and indirectly associated with changes in the musculoskeletal system, notably the bones, cartilage, joints and synovial tendon sheaths. It spreads itself onto the adjacent mucous membranes, destroys bones, makes teeth fall out etc.” (Translated by Author)[1] (14).

Earlier diagnosis of SLE was made possible in 1948 when abnormal ‘L.E.’ cells in bone marrow aspirate were first detected (15). The L.E. cells were subsequently found to be induced in healthy bone marrow, with the addition of plasma from SLE patients (16). Twenty years later the Schur’s and Sandson’s discovery of ‘precipitins’ to DNA (17), otherwise known as anti-DNA antibody, and reduced serum compliment in active SLE meant the onset and exacerbations of the disease became easier to predict (18).

1.2 Premature ‘Accelerated’ Atherosclerosis

1.2.1 Bimodal Mortality Pattern of SLE

By the mid-1970s new treatment regimes meant that there was a greater chance of surviving the acute illness that accompanied the onset of SLE. Similarly, subsequent flare-ups could be managed. Rapid fatality was no longer necessarily the course of SLE, however long-term survival had not dramatically changed (18).

At the time it was noted by Urowitz *et al.* that a significant contributing factor in the early mortality of SLE patients was infection, for which treatment with antibiotics could prolong survival (18). However, atherosclerotic vascular disease was the greatest risk factor for mortality in late-stage SLE. The death of women as young as 32 from myocardial infarction (MI) (Case 16, Table 1.1), without signs of active SLE, highlights how significant the impact of atherosclerotic disease is in prolonged disease (18).

[1] “[Lupus] er direkt und indirekt zu Veränderungen, speziell der Bewegungsapparate, vor allem der Knochen, Knorpel, Gelenke und Sehnenscheiden (Tscherning) führt, daß er per contiguitatem und per continuitatem auf die Schleimhaut übergreift, Knochen zerstört, Zähne zum Ausfallen bringt, etc.”

Table 1.1: SLE - late deaths

Case No.	Age at Death (yr)	Sex	Duration of Disease (yr)	Active Lupus	Active Lupus Nephritis	Sepsis	Myocardial Infarction
49	50	M	6	+	-	-	+
5	42	F	8	-	-	-	+
10	39	F	19.5	-	-	-	+
76	46	F	7	-	-	-	+
16	32	F	2.5	-	-	-	+

Source: Adapted from Urowitz (18)

1.2.2 Increased Risk of Coronary Heart Disease in SLE

A prospective longitudinal study of 229 SLE patients by Petri *et al.* (19) found that the predominant risk factors for developing CHD were patient age, duration of disease and prednisone use, hypercholesterolemia, and hypertension. These findings are concordant with speculation made by Urowitz *et al.* that corticosteroids may have contributed to the development of atherosclerotic lesions in patients with traditional comorbidities, including hypertension and dyslipidemia (18). Furthermore, multiple logistic regression performed in this study showed age at diagnosis as the only independent risk factor for developing CHD, whilst other positively identified risks were associated with prednisone treatment.

A large retrospective cohort study by Manzi *et al.* documented the increased incidence of cardiovascular events in women with SLE, compared with women of a similar age in the Framingham Offspring Study over the same time period (20) (21). The frequency of cardiovascular events occurring in relatively young women is described as “disturbingly high”, whilst the risk of suffering an MI was seven times greater overall in the women with SLE, and over 50 times greater in those aged 35-44 years (20). Only one the traditional risk factors measured, hypercholesterolemia, showed a significant correlation with cardiovascular events (rate ratio (RR) = 3.35, 95% CI 1.34-8.36).

1.3 Measuring SLE Activity

Indices of disease activity are designed to measure changes in disease pathology over time (22). This is necessary due to the variability of three factors: clinical

presentation, severity, and alternating phases of flares and remissions (1). During active SLE tissue inflammation is more likely to be present. Knowing which organs and tissues are involved can improve treatments, and provide researchers with a better understanding of specific disease sequelae (23).

Efforts to develop a criteria for classifying SLE began as early as th 1970s, with preliminary criteria produced by the American Rheumatism Association (ARA) that were subsequently updated in 1982 (24, 25). However, quantifying disease activity and distinguishing reversible organ dysfunction from irreversible organ damage is more problematic than simply defining it (26). Since the 1980s more than 60 methods have been developed for measuring disease activity in SLE (23). Two main types of disease activity measure can be categorised, one a global score that provides an assessment of overall disease activity, the other measuring damage in individual organ-systems, such as the British Isles Lupus Assessment Group (BILAG) index (22). There is no absolute gold standard for measuring disease activity (23), however the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) is the most frequently used global scoring index used in observational studies and clinical trials (27).

1.3.1 SLEDAI

The need to standardise outcome measures for SLE was determined at the *Conference on Prognosis Studies in SLE* in Toronto in 1985 (28). By 1992 rheumatologists had developed SLEDAI as a uniformly accepted index to measure disease activity in SLE. The SLEDAI is a global score designed to model a clinican's judgement based on 24 descriptors of 9 organ systems (29). SLEDAI is one of three components, including damage caused by disease, and the patient's health status, that provide a comprehensive representation of outcomes in SLE (29).

SLEDAI is validated against other indices for use with trained specialists, and with less experienced clinicians (30). A modified version of SLEDAI for the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) trial (31) redefined some of the disease definitions, but the overall scoring did not change (22). These revisions were incorporated into an updated index, known as SLEDAI-2000 (SLEDAI-2K), which was validated against the original SLEDAI in 18,636 patients and was found to describe disease activity in a comparable manner (32).

Table 1.2: Lipoprotein classification and properties

Class	*Density (g/mL)*	*Electrophoretic mobility*	*Diameter (nm)*	*Molecular weight (Da)*
Chylomicrons	0.93	Remain at origin	75-1200	(50-1000) x 10^6
Very low-density lipoproteins	0.93-1.006	Pre-β	30-80	(10-80) x 10^6
Intermediate-density lipoproteins	1.006-1.019	Slow pre-β	25-35	(5-10) x 10^6
Low-density lipoproteins	1.019-1.063	β	18-25	(2-3) x 10^6
High-density lipoproteins	1.063-1.21	α	5-12	(65-386) x 10^3

Source: Adapted from Choi (34).

1.4 Atherosclerosis and Lipidology

The term 'atherogenic dyslipidemia' has been used to describe the series of abnormal lipid levels in individuals with increase CHD risk. This dyslipidemia comprises of raised levels of triglycerides (TG), very low-density-lipoprotein (VLDL) and intermediate-density-lipoproteins (IDL), lower levels of high-density-lipoproteins (HDL), and an increase in the particle number of a type of low-density-lipoprotein (LDL) known as small dense LDL (SDLDL) (33). The variability in lipoprotein density is only slight, however, the change in particle size and molecular weight between lipoprotein molecules is considerable (Table 1.2). Although classified by their density, the physiological characteristics of lipoproteins are dependent on the apolipoproteins (Apo) that they carry (34).

This phenotype of atherogenic dyslipidemia is strongly associated with overall heart disease risk (33, 35). SDLDL is a good marker for this phenotype, however it is not the particle size directly that is atherogenesic. When particle size is accounted for its association with atherosclerosis is insignificant (36). This implies that SDLDL is likely to be a surrogate measure for something else such as metabolic derangement resulting from higher plasma TG concentration (37).

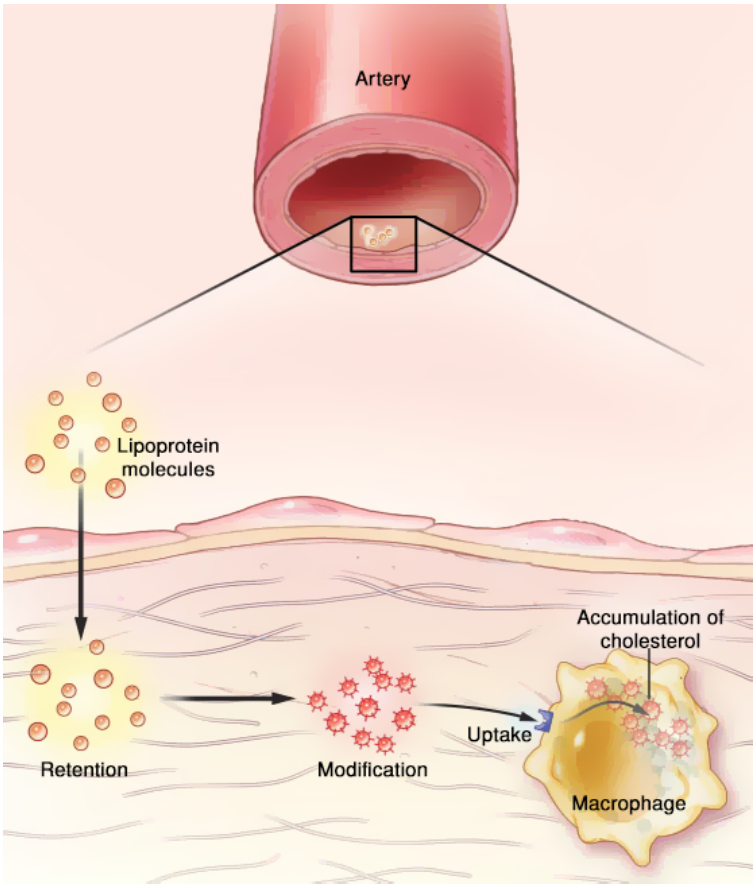


Figure 1.1: Atherosclerotic plaque formation begins with the retention of lipoprotein molecules in the tunica intima of the artery. Lipoproteins are modified by oxidative and enzymatic mechanisms, which are then taken up by scavenger receptors of macrophages. These then evolve into cholesterol rich foam cells (38).

1.4.1 Cholesterol

Cholesterol is necessary for the basic functioning of human biology, such as maintenance of cell membrane structures, cell growth, and hormonal function. However, if cholesterol builds up in arterial smooth muscle cells (SMC) this can lead to atherosclerotic plaque formation and increased CHD risk (Figure 1.1). The role of cholesterol is essentially health promoting, however if it cannot be cleared from arterial SMC before being modified and evolving into a foam cell, then it becomes disease inducing (34).

Delivering cholesterol to peripheral tissues is not a vital function of the body's lipid transport system, because it can be synthesised locally in cells where it is needed (39). The primary function of these lipoproteins, it has been argued (40), is to transport insoluble fatty acids (FA) and TG to muscle and adipose tissue for use in energy metabolism and storage. This can be seen in the proportion of TG

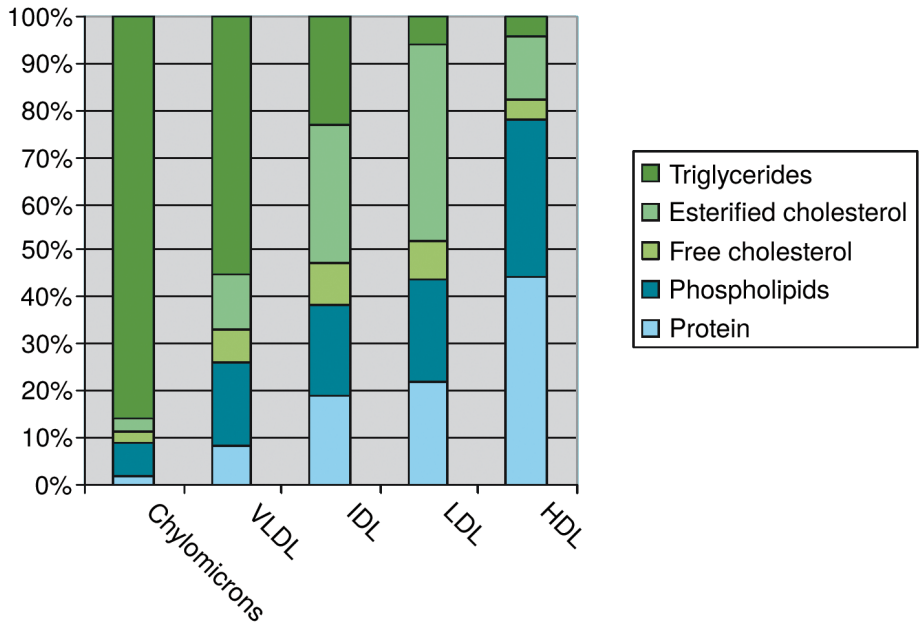


Figure 1.2: Lipoprotein composition as a percetage of overall mass. Adapted from Choi (34)

transported in the very large chylomicron and VLDL lipoproteins, compared to other components (Figure 1.2). The timely demand for FA in some tissues, for instance well-oxygenated cardiac muscle, makes rapid lipid transport from storage cells to the site of respiration a necessity (41). To some extent cholesterol transport can be viewed as subordinate to this primary function.

Cholesterol homoeostasis is controlled by balancing exogenous cholesterol absorption with endogenous cholesterol synthesis. Bile acid secretions accounts for 50-70% of absorbed cholesterol, with exogenous cholesterol from diet only providing 15-30%, and the rest coming for the turnover of intestinal mucosal epithelium (42). The liver is the main site of cholesterol synthesis, and is regulated by the enzyme 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA) (43). HMG-CoA is the rate limiting step in the cholesterol production, and the increase in levels of cellular cholesterol suppresses it by altering its activity and transcription. This is facilitated by LDL receptor- (LDLR) mediated endocytosis, which bind to the primary structural apolipoprotein, ApoB-100, of LDL molecules (44). Hence, serum cholesterol levels are dependant on expression of LDLR and clearance of LDL from the circulation.

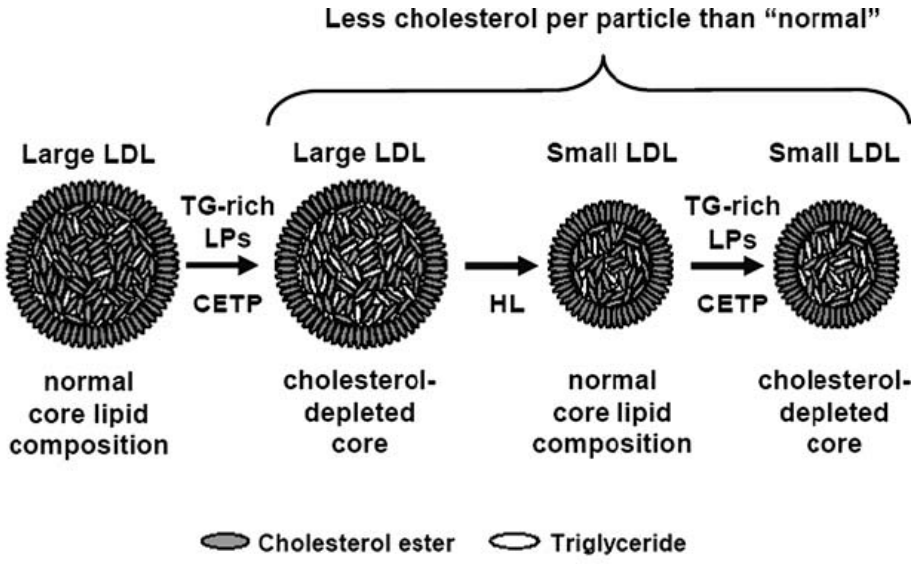


Figure 1.3: Metabolic origins of LDL particles with reduced cholesterol volume. Adapted from Chromwell and Otvos (45).

1.4.2 Low-Density Lipoproteins

Measurements of LDL-C are a summation of cholesterol in a range of different particles (Table 1.2). Variability in LDL particle size, a surrogate measure for atherogenic dyslipidemia, is driven by the composition of VLDLs synthesised in hepatocytes, which are precursors to LDLs (45). Elevated concentrations of hepatic TG stimulate the formation of large TG-rich VLDL within the hepatocyte. When plasma TG levels are raised, cholesterol ester transfer protein (CETP) catalyses a reaction that exchanges TG from the core of the TG-rich lipoproteins with cholesterol esters in cholesterol-rich LDLs. This restores the normal ratio of cholesterol and TG to the remnant of the VLDL, at the cost of depleting cholesterol from the core of the LDL. These abnormal LDL become a substrate for hepatic lipase (HL), which partially hydrolyses the core TGs and remodels the lipoprotein structure transforming it into a SDLDL (Figure 1.3).

Removing LDL particles from circulation can reduce CHD risk. This is evident in studies that show the effectiveness of LDL-cholesterol (LDL-C) lowering therapies (38, 46), in contrast with studies where raised HDL-cholesterol (HDL-C) does not have the same effect in reducing CHD risk (47). Genetic studies also suggest that CHD risk is closely associated with genetically determined LDL-C levels and exposure over time, whereas genetic influences affecting HDL lipoproteins were not strongly associated with CHD risk (48).

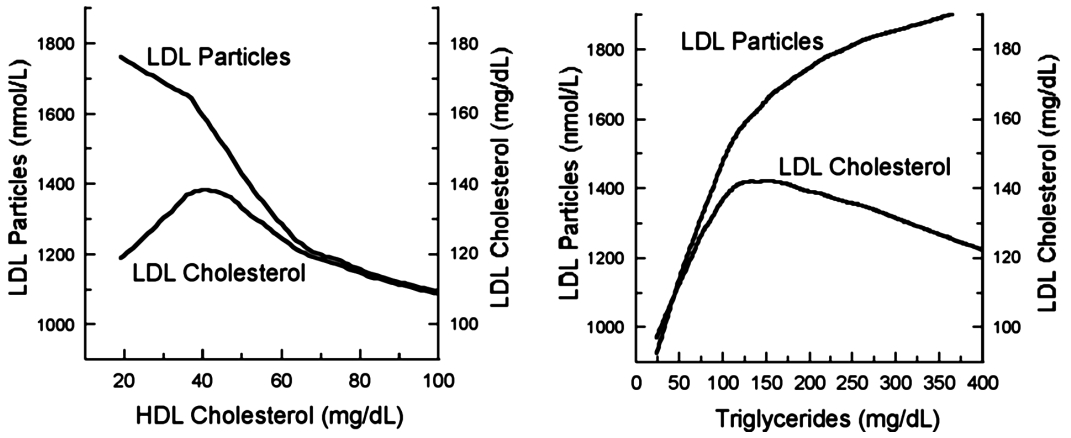


Figure 1.4: LDL particles measured with Nuclear magnetic resonance (NMR) spectroscopy and Friedewald calculated LDL-C compared with HDL-C and TG levels. Adapted from Cromwell and Otvos (45).

Lamarche *et al.* (49) showed that once particle size had been taken into account LDL-P was the "best and only significant predictor of ischemic heart disease (IHD) risk." In clinical practice, however, the CHD risk associated with atherogenic dyslipidemia is assessed, not by measuring lipoprotein particle size or number, but instead the amount of cholesterol bound to lipoprotein molecules. In the case of LDL-C, this measurement is not directly obtained but rather calculated using the Friedewald equation (50). This is problematic because this estimate of LDL-C tends to be an underestimate of the true circulating level, especially when TG is raised (51).

Nuclear magnetic resonance (NMR) analyses of 3,437 frozen plasma specimens from the Framingham Offspring Study show the relationship between LDL particle number (LDL-P) and LDL-C measured against HDL-C, known to be a strong predictor of CHD independent of LDL (52), and TG levels (figure 1.4). If LDL-C was used as a predictive measure of CHD risk, in cases where HDL-C was very low or TG was very high, a sign of profound dyslipidemia, LDL-C values may appear normal or only slightly elevated. In cases where LDL-C is elevated, HDL-C and TG may be much lower than would be interpreted from this perceived hyperbetalipoproteinemia. Elevated LDL-P is associated with SDLDLs, which carry the least amount of cholesterol relative to larger LDL particles. Hence, LDL-C may significantly underestimate the CHD risk posed by the increased particle number, and this ultimately makes LDL-C ineffective at predicting CHD risk in cases where it matters the most.

Table 1.3: HDL subpopulation classification by different methods

Separation Principle	HDL Subclasses
Density	HDL-2 (density range 1.063-1.125 g/mL) HDL-3 (density range 1.125-1.210 g/mL)
Electrophoretic mobility and size	HDL-2b (9.7 12 nm) HDL-2a (8.8-9.7 nm) HDL-3a (8.2-8.8 nm) HDL-3b (7.8-8.2 nm) HDL-3c (7.2-7.8 nm)
NMR and size range	Large HDL-P (9.4-14 nm) Medium HDL-P (8.2-9.4 nm) Small HDL-P (7.3-8.2 nm)
Apolipoprotein composition	ApoA-I ApoA-I:A-II

Source: Adapted from Rizzo, Otvos, Nikolic *et al.* (57)

Oxidative and enzymatic modifications of LDL also play an important role in the development of atherosclerosis. Reactive oxygen species in tissues produce oxidised LDL (oxLDL), which is taken up by macrophages more readily than unmodified lipoproteins (53). These oxLDL also interact directly with inflammatory mediators such as such as vascular adhesion molecule-1 (VCAM-1), promoting inflammation AlGadban2015. Several studies have indicated that small dense LDL are more susceptible to oxidative modification (54, 55), due to diminished LDLR affinity and, thus, a longer period of time spent in the circulating plasma (56).

1.4.3 High-Density Lipoproteins

HDLs are a family of particles that display considerable heterogeneity (Table 1.3). Using non-denaturing gradient gel electrophoresis it is possible to subdivide the HDL into five distinct subfractions, refining the classification of high- and low-density HDL. Categorisation by NMR spectroscopy yields particle sizes that can range from large to small. The majority of studies conducted looking at the relationship between HDL subfractions and CHD risk, found HDL particle size, whether it was measured by ultracentrifugation, gel electrophoresis, or NMR spectroscopy, was inversely correlated with CHD risk, and are potentially atheroprotective (57). This result is consistent with studies that have found HDL-C is inversely correlated with CHD risk, and a better predictor of adverse outcomes than LDL-C or total cholesterol (52).

A crucial function of HDL is in preventing the excess accumulation of lipids in peripheral tissues, which, unlike hepatocytes, are unable to catabolise cholesterol (58). Known as 'reverse cholesterol transport', ApoA-I stimulates the externalisation of cholesterol and phospholipids from the cell, and activates enzymes which esterify the cholesterol allowing it to diffuse in to the core of the HDL particle (58).

During phases of inflammation the HDL molecules are depleted of apoA-I, and may become themselves pro-inflammatory (piHDL). HDL's normal protective function is lost, and it becomes partially saturated with oxidised products of inflammation (59). In the presence of persistent inflammation, or with flares of active SLE, HDL's protective role may diminish over time and allow for the accrual of more proinflammatory mediators, and eventual tissue damage. PiHDL's contribution to atherosclerosis may be that in failing to prevent oxidation of LDL molecules, it further promotes the oxidative process (60, p. 131).

1.4.4 Lipoprotein Metabolism and Dyslipidemia

Lipoproteins undergo a complex series of synthesis and regulation. ApoB, an essential component of atherogenic lipoproteins, is unusual because it is under constant synthesis by the liver. The regulation of ApoB occurs through its degradation while, or after, it is produced (61). This regulation is inhibited by triglyceride synthesis, and when conditions are not acceptable its assembly and export ApoB's turnover is decreased (61).

TG synthesis is influenced by the supply of free fatty acids (FFA). Stored fat in adipose tissue, exogenous fats from dietary intake, and FFA sythesised *de novo* from carbohydrates and amino acids may all contribute to the over production of TG (62). Inflammatory cytokines such as TNFα are shown to increase circulation FFAs by increasing lipolysis of stored fats (63), while interleukin-6 (IL-6) has been show to directly increase lipolysis and FFA oxidation, and indirectly inhibit lipoprotein lipase (LPL) (64).

A disturbance in LPL activity has an effect of raising circulating chylomicrons and TGs. This has been shown in two autosomal recessive genetic disorders. In cases of LPL deficiency, a loss-of-function mutation of the *LPL* gene inactivates LPL and prevents the hydrolysis of TGs in chylomicrons. In familial apo C-II de-

ficiency, a mutation in the gene *APOC2* results in LPL inactivity secondary to apo C-II dysfunction, an essential cofactor for LPL activity (65, p. 186). This results in affected individuals developing familial chylomicron syndrome (FCS), a disease characterised by childhood presentation of elevated plasma TG $>$ 1000 mg/dL, and acute pancreatitis (66). In SLE patients an acquired disruption in LPL function is suggested, and several mechanisms have been proposed (67).

1.5 Treatment and Inflammatory Pathways in Lupus

Lupus patient experience considerable heterogeneity in their clinical presentation and severity of disease. This makes SLE a difficult disease to understand and to treat. The clinical presentation of lupus can be categorised into distinct phenotypes, such as renal involvement and thrombocytopenia, suggesting multiple mechanisms of disease aetiopathology (68). This is further complicated by the use of treatments, such as corticosteroid therapy, which is known to affect metabolic factors associated with atherogenesis, but also reduces inflammation. Thus, trying to distinguish isolated lupus pathologies associated with particular disease characteristics is ultimately very challenging (69). The cycle of inflammation that is associated with increased CHD risk in SLE must also account for other potential atherogenic risk factors.

1.5.1 Inflammatory cytokines

In SLE there is an increased production of IL-6 and tumor necrosis factor alpha (TNFα). Il-6 has been linked to higher visceral adiposity (70), and plays an important role in the recruitment of inflammatory cells (71). Glucocorticoids are known to reduce the synthesis of pro inflammatory cytokines such as IL-6 and TNFα (72), and are useful in treating the inflammation associated with SLE.

C-reactive protein (CRP), released by the liver in the acute phase response, is a component of the innate immune system that has been correlated with atherogenesis. CRP has been identified as an independent risk factor for developing atheroma (73), whilst evidence of CRP's binding to modified low density lipoproteins (74), and its accumulation and potential apoptotic effect within vascular smooth muscle points to a more direct involvement in atherogenesis (75) In the

late 1990s a high-sensitivity CRP (hsCRP) assay was developed that could determine CRP levels accurately below the standard reference range (76). Results of a multivariate regression analysis by Nikpour *et al.* has recently showed an increased likelihood of any CHD events in SLE patients with a hsCRP $\geq$ 1.6 mg/litre (77). However, McMahon *et al.* showed that hsCRP in women was only associated with carotid plaques in control subjects (60).

Vascular SMCs can express interferon gamma (IFNγ), which has been demonstrated in vivo to promote atherosclerosis directly via IL-18 receptor ligation (78). Levels of circulating free IL-18 are also elevated in the presence of increased IL-18 binding protein (IL-18BP) concentrations in lupus patients (79). IL-18BP acts as an antagonist for IL-18 activity by binding to the cytokine and neutralising its biological activity (80).

ApoE lipoproteins, known to be associated with an increased risk of dyslipidemia (81), are up-regulated in lupus patients and positively correlate with disease activity (72). ApoE is also associated with increased anti-dsDNA antibodies and related cytokines. In addition to the stimulation of cytokine expression by modified LDL and VLDL remnant particles (82), lipoproteins have a significant affect in modulating inflammation.

1.5.2 Adipokines

Adipokines are adipose derived cytokines, and have been linked to inflammation in adipose tissue (83). Raised adiponectin is associated with low levels of adipose tissue and insulin sensitivity (70), indicating a possible association with metabolic dysfunction. Adiponectin also acts as an anti-inflammatory and can improve overall energy homoeostasis (83). Low levels of adiponectin have reportedly been associated with proinflammatory states, however the evidence of its involvement with metabolic dysfunction is inconclusive (84).

Leptin has been widely associated with obesity and insulin resistance. Leptin has both peripheral and hypothalamic effects on energy homoeostasis. Leptin resistance in the ventral-medial hypothalamus has been linked with increased levels of insulin, suggesting that insulin is an antagonist of leptin signalling (85). Peripherally, elevated leptin levels can induce oxidative stress and accelerate

the formation of proinflammatory lipoproteins, such as piHDL, which leads to the formation of atherosclerotic plaques (60).

IL-6 and TNFα are also released by adipose tissue to improve insulin sensitivity and increase lipolysis (83). This may be interpreted as a metabolic response to an inflammatory signal, indicating the necessity for increasing circulating FFA and inducing dyslipidemia in response to acute inflammation.

1.5.3 Insulin Resistance and Metabolic Syndrome

Metabolic syndrome (MetS) is associated with an increased risk of CHD in the general population (86). This increased incidence of CHD in SLE cannot be accounted for with traditional risk factors alone (2) There is evidence to suggest that MetS is linked to low-grade inflammation in the general population (87), as it is significantly increased in other rheumatological disorders such as rheumatoid arthritis (88). Approximately 18% of lupus patients have metabolic syndrome (69), and there are several studies examining the prevalence of MetS in SLE (86). The metabolic factors associated with MetS are also highly prevalent in SLE, which may indicate an association between their underlying disease processes (89).

1.6 Effects of Treatments in SLE

1.6.1 Antimalarials

Antimalarial drugs such as hydroxychloroquine have a protective effect in SLE, and are associated with reduced morbidity and mortality (90). In a longitudinal study of over three thousand patient visits, hydroxychloroquine was associated with lower total cholesterol levels (91). Further studies have confirmed this finding, and also found hydroxychloroquine to be associated with lower VLDL-C and LDL-C levels, including for patients taking corticosteroid medication (92). In addition to it's disease modifying properties, hydroxychloroquine is also known to have anticoagulant effects (93), which may be further protective in SLE due to the activation of the coagulation cascade in systemic inflammation (94).

1.6.2 Corticosteroids

In Urowitz's original case series, six patients (mean age 39.6 yr) suffered MIs. Examination of risk factors for these six patients showed normal serum cholesterol, elevated blood pressure (BP), and increased triglycerides. In the discussion it was observed that corticosteroids had previously been associated with dyslipidemia, notably hypertriglyceridemia in asthmatics, and hypercholesterolemia in rheumatic disorders. However, the patients whom suffered MIs were on a significantly lower dose of prednisolone (mean dose 10.4 mg/day) compared with the overall cohort (18.1 mg/day), but had been taking it over a longer duration (18).

Bulkley and Roberts compared necroscopies of 36 SLE patients whom had died in the 'corticosteorid era', to necroscopy observations in 20 SLE patients before corticosteroids were used. This detailed analysis found significant changes in cardiac morphology of corticosteroid treated patient's compared to untreated SLE patients' hearts. it is also observed that corticosteroid treatment of longer than one year had a significant impact on coronary artery stenosis, with 42% of these patients having at least one major coronary artery narrowed by more than 50% (95). This correlation may be the results of confounding by indication, a bias due to association between the amount of steroid given and the severity of the disease.

A matched case-control study by Ettinger *et al.* investigated the 46 women with inactive SLE, none of whom had had any changes to their medication one month prior to enrolment, with 32 from this cohort being treated with prednisolone. A standard lipid panel and various lipoprotein subfractions were measured. The study found raised levels of total cholesterol, TG, LDL-C, and protein apo E in patients who were treated with prednisolone compared with either those without prednisolone treatment or controls. When comparing lipoprotein subfractions between groups, although the mean HDL particle size was the same across groups, it showed that the prednisolone treated patients had a lower proportion of the more cardio protective HDL-2. This study showed no correlation between lipid levels and duration of prednisolone use, but it did note that the dyslipoproteinemia in patients treated with prednisolone was likely due to elevated very-low-density lipoprotein (VLDL) levels in hypertriglyceridemic subjects (96).

Contrary findings were reached by Petri *et al.* in a prospective longitudinal study

that examined risk factors for CAD in SLE patients. Data on 229 patients was collected over a three year period, 19 of whom were identified as having CAD. This study found that CAD was more likely to occur in patients that had a longer duration of prednisolone use. Using multiple logistic regression analysis, it was also observed that other positive risk factors for CAD, including hypercholesterolemia, hypertension, and obesity, were either directly or indirectly related to prednisolone use. The only independent risk factor for CAD was age at diagnosis of SLE, which showed a positive correlation. The study, however, could not rule out the possibility that the association between CAD risk and duration of prednisone therapy was not a confounding by indication (19).

A consecutive case series by Borba and Bonfá investigated 19 newly diagnosed female patients with active lupus who were not on either prednisolone or chloroquine. This group was compared with 17 female patients with inactive or stable disease (SLEDAI $\leq$ 4) not treated with either prednisone or chloroquine for at least three months prior to enrolment, and a control group of 30 healthy females. The groups were assessed for clinical disease activity, and fasting blood samples were collected for analysis. Total cholesterol, TG, and HDL-C were measured directly, whilst LDL-C and VLDL-C were estimated using the Friedewald formula (50). The study also had strict exclusion criteria for enrolment. The lipid profile of the inactive group compared with the control group showed an increase in mean TG and VLDL-C, and a decrease in mean HDL-C levels. Compared with controls, and with the inactive group, active SLE patients also showed a significant increase in mean TG and VLDL-C levels, and reductions in mean HDL-C, and LDL-C. The patients with active lupus were more likely to have an 'undesirable' HDL-C and TGs compared with inactive and control groups (P leq 0.001), whilst those in the inactive group were also at higher risk of low HDL-C levels compared with controls (97).

This study suggests a similar phenotype to atherogenic dyslipidemia is present in SLE patients, a so called 'lupus pattern' of dyslipoproteinemia. It demonstrates that disease activity has a significant worsening effect on the lipoprotein status in SLE patients. Termed the 'active lupus pattern', we see a more striking derangement of the previous lipid levels and also a decrease in LDL-C level, which might be a result of a very high number of cholesterol-depleted SDLDLs (Figures 1.3, 1.4). This involvement is further supported by a positive correlation found

using logistic regression, between the ‘lupus pattern’ of dyslipoproteinemia and the presence of lupus vasculitis (P = 0.0001) (97).

1.7 Hypothesis

SLE is associated with atherogenic dyslipidemia, which is related to levels of disease activity, inflammation, and metabolic syndrome status.

1.8 Aims

1 To describe the pattern of lipid subfractions and the presence of MetS in an inception cohort of SLE patients.

2 To examine the levels of inflammatory cytokines in SLE patients.

3 To study how disease status and inflammation influences lipid subfractions in SLE and MetS.

4 To study how inflammatory cytokines, corticosteroids, and other treatments (notably antimalarials) influence lipid subfractions in SLE and MetS.

2 Materials and Methods

2.1 Study Design Overview

This is a prospective observational study based on a large international cohort of newly diagnosed SLE patients. Cross-sectional analysis of patients at enrolment provided a large sample from which comparisons could be made between sub-groups. A key feature of this analysis will be to demonstrate evidence of SLE’s association with atherogenic dyslipidemia observed in previous studies, and then go on to examine what effect SLE has on lipid subfractions in patients with and without MetS.

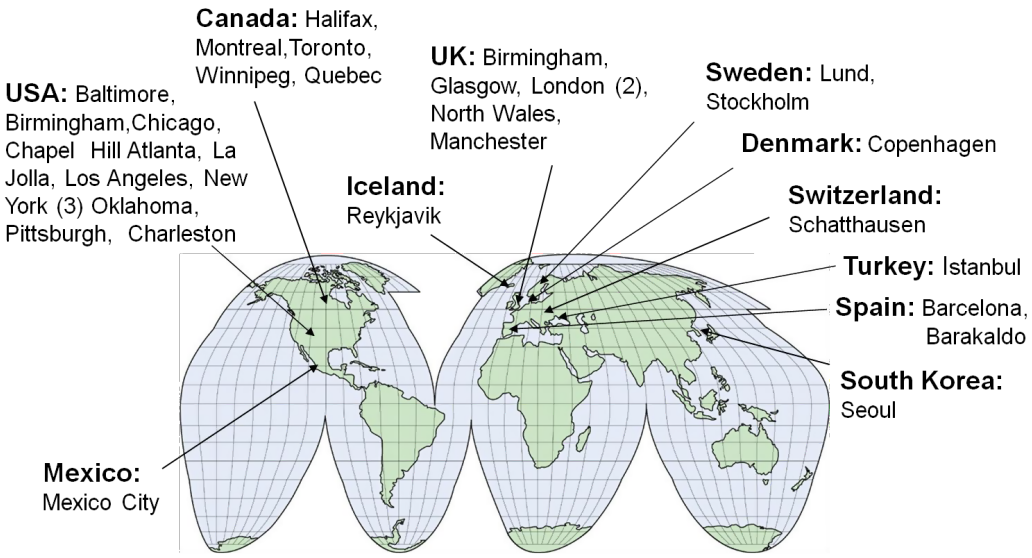


Figure 2.1: Map of participating centres in the SLICC group

2.2 Participants

2.2.1 SLICC Inception Cohort

The Systemic Lupus International Collaborating Clinics (SLICC) Atherosclerosis Registry (SLICC-RAS) is a well established international inception cohort registry of recently diagnosed patients with SLE from around the world. The methodology of this has been widely cited (68, 98, 99) since the registry was established in 2000. The SLICC group comprises of researchers at 33 centres in 11 countries across Central and North America, Europe, and Asia (Figure 2.1). SLICC-RAS is the only prospective study of its kind, set up with the explicit aim of determining the prevalence and risk factors of atherosclerosis in SLE patients.

Participating centres contributed clinical data and tissue samples for biochemical and genetic analysis. The University of Toronto Lupus Clinic served as the coordinating centre for all data and samples obtained at the local sites.

2.2.2 Ethical Approval

All participating centres are responsible for maintaining ethics committee approval from their respective institutional research ethics boards, and copies of consent forms (Appendix B) are kept centrally. Ethical approval for this study has

been given by Central Manchester Local Research Ethics Committee, July 2003 (Appendix A), and University Health Network Research Ethics Board, Toronto Canada, in accordance with the declaration of Helsinki (100).

2.2.3 Recruitment

At its inception the SLICC-RAS cohort intended to recruit 1800 patients, in order to provide sufficient statistical power to allow for multiple lines of inquiry into specific disease-related factors across a broad population (99). Enrolment into the SLICC-RAS began in October 1999, and continued until September 2011. Patients were eligible to enrol within 15 months of a diagnosis of SLE, which required the patient to meet four or more American College of Rheumatology (ACR) criteria for the classification of SLE (101). Patients were subsequently seen annually to collect follow-up data.

2.3 Data Collection

The data analysed in this study was collected from the commencement of the SLICC-RAS until the end of 2009. The dataset used in the analysis had previously formed the basis for several published papers (98, 102).

2.3.1 Clinical Assessment

Information was collected at enrolment and then at annual follow-up visits, according to standardised protocols (Appendix B). This information consisted of demographic data, family history and lifestyle, biometrics, classic cardiovascular risk factors and prior cardiovascular events, and current or previous therapy, particularly with corticosteroids, antimalarials, or immunosuppressives. Lupus symptoms and disease phenotype were assessed using the SLE disease activity index (SLEDAI-2K) (32), and SLICC/ACR SLE damage index (28).

Table 2.1: Definitions of lipid abnormalities

	Lipid Measurements	
	Plasma Biomarker	**mmol/L**
Lipid Abnormality		
Hypertriglyceridemia	Triglycerides	$\geq$2.26
Hypercholesterolemia	Total Cholesterol	$\geq$6.21
Hyperbetalipoproteinemia	LDL-C	$\geq$4.14
Hypoalphalipoproteinemia	HDL-C	$<$1.03

Source: NCEP ATP III (103)

2.3.2 Tissue samples

Routine laboratory tests were performed on blood samples taken from each patient. This give data for a standard lipid profile and glucose, which were either fasting or non-fasting samples. Additionally, C-reactive protein (CRP), homocysteine, creatinine, and erythrocyte sedimentation rate (ESR) were also measured. Additional samples were taken from patients whom were fasted, in order to provide plasma for additional analysis of various biomarkers, such as insulin. Tissue samples for genetic analysis were also obtained from some patients.

2.4 Defining Lipid Abnormalities

From the data available it was possible to clearly define the parameters of what constitutes an abnormal lipid profile in this cohort. This study utilises the National Cholesterol Education Program's (NCEP) Adult Treatment Panel (ATP) III report (103) when defining lipid abnormalities, similarly to previous studies (97). Table 2.1 shows the lipid levels of measured biomarkers indicative of dyslipidemia.

Total cholesterol, triglycerides, and HDL-C were used as markers for abnormal lipid levels. As discussed previously, estimated LDL-C is not a reliable biomarker for atherosclerotic risk, and as such was not a measured outcome of this study. In addition to the measured serum levels of plasma biomarkers, definitions for hypercholesterolemia and hypertriglyceridemia included patients using lipid-lowering medications (predominantly Statins), given the significant affect these can have on lowering serum cholesterol and triglyceride measurements (Table 2.2). Such

Table 2.2: Lipid-lowering therapies and their effects upon blood lipid levels

	Change in Lipid Measurement (%)		
	LDL-C	**Triglyceride**	**HDL-C**
Lipid-lowering Agent			
Statins	↓ 18-55	↓ 7-30	↑ 5-15
Nicotinic acids (niacin)	↓ 5-25	↓ 20-50	↑ 15-35
Fibric acids (fibrates)	↓ 5-20	↓ 20-50	↑ 10-20
Ezetimibe	↓ 17-22	↓ 4-11	↑ 2-5
Bile acide sequestrants	↓ 15-30	No change to increased	↑ 3-5

Source: Adapted from Bays and Stein (104)

profound effects of these medications are not seen in changes to HDL-C levels, thus, their use was not included in the definition for hypoalphalipoproteinemia

2.5 Defining Metabolic Syndrome

The dataset used in this study contained predefined variables for metabolic syndrome (MetS) diagnostic criteria that follow the recommendations adopted by the International Diabetes Federation (IDF) in 2009 (105). These criteria used the NCEP ATP III as the starting point for a new definition of MetS (106), adopting an approach that focuses more heavily on cardiovascular risk factors. The IDF 2009 definition states that any three of the five criteria in Table 2.3 is sufficent for a clinical diagnosis of MetS.

2.6 Laboratory Analysis of Biomarkers

All routine blood sample collection and analysis was done at the local participating clinics. Phil Pemberton of the biochemistry laboratory at Central Manchester Healthcare Trust oversaw the measurement and analysis of additional serum samples for insulin, adipocytokines and inflammatory cytokines (hereafter referred to as 'biomaker data'). There was no specific selection criteria for sample analyses, and sample selection also relied on data that had been obtained from these samples for previous studies.

Table 2.3: IDF 2009 criteria for metabolic syndrome

Measure	Categorical Cut Points
Elevated waist circumference	Population- and country-specific definitions
Elevated triglycerides (drug treatment for elevated triglycerides is an alternate indicator)	$\geq$150 mg/dL (1.7 mmol/L)
Reduced HDL-C (drug treatment for reduced HDL-C is an alternate indicator)	$<$40 mg/dL (1.0 mmol/L) in males; $<$50 mg/dL (1.3 mmol/L) in females
Elevated blood pressure (antihypertensive drug treatment in a patient with a history of hypertension is an alternate indicator)	Systolic $\geq$130 and/or diastolic $\geq$85 mm Hg
Elevated fasting glucose (drug treatment of elevated glucose is an alternate indicator)	$\geq$100 mg/dL (5.6 mmol/l)

Source: IDF 2009 definition (105)

2.6.1 Commercially Prepared Kits

The analyses of 7 different biomarkers - interleukin 6, tumor Necrosis factor alpha, interleukin 18, interleukin 18 binding protein, B-lymphocyte stimulator, leptin, and adiponectin - were performed using the sandwich ELISA principle outlined in Figure 2.2. Insulin and oxidised LDL were analysed as per the kit instructions. High-sensitivity C-reactive protein was measured using an in-house ELISA method at the University of Manchester (107) (Appendix C).

In many cases, the sample (antigen) was diluted prior to analysis in order that the absorbance value of the colour change fell within the standard curve. The absorbance values were converted into corresponding units of concentration, and then multiplied by the original dilution factor to give a sample measurement. If the absorbance value fell outside the highest standard specified for a given assay, then the analysis was repeated at a greater sample dilution.

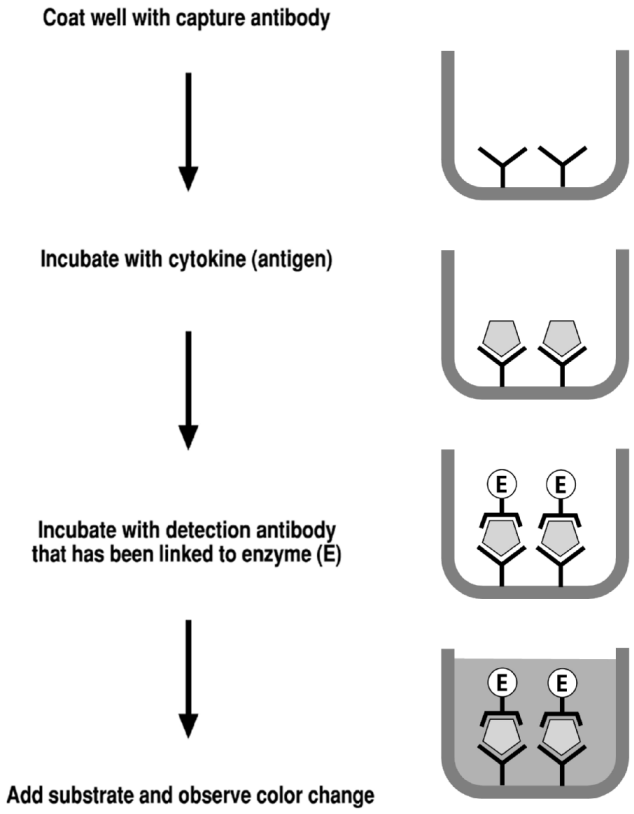


Figure 2.2: Basic protocol for traditional double-antibody sandwich ELISA, Leng *et al.* (108)

2.7 Statistical Analysis

2.7.1 General Statistical Approach

Stata® 13 data analysis and statistical software was used exclusively throughout this study. To describe patterns in the data, frequency distributions, means and standard deviations (SD) for normally distributed samples, and medians and inter-quartile ranges (IQR) for non-normal distribution were used initially. Graphical tests were analysed visually to determine the distribution of individual variables. P-P and Q-Q plots were used for primary evaluation of the distributions, after which histograms were used to verify the results (Appendix D). Significant differences between sample groups were analysed using χ^2 tests for categorical variables, independent samples t-tests for normally distributed continuous data, and Wilcoxon–Mann–Whitney tests for non-normally distributed ordinal data. The initial preparation and statistical methodology for the dataset are detailed in the PhD dissertation from which it derived (109).

2.7.2 Data Extraction and Study Samples

The first stage in preparing the data for analysis was merging the biomarker data obtained directly from Central Manchester Healthcare Trust's laboratory with the existent SLICC-RAS dataset (hereafter referred to as 'SLICC-bio data'). Not all patients in the SLICC-RAS had biomarker data, but these were not excluded from the primary SLICC-bio study sample. This study sample represents the population of individuals whom were diagnosed with SLE and eligible to join the SLICC-RAS cohort study. Two specific subsets of the SLICC-bio data were classified. All patients with biomaker data at enrolment formed a biomarker cohort, whilst all patients at enrolment for whom MetS status could be determined formed a SLICC-MetS cohort. Although the SLICC-bio study sample contains two years of follow-up data, the study was restricted to a cross-sectional analysis of enrolment data. The exception to this was a descriptive analysis of the biomarker data over the follow-up period.

2.7.3 Selecting Predictor Variables

Significant disease aspects likely to influence the levels of lipid subfractions in SLE patients were considered on the basis of existing literature. Classic CHD risk factors such as blood pressure and smoking were included. Previous studies have provided evidence of associations between lipid abnormalities and disease status and inflammation, disease phenotype, and corticosteroid and antimalarial therapies (97, 98). Variables representing these characteristics were used to determine SLE's influence on patients' lipid profiles. Additionally, the research proposal for the SLICC-RAS study (110, 111) included a rationale for investigating specific inflammatory biomarkers, namely hsCRP, Il-18, leptin, and oxLDL in relation to their relationship with MetS and disease activity. The primary analysis of the biomarker cohort focused on these particular inflammatory biomarkers, after which a secondary analysis was performed using other biomarker data and clinical features to further investigate the study sample.

2.7.4 Identifying Confounding Variables

The association of predictors with disease can fail to accurately reflect a causal relationship due to the presence of one or more confounding variables or covariates. This is especially problematic in epidemiological research where causal relationship may be poorly defined, and the number of variables contributing to measured outcomes is vast. Statistically, a covariate is defined as a variable can determine a given causal parameter, but only if the outcome data is known (112). When determining whether a predictor variable is causally related to a given outcome, the confounding variable is then independently associated with both predictor and outcome. There are known associations between SLE, socioeconomic, and demographic factors (9), and thus all regression analysis was adjusted for age, gender, and ethnic classification. A grounded theory approach (113) was used to determine other potential covariates *a priori*. These included measures of CHD risk and metabolic health not used as part of the 2009 IDF definition of MetS, and drug therapies known to influence lipid metabolism and transport.

2.7.5 Analytic Strategy

After the study sample was described and comparisons between sample groups analysed, univariate analysis was employed to examine the relationship between lipid abnormalities and disease factors across the entire SLICC-bio sample. A purposeful selection process of potential covariates and predictors for each outcome determined candidate variables for a subsequent multivariate analysis (114). A significant univariate test was defined as p-value < 0.2. These variables were then analysed using an iterative process of backward stepwise multivariate logistic regression, and a significance value of $p < 0.05$ was set. This process was repeated for the SLICC-MetS cohort using the same outcome variables, categorised by the presence or absence of MetS. A similar analysis was performed on the biomarker cohort, where the outcome measured in this instance was MetS status.

2.7.6 Assessing Covarience

Covariance of predictors and potential confounders in the multivariate analysis were identified using pairwise correlation, displayed in a correlation matrix. The null hypothesis was that pairs of variables were not correlated, and a significant correlation was determined if $p < 0.05$. Any covariance identified could then be adjusted for in the multivariate model by including an interaction term between these variables.

2.7.7 Diagnostics and Evaluation

Each multivariate model was assessed for goodness of fit using the Hosmer–Lemeshow test, and the performance of the model judged by receiver operating characteristic (ROC) curves of each predictor variable. The ROC gives a figure for the area under the curve (AUC) that can be roughly categorised as follows (115):

- 0.9–1.0 = excellent
- 0.8–0.9 = good
- 0.7–0.8 = fair
- 0.6–0.7 = poor
- 0.5–0.6 = fail

An AUC of 0.5 means there is just as much likelihood of differentiating between outcome through chance as it would be relying on the predictor (116). In the case that there were two competing models for a given outcome variable, the best model would be selected on the basis of these diagnostics and evaluations.

3 Results

3.1 Fasting lipids in SLE at Enrolement

The data analysed in this study has been described in previous publications (102, 109) under the SLICC-RAS name. However, for this analysis the entire dataset was re-examined and analyses were re-run. For this reason, even though the

initial dataset for the SLICC-bio cohort uses the same data published for the SLICC-RAS and might overlap to some degree, these analyses are independent of each other. Hence, all future reference to this dataset will use the term 'SLICC-bio' to distinguish it from these previous analyses.

3.1.1 Demographic Features

Baseline characteristics of patients in the SLICC-bio dataset are summarised in Table 3.1. Enrolment Data was initially available on 1687 patients, whom were enrolled into the SLICC-RAS (registry for atherosclerosis) up until the end of 2009. All patients had a diagnosis of SLE in accordance with the American College of Rheumatology (ACR) revised criteria (101). This was a young cohort of patients with a mean (SD) age at of 35.2 years (13.4) and a short median disease duration of 21.1 weeks (approximately 5 months). As expected, there was a higher proportion of women 1505/1687 (89.3%) compared to males at enrolment, a ratio of 8.36:1. The majority of women 1328/1505 (88.2%) were premenopausal.

There was significant ethnic heterogeneity in the cohort (Figure 3.1), consistent with the broad international catchment of the collaborating clinics. Four patients had missing ethnicity data, three from the Toronto clinic in Canada, and one from Birmingham, UK. Despite this ethnic variation, the cohort comprises of a large proportion of Caucasians (45.2%), nearly three times the second most frequent ethnicity, Hispanics (15.3%). Europe and the USA recruited more than half of the patients (937/1687), and had the majority of the participating centres (12 and 11 respictively). There were three centres in Canada, and only one in Korea, and one in Mexico. The Korean centre was located in Seoul, and of the patients recruited 166/169 (98.2%) were Korean. Similarly, the Mexican centre was located in Mexico city, and 201/203 (99%) of the recruited patients were Hispanic. The ethnicity of those classified as 'Other' constituted roughly 10 percent of the cohort, and comprised of 6 separate ethnic groups (109).

No Caucasian, Indo-Asian, Black African, or Black Caribbean, and only 5/166 (3.0%) of 'Other' ethnicities came from Mexico or Asia (Figure 3.2). Only 5/171 Korean patients came from outside of Korea, and whilst 56/257 (21.8%) of Hispanics came from outside of Mexico, the majority of them 47/56 (83.9%) from the USA.

Table 3.1: Baseline characteristics of patients in SLICC-bio

No. of patients	1687	
Age (years) (mean (SD))	35.2	(13.4)
Gender (%)		
Male	180	(10.7)
Female	1505	(89.3)
Premenopausal*	1328	(88.2)
Ethnic classification[†] (%)		
Caucasian	760	(45.2)
Indo-Asian	57	(3.4)
Black African	158	(9.4)
Black Caribbean	114	(6.8)
Korean	171	(10.2)
Hispanic	257	(15.3)
Other	166	(9.9)
CHD risk factors (mean (SD))		
BP systolic (mm Hg)	119.5	(16.7)
BP diastolic (mm Hg)	75.0	(11.0)
Glucose (mmol/L)	5.03	(1.63)
Waist (cm)	83.0	(14.0)
BMI (kg/m^2)	25.2	(5.92)
Smoker current (%)	252	(15.0)
Fulfils IDF criteria for MetS[†] (%)	404[†]	(35.3)
Lipid profile (mean (SD))		
Triglycerides (mmol/L)	1.60	(1.02)
Total cholesterol (mmol/L)	4.93	(1.48)
HDL-cholesterol (mmol/L)	1.39	(0.60)
Disease status		
Disease duration (weeks)[‡]	21.1	(8.9, 39.4)
SLEDAI[‡]	4	(2, 8)
Active disease (SLEDAI $\geq$6) (%)	650	(38.5)
SLICC/ACR DI $\geq$1 (%)	130	(7.7)
Disease phenotype (%)		
Active renal disease	365	(21.6)
Anti-dsDNA positive	579	(34.3)
Low Complement	569	(33.7)
Using medication (%)		
Antihypertensive	484	(28.7)
Lipid-lowering	171	(10.1)
Oral corticosteroid	1165	(69.1)
Average CS dose (mg)*[‡]	20	(10, 32)
Highest CS dose (mg)*[‡]	40	(20, 60)
Cumulative CS dose (g)*[‡]	2.5	(1.0, 4.9)
Antimalerial	1115	(66.1)
Immunosuppressant	657	(38.9)

See list of abbreviations for clarification of acronyms.
* For patients identified in the preceding variable.
[†] Ethnic classification/1683, Fulfils IDF Criteria for MetS/1145
[‡] Median (IQR)

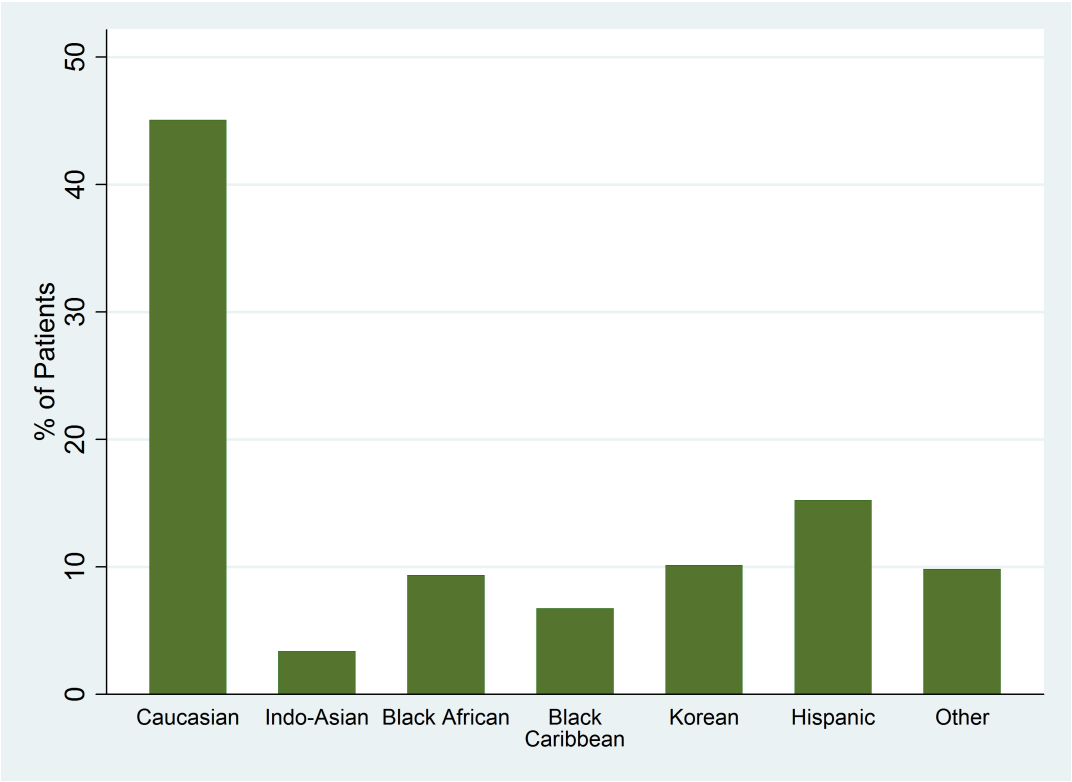


Figure 3.1: Ethnic classification of SLICC-bio cohort at enrolment.

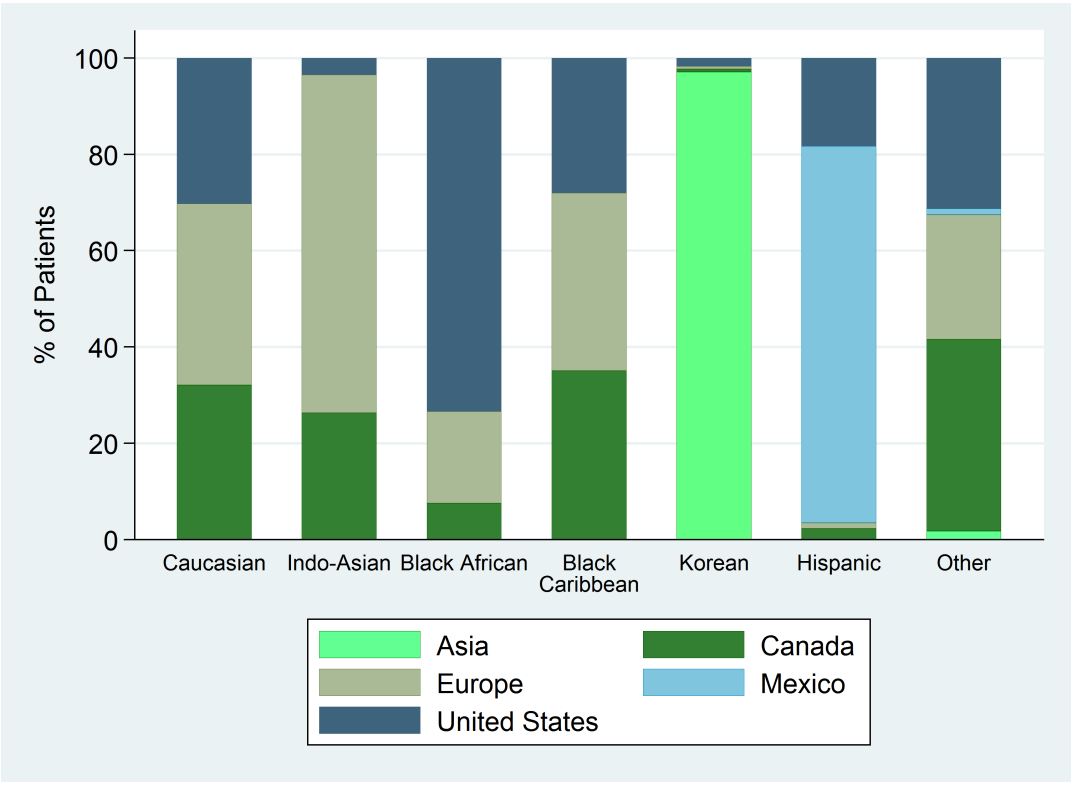


Figure 3.2: Ethnic classification of SLICC-bio cohort at enrolment.

3.1.2 Clinical Features and Disease Activity

The cohort showed was not overall hypertensive, although 484/1687 (28.7%) were taking antihypertensive medication. For the 252 (15%) patients whom smoked, the median (IQR) number of pack-years smoked was 14 (5.5, 22). The mean (SD) body mass index (BMI) of the cohort was 25.2 (5.92) kg/m^2, indicating that the patients were likely to be classed as overweight. There were two measures of disease activity used, overall SLEDAI score which gave a raw total out of 105, and number of patients with SLEDAI $\geq$6, which would equate to a moderate or severe disease flare (97). At enrolment a high proportion of patients 650/1687 (38.5%) were experiencing moderate or severe disease symptoms. The median SLEDAI of 4 meant more than half of all patients at enrolment were experiencing a flare-up in their lupus (117).

The cohort was not dyslipidemic, including 171 patients (10.1%) on lipid-lowering therapy. 1401 (83.1%) patients had some fasting lipid data available at enrolment, which classed as one or more of total cholesterol, TG, and/or HDL-C. Only 751 patients at enrolment, 550 at year 1, and 454 at year 2 had fasting lipid data for all three standard lipid profile markers. HDL-C had the highest frequency of missing lipid data, where less that half of patients 757/1687 had recorded values.

3.1.3 Comparison of Patients' Baseline Characteristics by Missing Lipid Data

286 patients did not have any lipid data. There were significant difference between the patients with and without lipid data. There were differences in race (p=$<$0.001), where fewer Black African (8.5%) and Hispanic (13.4%) ethnicities had lipid data than those with missing data (13.4%, and 24.5% respectively). Koreans made up only 1.1% of patients with missing lipid data, compared to 12.0%. Geographically, the USA made up 24.0% of patients with lipid data, but constituted more than half (51.1%) of patients missing data (p=$<$0.001). Both waist size [95% CI 1.07-7.14 (cm)] and BMI [95% CI 0.40-1.97 (kg/m^2)] were lower in those with lipid data. More patients with lipid data (11.1% versus 5.2%) were taking lipid lowering medications. Active disease was also significantly higher in more than one variable in those patients with lipid data (Table 3.2)

Table 3.2: Differences in disease activity between SLICC-bio patients whom are missing lipid data and the rest of the cohort*

	Patients with lipid data		Without lipid data		p Value [95% CI]
No. of patients	1401		286		
SLEDAI (Mdn (IQR))	4	(2, 8)	2	(0, 6)	0.001‡
Active disease (SLEDAI $\geq$6)	570	(40.8)	80	(30.1)	0.001
Active renal disease	317	(22.7)	48	(16.8)	0.029
Low Complement	498	(33.7)	71	(24.8)	$<$0.001
Anti-dsDNA positive	506	(36.1)	73	(25.5)	0.001

*Unless otherwise stated, values are n (%) of patients; p Values calculated using χ^2 test.
‡ Two-sample Wilcoxon rank-sum (Mann-Whitney) test.

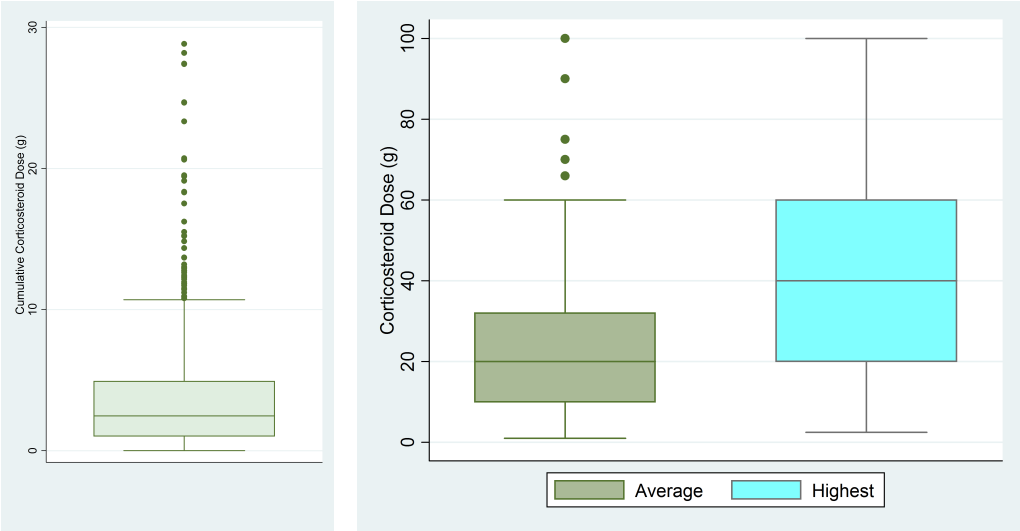
3.1.4 Corticosteroid Exposure in SLICC-bio Cohort

Three measures of corticosteroid therapy were used to evaluate steroid exposure at enrolment (Figure 3.4(b)). 69.3% of patients were taking oral corticosteroid, with a median average dose of 20mg and a largest average dose of 100 mg/day. As we would expect the highest median dose taken by patients is higher than the average dose, 40 mg/day. The final measure of steroid exposure is the cumulative corticosteroid dose (Figure 3.4(a)). The distribution of this variable was significantly positively skewed (Figures D.2(l) and D.3(d)), with most patients averaging around 2.5 grams of cumulative oral corticosteroid consumed, but the 95th and 99th percentiles (not shown in graph) were greater by several orders of magnitude at 71.4 and 169 grams, respectively.

3.1.5 Logistic Regression of Hypercholesterolemia in SLE

We performed univariate logistic regression analyses with total cholesterol $\geq$6.21 mmol/l as our outcome measure, and several predictor variables associated with disease status and inflammation. These were disease duration, SLEDAI score, and the presence of active renal disease, low complement, and anti-double stranded DNA antibodies. The confounders were age, gender, ethnic classification, BMI, presence of clinically defined hypertension, number of cigarette pack-years smoked, current drug therapy with antimalarials and oral corticosteroids, and cumulative steroid dose. Significant p-values of < 0.2 (Table 3.3) were used in a multivariate model.

Figure 3.3: Steroid treatment at enrolment



(a) Cumulative dose

(b) Average and highest doses

Table 3.3: Significant univariate analyses of hypercholesterolemia at enrolment

	p-Value	Odds Ratio [95% CI]
Predictor		
Age	<0.001	1.014 [1.005 - 1.023]
Gender	0.003	1.743 [1.210 - 2.510]
Ethnicity	0.012	1.031 [1.007 - 1.055]
BMI (kg/m^2)	0.147	1.016 [0.995 - 1.038]
Hypertension	<0.001	3.776 [2.892 - 4.930]
On antimalarial	<0.001	0.343 [0.266 - 0.446]
On oral CS	<0.001	2.245 [1.656 - 3.043]
Cumulative oral CS (g)	<0.001	1.073 [1.004 - 1.105]
Disease duration	0.153	0.995 [0.988 - 1.002]
SLEDAI	<0.001	1.076 [1.053 - 1.100]

Abbreviations: CI confidence interval; BMI body mass index; CS corticosteroid; dsDNA double-stranded DNA antibody

Table 3.4: Significant multivariate analyses of hypercholesterolemia at enrolment

	Odds Ratio	**[95% CI]**
Predictor, n = 1381		
Age (years)	1.024	[1.013 - 1.034]
Hypertension	2.108	[1.564 - 2.841]
On antimalarial	0.507	[0.382 - 0.674]
On oral CS	1.451	[1.034 - 2.038]
Active renal disease	3.944	[2.836 - 5.486]

Age is a continuous variable with risk of hypercholesterolemia increasing by 1 year.
Abbreviations: CI confidence interval; CS corticosteroid

In the univariate analyses number of pack-years smoked, low complement, and anti-dsDNA positive patients were not associated with raised cholesterol levels. Stepwise backwards logistic regression was used to assess which of the significant remaining predictors were associated with hypercholesterolemia at enrolment. Before the final model was determined a significant covariance between active renal disease, SLEDAI, and disease duration ($p=<0.001$) had no effect on the independence of these predictors.

The final model (Table 3.4) showed that increasing age (years), hypertension, current use of oral corticosteroids, and active renal disease were associated with an increased likelihood of hypercholesterolemia. Current use of antimalarial medication was associated with lower odds of having a high total cholesterol. The Hosmer-Lemeshow diagnostic test showed that the model was a good fit, and the ROC-AUC for this model of 0.77 indicates that the model is 'fair' to 'good' at determining hypercholesterolemia at enrolment.

3.1.6 Logistic Regression of Hypertriglyceridemia in SLE

Next we performed a similar logistic regression analysis to the previous one, this time using high triglyceride levels as our outcome. We used the same group of predictors associated with disease status and inflammation, and the same group of confounding variables (Table 3.5).

We did not find a significant enough association between age, number of pack-years smoked, disease duration, low complement, or patients whom were anti-dsDNA positive and high triglyceride levels in univariate analyses. Stepwise backwards logistic regression was used again to create a multivariate model of inde-

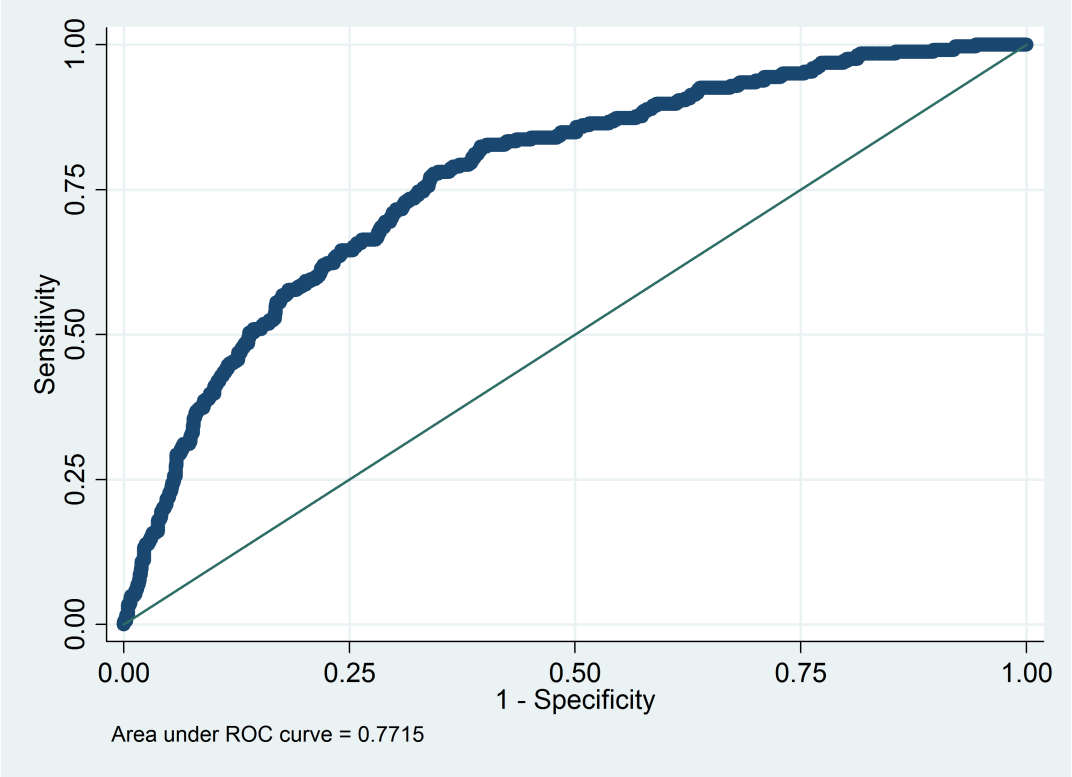


Figure 3.4: ROC curve for multivariate model (Table 3.4) of the predictors of hypercholesterolemia.

Table 3.5: Significant univariate analyses of hypertriglyceridemia at enrolment

	p-Value	Odds Ratio [95% CI]
Predictor		
Gender	<0.001	2.370 [1.659 - 3.387]
Ethnicity	<0.001	1.045 [1.021 - 1.070]
Hypertension	<0.001	2.856 [2.209 - 3.692]
On antimalarial	<0.001	0.405 [0.314 - 0.522]
Pack years smoked	0.122	1.010 [0.997 - 1.023]
On oral CS	<0.001	1.908 [1.427 - 2.550]
Cumulative oral CS	0.082	1.002 [0.998 - 1.043]
Disease duration	0.028	0.992 [0.985 - 0.999]
SLEDAI	<0.001	1.085 [1.061 - 1.110]
Active renal disease	<0.001	4.058 [3.076 - 5.353]

Abbreviations: CI confidence interval; BMI body mass index; CS corticosteroid

Table 3.6: Significant multivariate analyses of hypertriglyceridemia at enrolment

	Odds Ratio	[95% CI]
Predictor, n = 1672		
Gender	1.705	[1.153 - 2.521]
Korean ethnicity	1.774	[1.025 - 3.071]
Hypertension	2.070	[1.556 - 2.753]
On antimalarial	0.555	[0.420 - 0.733]
SLEDAI	1.041	[1.014 - 1.069]
Active renal disease	1.923	[1.358 - 2.722]

Abbreviations: CI confidence interval

pendently significant predictors of hypertriglyceridemia (Table 3.6). Interaction terms to account for the covariance between disease duration, SLEDAI score, and active renal disease were also included.

Ethnic classification was significant at the univariate level, and the multiple indicators contained with this factor variable were treated individually. In this analysis (Table 3.6) we found that Korean ethnicity conferred an increased likelihood of having a high triglyceride level. Gender, hypertension, active real disease, SLEDAI score, and current use of antimalarial medication were also independently associated with hypertriglyceridemia. The AUC was 0.73, indicating a 'fair' predictive power for determining high triglycerides in patients at enrolment.

3.1.7 Logistic Regression of Hypoalphalipoproteinemia in SLE

The final logistic regression for the SLICC-bio cohort was looking at the association of SLE disease and inflammation with low levels of HDL-C. The association of ethnic classification with low HDL-C at the univariate level (Table 3.7) was investigated in the multivariate model by expanding this factor variable and analysing the indicators using stepwise backwards logistic regression, alongside other significant univariate predictors.

In the final multivariate model (Table 3.8) Korean and Hispanic ethnicities, active renal disease or anti-dsDNA positive phenotypes, current use of antimalarial medication, and SLEDAI score were independently associated with hypoalphalipoproteinemia. The ROC-AUC of 0.69 suggests the model makes a 'fair' to 'poor' determination of whether a patient has low HDL-C at enrolment.

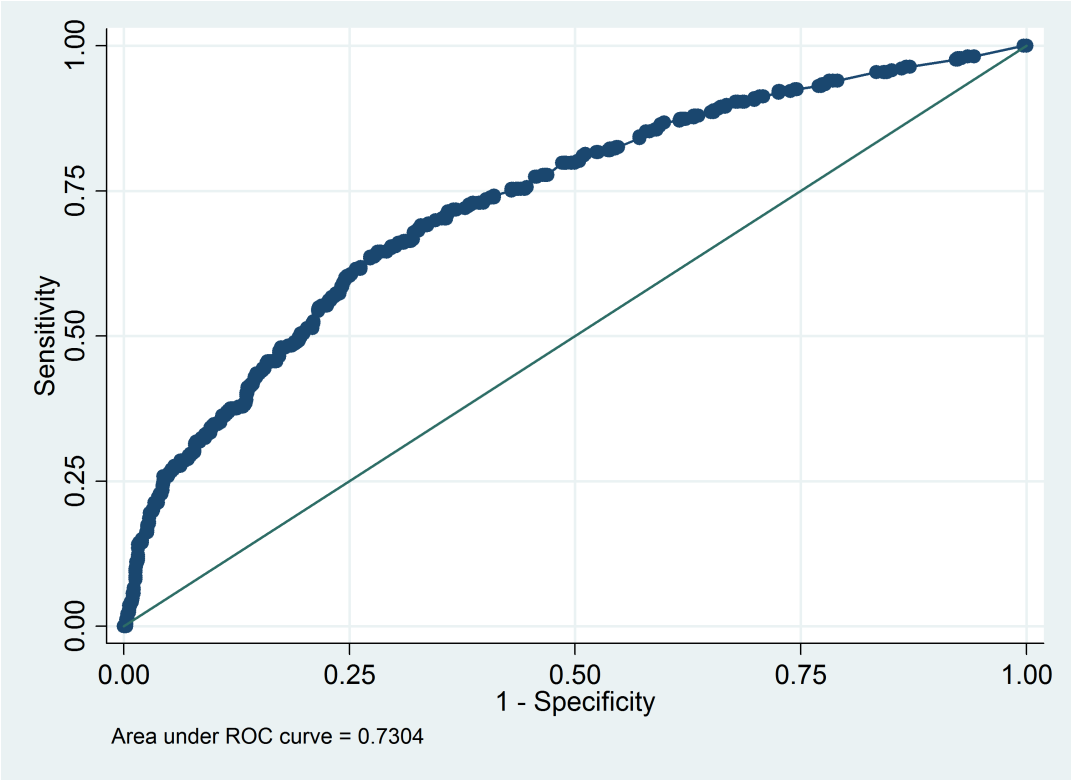


Figure 3.5: ROC curve for multivariate model (Table 3.6) of the predictors of hypertriglyceridemia.

Table 3.7: Significant univariate analyses of hypoalphalipoproteinemia at enrolment

	p-Value	Odds Ratio [95% CI]
Predictor		
Age	0.006	0.982 [0.970 - 0.995]
Ethnicity	0.007	1.058 [1.011 - 1.076]
BMI (kg/m^2)	0.006	0.954 [0.923 - 0.986]
Hypertension	0.084	0.749 [0.540 - 1.040]
Pack years smoked	0.121	0.985 [0.967 - 1.004]
On antimalarial	0.047	0.715 [0.513 - 0.996]
Cumulative oral CS	0.102	0.956 [0.903 - 1.009]
Disease duration (weeks)	0.005	0.986 [0.977 - 0.996]
SLEDAI	$<$0.001	1.056 [1.026 - 1.087]
Low complement	$<$0.001	1.834 [1.325 - 2.539]
Anti-dsDNA positive	$<$0.001	2.172 [1.567 - 3.013]

Age is a continuous variable with risk of hypoalphalipoproteinemia increasing by 1 year.
Abbreviations: CI confidence interval; BMI body mass index; CS corticosteroid; dsDNA double-stranded DNA antibody

Table 3.8: Significant multivariate analyses of hypoalphalipoproteinemia at enrolment

	Odds Ratio	[95% CI]
Predictor, n = 753		
Korean ethnicity	4.414	[1.916 - 10.169]
Hispanic ethnicity	3.503	[1.499 - 8.185]
On antimalarial	0.657	[0.455 - 0.950]
Active renal disease	0.409	[0.246 - 0.681]
SLEDAI	1.062	[1.021 - 1.069]
Anti-dsDNA positive	2.130	[1.551 - 2.926]

Abbreviations: CI confidence interval; dsDNA double-stranded DNA antibody

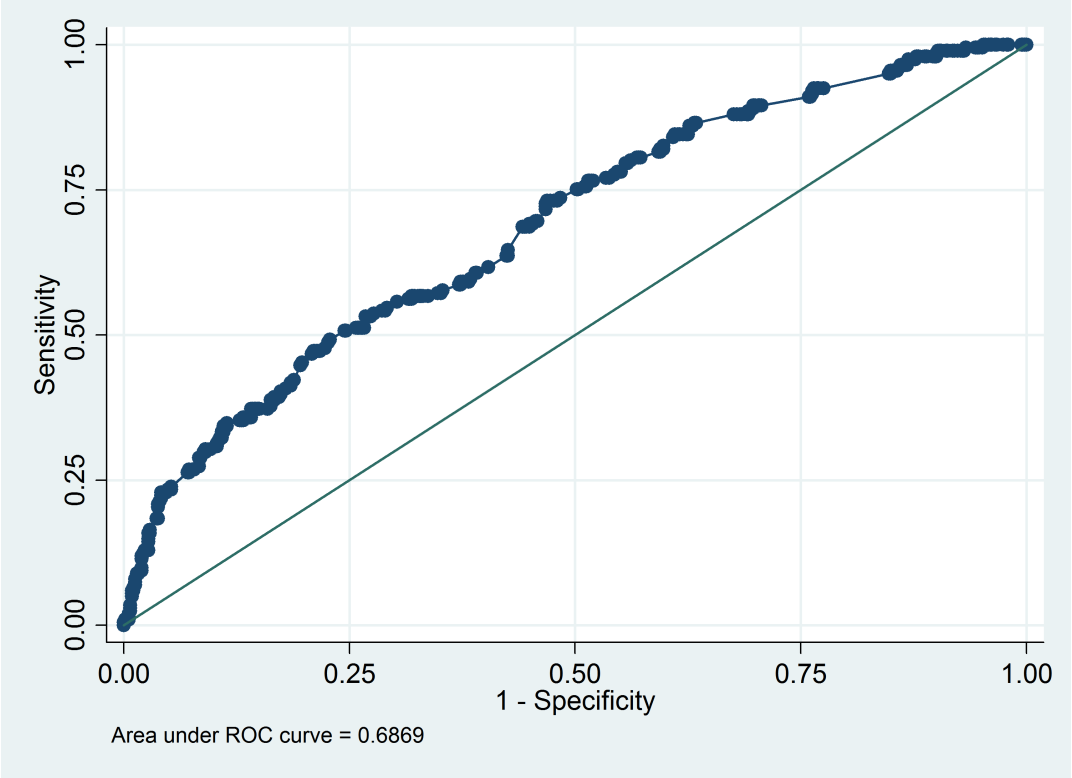


Figure 3.6: ROC curve for multivariate model (Table 3.8) of the predictors of hypoalphalipoproteinemia.

Table 3.9: Inflammatory and metabolic biomarker measurements in a subset of patients from the SLICC-RAS

	Enrolment			Year 1			Year 2		
	n	Mdn	IQR	n	Mdn	IQR	n	Mdn	IQR
Inflammatory biomarkers									
Interleukin 18 (ng/ml)	319	0.36	(0.29)						
Interleukin 18 Binding Protein (ng/ml)	319	19.1	(17.1)						
Tumor Necrosis Factor Alpha (pg/ml)	538	16.5	(56.5)	296	16.7	(80.5)	99	20.1	(96.1)
B Lymphocyte Stimulator (ng/ml)	318	0.44	(0.30)						
High-Sensitivity C-Reactive Protein (mg/l)	548	0.94	(3.61)	386	0.92	(3.04)	103	0.96	(3.37)
Interleukin 6 (pg/ml)	389	2.31	(5.25)	303	1.55	(3.33)	103	2.48	(5.41)
Metabolic biomarkers									
Insulin (mU/l)	418	20.7	(23.3)	202	18.6	(16.7)	64	16.0	(14.2)
Lepin (ng/ml)	548	16.7	(25.0)	386	19.7	(29.6)	103	19.3	(34.8)
Adiponectin (mg/l)	548	3.07	(2.26)	386	3.24	(2.14)	103	2.92	(1.89)
Oxidised Low-Density-Lipoprotein (U/l)	233	25.8	(13.6)						
Total (%)	4178 (62.2)			1959 (29.2)			575 (8.6)		

3.2 Biomarker Cohort Analysis

3.2.1 Description of Biomarker Data

A total of 650/1687 (38.5%) patients had additional biomarker data at enrolment. In addition the SLICC-bio dataset contained two years worth of follow-up data on patients and biomarker results (Table 3.9). 57 patients had biomarker data for one or more follow-up visits, and were included in the biomarker cohort, but did not have biomarker data at enrolment. These patients were excluded from any analyses of baseline measurements. There was complete biomarker data for 118 patients at enrolment, however, none of the follow-up data was complete. The most frequent missing biomarker data at enrolment was oxLDL, 417/650 (64.2%), followed by blys, IL-18 and IL-18BP, and IL-6.

Table 3.10: Key differences between patients with and without biomarker data at enrolment*

	Bio data	No bio data	p Value [95% CI]
No. of patients	650	1035	
Country			<0.001
Asia	124 (19.1)	45 (3.34)	
Canada	227 (34.9)	160 (15.4)	
Europe	175 (26.9)	271 (26.1)	
Mexico	0 (0)	203 (19.6)	
United States	124 (19.1)	358 (34.5)	
Ethnic classification			<0.001
Caucasian	326 (50.2)	434 (42.0)	
Indo-Asian	25 (3.39)	32 (3.10)	
Black African	37 (5.70)	121 (11.7)	
Black Caribbean	49 (7.54)	65 (6.29)	
Korean	122 (18.8)	49 (4.29)	
Hispanic	18 (2.77)	239 (23.1)	
Other	73 (11.23)	93 (9.00)	
CHD risk factors (mean (SD))			
Waist circumference (cm)	81.6 (13.4) *n* = 612	83.9 (14.3) *n* = 819	0.002 [0.84 to 3.76]†
BMI (kg/height^2)	24.8 (5.89) *n* = 646	25.5 (5.93) *n* = 960	0.023 [0.09 to 1.27]†
HDL-cholesterol (mmol/L)	1.44 (0.62) *n* = 414	1.34 (0.58) *n* = 343	0.019 [-0.19 to -0.02]†
Disease status			
SLEDAI (Mdn (IQR))	4 (2, 8) *n* = 650	4 (1, 8) *n* = 1037	<0.001‡
Active disease (SLEDAI ≥6)	282 (43.5)	368 (36.3)	0.004
Anti-dsDNA positive	267 (41.1)	312 (30.1)	<0.001
Low complement	262 (40.3)	307 (29.6)	<0.001
Using medication			
Antihypertensive	166 (25.5)	318 (30.7)	0.023
Lipid-lowering	90 (13.9)	81 (7.81)	<0.001
Oral corticosteroid (Mdn (IQR))			
Cumulative CS dose (g)	2.2 (0.9, 4.4) *n* = 429/445	2.6 (1.1, 5.3) *n* = 683/720	0.009‡

*Unless otherwise stated, values are n (%) of patients; p Values calculated using χ^2 test.
† Two-tailed T-test.
‡ Two-sample Wilcoxon rank-sum (Mann-Whitney) test.

3.2.2 Comparison with SLICC-bio cohort

The key differences between the biomarker and SLICC-bio cohorts are outlined in Table 3.10. There were no significant differences between these two groups in terms of age, gender, or disease duration, however, there was notable differences in country and ethnicity. There was no biomarker data for any patients from Mexico, a disproportionally high number of Asian (19.1%) and Canadian (34.9%) patients with biomarker data, and a comparatively low number of patients from the United States (19.1%). This is reflected in the ethnic classification, with only 18/650 (2.83%) of patients with biomarker data identifying as Hispanic compared with 239/1035 (23.1%) patients without biomarker data. Korean ethnicity is over represented in the biomarker group where they make up 122/650 (18.8%) of patients, compared with 49/1035 (4.29%) in the non-biomarker group.

Due to relatively limited data from some countries in the biomarker cohort, for the purposes of analysis we grouped together Caucasians (50.2%), Koreans (18.8%), individuals with African ancestry (13.2%), and others (17.8%) into separate categories. The 'Other' group comprised of Hispanics, Indo-Asian, non-Korean Asians, individuals of mixed race, and other small minority or native aboriginal ethnicities.

Key differences were also noted in CHD risk factors, disease status, and medication use between patients with and without biomarker data at enrolment. The proportion of patients in the biomarker group with signs of active disease were all significantly higher than those without biomarker data. In the biomarker cohort patients were on average prescribed more antihypertensive and lipid-lowering medication than those without biomarker data. However this was not true of the cumulative dose of corticosteroids, which was lower in the biomarker cohort (Figure 3.7).

3.2.3 Comparison of Patients With and Without MetS

In the SLICC-bio cohort, there were 1145 patients at enrolment with sufficient data to define the presence or absence of MetS (Table E.1). A further 542 patients in the group at enrolment had data for MetS diagnostic criteria, but these were insufficient to confirm the presence of MetS. In the biomarker cohort (Ta-

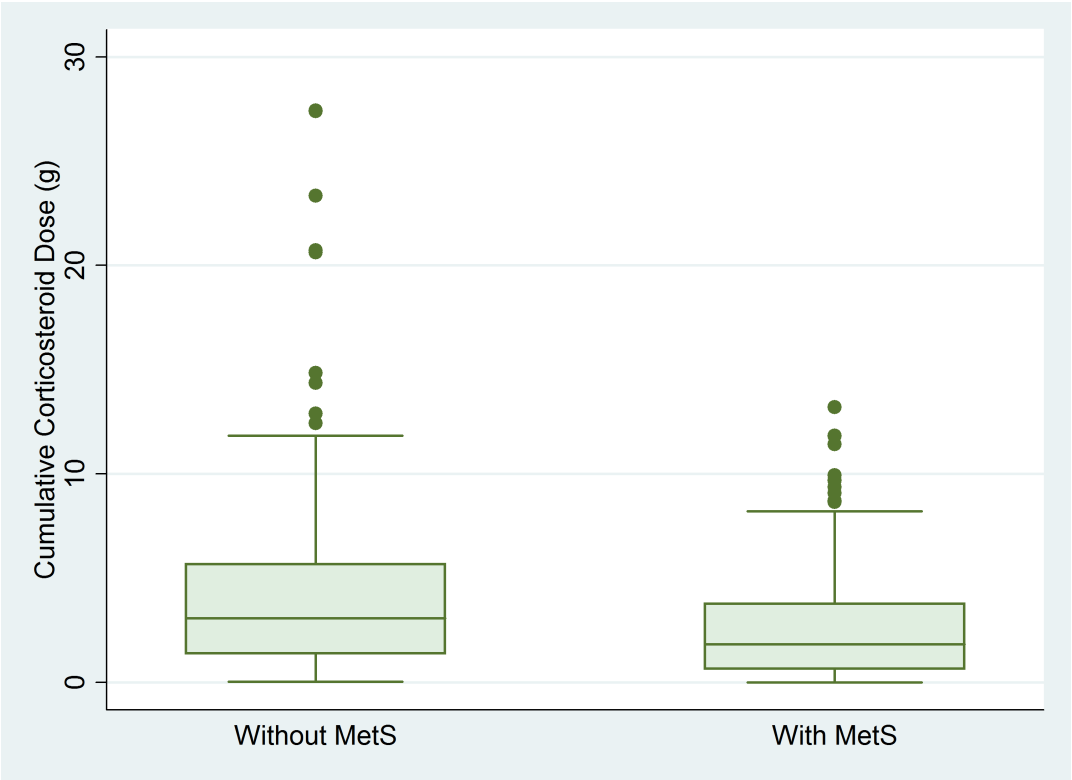


Figure 3.7: Cumulative steroid dose compared in biomarker cohort patients whom either fulfil, or do not fulfil the IDF 2009 criteria for MetS at enrolment.

ble 3.11), 630 patients had sufficient data to categorise their MetS status, of whom 32.5% met the IDF 2009 criteria at baseline. MetS was overrepresented in males compared with females. Disease activity, and the quantity and strength of medications was higher in those with MetS, whom were also more likely to have dyslipidemia. Fasting glucose, raised blood pressure, and waist circumference, which all form part of the IDF 2009 criteria, were unsurprisingly raised in patients with MetS, as was BMI, which is closely related but not part of the MetS diagnosis.

Table 3.11: Key differences between 686 patients with additional biomarker data, whom either fulfil or do not fulfil the IDF 2009 criteria for MetS at enrolment*

	With MetS	Without MetS	p Value [95% CI]
No. of patients	205	425	
Gender			0.017
Male	27 (13.2)	31 (7.3)	
Female	178 (86.8)	394 (92.7)	
CHD risk factors (mean (SD))			
BP systolic (mm Hg)	125.4 (16.2) *n* = 205	115.0 (13.9) *n* = 425	<0.001 [-12.9 to -8.01]†
BP diastolic (mm Hg)	78.4 (10.6) *n* = 205	72.4 (9.29) *n* = 425	<0.001 [-7.63 to -4.38]†
Glucose (mmol/L)	5.80 (2.04) *n* = 191	4.64 (0.73) *n* = 407	<0.001 [-1.38 to -0.93]†
Waist (cm)	88.5 (13.7) *n* = 191	78.2 (11.8) *n* = 407	<0.001 [-12.4 to -8.12]†
BMI (kg/height^2)	27.1 (6.48) *n* = 203	23.6 (5.04) *n* = 423	<0.001 [-4.42 to -2.56]†
Triglycerides (mmol/L)	2.14 (1.21) *n* = 198	1.25 (0.68) *n* = 412	<0.001 [-1.04 to -0.74]†
Total cholesterol (mmol/L)	5.40 (1.78) *n* = 198	4.63 (1.19) *n* = 412	<0.001 [-1.00 to -0.52]†
HDL-cholesterol (mmol/L)	1.31 (0.58) *n* = 140	1.51 (0.63) *n* = 269	<0.001 [0.08 to 0.33]†
Disease status			
SLEDAI (Mdn (IQR))	6 (2, 10) *n* = 205	4 (2, 8) *n* = 425	0.005‡
Active disease (SLEDAI ≥6)	103 (50.2)	164 (38.7)	0.006
Active renal disease	73 (36.0)	63 (14.8)	<0.001
Using medication			
Antihypertensive	120 (58.5)	41 (9.7)	<0.001
Lipid-lowering	84 (41.0)	5 (1.2)	<0.001
Antimalerial	115 (56.1)	314 (74.1)	<0.001
Immunosuppressant	107 (52.5)	145 (34.1)	<0.001
Oral corticosteroid	157 (76.6)	277 (65.2)	0.004
Average CS dose (mg, Mdn (IQR))	25 (15, 40) *n* = 155/157	16.5 (10, 30) *n* = 276/277	<0.001‡
Highest CS dose (mg, Mdn (IQR))	45 (30, 60) *n* = 154/157	30 (15, 50) *n* = 274/277	<0.001‡
Cumulative CS dose (g, Mdn (IQR))	3.1 (1.4, 5.7) *n* = 152/157	1.8 (0.7, 3.8) *n* = 267/277	<0.001‡

*Unless otherwise stated, values are n (%) of patients; p Values calculated using χ^2 test.
† Two-tailed T-test.
‡ Two-sample Wilcoxon rank-sum (Mann-Whitney) test.

Table 3.12: Significant univariate analyses of the initial biomarkers in the biomarker cohort at enrolment

	p-Value	Odds Ratio [95% CI]
Predictor		
Age	<0.001	1.037 [1.024 - 1.049]
Gender	0.003	2.196 [1.317 - 3.662]
Leptin	<0.001	1.023 [1.014 - 1.032]
oxLDL	0.008	1.029 [1.008 - 1.051]

Age is a continuous variable with risk increasing by every 1 year.
Abbreviations: CI confidence interval; oxLDL oxidised LDL

3.2.4 Logistic Regression of MetS Status

Due to the limited number of patients with complete biomarker data, and the relatively small number of observations for some of the biomarkers, such as oxLDL, it was decided to run logistic regression analyses in stages. This allowed for a better chance that a weak signal may be observed, which may otherwise be indistinguishable in a multivariate analysis that included more variables (118). We first examined the four biomarkers highlighted in the research proposal (110, 111). Univariate analyses were performed on these predictor variables, and age, gender, and ethnic classification were also tested as potential confounders. The outcome variable was whether the patient had been diagnosed with MetS. Significant predictors in univariate models (Table 3.12) were then tested using stepwise backwards logistic regression in a multivariate model.

Race, IL-18, and hsCRP were all insignificant at the univariate level. The remaining predictor variables, leptin and oxLDL, were significantly covariant (p=0.0368) that an interaction term between them was included in the multivariate model. The covariance between lepin and oxLDL was significant and was not included in the final model (Table 3.13). None of the variables that were significant at the univariate level dropped out of the multivariate regression. The Hosmer-Lemeshow test showed the model was a good fit, and the ROC-AUC of 0.76 showed that the model was fairly good at predicting MetS status at enrolment.

Following on from this analysis, the next step was to introduce treatment variables to the multivariate regression and observe the effect they have on the model (Table 3.14). In univariate analyses current treatment with antimalarials or corticosteroids, and cumulative steroid dose were all significant. An interaction term

Table 3.13: Multivariate analyses of the initially significant biomarkers in the biomarker cohort at enrolment

	Odds Ratio	[95% CI]
Predictor, n = 228		
Age	1.038	[1.0134 - 1.062]
Gender	3.642	[1.241 - 10.69]
Leptin	1.033	[1.015 - 1.051]
oxLDL	1.31	[1.007 - 1.055]

Age, leptin, and oxLDL are continuous variable with risk increasing by every, 1 year, 1 ng/ml, and 1 U/L respectively.
Abbreviations: CI confidence interval; oxLDL oxidised LDL

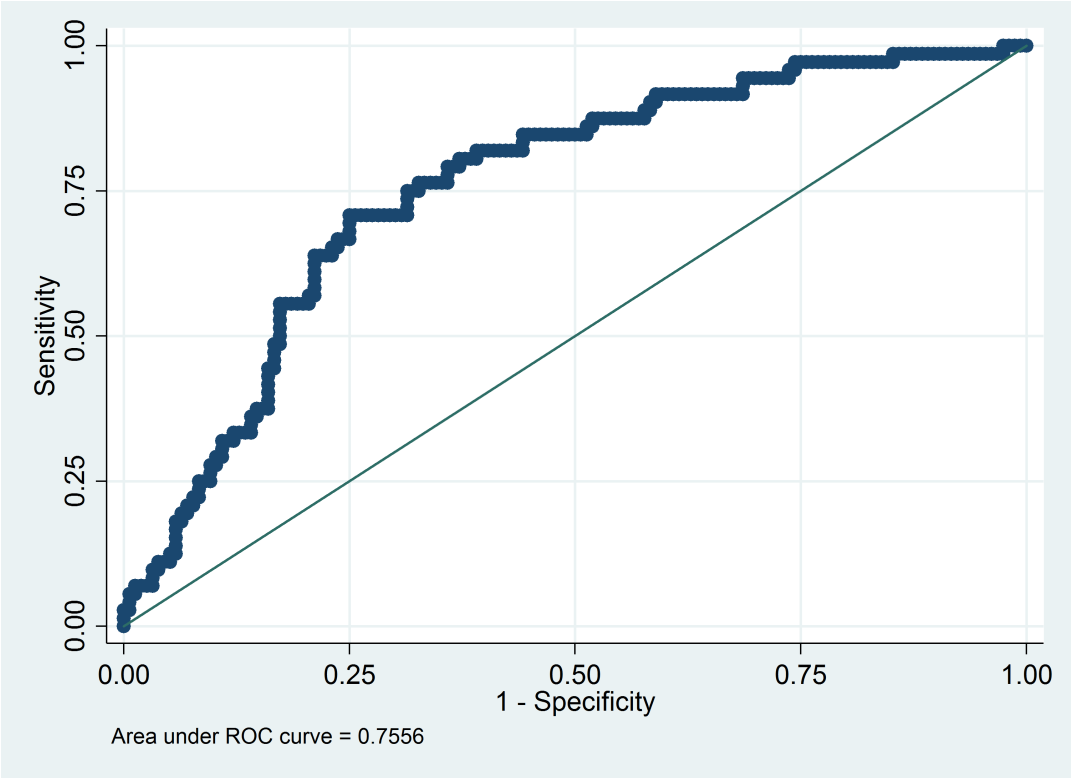


Figure 3.8: ROC curve for multivariate model (Table 3.13) of initially significant biomarkers in the biomarker cohort at enrolment.

Table 3.14: Multivariate analyses of significant initial biomarkers and treatment variables in the biomarker cohort at enrolment

	Odds Ratio	**[95% CI]**
Predictor, n = 517		
Age	1.037	[1.022 - 1.053]
Gender	2.152	[1.090 - 4.248]
Leptin	1.023	[1.013 - 1.033]
On antimalarial	0.475	[0.311 - 0.726]
Cumulative oral CS	1.071	[1.014 - 1.128]

Age, and leptin are continuous variable with risk increasing by every, 1 year, and 1 ng/ml respectively.
Abbreviations: CI confidence interval; CS corticosteroid

between leptin and oxLDL was again added, alongside the treatment variables to stepwise backwards logistic regression.

Current antimalarial treatment, and cumulative steroid dose both remained in the model, whilst oxLDL was no longer significant in their presence. This also had the effect of increasing the number of observations from 228 to 517, because the analysis was no longer limited by the number of oxLDL observations, which initially restricted the multivariate analysis to 34% of the cohort. The model passed the goodness-of-fit test, and the ROC-AUC for predicting MetS status at enrolment was 0.75 (Figure 3.9)

The final stage of this progressive analysis was to perform univariate analyses for the remaining biomarkers, and include all significant variables into a final model. Insulin and IL-18BP reached significance at the univariate level. A stepwise backwards logistic regression was then performed. Whilst this time age, gender, and leptin remained in the model, insulin was favoured over the treatment variables in the previous model (Table 3.14) The best final model included insulin level as an independent predictor of MetS status. This model had a Hosmer-Lemeshow p-value of 0.82 and the ROC-AUC signified it was fairly good at predicitng MetS status at enrolment.

4 Discussion

In this thesis we have described that the level of lipid subfractions in patients with SLE is influenced by disease phenotype and level of disease activity. Furthermore an investigation of novel biomarker data suggest that leptin and oxLDL my

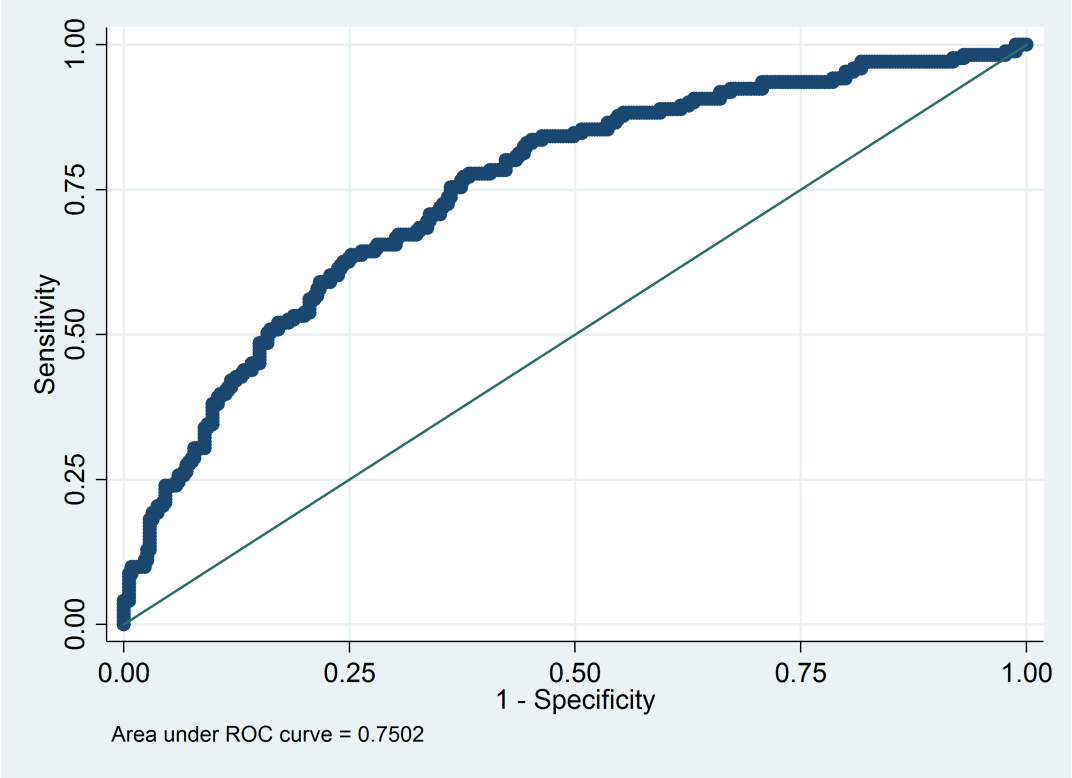


Figure 3.9: ROC curve for multivariate model (Table 3.14) of significant biomarkers and treatment variables in the biomarker cohort at enrolment.

Table 3.15: Multivariate analyses of all significant univariate predictors of MetS in the biomarker cohort at enrolment

	Odds Ratio	**[95% CI]**
Predictor, n = 312		
Age	1.042	[1.022 - 1.064]
Gender	2.976	[1.172 - 7.558]
Leptin	1.026	[1.012 - 1.040]
Insulin	1.012	[1.001 - 1.022]

Age, leptin, and insulin are continuous variable with risk increasing by every, 1 year, 1 ng/ml, and 1 mU/L respectively.
Abbreviations: CI confidence interval; CS corticosteroid

have an effect on the outcome of MetS status, but these associations are weakened by the inclusion of confounding variables that may mask the significance of these finding. Biochemical insulin levels are associated with MetS status, and are a driving force in developing metabolic dysfunction. Despite insulin's strong association with MetS, leptin remained significant in the final multivariate analysis. This analysis also demonstrates that when adjusted for age and gender, leptin levels seem to remain a fairly good independent predictor of MetS.

The baseline characteristics of the cohort were not abnormal compared with previous analysis of this dataset (102). The cohort was relatively young, but not overburdened with disease. There was significant difference in the ethnic and geographical regions represented in the cohort. Koreans and Mexicans predominantly are from single centres in Korea and Mexico, respectively. The predominance of Caucasian ethnicity in the SLICC-bio cohort also means that the results are less applicable to other ethnic classifications, but the overall cohort remains significantly diverse.

The key differences in the subgroup analysis of biomarker data (Table 3.10) show a significant change in the ethnic and geographical make up of the biomarker cohort. There are relatively more Korean samples in the biomarker cohort (18.8%) compared with patients whom did not have fasting samples (4.3%). In contrast, there were no fasting samples obtained from the centre in Mexico, and this has a profound affect on the proportion of Hispanic patients included in the biomarker cohort, 2.8% versus 23.1% in patients without fasting samples. Both the USA and Mexico are poor at obtaining fasting blood samples, whereas the majority of Korean patients do have fasting samples (109). Hispanic or Black African ethnicity has previously shown an independent association with MetS (98), and the lack of representation by Hispanic patients has had an effect on the overall make up of the biomarker cohort. Hispanic patients have a higher rate of central obesity compared with Korean patients, whom have a greater prevalence of MetS at baseline (98). This can be seen in increased odds of low HDL-C levels, which are independently associated with both of these ethnicities (Table 3.8. Hispanic patients also have a higher cumulative steroid dose at enrolment compared with Koreans whom have relatively low exposures to corticosteroid at enrolment (102), which accounts for the paradoxical increase in measures of disease activity and CHD risk factors in the biomarker cohort, while the cumulative steroid dose is

significantly lower (Figure 3.7). This also provides an explanation as to the difference in disease activity between patients with and without fasting blood samples (Table 3.2).

We show that the multivariate analysis of hypercholesterolemia (Table 3.4) demonstrate significant independent associations with age, hypertension, current antimalarial and corticosteroid treatment, and the presence of active renal disease. The involvement of renal disease profoundly increases the risk of raised total cholesterol (odds ratio (95% CI)) (3.944 (2.836 - 5.486)), and is associated also with increased disease severity and activity. Active renal disease is also positively associated with raised triglyceride levels (1.923 (1.358 - 2.722)) and low HDL-C (2.130 (1.551 - 2.926)). Antimalarial treatment was the other variable that showed a significant independent negative correlation across all three lipid outcome. This evidence support the already well established theory that Antimalarial therapy is affective in preventing dyslipidemia.

Interestingly, corticosteroids do not appear to have an effect on high fasting triglyceride levels or low HDL-C. Previous studies examining the relationship between CHD risk factors and glucocorticoid therapy use in lupus patients (119), have found that higher corticosteroid dose over the preceding year was associated with raised serum triglyceride levels. Due to the short average disease duration, 21.1 weeks, of patients in the SLICC register, it may be that sufficient exposure to steroids over a longer time frame is necessary to induce hypertriglyceridemia. Current use of corticosteroid is associated with high cholesterol levels (1.451 (1.034 - 2.038)) in the multivariate analysis, but there were no effects of cumulative steroid dose on lipid subfractions outside of the univariate analysis. The increase in SLEDAI score associated with risk of hypertriglyceridemia (1.041 (1.014 - 1.069)) and hypoalphalipoproteinemia (1.062 (1.021 - 1.069)) is in keeping with the known affects of disease activity and inflammation on lipid profiles in lupus (97).

We took into account that lipid levels may be more normal due to lipid-lowering medication prescribed to treat dyslipidemia. The analysis of lipid subfractions was re-run with the exclusion of patients taking lipid-lowering medication. In the multivariate analysis of hypercholesterolemia (n = 1227) and hypertriglyceridemia (n = 1183), hypertension was no longer significantly associated with those out-

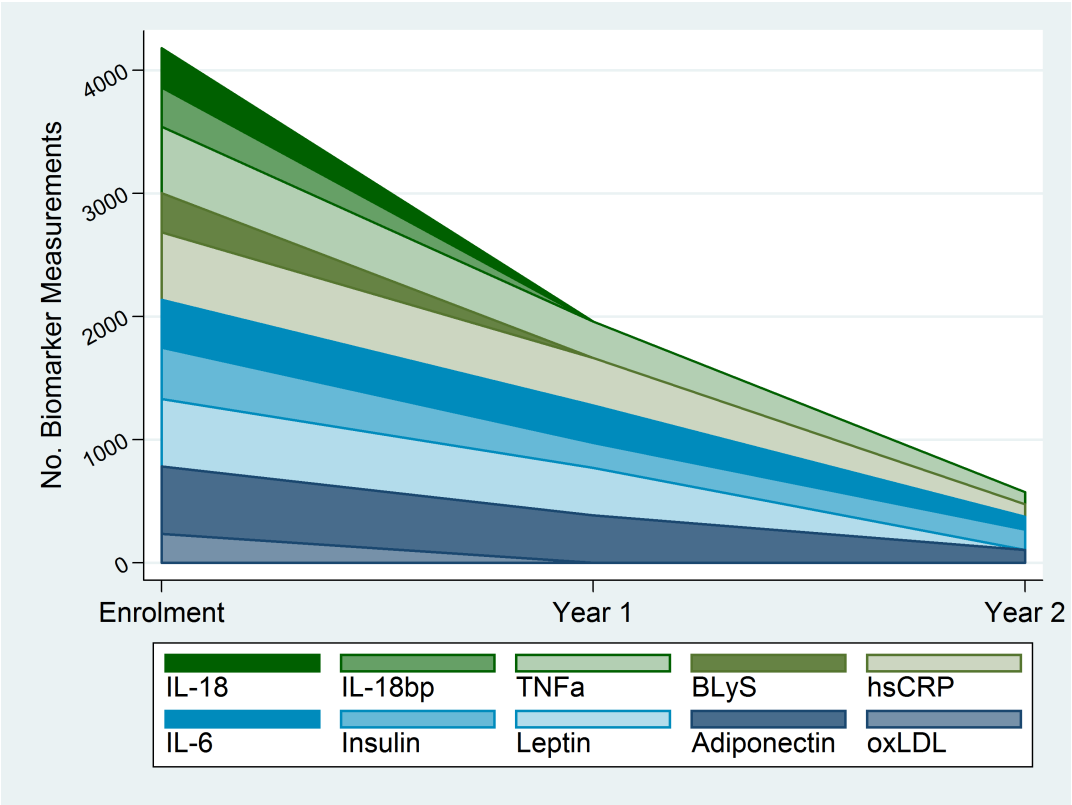


Figure 4.1: Number of biomarker samples at enrolment and follow-ups one and two.

comes. However, each analysis suffers form a reduced number of patients, and the ROC-AUCs are less predictive overall of the the lipid outcome. The use of a binary measure of hypertension does also weaken the impact of its association with the outcome variables, because it condenses all the variance in hyper- and normotensive readings down to a single measure for each.

Initially we planned to perform a longitudinal analysis on the relationship between the inflammatory biomarkers and their association with MetS status. Unfortunately the quantity of biomarker samples processed for the follow-up visits was significantly fewer than were available at enrolment (Figure 4.1). It was necessary in the given time frame to adapt the study to look only at the cross-sectional data at enrolment. Whilst this maintains statistical power in the analyses, it significantly reduces the scope of the investigation.

We found that leptin levels were significantly associated independently with MetS in SLE. Leptin was a significant predictor in all three multivariate analyses. oxLDL was showed initial significance (1.31 (1.007 - 1.005)) in the first multivariate analysis, but this association did not hold with the introduction of the treatment vari-

ables to the model. The degree of separation of oxLDL from the outcome may be a factor in this. Where the underlying pathology caused oxLDL to initiate tissue inflammation, which is then subject to treatment with drug therapies. The original cause of this cascade is already several steps removed from other confounders which may influence the outcome. This may be the case also with the inclusion of insulin into the multivariate model, where we see oxLDL and the treatment variables become insignificant in comparison. If oxLDL leads to corticosteroid treatment which leads to insulin resistance, then whilst each part of the causal chain is associated with the development of metabolic syndrome, the evidence of this is too weak to link oxLDL and corticosteroid treatment independently with MetS while insulin exerts such a dominant effect.

4.1 Conclusions and Future Research

The primary objective of this thesis was to examine the association between inflammatory related disease process in SLE, and metabolic outcomes to identify possible disease pathways. We took the approach of using the statistical power that comes with a large sample size, and attempted to uncover subtle influences of disease processes that may not have previously been observed.

There is clear evidence that leptin is associated with MetS in SLE, and that an increase in leptin resistance associated with increased leptin levels may be influential in modulating inflammatory cytokines such as IL-6 and TNFα. The theoretical basis for this is the known association of inflammation with dyslipidemia, which we have also shown in our results. The increase in lipolysis due to activation of inflammatory cytokines increases FFA concentrations in the liver and inhibits LPL from removing circulating remnant lipoproteins. This can initiate de novo lipogenesis in the hepatocytes, which contributes to increased levels of dyslipidemia, which feed back into an inflammatory cycle. Active lupus is tied heavily with treatment variables in the real world, and it is challenging to tease apart the various factors that may have an overall influence on the disease cycle.

Future work will consist of completing the biomarker data with the processing of all enrolment and follow-up samples. This will enable research into the longitudinal affects of these biomarkers, and open up a wider field of investigation. Additionally, linear regression analysis of the current data would provide a more robust

analysis than simple logistic regression. Following on from this work, submission of this research for publication in association with the Centre for Musculoskeletal Research at the University of Manchester will be considered.

5 References

1 Agmon-Levin N, Mosca M, Petri M, Shoenfeld Y. Systemic lupus erythematosus one disease or many? Autoimmunity Reviews. 2012 jun;

2 Esdaile JM, Abrahamowicz M, Grodzicky T, Li Y, Panaritis C, Berger RD, et al. Traditional Framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus. Arthritis and Rheumatism. 2001;

3 Cervera R, Khamashta MA, Font J, Sebastiani GD, Gil A, Lavilla P, et al. Systemic lupus erythematosus: clinical and immunologic patterns of disease expression in a cohort of 1,000 patients. The European Working Party on Systemic Lupus Erythematosus. Medicine. 1993 mar;

4 Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, et al. Development of Autoantibodies before the Clinical Onset of Systemic Lupus Erythematosus. New England Journal of Medicine. 2003 oct;

5 Dubois EL, Tuffanelli DL. Clinical Manifestations of Systemic Lupus Erythematosus. JAMA. 1964 oct;

6 Grennan DM, Parfitt A, Manolios N, Huang Q, Hyland V, Dunckley H, et al. Family and twin studies in systemic lupus erythematosus. Disease markers. 1997 apr;

7 Petri Ma, Lahita RG, Van Vollenhoven RF, Merrill JT, Schiff M, Ginzler EM, et al. Effects of prasterone on corticosteroid requirements of women with systemic lupus erythematosus: a double-blind, randomized, placebo-controlled trial. Arthritis Rheum. 2002;

8 Danchenko N, Satia J, Anthony M. Epidemiology of systemic lupus erythematosus: a comparison of worldwide disease burden. Lupus. 2006 may;

9 Ward MM. Clinical Manifestations of Systemic Lupus Erythematosus. Archives of Internal Medicine. 1990 apr;

10 Biett D, Theodore L, Cazenave PLA, Schedel HÉ. Abrégé pratique des maladies des la peau: d'après les auteurs les plus estimés, et surtout d'apres des documens puisés dans les leçons cliniques de le docteur Biett. Bechet

11 Kaposi MK. Neue Beiträge zur Kenntniss des Lupus erythematosus. Archiv für Dermatologie und Syphilis. 1872 mar;

12 Harvey AM, Shulman LE, Tumulty PA, Conley CL, Schoenrich EH. Systemic lupus erythematosus: review of the literature and clinical analysis of 138 cases. Medicine. 1954 dec;

13 Osler W. On the visceral complications of erythema exudativum multiforme. The American Journal of the Medical Sciences. 1895 dec;

14 Mraĉek F, editor. Handbuch Der Hautkrankheiten. 2nd ed. Vienna: Alfred Hölder

15 Hargraves MM, Richmond H, Morton R. Presentation of two bone marrow elements; the tart cell and the LE cell. In: Proceedings of the staff meetings. Mayo Clinic. vol. 23; 1948.

16 Haserick JR, Bortz DW. A new diagnostic test for acute disseminated lupus erythematosus. Cleveland Clinic quarterly. 1949 jul;

17 Schur PH, Sandson J. Immunologic Factors and Clinical Activity in Systemic Lupus Erythematosus. New England Journal of Medicine. 1968 mar;

18 Urowitz MB, Bookman aaM, Koehler BE, Gordon Da, Smythe Ha, Ogryzlo Ma. The bimodal mortality pattern of systemic lupus erythematosus. American Journal of Medicine. 1976;

19 Petri M, Perez-Gutthann S, Spence D, Hochberg MC. Risk factors for coronary artery disease in patients with systemic lupus erythematosus. The American journal of medicine. 1992;

20 Manzi S, Meilahn EN, Rairie JE, Conte CG, Medsger TA, Jansen-McWilliams L, et al. Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. American journal of epidemiology. 1997;

21 Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham offspring study. American journal of epidemiology. 1979;

22 Strand V, Gladman D, Isenberg D, Petri M, Smolen J, Tugwell P. Outcome measures to be used in clinical trials in systemic lupus erythematosus. The Journal of rheumatology. 1999 feb;

23 Griffiths B, Mosca M, Gordon C. Assessment of patients with systemic lupus erythematosus and the use of lupus disease

activity indices. Best Practice & Research Clinical Rheumatology. 2005 oct;

24 Cohen A, Reynolds W, Franklin E, Kulka J, Ropes M, Shulman L, et al. Preliminary criteria for the classification of systemic lupus erythematosus. Bulletin on the Rheumatic Diseases. 1971;

25 Tan EM, Cohen AS, Fries JF, Masi AT, Mcshane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythrematosus. Arthritis and Rheumatism. 1982;

26 Hay EM, Bacon PA, Gordon C, Isenberg DA, Maddison P, Snaith ML, et al. The BILAG index: a reliable and valid instrument for measuring clinical disease activity in systemic lupus erythematosus. QJ Med. 1993;

27 Mikdashi J, Nived O. Measuring disease activity in adults with systemic lupus erythematosus: the challenges of administrative burden and responsiveness to patient concerns in clinical research. Arthritis Research & Therapy. 2015;

28 Gladman D, Ginzler E, Goldsmith C, Fortin P, Liang M, Sanchez-Guerrero J, et al. The development and initial validation of the systemic lupus international collaborating clinics/American college of rheumatology damage index for systemic lupus erythematosus. Arthritis & Rheumatism. 1996 mar;

29 Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis and rheumatism. 1992;

30 Hawker G, Gabriel S, Bombardier C, Goldsmith C, Caron D, Gladman D. A reliability study of SLEDAI: a disease activity index for systemic lupus erythematosus. The Journal of rheumatology. 1993 apr;

31 gov C. Safety of Estrogens in Lupus: Hormone Replacement Therapy

32 Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. The Journal of rheumatology. 2002 feb;

33 Austin MA. Low-Density Lipoprotein Subclass Patterns and Risk of Myocardial Infarction. JAMA: The Journal of the American Medical Association. 1988 oct;

34 Choi BG, Badimon JJ, Fuster V. Lipoprotein Metabolism and Vascular Biology. In: Davidson MH, Toth PP, Maki KC, Gotto AM, editors. Therapeutic Lipidology. Contemporary Cardiology. Totowa, NJ: Humana Press; 2008.

35 Grundy SM. Hypertriglyceridemia, Atherogenic Dyslipidemia, and the Metabolic Syndrome. The American Journal of Cardiology. 1998 feb;

36 Mora S, Szklo M, Otvos JD, Greenland P, Psaty BM, Goff DC, et al. LDL particle subclasses, LDL particle size, and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis. 2007 may;

37 McNamara JR, Jenner JL, Li Z, Wilson PW, Schaefer EJ. Change in LDL particle size is associated with change in plasma triglyceride concentration. Arteriosclerosis, Thrombosis, and Vascular Biology. 1992 nov;

38 Hansson GK. Inflammation, Atherosclerosis, and Coronary Artery Disease. New England Journal of Medicine. 2005 apr;

39 Hall JE, Guyton AC. Metabolism of Carbohydrates, and Formation of Adenosine Triphosphate. In: Guyton and Hall textbook of medical physiology. 12th ed. Philadelphia: Saunders Elsevier; 2011.

40 Fredrickson DS, Levy RI, Lees RS. Fat Transport in Lipoproteins — An Integrated Approach to Mechanisms and Disorders. New England Journal of Medicine. 1967 jan;

41 Hamilton JA. Fatty acid transport: difficult or easy? Journal of lipid research. 1998;

42 Lammert F, Wang DQ. New insights into the genetic regulation of intestinal cholesterol absorption. Gastroenterology. 2005;

43 Berg JM, Tymoczko JL, Stryer L. The complex regulation of cholesterol biosynthesis takes place at several levels.

44 Go Gw, Mani A. Low-density lipoprotein receptor (LDLR) family orchestrates cholesterol homeostasis. The Yale journal of biology and medicine. 2012;

45 Cromwell WC, Otvos JD. Utilization of Lipoprotein Subfractions. In: Davidson MH, Toth PP, Maki KC, Gotto AM, editors. Therapeutic Lipidology. Contemporary Cardiology. Totowa, NJ: Humana Press; 2008.

46 Davidson MH. Pharmacological Therapy for Cardiovascular Disease. In: Davidson MH, Toth P, Maki KC, editors. Therapeutic Lipidology. Totowa, NJ: Humana Press; 2008.

47 Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. The Lancet. 2012 aug;

48 Hegele RA. Plasma lipoproteins: genetic influences and clinical implications. Nature Reviews Genetics. 2009 feb;

49 Lamarche B, Tchernof A, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, et al. Small, Dense Low-Density Lipoprotein Particles as a Predictor of the Risk of Ischemic Heart Disease in Men. Circulation. 1997;

50 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry. 1972 jun;

51 Martin SS, Blaha MJ, Elshazly MB, Brinton EA, Toth PP, McEvoy JW, et al. Friedewald-Estimated Versus Directly Measured Low-Density Lipoprotein Cholesterol and Treatment Implications. Journal of the American College of Cardiology. 2013;

52 Castelli WP, Garrison RJ, Wilson PW, Abbott RD, Kalousdian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels: the Framingham Study. Jama. 1986;

53 Lopes-Virella MF, Virella G. Pathogenic Role of Modified LDL Antibodies and Immune Complexes in Atherosclerosis. Journal of Atherosclerosis and Thrombosis. 2013;

54 Tribble DL, Holl LG, Wood PD, Krauss RM. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. Atherosclerosis. 1992 apr;

55 Chait A, Brazg RL, Tribble DL, Krauss RM. Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. The American journal of medicine. 1993 apr;

56 Gardner CD, Fortmann SP, Krauss RM. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. JAMA. 1996 sep;

57 Rizzo M, Otvos J, Nikolic D, Montalto G, Toth PP, Banach M. Subfractions and subpopulations of HDL: an update. Current medicinal chemistry. 2014;

58 Toth PP. High-Density Lipoproteins. In: Davidson MH, Toth PP, Maki K, editors. Therapeutic Lipidology. Totowa, NJ: Humana Press; 2008.

59 Hahn BH, Grossman J, Chen W, McMahon M. The pathogenesis of atherosclerosis in autoimmune rheumatic diseases: Roles of inflammation and dyslipidemia. Journal of Autoimmunity. 2007;

60 McMahon M, Skaggs BJ, Grossman JM, Sahakian L, Fitzgerald J, Wong WK, et al. A panel of biomarkers is associated with increased risk of the presence and progression of atherosclerosis in women with systemic lupus erythematosus. Arthritis & rheumatology (Hoboken, NJ). 2014 jan;

61 Fisher EA, Ginsberg HN. Complexity in the Secretory Pathway: The Assembly and Secretion of Apolipoprotein B-containing Lipoproteins. Journal of Biological Chemistry. 2002 may;

62 Jeschke M. The Hepatic Response to Thermal Injury: Is the Liver Important for Postburn Outcomes? Molecular Medicine. 2009;

63 Boden G. Role of Fatty Acids in the Pathogenesis of Insulin Resistance and NIDDM. Diabetes. 1997;

64 Lyngsø D, Simonsen L, Bülow J. Metabolic effects of interleukin-6 in human splanchnic and adipose tissue. The Journal of Physiology. 2002;

65 Surendran RP, Visser ME, Heemelaar S, Wang J, Peter J, Defesche JC, et al. Mutations in LPL , APOC2 , APOA5 , GPIHBP1 and LMF1 in patients with severe hypertriglyceridaemia. Journal of Internal Medicine. 2012 aug;

66 Cuchel M, Qasim A, Rader DJ. Genetic Disorders of Lipoprotein Metabolism. In: Davidson MH, Toth PP, Maki KC, Gotto AM, editors. Therapeutic Lipidology. Contemporary Cardiology. Totowa, NJ: Humana Press; 2008.

67 de Carvalho JF, Bonfá E, Borba EF. Systemic lupus erythematosus and "lupus dyslipoproteinemia". Autoimmunity Reviews. 2008;

68 Bruce IN, O'Keeffe AG, Farewell V, Hanly JG, Manzi S, Su L, et al. Factors associated with damage accrual in patients with systemic lupus erythematosus: results from the Systemic Lupus International Collaborating Clinics (SLICC) Inception Cohort. Annals of the rheumatic diseases. 2014;

69 Bruce IN. 'Not only...but also': factors that contribute to accelerated atherosclerosis and premature coronary heart disease in

systemic lupus erythematosus. Rheumatology (Oxford, England). 2005;

70 Parker B, Bruce I. SLE and metabolic syndrome. Lupus. 2013;

71 Asanuma Y, Chung CP, Oeser A, Shintani A, Stanley E, Raggi P, et al. Increased concentration of proatherogenic inflammatory cytokines in systemic lupus erythematosus: relationship to cardiovascular risk factors. The Journal of rheumatology. 2006 mar;

72 Song LJ, Liu WW, Fan YC, Qiu F, Chen QL, Li XF, et al. The positive correlations of apolipoprotein E with disease activity and related cytokines in systemic lupus erythematosus. Diagnostic Pathology. 2013;

73 Binder CJ, Chang MK, Shaw PX, Miller YI, Hartvigsen K, Dewan A, et al. Innate and acquired immunity in atherogenesis. Nature Medicine. 2002 nov;

74 Singh SK, Suresh MV, Voleti B, Agrawal A. The connection between C-reactive protein and atherosclerosis. Annals of medicine. 2008 jan;

75 Blaschke F. C-Reactive Protein Induces Apoptosis in Human Coronary Vascular Smooth Muscle Cells. Circulation. 2004 aug;

76 Ridker PM. High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. Circulation. 2001 apr;

77 Nikpour M, Harvey PJ, Ibanez D, Gladman DD, Urowitz MB. High-sensitivity C-reactive protein as a marker of cardiovascular risk in systemic lupus erythematosus. Arthritis & Rheumatism. 2012 sep;

78 Gerdes N, Sukhova GK, Libby P, Reynolds RS, Young JL, Schönbeck U. Expression of Interleukin (IL)-18 and Functional IL-18 Receptor on Human Vascular Endothelial Cells, Smooth Muscle Cells, and Macrophages. The Journal of Experimental Medicine. 2002 jan;

79 Novick D, Elbirt D, Miller G, Dinarello CA, Rubinstein M, Sthoeger ZM. High circulating levels of free interleukin-18 in patients with active SLE in the presence of elevated levels of interleukin-18 binding protein. Journal of Autoimmunity. 2010;

80 Favilli F, Anzilotti C, Martinelli L, Quattroni P, Martino SD, Pratesi F, et al. IL-18 Activity in Systemic Lupus Erythematosus. 2009;

81 Gonzàlez M, Ribalta J, Vives G, Iftimie S, Ferré R, Plana N, et al. Nuclear magnetic resonance lipoprotein subclasses and the APOE genotype influence carotid atherosclerosis in patients with systemic lupus erythematosus. The Journal of rheumatology. 2010 nov;

82 Schanberg LE, Sandborg C. Dyslipoproteinemia and premature atherosclerosis in pediatric systemic lupus erythematosus. Current rheumatology reports. 2004;

83 Piya MK, McTernan PG, Kumar S. Adipokine inflammation and insulin resistance: The role of glucose, lipids and endotoxin. Journal of Endocrinology. 2013;

84 Grundy SM. Diagnosis and Management of the Metabolic Syndrome: An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005 oct;

85 Kellerer M, Lammers R, Fritsche A, Strack V, Machicao F, Borboni P, et al. Insulin inhibits leptin receptor signalling in HEK293 cells at the level of janus kinase-2: a potential mechanism for hyperinsulinaemia-associated leptin resistance. Diabetologia. 2001;

86 Parker B, Bruce IN. The metabolic syndrome in systemic lupus erythematosus. Rheumatic Disease Clinics of North America. 2010;

87 Yudkin JS, Juhan-Vague I, Hawe E, Humphries SE, di Minno G, Margaglione M, et al. Low-grade inflammation may play a role in the etiology of the metabolic syndrome in patients with coronary heart disease: the HIFMECH study. Metabolism: clinical and experimental. 2004 jul;

88 Skeoch S, Bruce IN. Atherosclerosis in rheumatoid arthritis: is it all about inflammation? Nature reviews Rheumatology. 2015;

89 Huang D, Aghdassi E, Su J, Mosko J, Hirschfield GM, Gladman DD, et al. Prevalence and risk factors for liver biochemical abnormalities in Canadian patients with systemic lupus erythematosus. The Journal of rheumatology. 2012;

90 Shinjo SK, Bonfá E, Wojdyla D, Borba EF, Ramirez LA, Scherbarth HR, et al. Antimalarial treatment may have a time-dependent effect on lupus survival: Data from a multinational Latin American inception cohort. Arthritis & Rheumatism. 2010 feb;

91 Petri M, Lakatta C, Magder L, Goldman D. Effect of prednisone and hydroxychloroquine on coronary artery disease risk factors in systemic lupus erythematosus: A longitudinal data analysis. The American Journal of Medicine. 1994 mar;

92 Tam L, Gladman DD, Hallett DC, Rahman P,

Urowitz MB. Effect of antimalarial agents on the fasting lipid profile in systemic lupus erythematosus. The Journal of rheumatology. 2000;

93 Johansson E, Forsberg K, Johnsson H. Clinical and experimental evaluation of the thromboprophylactic effect of hydroxychloroquine sulfate after total hip replacement. Pathophysiology of Haemostasis and Thrombosis. 1981;

94 van Leuven SI, Franssen R, Kastelein JJ, Levi M, Stroes ESG, Tak PP. Systemic inflammation as a risk factor for atherothrombosis. Rheumatology. 2008 jan;

95 Bulkley BH, Roberts WC. The heart in systemic lupus erythematosus and the changes induced in it by corticosteroid therapy. A study of 36 necropsy patients. The American journal of medicine. 1975;

96 Ettinger WH, Goldberg AP, Applebaum-Bowden D, Hazzard WR. Dyslipoproteinemia in systemic lupus erythematosus. The American Journal of Medicine. 1987 sep;

97 Borba E, Bonfa E. Dyslipoproteinemias in systemic lupus erythematosus: influence of disease, activity, and anticardiolipin antibodies. Lupus. 1997 jan;

98 Parker B, Urowitz MB, Gladman DD, Lunt M, Donn R, Bae Sc, et al. Impact of early disease factors on metabolic syndrome in systemic lupus erythematosus: data from an international inception cohort. Annals of the Rheumatic Diseases. 2015;

99 Urowitz MB, Gladman DD, Anderson NM, Su J, Romero-Diaz J, Bae SC, et al. Cardiovascular events prior to or early after diagnosis of systemic lupus erythematosus in the systemic lupus international collaborating clinics cohort. Lupus Science & Medicine. 2016;

100 64th WMA General Assembly. WMA Declaration of Helsinki; 2013. Available from: http://www.wma.net/en/30publications/10policies/b3/.

101 Hochberg MC. Updating the American college of rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis & Rheumatism. 1997 sep;

102 Parker B, Urowitz MB, Gladman DD, Lunt M, Bae SC, Sanchez-Guerrero J, et al. Clinical associations of the metabolic syndrome in systemic lupus erythematosus: data from an international inception cohort. Annals of the Rheumatic Diseases. 2012;

103 Expert Panel on Detection, Evaluation, of High Blood Cholesterol in Adults T. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA: The Journal of the American Medical Association. 2001 may;

104 Bays H, Stein EA. Pharmacotherapy for dyslipidaemia – current therapies and future agents. Expert Opinion on Pharmacotherapy. 2003;

105 Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the Metabolic Syndrome: A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International . Circulation. 2009 oct;

106 Alberti KGM, Zimmet P, Shaw J. The metabolic syndrome—a new worldwide definition. The Lancet. 2005 sep;

107 Pemberton PW, Aboutwerat A, Smith A, Warnes TW. Ursodeoxycholic acid in primary biliary cirrhosis improves glutathione status but fails to reduce lipid peroxidation. Redox Report. 2006;11(3):117–123.

108 Leng SX, McElhaney JE, Walston JD, Xie D, Fedarko NS, Kuchel GA. ELISA and Multiplex Technologies for Cytokine Measurement in Inflammation and Aging Research. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2008 aug;

109 Parker BJ. Determining the relationship between inflammation , therapeutic exposure and cardiovascular risk in patients with systemic lupus erythematosus. PhD [dissertation]. The University of Manchester; 2012.

110 Canadian Institute of Health Research. Project Information: The Metabolic Syndrome in Patients with Systemic Lupus Erythematosus (SLE); 2012. Available from: http://webapps.cihr-irsc.gc.ca/funding/detail_e?pResearchId=6039631&p_version=CIHR&p_language=E&p_session_id=.

111 International Aging Research Portfolio. Details of CIHR-942949: The metabolic syndrome in patients with systemic lupus erythematosus (sle); 2009. Available from: http://agingportfolio.org/projects/project/CIHR-942949.

112 Robins J. The control of confounding by

intermediate variables. Statistics in Medicine. 1989 jun;

113 Starks H, Brown Trinidad S. Choose Your Method: A Comparison of Phenomenology, Discourse Analysis, and Grounded Theory. Qualitative Health Research. 2007;

114 Bursac Z, Gauss CH, Williams DK, Hosmer DW. Purposeful selection of variables in logistic regression. Source code for biology and medicine. 2008;

115 Xia J, Broadhurst DI, Wilson M, Wishart DS. Translational biomarker discovery in clinical metabolomics: an introductory tutorial. Metabolomics. 2013;

116 Hajian-Tilaki K. Receiver operating characteristic (ROC) curve analysis for medical diagnostic test evaluation. Caspian journal of internal medicine. 2013;

117 Yee CS, Farewell VT, Isenberg DA, Griffiths B, Teh LS, Bruce IN, et al. The use of Systemic Lupus Erythematosus Disease Activity Index-2000 to define active disease and minimal clinically meaningful change based on data from a large cohort of systemic lupus erythematosus patients. Rheumatology.

118 Stanislaw H, Todorov N. Calculation of signal detection theory measures. Behavior research methods, instruments, & computers. 1999;

119 Karp I, Abrahamowicz M, Fortin PR, Pilote L, Neville C, Pineau CA, et al. Recent corticosteroid use and recent disease activity: Independent determinants of coronary heart disease risk factors in systemic lupus erythematosus? Arthritis & Rheumatism. 2008 feb;

Appendices

A Ethics Approval

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

Our ref: *03/NM/288*
Your ref:

17 July 2003

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

Dear Dr Bruce

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

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

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

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

Conditions of Approval

- You do not recruit any research subjects within a research site unless favourable opinion has been obtained from the relevant local research ethics committees.
- You do not undertake this research in an NHS organisation until the relevant NHS management approval has been gained as set out in the *Framework for Research Governance in Health and Social Care.*

Chair: Philip Smith
Chief Executive: Neil Goodwin

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

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

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

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

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

Submissions to other LRECs in Greater Manchester

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

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

and one copy of

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

Yours sincerely

Mrs G Rimington
Chairman, North Manchester Local Research Ethics Committee

Enclosures *Annual Report Form*
Membership List

Greater Manchester Local Ethics Committees
North Manchester LREC

Response form for Applicants

Details of Applicant

Name and address of principal researcher

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

Title of project

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

Name and address of Sponsor:

N/A

Details of REC

Name and address of LREC

North Manchester LREC, Room 181, Gateway House, Piccadilly South, Manchester, M60 7LP.

LREC reference number

03/NM/288

Listed below is a complete record of the review undertaken by LREC with the decisions made, dates of decisions and the requirements at each stage of the review:

Date of meeting, Decision made, Issues raised

26th June 2003, conditional approval subject to:

- **When asking for consent for the future use of genetic material the following phrase should be added to the consent form -: *'I offer my tissue or blood sample as a gift that may be used for future research'*.**
- **The committee suggests incorporating the use of a depression measure into the study.**
- **Due to the increase in workload of the Manchester Ethics Committees it was necessary for this study to be transferred to North Manchester Ethics Committee for consideration. The reference to Central Manchester Ethics Committee in the Patient Information sheet should be**

changed to read *'The study has been reviewed by one of the Manchester Local Research Ethics Committees, who agree it may go ahead'.*

The committee would like it noted that the information sheet was well written.

14th July 2003, response received from Dr IN Bruce addressing the above points.

The final documents and arrangements approved by the LREC

The following items have been approved by the North Manchester LREC:

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

Date of Approval 17th July 2003

Date 17th July 2003

Mrs G Rimington
Chairman
North Manchester LREC

B Consent Form

Central Manchester University Hospitals NHS
NHS Foundation Trust

Version 3, 04/07/2011

Centre Number: ☐ Study Number: ☐

CONSENT FORM

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

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

Please initial box

1.	I confirm that I have read and understand the information sheet dated 04/07/2011 (Version 3) for the above study and have had the opportunity to ask questions.	☐
2.	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
3.	I agree to a minimum of 5 years follow up.	☐
4.	I agree that my GP will be informed about my involvement in this study.	☐
5.	I understand that I will be informed if any of the results of the medical tests done as part of the research are important for my health, and with my permission, my GP and/or consultant will also be informed.	☐
6.	I understand that sections of any of my medical notes may be looked at by responsible individuals from Manchester Royal Infirmary, Department of Rheumatology or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.	☐
7.	I agree for a blood sample to be sent to the central co-ordinating laboratories in the University of Toronto and Dalhousie University, Halifax, Canada for use in this study.	☐
8.	I agree for a genetic sample to be stored in the central co-ordinating laboratory in The University of Toronto, Canada. I understand that it will be used for studies relating to this Registry and that only researchers approved by the SLICC group will have access to this sample. I also understand that this genetic sample will be stored for the duration of the study. If at any time I withdraw consent for its use, the sample will be destroyed.	☐
9.	I agree to take part in the study.	☐
10.	I offer my blood sample as a gift that may be used for future research and that further ethics committee approval will be sought for any additional use of it in future research.	☐

Please turn over...

1 x copy for patient; 1 x copy for researcher; 1 x copy for medical notes

Version 3, 04/07/2011

Centre Number: [ ] Study Number: [ ]

CONSENT FORM

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

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

Please sign below:

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

1 x copy for patient; 1 x copy for researcher; 1 x copy for medical notes

C Assay Validation

IL-18 Source: MBL, code no. 7620
Assay Dynamic Range: up to 1ng/ml
The sensitivity of the assay is 12.5pg/ml
Inter-assay variation: n = 40, CV = 7.3Inter-assay variation: n = 25, CV = 7.5

TNFα Source: R&D Systems DuoSet development kit, DY210.
Assay Dynamic Range: up to 1ng/ml
Analytical sensitivity: minimum detection limit calculated from the mean plus two standard deviations of 11 replicate analyses of reagent blank was found 2pg/ml.
Intra-assay variation: n = 8, CV = 5.9Inter-assay variation: n = 8, CV = 13.1

IL18 Bp Source: R&D Systems DuoSet development kit, DY119.
Assay Dynamic Range: up to 6ng/ml
Analytical sensitivity: minimum detection limit, calculated from the mean plus two standard deviations of 16 replicate analyses of reagent blank, was 0.013ng/ml.
Intra-assay variation: n = 32, CV = 3.7Inter-assay variation: not determined

Insulin Source: DRG, code EIA-2935
Assay Dynamic Range: up to 100μIU/ml.
Analytical sensitivity: minimum detection limit was 1.8 μIU/ml.
Intra-assay variation: n = 40, CV = 2.2Inter-assay variation: n = 24, CV = 4.5

BLyS Source: R&D Systems DuoSet development kit DY124-05
Assay Dynamic Range: up to 1ng/ml
Analytical sensitivity: minimum detection limit, calculated from the mean plus two standard deviations of 16 replicate analyses of reagent blank, was 2.9pg/ml.
Intra-assay variation: not determined
Inter-assay variation: not determined

Adiponectin Source: R&D Systems DuoSet development kit DY1065.
Assay Dynamic Range: up to 3ng/ml
Analytical sensitivity: minimum detection limit calculated from the mean plus two standard deviations of 16 replicate analyses of reagent blank and was found to be 0.02ng/ml.
Intra-assay variation: n = 40, CV = 3.2Inter-assay variation: n = 20, CV = 9.3

Leptin Source: R&D Systems DuoSet development kit DY398.
Assay Dynamic Range: up to 2ng/ml
Analytical sensitivity: minimum detection limit calculated from the mean plus two standard deviations of 13 replicate analyses of reagent blank and was found to be 0.04ng/ml.
Intra-assay variation: n = 14, CV = 5.3Inter-assay variation: n = 11, CV = 7.2

hs-CRP Source: in house assay
Assay Dynamic Range: up to 15μg/L
Analytical sensitivity: minimum detection limit calculated from the mean plus two standard deviations of 8 replicate analyses of reagent blank was 0.1μg/L.
Intra-assay variation: n = 14, CV = 4.7Inter-assay variation: n = 19, CV = 6.2

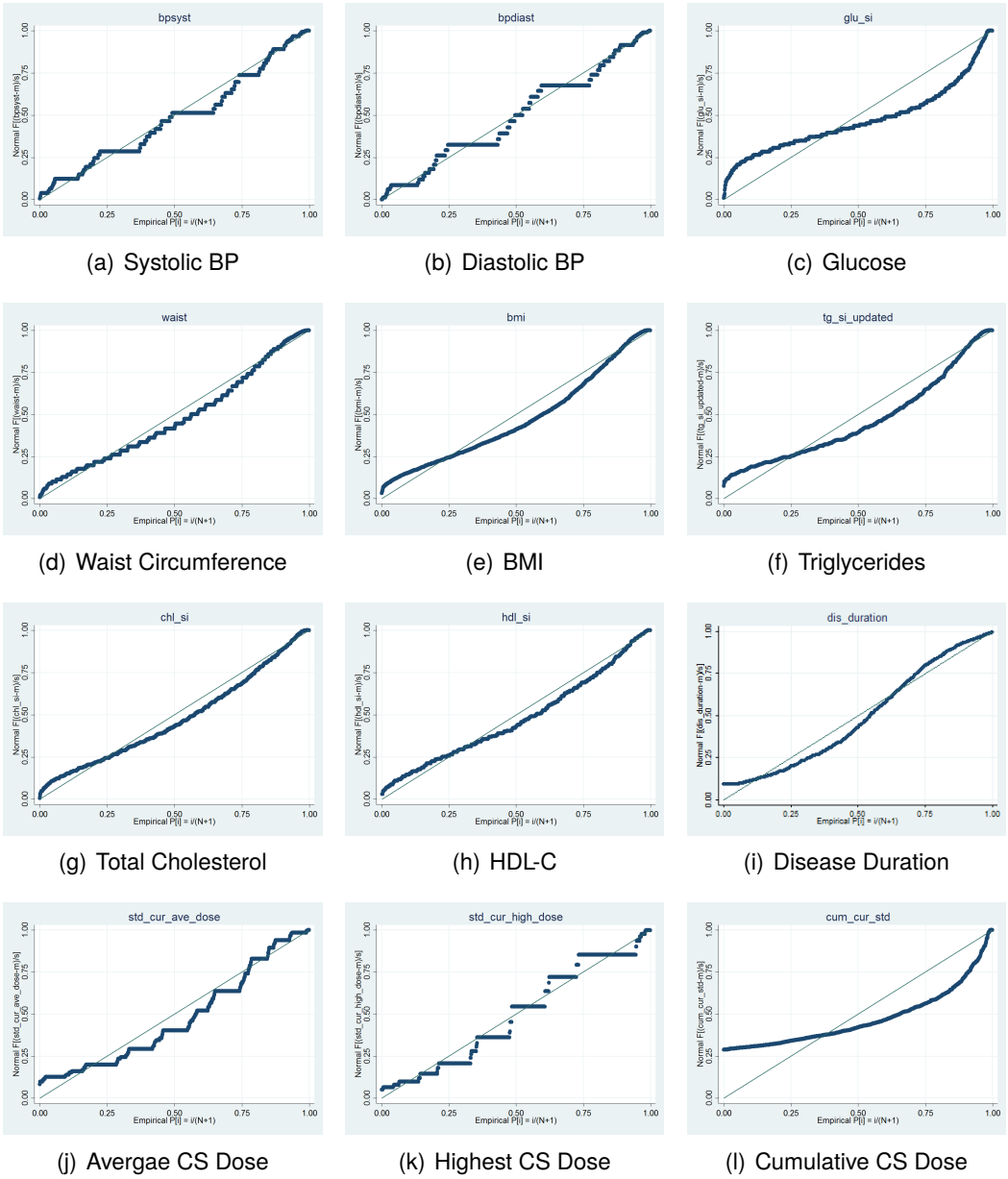
IL-6 Source: R&D Systems DuoSet development kit DY206.
Assay Dynamic Range: up to 600pg/ml
Analytical sensitivity: minimum detection limit calculated from the mean plus two standard deviations of 9 replicate analyses of reagent blank was 0.5pg/ml.
Inter-assay variation: n = 28, CV = 17.18Inter-assay variation: not determined

Ox-LDL Source: Mercodia 10-1143-01.
Assay Dynamic Range: up to 25mU/l.
Analytical sensitivity: minimum detection limit was calculated from the mean plus two standard deviations of 8 analyses of reagent blank and was found to be 0.037mU/l.
Inter-assay variation: n = 5, CV = 5.8Inter-assay variation: not determined

D Graphical Tests for Normality

D.1 Theoretical Plots

D.1.1 P-P Plots



(a) Systolic BP (b) Diastolic BP (c) Glucose

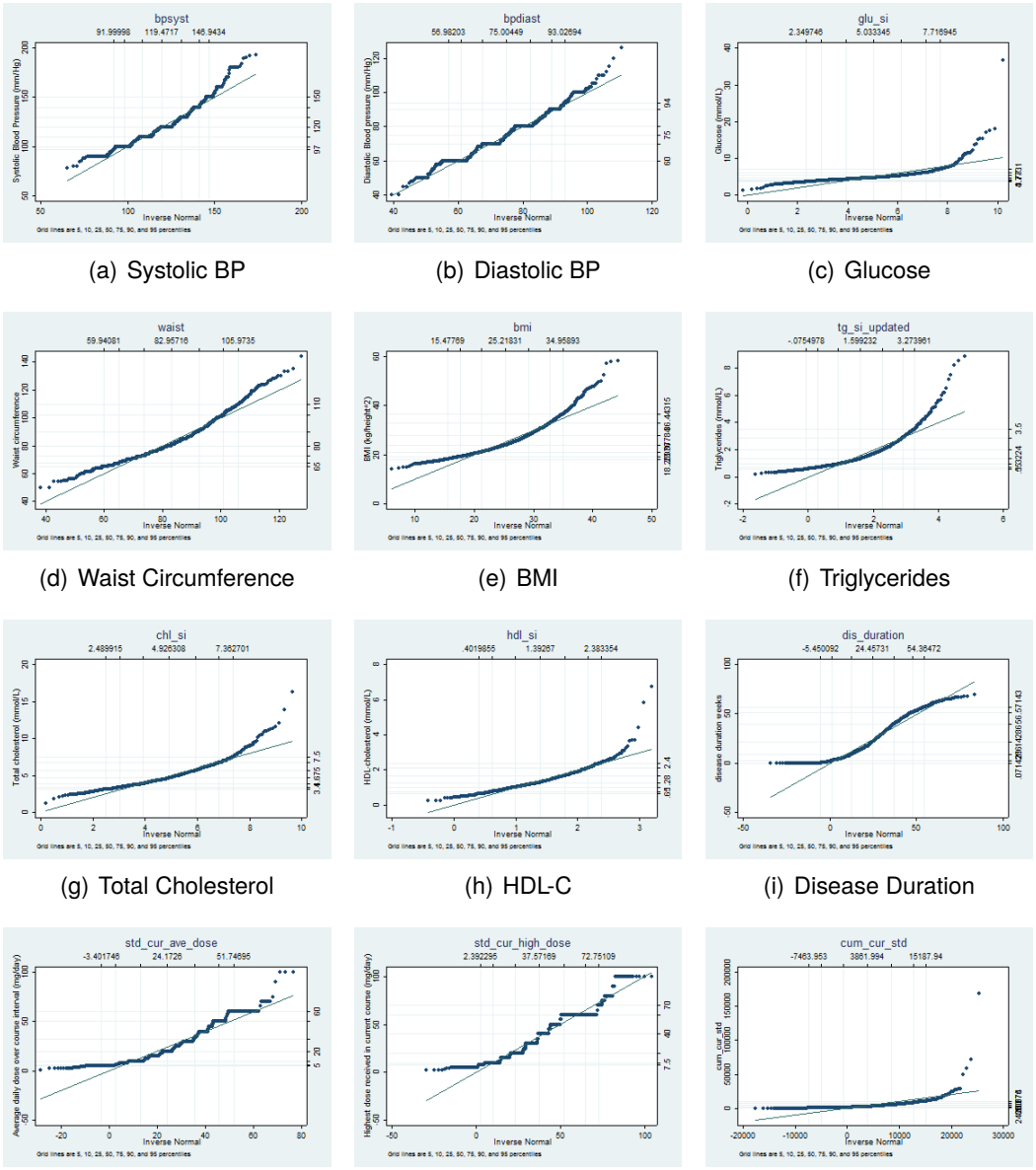
(d) Waist Circumference (e) BMI (f) Triglycerides

(g) Total Cholesterol (h) HDL-C (i) Disease Duration

(j) Avergae CS Dose (k) Highest CS Dose (l) Cumulative CS Dose

Figure D.1: The probability-probability plot (P-P plot) compares the empirical cumulative distribution function of a variable with the standard normal distribution function.

D.1.2 Q-Q Plots



(a) Systolic BP (b) Diastolic BP (c) Glucose

(d) Waist Circumference (e) BMI (f) Triglycerides

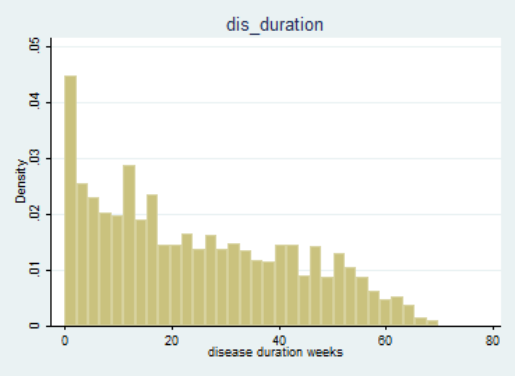
(g) Total Cholesterol (h) HDL-C (i) Disease Duration

(j) Avergae CS Dose (k) Highest CS Dose (l) Cumulative CS Dose

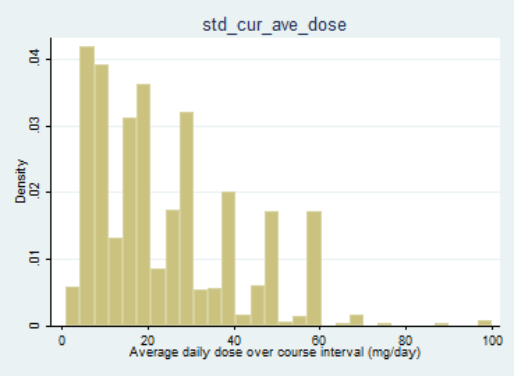
Figure D.2: The quantile-quantile plot (Q-Q plot) compares ordered values of a variable with quantiles of the standard normal distribution function.

D.2 Descriptive Plots

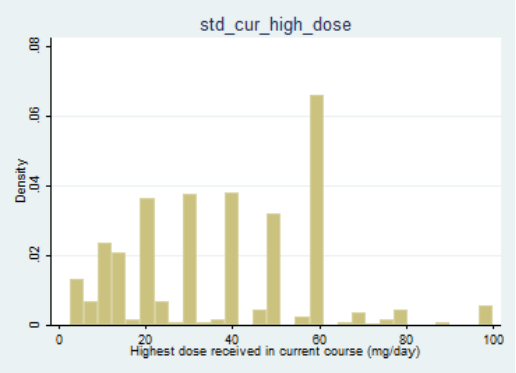
D.2.1 Histograms



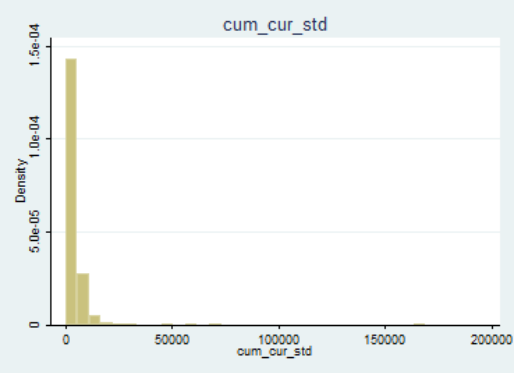
(a) Disease Duration



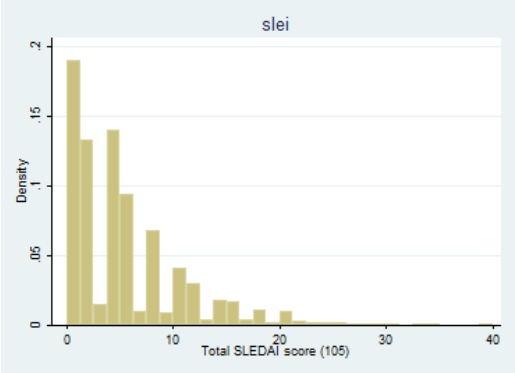
(b) Avergae CS Dose



(c) Highest CS Dose



(d) Cumulative CS Dose



(e) SLEDAI

Figure D.3: The histogram shows bars with a proportional area to the frequency of a variable, and bar widths equal to bin/class intervals. These variables demonstrate non-normal distributions.

E Comparison of MetS status in SLICC-bio

Table E.1: Key differences between SLICC-bio patients whom either fulfil, or do not fulfil the IDF 2009 criteria for MetS at enrolment*

	With MetS	Without MetS	p Value [95% CI]
No. of patients	404	741	
Gender			<0.001
Male	57 (14.1)	55 (7.42)	
Female	347 (85.9)	686 (92.6)	
Country			<0.001
Asia	58 (14.4)	94 (12.7)	
Canada	101 (25.0)	206 (27.8)	
Europe	75 (18.6)	207 (27.9)	
Mexico	69 (17.1)	83 (11.2)	
United States	101 (25.0)	151 (20.4)	
Ethnic classification			<0.001
Caucasian	175 (43.3)	342 (46.3)	
Indo-Asian	10 (2.48)	27 (3.65)	
Black African	38 (9.41)	38 (5.14)	
Black Caribbean	19 (4.70)	55 (7.44)	
Korean	57 (14.1)	94 (12.7)	
Hispanic	78 (19.3)	103 (13.9)	
Other	27 (6.68)	80 (10.8)	
CHD risk factors (mean (SD))			
BP systolic (mm Hg)	125.9 (17.2) *n* = 404	114.2 (14.2) *n* = 741	<0.001 [-13.5 to -9.81]†
BP diastolic (mm Hg)	79.3 (10.9) *n* = 404	72.1 (9.70) *n* = 740	<0.001 [-8.43 to -5.98]†
Glucose (mmol/L)	5.65 (2.04) *n* = 360	4.68 (1.41) *n* = 705	<0.001 [-1.18 to -0.76]†
Waist (cm)	89.5 (14.5) *n* = 373	77.7 (11.6) *n* = 687	<0.001 [-13.5 to -10.2]†
BMI (kg/height^2)	27.1 (6.28) *n* = 397	23.5 (4.96) *n* = 724	<0.001 [-4.29 to -2.95]†
Triglycerides (mmol/L)	2.25 (1.26) *n* = 374	1.23 (0.65) *n* = 701	<0.001 [-1.13 to -0.90]†
Total cholesterol (mmol/L)	5.45 (1.85) *n* = 377	4.60 (1.18) *n* = 705	<0.001 [-1.03 to -0.66]†
HDL-cholesterol (mmol/L)	1.27 (0.62) *n* = 271	1.47 (0.58) *n* = 459	<0.001 [0.11 to 0.29]†
Disease status			
SLEDAI (Mdn (IQR))	4 (2, 9) *n* = 404	4 (2, 7) *n* = 741	<0.001‡
Active disease (SLEDAI ≥6)	186 (46.0)	272 (36.9)	<0.001
SLICC/ACR DI ≥1	47 (11.6)	45 (6.1)	0.001
Active renal disease	151 (37.7)	109 (14.7)	<0.001
Low complement	130 (32.2)	287 (38.7)	0.028
Using medication			
Antihypertensive	242 (59.9)	80 (10.8)	<0.001
Lipid-lowering	156 (38.6)	12 (1.6)	<0.001
Antimalerial	213 (52.7)	542 (73.3)	<0.001
Immunosuppressant	216 (53.7)	248 (33.5)	<0.001
Oral corticosteroid	314 (77.7)	476 (64.6)	<0.001
Average CS dose (mg, Mdn (IQR))	25 (15, 40) *n* = 308/314	17.5 (10, 30) *n* = 472/479	<0.001‡
Highest CS dose (mg, Mdn (IQR))	50 (30, 60) *n* = 306/314	30 (15, 50) *n* = 471/479	<0.001‡
Cumulative CS dose (g, Mdn (IQR))	3.3 (1.5, 5.9) *n* = 302/314	2.0 (0.8, 4.0) *n* = 461/479	<0.001‡

*Unless otherwise stated, values are n (%) of patients; p Values calculated using χ^2 test.
† Two-tailed T-test.
‡ Two-sample Wilcoxon rank-sum (Mann-Whitney) test.