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STUDIES OF ATHEROSCLEROSIS IN SYSTEMIC LUPUS ERYTHEMATOSUS

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ABSTRACT

The role of inflammation in the development of atherosclerosis is now accepted and a focus of many studies because of its complex mechanisms. The risk of cardiovascular disease (CVD) and atherosclerosis is reported to be increased in systemic lupus erythematosus (SLE), especially in the group of young women. The introduction of statins in the 1990's lowered considerably the morbidity and mortality in CVD. In the last decade, research efforts were concentrated on the immunological mechanisms of atherosclerosis and on the possibility to influence these mechanisms.

Our group recently reported a negative association between natural IgM-antibodies against phosphorylcholine (IgM anti-PC) and CVD outcome in the general population. Potential mechanisms considered include anti-inflammatory properties and inhibition of uptake of oxidized low density lipoprotein (oxLDL) in macrophages. The objective herein was to study mechanisms of atherosclerosis in SLE and the relation to traditional and non-traditional risk factors in an SLE cohort, in comparison with an age and sex matched control group. As systemic endothelial dysfunction is one of the earliest signs of atherosclerosis in the general population, we also assessed skin microvascular endothelial function in SLE patients and controls.

A total of 114 patients with SLE were compared with 122 age and sex-matched population-based controls. Common carotid intima-media thickness (IMT), calculated intima-media area (cIMa) and plaque occurrence were determined by B-mode ultrasound. Plaques were graded according to echogenicity. Anti-PC was assessed by enzyme-linked immunosorbent assay (ELISA). Endothelial function in skin was tested with local application of acetylcholine (ACh) and any concomitant increase in skin perfusion was measured with Laser Doppler Fluxmetry (LDF) in 84 of the SLE-patients and 81 of the age- and sex-matched controls.

Incidence of hypertension, presence of insulin resistance (determined by homeostasis model assessment of insulin resistance, HOMA-IR) and the levels of triglycerides and C-reactive protein (CRP) were increased in the SLE patients, while smoking, cholesterol and high density lipoprotein (HDL) did not differ from controls. Low levels of IgM anti-PC were more common in the SLE patients than in the controls. IMT and cIMa did not differ significantly between groups. However, plaques were more often found in the SLE patients. Age, LDL and IgM anti-PC were independently associated with plaque occurrence in the SLE patients. Furthermore, in the left carotid arteries echolucent plaques were more prevalent in SLE when compared to controls. There were no significant differences in skin microvascular endothelial function between SLE patients and controls. In the SLE group, endothelial function did not vary in relation to presence of skin manifestations, Raynaud's phenomenon, nephritis or plaque occurrence. In SLE patients with CVD, however, endothelial function was impaired.

Conclusion: Plaque occurrence in the carotid arteries was increased in SLE and was independently associated with age, LDL and low anti-PC levels. Vulnerable plaques were more common in SLE than in controls. Anti-PC could be a novel risk marker for atherosclerosis with therapeutic potential in SLE. Skin microvascular endothelial function was associated with CVD but not with early signs of atherosclerosis in SLE-patients. The endothelial function was not different in SLE-patients, as compared to controls.

LIST OF PUBLICATIONS

I. *Increased prevalence of vulnerable atherosclerotic plaques and low levels of natural IgM antibodies against phosphorylcholine in patients with systemic lupus erythematosus.*

Cristina Anania, Thomas Gustafsson, Xiang Hua, Jun Su, Max Vikström, Ulf de Faire, Mikael Heimbürger, Tomas Jogestrand, Johan Frostegård
Arthritis Research & Therapy 2010, 12:R214

II. *Microcirculation as determined by iontophoresis in SLE-patients and controls*

Cristina Anania, Mikael Norman, Mikael Heimburger, Thomas Gustafsson, Tomas Jogestrand, Ingiäld Hafström and Johan Frostegård
Submitted manuscript

CONTENTS

LIST OF ABBREVIATIONS

ACh	Acetylcholine
aCL	Anti cardiolipin antibodies
aPL	Anti-phospholipid antibodies
AMI	Acute myocardial infarction
BMI	Body mass index
CABG	Coronary artery bypass graft
CRP	C-reactive protein
CVD	Cardiovascular disease
EC	Endothelial cells
ESR	Erythrocytes sedimentation rate
FMD	Flux mediated dilatation
HDL	High density lipoprotein
HOMA-IR	Homeostasis model assessment of insulin resistance
Ig	Immunoglobulins
IL	Interleukin
IMT	Intima-media thickness
cIMa	calculated intima-media area
IFN	Interferon
LD	Laser Doppler
LDF	Laser Doppler fluxmetry
LDL	Low density lipoprotein
MMP	Matrix metalloproteinase
MSPR	Maximum skin perfusion response
Nab	Natural antibodies
NO	Nitric oxide
OxLDL	Oxidized low density lipoprotein
PC	Phosphorylcholine
PGI2	Prostaglandin I2
SCORE	Systematic Coronary Risk Evaluation
SLAM	SLE Activity Measure
SLE	Systemic Lupus Erythematosus
SLEDAI	SLE Disease Activity Index
SLICC	Systemic Lupus International Collaborating Clinics
TG	Triglycerides
TNF	Tumor necrosis factor

1 SYSTEMIC LUPUS ERYTHEMATOSUS

1.1 INTRODUCTION

Systemic Lupus Erythematosus (SLE) is an inflammatory, autoimmune disease with multiorgan involvement, a wide variety of manifestations and an unpredictable course. It is characterized by excessive autoantibody production, immune complex formation and immunologically mediated tissue injury.

1.2 HISTORY

Pierre Louis Alphée Cazenave is credited with the introduction of the term "lupus erythematosus", in 1852. Moriz Kaposi, in 1872, recognized the systemic nature of the disease. In the beginning of the 20th century, Emanuel Libman and Benjamin Sacks described an endocarditis of a peculiar type in SLE. Paul Klemperer first used the term "diffuse connective tissue disease" in 1941. The history of SLE continued with Harris, who described in 1987, the Antiphospholipid Syndrome, a disorder that is manifested clinically as recurrent venous or arterial thrombosis and/or fetal loss and is characterised by antibodies against cardiolipin and beta 2 glycoprotein I.

1.3 CLASIFICATION CRITERIA

Already in 1971 the American College of Rheumatology proposed 14 criteria to diagnose SLE. The list was revised in 1982 and 1997, and currently includes 11 criteria: malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, serositis, renal involvement, central nervous system involvement, hematologic abnormalities and immunologic markers (Table 1). The presence of at least four criteria is required to establish a diagnosis of SLE (Tan et al., 1982, Hochberg, 1997).

Table 1. Classification Criteria for the Diagnosis of SLE (Smith and Shmerling, 1999)

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

1.4 INCIDENCE AND DISEASE COURSE

The *incidence rate* is approximately 4 per 100000 person year. The disease is 2 to 4 times more frequent and more severe among nonwhite populations around the world. Men, pediatric patients and late-onset lupus patients tend to be more severely affected (Pons-Estel et al., 2010, Abu-Shakra et al., 2004).

Survival in patients with SLE has improved over the last decades. Since the 1950's, the estimated 5-year survival of SLE patients in developed countries rose from <50% to >95% and similar increases were seen in 10-year survival (Borchers et al., 2004). Currently the approximate 5-, 10-, and 15-year survival rates are 96%, 93%, and 76%, respectively (Doria et al., 2006).

Lupus patients have a 3 to 5 times higher *mortality* compared with the general population (Abu-Shakra et al., 2004). Age at onset, sex and race are associated with different *mortality rates* (Bernatsky et al., 2006). Most notably the proportionate mortality from vascular disease, particularly accelerated atherosclerosis increased dramatically in SLE patients (Stahl-Hallengren et al., 2000, Borchers et al., 2004).

In 1976 Urowitz et al. described the bimodal pattern of mortality in SLE with an early phase due to renal and central nervous system (CNS) involvement and a late phase due to complications of the disease or treatment (Urowitz et al., 1976).

In the mid 1980's Rubin, Gladman and Urowitz started to report increased prevalence of cardiovascular disease (CVD) in SLE cohorts. Traditional risk factors, such as hypertension, metabolic syndrome and cigarette smoking could not entirely explain the 5 times increased risk to develop atherosclerosis and CVD, even if they were higher in SLE patients compared to the general population (Rubin et al., 1985, Gladman and Urowitz, 1987). Furthermore, in the group of 35-45 years old women with SLE, the risk of developing atherosclerosis was shown to be 50 times higher than in the age and sex matched controls (Manzi et al., 1997).

Several types of vascular lesions can affect diverse organs in SLE and seem to be crucial for the majority of clinical manifestations. The most common mechanism of vascular involvment is immune complex deposition in the walls of small arteries and arterioles. Thrombotic microangiopathic changes can also occur in SLE, especially in the presence of anticardiolipin (aCL) and beta 2 glycoprotein antibodies.

1.5 AUTOANTIBODIES

The complex etiopathogenesis of SLE still remains unsolved. A failure of the immune regulatory mechanisms with T-cell dysfunction, B-cell activation and an imbalance in the production of cytokines characterize the disease. A consequent feature is the overproduction of autoantibodies.

Autoantibodies are immunoglobulins (Ig) that bind via their combining sites to antigens in the same individual or species. In SLE they target nuclear and cytoplasmic components of the cell. The presence of antinuclear antibodies (ANA) and complement activation are the hallmarks of SLE (Sawalha and Harley, 2004). More than 100 antibodies are described in SLE, but only a few have clinical relevance: ANA, anti double stranded DNA antibodies (anti ds-DNA), anti-Smith antibodies (anti-Sm), anti ribonucleoprotein antibodies (anti RNP), Sjögren`s syndrome antibodies A and B (anti-SSA, anti-SSB) and antiphospholipid antibodies (aPL). The presence of one or more of these serologic markers is considered a criterion for SLE diagnosis.

Natural antibodies (Nab) are secreted mostly by B1-cells in the peritoneum and mucosal sites, as well as by marginal zone B cells. They are present in individuals without apparent immunization and bind to a variety of microbial polysaccharides and lipids. They are predominantly of the IgM isotype (Boes, 2000). IgM antibodies reflect a primary immune response, are pentavalent and usually of low affinity.

2 ATHEROSCLEROSIS

Atherosclerosis is a pathologic process in the blood vessels which can lead to CVD. It consists of accumulation of foam cells in the arterial wall and atherosclerotic plaque formation. Plaques that are unstable, vulnerable and prone to rupture induce thrombus formation in the lumen of the artery by exposing thrombogenic material to blood circulation. CVD is the most common cause of mortality in developed countries.

Risk factors, such as age, male gender, smoking, hypertension, diabetes, abdominal obesity, hypercholesterolemia and psychosocial factors can statistically explain 90% of CVD (Yusuf et al., 2004). In the last decade other risk factors for CVD started to receive clinical attention: homocysteinemia, hypertriglyceridemia, low levels of HDL, insulin resistance, high levels of CRP and apoB/apoA1 and low levels of natural antibodies. The European Society of Cardiology guidelines on CVD prevention, published in 2007, address these factors and markers (Graham et al., 2007). Already in 2003 The European Society of Cardiology developed SCORE (**S**ystematic **CO**ronary **R**isk **E**valuation) as an instrument for evaluation of the total fatal risk due to CVD (Conroy et al., 2003).

2.1 ENDOTHELIUM

The wall of large arteries consists of 3 layers: intima, media and adventitia. The internal layer – *intima* - comes in direct contact with the blood and its forces. That includes a thin lining of endothelial cells (EC), a sub-endothelial layer of connective tissue and an internal elastic membrane. *Tunica media* consists of smooth muscle cells and collagen fibres. *Adventitia*, the outer layer contains fibroblasts, collagen and elastic fibres.

The endothelium, focus of many studies, is considered the main regulator of the vascular homeostasis. It maintains the balance between vasodilation and vasoconstriction by secreting vasoactive substances (Davignon and Ganz, 2004). The healthy endothelium is both anticoagulant and antithrombotic. EC produce nitric oxide (NO), which is a major vasodilator, and inhibit the proliferation of the smooth cells and the interaction between circulating blood cells and the arterial wall. EC produce prostacyclin (PGI2) which inhibits platelet aggregation, as well as endothelin and angiotensin, which are potent vasoconstrictors (Luscher and Barton, 1997, Deanfield et al., 2007). Endothelium damage and the factors which inhibit NO production disturb the balance between vasodilation and vasoconstriction and initiate a number of events that promote atherosclerosis.

Altered NO production by EC is considered the hallmark of endothelial dysfunction and a precursor of atherosclerosis. NO is produced in EC from its precursor L-arginine via enzymatic action of endothelial NO-synthase (e-NOS). Shear stress increases the expression of e-NOS whereas statins increase the availability of e-NOS (John et al., 1998). Asymmetric dymethylarginin (ADMA) inhibits NO production (Cooke, 2000). NO prevents oxidation of LDL (Rubbo et al., 2002).

2.2 LIPIDS

In the early 1950's J.W. Gofman recognized the importance of lipids and lipoproteins in atherosclerosis (Gofman and Lindgren, 1950). More than twenty years later, J.L. Goldstein and co-workers discovered the scavenger receptor and described its role in atherosclerosis (Goldstein et al., 1979). Meanwhile, the research on lipid lowering drugs in the 1990's led to the broad use of statins in clinical practice. Since then, the mortality in CVD declined by 40%, but it is still the major cause of mortality in the industrialised countries (Bjorck et al., 2009).

Cholesterol and triglycerides (TG) are transported in the blood as lipoproteins: very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), high density lipoprotein (HDL) and chylomicrons. The first three have on the surface an apoB-protein and they are atherogenic. HDL has apoA1 on its surface and it is anti-atherogenic, transports the cholesterol to the liver and has anti-inflammatory and vasodilatatory effects (Walldius and Jungner, 2006). The mature chylomicron has apoA; -B; -C and -E on its surface. Some LDL particles from the blood enter the endothelium, get oxidized to oxLDL, which is recognized and taken up by macrophages. By quantifying apoB an indirect measure for potential atherogenic particles is obtained. Meanwhile, the apoA1 level reflects the anti-atherogenic HDL particles. Higher apoB/apoA1 ratio is considered a risk factor for CVD development (Walldius and Jungner, 2006). For SLE patients however, this ratio has little predictive value since their lipid profile is different (Svenungsson et al., 2003).

2.3 ATHEROSCLEROSIS AS AN INFLAMMATORY DISEASE

During the 1980's and at the beginning of the 1990's the association between inflammatory variables/markers and atherosclerosis was first reported. Since then, the inflammatory nature of atherosclerosis was recognized and described (Ross, 1999, Hansson, 2005). The formation of fatty streaks under the endothelium starts during childhood. Some streaks disappear and others transform into atherosclerotic plaques,

but the exact mechanism of these changes is not fully understood. There is evidence that cytokines, such as interferon gamma (IFN-γ), tumor necrosis factor (TNF), interleukin (IL) 1 and IL 6, some produced by activated CD4+ T-cells, are involved in this process. Once the plaque is formed, matrix metalloproteinases (MMP) and cysteine protease contribute to the rupture of the fibrous cap and thrombus formation, with consequent partial or total obliteration of the blood vessel.

Shear stress, determined by blood and plasma viscosity, hematocrit and wall flow velocity gradient, activates the endothelial wall of the large and medium arteries and regulates the production of NO in vascular wall (Hightower et al., 2011). NO bioavailability regulates the contractility of the blood vessels (Forstermann, 2010).

EC produce leukocyte adhesion molecules, which stimulate monocytes to migrate from the blood to the blood vessel wall where they transform into macrophages. Also LDL accumulates in the vessel wall and oxidizes to oxLDL and is incorporated into the macrophages. Further, activated CD4+ T-cells, which produce IFN-γ, TNF and IL-1, are implicated. CD8+ T-cells present in the plaque can be activated by other antigens such as viruses and have a regulatory, anti-atherosclerotic role, but can also contribute to aneurysm formation by producing elastolytic enzymes (Binder and Silverman, 2005).

The B-cells present in the plaque are considered to have anti-atherosclerotic role by secreting antibodies against: oxLDL, apoptotic cells and Streptococcus like species. These antibodies bind to phosphorylcholine (PC) on respective surfaces and facilitate the clearance of these structures from the blood vessel wall (Caligiuri et al., 2003). These antibodies, so called natural antibodies (Nab), are secreted by B1 cells and marginal zone B-cells in the spleen and are both IgG but mainly IgM antibodies (Baumgarth et al., 1999). They arise spontaneously in the perinatal period and have an important role in immediate host defense (Chou et al., 2008). Because of their broad specificity and low affinity (especially IgM) they are presumed to have an important role in the prevention of the autoimmune reactions and in the clearance of immune complexes (Shoenfeld et al., 2004).

In atherosclerosis oxLDL presents a PC epitope that Nab against PC can bind to, thus blocking the incorporation of oxLDL into the macrophage and in this way stopping the development of atheromathosis (de Faire et al., 2010).

3 CARDIOVASCULAR DISEASE AND SLE

CVD is a consequence of atherosclerosis. It includes coronary artery disease, manifested as acute myocardial infarction (AMI) or angina pectoris, cerebral artery disease, manifested as transient ischemic attack (TIA) or stroke and peripheral artery disease manifested as claudicatio or peripheral ischemia.

The first reports of the bimodal pattern of morbidity in SLE came in 1976 from Urowitz and co-workers (Urowitz et al., 1976), and were later followed by studies from Gladman (Gladman and Urowitz, 1987) and Petri (Petri et al., 1992). The latter described a cohort of SLE patients, with an average age of 38.3 years, in whom the incidence of Coronary Artery Disease (CAD) was 8%! In 1997 Manzi and co-workers compared the incidence of CAD between a group of 498 women with SLE and 2208 women participating in the Framingham study (Manzi et al., 1997). The authors reported a 50 times increase in the risk of AMI in SLE patients in the 35-44 year old age group.

Since atherosclerosis is a chronic inflammatory disease and SLE is a multiorgan inflammatory disease with increased rate of CVD, many researchers have in the last 10 years focused on studying the atherosclerotic process in SLE, with the hope of discovering the mechanisms of this comorbidity. Generally, the studies have reported increased plaque frequency and increased CVD incidence in the SLE patients. Similarly significantly increased IMT as well as endothelial dysfunction, measured by flow mediated dilation (FMD) have also been reported in SLE (Tyrrell et al., 2010). Selection bias, study design and the measurement technique used could have influenced the outcomes of these studies.

Hypertension, diabetes mellitus, smoking, dyslipidemia and male gender are widely recognized risk factors for CVD development. However, these factors alone cannot fully account for the atherosclerotic process in SLE. Other factors, first described by Petri (Petri et al., 1992), such as the age at SLE diagnosis, long disease duration and the duration of prednisolone treatment, are currently accepted as significant CVD risk factors in SLE. The importance of these factors for the course of the CVD in women diagnosed with SLE was later confirmed. Thus, Svenungsson et al. reported in 2001 a significant association between CVD in SLE and circulating oxLDL, high levels of triglycerides (TG), low levels of HDL and raised alpha 1-antitrypsin and homocysteine (Svenungsson et al., 2001). Doria and co-workers found in 2003 that cumulative doses of prednisolone are also important (Doria et al., 2003). Selzer et al. noted in 2004 that a

higher level of CRP is associated with increased IMT (Selzer et al., 2004). Frostegård concluded in 2005 that a combination of traditional and nontraditional risk factors (e.g. inflammation, antiphospholipid antibodies (aPLs), and lipid oxidation) contribute to CVD in autoimmune diseases (Frostegard, 2005). Hansson speculated in 2005 that high levels of CRP are more likely a consequence of increased IL6, then a direct marker of atherosclerosis progress (Hansson, 2005). Later studies demonstrated that only in SLE, CRP has a direct pathologic pattern (Barnes et al., 2005).

Similarly, age, systolic blood pressure and disease duration are important factors for increased IMT, as described by Bhatt et al. for an Asian Indian SLE cohort (Bhatt et al., 2006). De Leeuw found in 2006 that inflammatory endothelial markers, such as vascular cell adhesion molecule (VCAM) 1, MMP 3, von Willebrand factor, were all significantly higher in SLE patients than in controls. However, in a multivariate analysis age and SCORE remained the only independent predictors for increased IMT in SLE patients (de Leeuw et al., 2006).

Rho et al. described in 2008 that higher concentrations of adhesion molecules (E-selectin, VCAM, inter-cellular adhesion molecule (ICAM)) and TNF-alpha are associated with coronary atherosclerosis in SLE, independent of the Framingham risk score (Rho et al., 2008). The same year Svenungsson et al. reported that high levels of sVCAM-1, associated with systemic TNF-alpha activity, were identified as a novel discriminator for SLE-related CVD (Svenungsson et al., 2008). Su and co-workers reported that low levels of IgM anti PC were associated with the development of CVD in the same cohort (Su et al., 2008).

There is no strong evidence to link disease modifying anti rheumatic drugs with development of CVD. For example, for chloroquine, which lowers the concentration of circulating lipids, the association with CVD development is poor (Sachet et al., 2007, Becker-Merok and Nossent, 2009)

The use of statins in SLE patients did not significantly affect the incidence of CVD events in a randomized controlled study over two years. The anti-inflammatory effect of statins observed in the general population has not been replicated in SLE (Petri et al., 2011)

4 AIMS

- To study atherosclerosis in SLE patients, including plaque vulnerability, as compared to control subjects.

- To study traditional and novel risk factors, including the role of anti-PC, in relation to atherosclerosis and CVD, in patients with SLE.

- To study microvascular reactivity in SLE patients compared with controls, in relation to atherosclerosis and CVD.

5 PATIENTS AND METHODS

5.1 PATIENTS

All 160 patients with SLE aged between 18 and 70 years, under observation and treatment at Karolinska University Hospital Huddinge, where invited in 2006 to participate in the study called SLEVIC (SLE vascular impact cohort). Age and sex matched controls, free of systemic inflammatory diseases, recruited from the Population Register of the South Stockholm catchment area were asked to participate in the study as controls. Finally, at the end of 2007, 118 patients and 122 controls had been included in the study. Four of these patients did not fulfil the revised ACR criteria for SLE and were later excluded from the analyses.

All 114 patients and 122 controls gave their written consent to participate in the study. The study was approved by the research ethics committee at Karolinska Institutet.

The first study (Study I) included all 114 patients and 122 controls enrolled. The demographic and baseline characteristics of the groups are presented below (Table 2).

Table 2. Demographic and baseline characteristics of the study groups – study I

Characteristics	SLE Patients (n=114)	Controls (n=122)	P level
Age, years	48±13	49±13	NS
Male gender	12.3% (n=14)	10.6% (n=13)	NS
Current smokers	14.0% (n=16)	15.5% (n=19)	NS
BMI, kg/m^2	24.9 (21-28)	24.7 (22.5-27.8)	NS

n = number; NS = non significant

The SLE-patients had a disease duration mean (SD) 12±10 years and mean (range) SLE activity measure, SLAM, 6 (0-20), SLE disease activity index, SLEDAI 2 (0-27), and Systemic Lupus International Collaborating Clinics damage index, SLICC 1 (0-8).

In the second study (Study II) 84 patients and 81 sex and age matched controls from the same cohort were included. Demographic and baseline characteristics of the groups are presented in Table 3.

Table 3. Demographic and baseline characteristics of the study groups – study II

Characteristics	SLE patients (n=84)	Controls (n=81)	P level
Age, years	47±14	51±13	NS
Male gender	17% (n=14)	14% (n=11)	NS
Current smokers	9.52 (n=8)	14.29 (n=12)	NS
BMI, kg/m^2	25.14±4.54	25.68±5.62	NS

n = number; NS = non significant

The SLE disease duration was of mean (range) 8 years (0-40), SLAM 6 (0-18), SLEDAI 2 (0-27) and SLICC 1 (0-8).

5.2 METHODS

5.2.1 General facts

Blood samples were collected between 7:00-10:00 AM, after 12 hours of fasting. The patients and controls had the weight, height and waist circumference measured. The body mass index (BMI) was calculated for the study participants. All subjects met with a medical doctor, who obtained a CVD focused medical history and proceeded with clinical investigation. Subsequently they, except for 3 patients, were investigated by Doppler carotid ultrasonography to determine IMT, cIMa and plaque presence. For the SLE patients SLAM; SLEDAI; SLICC were calculated (Liang et al., 1988, Bombardier et al., 1992, Gladman et al., 1996).

Medication: We calculated the doses of glucocorticoids (GC) taken by the patients up to the time of inclusion in the study. We looked at the total intake, last year's intake, as well as current and past dose per month, when available in the patient's chart (Table 4).

Table 4. Glucocortiocoids treatment characteristics for the SLE patients

Characteristic	Study I	Study II
GC total intake (gram)	13.18 (4.53-29.72)	13 (4.3-27.37)
GC last year intake (gram)	1.42±1.7	1.45±1.53
GC total intake/month (gram)	0.21 (0.15-0.30)	0.21 (0.15-0.30)
GC last year intake/month (gram)	0.12 (0-0.22)	0.11 (0-0.19)

The biochemical variables were determined by standard laboratory methods. Serum and cells were separately prepared before storage at -80°C.

Immunological analyses of aCL antibodies and beta 2 glycoprotein antibodies were run by Karolinska Immmunlaboratory, Solna using an enzyme-linked immunosorbent assay (ELISA).

Anti PC IgM were determined using a commercial ELISA kit (Athera CVDefine-TM, Stockholm, Sweden) as described by the manufacturers. Anti PC IgG were also determined using the CVDefine kit, but the secondary antibody was switched to detect IgG (i.e. Horseradish Peroxidase – goat - antihuman IgG, Invitrogen, Sweden).

Insulin resistance was determined using the homeostasis model assessment of insulin resistance (HOMA-IR) according to the following formula: HOMA-IR = fasting insulin (μU/ml)*fasting glucose (mmol/L)/22.5.

5.2.2 Carotid ultrasound

The right and left carotid arteries were examined with a duplex scanner (Sequoia, Siemens Acuson, Mountain View, CA, USA) using a 6 MHz linear array transducer. The far wall of the common carotid artery (CCA), 0.5 to 1.0 cm proximal to the beginning of the carotid bulb, was used for measurements of the IMT and lumen diameter. The IMT was defined as the distance between the leading edge of the lumen-intima echo and the leading edge of the media-adventitia echo. The lumen diameter was defined as the distance between the leading edge of the intima-lumen echo of the near wall and the leading edge of the lumen-intima echo of the far wall. The mean values of the IMT and lumen diameter within the 10 mm long section were calculated. When a plaque was observed in the region of the CCA measurements, the IMT was not measured.

Carotid plaque was defined as a localized intima-media thickening of greater than 1 mm and at least a 100% increase in thickness compared with adjacent wall segments. We screened for plaque in the common, internal and external carotid arteries. Plaque occurrence was scored as the absence of plaque, the presence of unilateral plaque, and the presence of bilateral plaque. Plaque morphology in terms of echogenicity was assessed using a modified version of Gray-Weale classification (Gray-Weale et al., 1988) and graded from 1 to 4 as echolucent, predominantly echolucent, predominantly echogenic and echogenic. Echolucency was defined with the arterial lumen as a reference and echogenicity with the far wall media-adventitia echo as a reference.

To compensate for the stretching effect of arterial distension on the wall thickness (secondary to increased arterial pressure), the cross-sectional intima-media area was calculated using the formula: Cross sectional intima media area = 3.14*(lumen diameter/2 + IMT) ^{2}- (lumen diameter/2)^2. This calculated intima-media area (cIMa), but not the IMT, has been shown to be unaffected by variations in artery distension secondary to changes in blood pressure (Jogestrand et al., 1995).

5.2.3 Laser Doppler fluxmetry and ionthophoresis

The studies were conducted in a dedicated microvascular measurement facility, with stable ambient temperature (23±1°C) and with no detectable circulating draught. The subjects were lying supine with both arms comfortably besides the body. Prior to the start of flux recordings, subjects were acclimated to room temperature for 20 min.

Acetylcholine (ACh) iontophoresis. Our protocol was based on the protocol previously described by Hansell and co-workers (Hansell et al., 2004). An iontophoresis electrode chamber was attached on the dorsal side of the non-dominant hand between fingers 1 and 2, using a double-sided adhesive disc. The chamber held the laser probe vertically enabling the skin perfusion to be measured directly over the site of drug application. An indifferent electrode was attached on the forearm at 15 cm distance from the probe. The chamber was filled with 1% ACh, which induces endothelium-dependent dilation and smooth muscle–mediated constriction. A PeriIont System 480-1 battery-powered iontophoresis controller provided the direct current for iontophoresis. A thermostatic probe holder helped to stabilise the skin temperature at 32°C. Iontophoresis allows charged substances to cross the skin by means of a small electrical current. After a baseline recording of 2 minutes, ACh was delivered using an anodal current of 0.1 mA for 20 seconds with iontophoretic stimulation, repeated six times at 60 seconds interval between doses. The flux response to ACh was continuously recorded for 5 minutes following the final sixth stimulation dose. A Laser Doppler (LD) instrument (Periflux 4001TM, wavelength 780 nm, Stockholm, Sweden) was used to measure perfusion changes during vascular provocations (Perimed AB, Stockholm, Sweden). The LD signal is proportional to the number and velocity of moving blood cells in illuminated superficial skin microvessels. The LD output is semi quantitative and expressed in perfusion units of output voltage.

5.3 STATISTICS

Variables were dichotomized or considered as continuous variables as indicated. Some variables were logarithmically transformed to minimize the skewness. We have calculated percentiles based on distributions in the whole study group. Age, gender and geographic area were matched through the design of the study. Taken into account the normality of the distribution, comparisons between groups were made using the Mann-Whitney U-test or Student's *t*-test.

In order to establish the association between potential risk factors for atherosclerosis and atherosclerotic plaque, logistic regression was applied with adjustment for covariates. Spearman correlation coefficient test was used for univariate correlations between continuous variables in study II, as well as linear general models and analysis of covariance.

Differences were considered statistically significant if the p-value was less than 0.05.

6 RESULTS

6.1 PAPER I

As shown in Table 5, the patient group had increased prevalence of hypertension ($p < 0.001$), higher levels of TG ($p < 0.01$), CRP ($p < 0.001$), HOMA-IR ($p < 0.05$) and apoE ($p< 0.01$) than the control group. Low IgM anti-PC levels occurred more often in SLE patients (lowest tertile; $p < 0.01$ anti-PC determinations available for 111 SLE-cases and 118 controls). We also found lower LDL levels ($p < 0.05$), as previously described. There were no significant differences regarding smoking habits, diabetes (few cases), total cholesterol, HDL, apoB/apoA levels or BMI.

Table 5. Clinical and laboratory characteristics of patient and controls

Characteristic	Cases (n=114)	Controls (n=122)	P level
Presence of diabetes	5.26% (n=6)	2.45% (n=3)	NS
Presence of hypertension % (n)	57.89% (n = 66)	26.22% (n = 32)	<0.001
Total cholesterol (mmol/L)	4.7±1.1	4.8±1.0	NS
LDL (mmol/L)	2.5±0.88	2.8±0.80	<0.05
HDL(mmol/L)	1.6 (1.3-1.8)	1.6 (1.3-1.9)	NS
Triglicerides (mmol/L)	0.99 (0.7-1.4)	0.78 (0.55-1.10)	<0.01
CRP (mg/L)	4.44 (0.8-4.8)	2.04 (0.5-2.5)	<0.001
ApoE (mg/L)	42.69±14.7	38.84±12.05	<0.05
ApoB/ApoA1, ratio	0.55 (0.50-0.70)	0.60 (0.50-0.70)	NS
BMI (kg/m^2)	24.89 (20.96-27.85)	24.67 (22.41-27.82)	NS
HOMA – IR	1.34 (0.80-1.96)	1.05 (0.69-1.48)	<0.05
IgM antiPC, lowest tertile	30.70% (n = 35)	16.39% (n = 20)	<0.01
IgG antiPC (U/ml)	9.31 (5.42-14.72)	7.45 (4.62-11.83)	<0.05

n = number; NS = non significant

As shown in Table 6, IMT and cIMa did not differ between groups, whereas a difference between the groups in occurrence of atherosclerotic plaques ($p < 0.05$) was found. In addition, left-sided echolucent plaques were more prevalent in the SLE

patients as compared to controls ($p < 0.05$), but there was no significant difference in echolucent plaque on the right side. CVD occurrence was increased in SLE patients ($p < 0.01$) when CVD was defined as a history of cerebrovascular events, AMI, coronary artery by-pass graft (CABG), heart valve prosthesis/impairment, peripheral arterial surgery or claudicatio.

Table 6. Carotid measurements in SLE-patients and controls

Measure	Cases	Controls	P level
IMT R (mm)	0.60±0.13	0.62±0.13	NS
IMT L (mm)	0.59 (0.50-0.71)	0.60 (0.52-0.70)	NS
cIMarea R (mm^2)	11.39 (9.60-14.34)	11.90 (9.93-14.01)	NS
cIMarea L (mm^2)	11.81 (9.41-13.43)	11.64 (9.88-13.83)	NS
Plaque presence	42.98% (n=49)	30.32% (n=37)	<0.05
Low-echogenic plaques (grade 1) **left** carotid artery	n=25	n=13	<0.05
Low-echogenic plaques (grade 1) **right** carotid artery	n=19	n=18	NS
CVD	21.92% (n=25)	2.45% (n=3)	<0.001

n = number; NS = non significant; R = right side; L = left side

We further compared the SLE-patients with and without plaques. In univariate analyses, age, SLE duration, hypertension, fasting glucose-level but not HOMA-IR, LDL-cholesterol, total cholesterol, apoB/apoA1 ratio, BMI, and IgM anti PC-levels were significantly different between SLE-patients with and without plaques. The total GC intake was not associated with atherosclerotic plaques (Table 7).

Table 7. Characteristics of SLE patients with and without atherosclerotic plaques

	SLE patients		
	With plaque (n=49)	Without plaque (n=62)	P level
Age, years	55.79±9.23	41.7±12.76	<0.001
Average disease duration (years)	14.00	9.82	<0.05
Current smokers % (n)	7.2% (n=8)	6.3% (n=7)	NS
Presence of hypertension % (n)	31.5% (n=35)	27% (n=30)	<0.05
Presence of diabetes % (n)	4.5% (n=5)	0.9% (n=1)	<0.05
Glucose (mmol/L)	4.70 (4.30-5.00)	4.35 (3.90-4.70)	<0.01
LDL (mmol/L)	2.87±1.05	2.28±0.66	<0.01
Cholesterol (mmol/L)	5.05±1.26	4.35±0.98	<0.01
ApoB/ApoA1,	0.60 (0.50-0.80)	0.50 (0.45-0.60)	<0.01
Triglycerides (mmol/L)	1.00 (0.74-1.50)	0.94 (0.61-1.30)	0.065
BMI (kg/m2)	25.95 (22.28-28.41)	24.16 (20.08-26.84)	<0.05
Cumulative lifetime GC dose/year (gram/year)	2.49 (1.75-3.68)	2.59 (1.87-3.49)	NS
Glucocorticoid last year (gram)	0.9 (0-2.19)	1.8 (0.07-2.70)	NS
Chloroquin/hydroxychlorochine	42.85% (n=21)	54.83% (n=34)	NS
IgM anti PC	48.75 (25.79-104.78)	85.94 (50.02-231.52)	<0.001
IgM antiPC<=33th percentile (%)	57.45	28.81	<0.01

n = number; NS= non significant; 25th and 75th quartile presented between brackets

In a multivariate analysis only age, hyperlipidemia (LDL ≥ 3 mmol/L) and IgM anti-PC (lowest tertile) remained significant and independently associated with plaque occurrence. If patients with previous CVD were excluded, the associations between low anti-PC and plaque prevalence remained significant (OR 4.4; CI 1.34 to 14.88; $p < 0.05$).

Conclusions: Taken together, our data indicate that atherosclerotic plaques, but not general atherosclerosis as indicated by IMT measurements, were more prevalent in SLE patients when compared to controls. Furthermore, vulnerable atherosclerotic plaques were more common in SLE. Age, LDL and low levels of IgM anti-PC were independently associated with the prevalence of atherosclerotic plaques in SLE.

6.2 PAPER II

In study II the smaller groups of 84 patients and 81 controls had nearly the same characteristics as in the first study. The frequency of hypertension and CVD was significantly higher in SLE patients compared with controls and there were also differences in HOMA-IR, CRP, ESR and homocysteine levels (Table 8).

Concerning the lipid profile, the patients had significantly lower cholesterol and LDL but higher TG.

Table 8. Clinical and disease characteristics for patients and controls in study II

Characteristic	Case n = 84	Controls n = 81	P level
Age, years	47±14	51±13	NS
Sex (women)	83% (n=70)	86% (n=70)	
Present smokers	n=8	n=12	NS
Ever smokers	n=43	n=46	NS
Pack years	7.64±12.53	6.73±9.08	NS
Hypertension	60% (n=50)	24% (n=10)	<0.001
CVD	19% (n=16)	2% (n=2)	<0.001
HOMA-IR	1.56±0.96	1.26±1.07	0.056
CRP, mg/L	4.55±6.29	1.96±2.52	<0.001
ESR, mm/h	23±17	9±5	<0.001
apoB/apoA1	0.59±0.22	0.65±0.33	NS
TG, mmol/L	1.08±0.64	0.90±0.46	<0.05
Cholesterol, mmol/L	4.63±1.14	4.97±1.01	<0.05
HDL, mmol/L	1.60±0.45	1.72±0.59	NS
LDL, mmol/L	2.53±0.87	2.91±0.82	<0.01
Homocysteine, µmol/L	13.42±5.47	11.57±4.80	<0.01

n = number; NS= non significant

6.2.1 Carotid measurements

There were no differences between IMT on either sites (right and left) between patients and controls. In all, 40% of the patients and 30% of controls had carotid plaques, p = 0.004 (when controlled for age).

6.2.2 Laser Doppler Fluxmetry (LDF)

Neither basal skin perfusion nor maximal skin perfusion response (MSPR) to iontophoresis of ACh was significantly different between patients and controls, when measured in perfusion units. The percentage change, defined as (MSPR-Basal flux)/Basal flux * 100) was found to be significantly higher in the patient group ($p < 0.05$).

We further analyzed if MSPR differed between the subgroups of SLE patients. It was obvious that patients treated for hypertension and those with CVD had significantly lower MSPR than those not treated for hypertension and those without CVD, respectively (Table 9).

No correlation was found between MSPR and the measures of disease activity and damage (SLAM, SLEDAI and SLICC).

We further checked whether differences in microcirculation existed between patients treated with different antihypertensive agents and those not treated. No significant differences in MSPR were found between patients on angiotensin converting enzyme (ACE) inhibitors vs. those not on ACE inhibitors, (89 vs. 73 PU; $p = 0.378$), nor for those on angiotensin receptor-blockers (ARB) vs. those not (87 vs. 24 PU; $p = 0.131$), nor between those on beta-blockers vs. those not taking them (89 vs. 61 PU; $p = 0.194$) and nor in those on Ca-antagonist vs. those not on the medication (87 vs 71 PU; $p = 0.48$).

No significant differences were found regarding MSPR values between patients treated or not treated with chloroquine ($p = 0.623$), or those on or off GC ($p = 0.273$).

Although smoking status (actual and previous) had no significant effect on MSPR, we found negative correlations between pack years and MSPR (Spearman $r = -0.234$, $p < 0.05$), as well as percentage response ($r = -0.251$, $p < 0.05$).

The percentage response to ACh correlated negatively with IMT left ($r = -0.336$, $p < 0.01$), whereas the other LDF measures did not significantly correlate with IMT.

Table 9. Maximum skin perfusion response with LDF for 84 SLE patients, when separated into presence = 1, or not = 0 of disease manifestations and co-morbidities

		Number	MSPR (PU)	P level
Skin	0	15	95.77±54.88	NS
	1	69	82.37±73.51	
Raynaud	0	45	90.76±76.20	NS
	1	39	77.84±63.43	
Nephritis	0	54	83.77±78.03	NS
	1	30	86.54±55.41	
Hyperlipidemia[1]	0	57	90.41±69.63	NS
	1	27	72.83±71.99	
Hypertension	0	34	99.77±79.50	NS
	1	50	74.56±62.34	
HT treatment	0	48	99.31±79.56	<0.05
	1	36	65.37±50.89	
CVD total	0	68	92.53±73.14	<0.05
	1	16	51.57±46.10	
Plaque	0	48	90.05±69.76	NS
	1	34	80.35±72.93	
Present smokers	0	76	87.38±72.09	NS
	1	8	59.89±48.71	
Ever smokers	0	41	94.16±66.78	NS
	1	43	75.80±73.43	

MSPR = Maximum skin perfusion response; NS = non significant;

PU = perfusion units

Conclusions: Skin microvascular endothelial function is associated with CVD, but not with early signs of atherosclerosis in SLE-patients. The endothelial function is not different in SLE-patients as compared to controls.

[1] LDL ≥ 3mmol/L and/or lipid lowering treatment

7 DISCUSSION

The patients with SLE had an increased number of carotid atherosclerotic plaques, as compared to sex- and age-matched controls from the same geographic area. Common carotid IMT and cIMa did not differ compared with controls.

The observation of increased occurrence of atherosclerotic plaques is in accordance with other observational and/or prospective studies (Doria et al., 2003, Manzi et al., 1999) and controlled studies (Roman et al., 2003, Asanuma et al., 2003, Svenungsson et al., 2001), where increased atherosclerosis has been reported as a feature of SLE. The finding that plaque occurrence but not IMT or cIMa was increased in SLE is in line with previous publications (Roman et al., 2003, Svenungsson et al., 2001).

Furthermore, in our present study patients with SLE had echolucent plaques more often than controls in the left carotid artery. Carotid echolucent plaques are considered to represent more vulnerable atherosclerotic lesions than echogenic plaques (Kwee et al., 2008). Thus, individuals with carotid stenosis and echolucent plaques were reported to have an increased risk of stroke and cerebrovascular events compared with individuals with stenosis and more echogenic plaques (Mathiesen et al., 2001).

In the present study nine SLE patients had reported stroke/TIA in medical history, compared with one control. The reason for the increased occurrence of echolucent plaques on the left but not on the right side in SLE-patients is not clear. One explanation might be the difference in gross anatomy of the left and right common carotid artery (CCA) which might create different shear stress conditions. Shear stress has been shown to be related to both intima-media thickness and echogenecity (Lind et al., 2009).

We found that low levels of natural IgM anti-PC antibodies were more prevalent in the SLE-patients group as compared with controls. This correlates with similar findings in a nested case-control study, where SLE patients with CVD were compared to those without CVD and with controls (Su et al., 2008). We also noted that IgG anti-PC were higher in SLE patients than in controls in the present study, which did not confirm previous data (Su et al., 2008). However, differences in study design, higher mean age, and only female SLE-patients in the Su et al. study, could explain the inconsistency.

High levels of IgM antiPC were previously associated with decreased prevalence of CV events in a group of hypertensive subjects (Su et al., 2006). Furthermore, low levels of IgM anti-PC predicted CVD in 60 years old men (de Faire et al., 2010) as well as AMI (Gronlund et al., 2009), stroke (Sjoberg et al., 2009) and mortality risk in patients on hemodialysis (Carrero et al., 2009).

In a study from 2006 Faria-Neto reported that passive immunization with monoclonal IgM antiPC reduced the vein graft atherosclerosis in apoE-null mice (Faria-Neto et al., 2006). In another mouse-model IgM anti-PC were shown to be protective against lethal meningococcal infections (Briles et al., 1982). Anti-PC antibodies have anti-inflammatory properties, inhibiting inflammatory phospholipids such as platelet activating factor (Su et al., 2008). Thus, low IgM anti-PC could represent an immune deficiency state, predisposing to CVD and inflammatory diseases (SLE included). Interestingly, platelet activating factor is a potent pro-inflammatory mediator implicated in active SLE (Tetta et al., 1990) but also in a diverse range of other human pathologies including shock, ischemia, asthma, CNS and renal disorders. Platelet activating factor induces leakage supposed to be mediated by an interaction between ROS and NO (Klabunde and Anderson, 2002).

Furthermore, human anti-PC inhibits the uptake of oxLDL in macrophages, implying another non-mutually exclusive possible mechanism by which IgM anti-PC could be atheroprotective (de Faire et al., 2010). We hypothesized that this may contribute to the low incidence of CVD, in addition to more favorable metabolic and other risk factors. Diet factors and exposure to infectious agents, including nematodes and parasites may contribute, but the reasons for differences in anti-PC levels among cohorts are not well understood (Frostegard et al., 2007). The possibility that diet factors contribute to anti-PC levels is supported by recent findings from our group showing that for patients with rheumatoid arthritis, a gluten-free vegan diet (Elkan et al., 2008) and self-reported Mediteranian diet (Elkan et al., 2009) led to increased levels of IgM anti PC.

Among traditional risk factors, hypertension and increased TG were significantly more common in SLE, while smoking was not, which is in line with previous publications (Svenungsson et al., 2001). LDL is not known to be commonly raised in SLE, and in this study, the frequency of hyperlipidemia was even higher among controls. An interesting finding is that HOMA-IR, a measure of insulin resistance, was increased in SLE. This confirms findings in a previous report (El Magadmi et al., 2006) and implies that SLE in general is characterized by early metabolic changes.

Within the SLE group, plaque occurrence was independently and positively associated with age and LDL and negatively with IgM anti-PC. This finding is in accordance with our previous study reporting a negative association between IgM anti-PC and atherosclerosis development in hypertensive patients (Su et al., 2006). Thus, traditional risk factors in combination with low levels of IgM anti-PC may explain the observed increased occurrence of plaques in SLE as seen in study I.

Cumulative or present doses of prednisolone (or other GC) were not associated with the occurrence of atherosclerotic plaques. Prednisolone has been much discussed in autoimmune diseases and has often been described as pro-atherogenic due to its unfavorable effects on metabolic factors. However, in a recent report on rheumatoid arthritis, no association between low-dose prednisolone intake and atherosclerosis development was found (Hafstrom et al., 2007). Our present data argue against the possibility that prednisolone is proatherogenic in SLE.

In study II we could not determine any significant difference in endothelial response to ACh iontophoresis in microcirculation, measured by LDF, between SLE-patients and controls. Our finding is in line with two previous smaller studies which also failed to find a difference in microcirculation, in SLE patients as compared to controls (Bengtsson et al., 2010, de Leeuw et al., 2008).

LDF has been analyzed also in other rheumatic diseases, for small patients groups: microvascular circulation was impaired in fibromyalgia and systemic sclerosis (Morf et al., 2005), also in ankylosing spondylitis (van Eijk et al., 2009) but not in early rheumatoid arthritis (van Eijk et al., 2011).

Previous studies on endothelial function as an early sign of CVD showed a correspondence between results obtained using invasive and non-invasive measurement methods. Thus, in 1995 Anderson et al. described a positive correlation between coronary vasoconstriction, produced by ACh (as a marker of damaged endothelium) and impaired FMD in the brachial artery (Anderson et al., 1995). Hansell and co-workers later described a correlation between FMD and LDF after ACh iontophoresis in healthy individuals (Hansell et al., 2004), though this finding was not subsequently confirmed in a later study (Gori et al., 2006).

ACh is a vasodilator agonist used in studies to stimulate endogen production of NO. In the coronary artery ACh may lead to vasoconstriction of the smooth cells (Davignon and Ganz, 2004, Ludmer et al., 1986). ACh applied on the skin stimulates the production of prostanoids (Berghoff et al., 2002). The cutaneus blood flow measured by LDF is regulated by endothelial, neuronal and humoral factors. ACh

increases the endothelial production of NO, but the iontophoresis is produced by a current which has been reported to stimulate the neuronal mechanisms (Berghoff et al., 2002). However, it is unlikely that the low current used herein produce such an effect (Hu et al., 1998, Martin et al., 2000). Localization and dimension of the iontophores probe may also influence the results of the measurements (Gardner-Medwin et al., 1997). Using our protocol, unspecific neurogenic vasodilation in response to the current used for iontophoretic stimulation is unlikely. Anodal iontophoresis must be used for longer periods with higher current strength and more concentrated ACh solutions to elicit axon reflexes (Martin et al., 2000).

The most commonly used method to determine endothelial function is FMD, and many if not all studies indicate that by use of this method, an impaired endothelial function is implicated in SLE (El Magadmi et al., 2004, Lima et al., 2002, Valdivielso et al., 2008, Kiss et al., 2006). However, in other studies, including our own, such a difference was not statistically significant (Cypiene et al., 2009, Aizer et al., 2009, Svenungsson et al., 2008). Differences in methodology and study design could possibly account for opposing results. However, in line with an impaired endothelial function in SLE are studies using other techniques, but with similar results (Nienhuis et al., 2010).

In this second study based on smaller sample of the same patient cohort (since not all patients were investigated with LDF), the prevalence of plaques was raised among SLE patients when we adjusted for age. However, we could not determine an association between atherosclerosis measurements and LDF, indicating that microvascular dysfunction is not an important correlate of atherosclerosis which occurs mainly in large arteries.

In the SLE group investigated with LDF, previously reported differences between patients with and without Raynaud's disease and smokers or non-smokers, could not be confirmed (Bengtsson et al., 2010, de Leeuw et al., 2008). This is possibly related to differences in group characteristics between the studies. Furthermore, we could not detect any association between medication (including chloroquine) and LDF.

The response to ACh differed between patients with and without CVD and with or without anti-hypertensive medication. In both of these groups a less favorable LDF response was documented. This finding indicates that in SLE patients with CVD there is a disturbance in the microvasculature. We also found a negative correlation between pack years and MRSP, a finding in line with previous reports (de Leeuw et al., 2008, Bengtsson et al., 2010).

The measurement of blood flow in the microcirculation as a response to ACh iontophoresis has the advantage of being an easy to use and non invasive method. Therefore it can be used to study ACh response during pregnancy and in newborn children. Associations between impaired ACh response, low maternal folate and low birth weight have been previously reported (Martin et al., 2000, Martin et al., 2007). Similarly, in patients with diabetes a correlation between artery stiffness and impaired response to ACh in microvascular bed was previously found (Hu et al., 1998).

Taken together, our data indicate that the prevalence of atherosclerotic plaques is increased in SLE, while IMT did not differ. Furthermore, a novel finding is that vulnerable plaques are more common among SLE patients. Low IgM anti-PC was, for the first time, determined to be an independent risk factor for prevalence of atherosclerotic plaques in SLE, together with age and LDL. Microcirculation as determined by iontophoresis did not differ between cases and controls, but was associated with CVD among SLE patients.

8 SVENSK SAMMANFATTNING

Senaste åren, i takt med teknologins utveckling och möjligheten att mäta mindre och mindre strukturer, har immunologins (läran om immunförsvaret) kunskaperna utvidgats avsevärt. Kroppens försvarsmekanismer bedöms vara grund till förekomst av många sjukdomar. SLE är en av dem, ett inflammatoriskt tillstånd som kan uttrycka sig i flera organ. Kroppens egen försvarsmekanism utvecklar antikroppar mot egna celldelar i kroppen. En uppkomst teori till sjukdomen är att immunsystemet aktiveras vid celldöd, då kärndelar bland annat exponeras för immunförsvarets celler; detta på grund av att antikroppar är riktade mot partiklar i cellens kärna. Man har kunnat identifiera mer än 100 antikroppar hos patienter med SLE.

Ateroskleros är en sjukdom i kroppens större kärl, på svenska kallad åderförkalkning eller åderförfettning. Åderförfettning kan starta redan i barndomen, men tar fart framför allt efter 45 års ålder och karakteriseras av plack bildning i större blodkärl. Plack kan leda till förträngningar och, under vissa omständigheter, tilltäppning av viktiga kärl och avstängning av blod försörjelse i livsviktiga organ som hjärta och hjärna, med hjärtinfarkt och hjärninfarkt som följd. Detta är fortfarande främsta orsaken till sjuklighet och dödlighet i västvärlden. När man tittar inne i plackens struktur hittar man bland annat aktiverade immunförsvarsceller. Denna aktivering och påverkan på blodkärl bedöms vara orsak till plackutvecklingen.

Patienter med SLE har mycket ökat risk att utveckla åderförkalkning i jämförelse med normal befolkning. För att hitta möjligheter till att blockera eller till och med förhindra åderförkalknings utveckling, jämför forskare SLE patienter med ålder och sexmatchade friska individer.

Ett problem är att hitta orsaken till att åderförkalkningen startar, en annan är att hitta individerna som har tendens till den. Det är också målet med våra två studier.

I vår första studie har vi jämfört 114 SLE patienter med 122 ålders och sex matchade individer. Vi har kommit fram till att SLE patienter har mera plaque, mera tendens att utveckla hjärtkärlsjukdomar och att låga nivåer av antikroppar mot phosphorylcholine (ett ämne som finns naturligt i människans cellmembran) är associerade med åderförkalkning.

Med ultraljuds teknik har vi, i vår andra studie, undersökt reaktiviteten i hudens små blodkärl, som ett tänkbart mått på kärlsjukdom, efter stimulering med en indirekt kärlvidgande substans. Vi hittade dock inga skillnader mellan patienter och kontroller beträffande reaktiviteten, men sämre reaktivitet var associerad med redan befintligt hjärtkärlsjukdom hos pat.

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