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Cardiovascular Disease in Systemic Lupus Erythematosus

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MD

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Cover : *Parthenos sylvia philippensis* (butterfly) approaching bleeding hearts
Parthenos sylvia philippensis (fjäril) som närmar sig en kvist med löjtnantshjärtan
Back: Butterflies from Butterfly house in Haga, Stockholm, and from Malaysia.
Fjärilar från Fjärilshuset i Haga och från Malaysia.

Till minne av Mor

"Att våga är att förlora fotfästet en stund.
Att inte våga är att förlora sig själv."

Sören Kirkegaard

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which will be referred to by their Roman numerals.

I **Svenungsson E,** Jensen-Urstad K, Heimbürger M, Silveira A, Hamsten A, de Faire U, Witztum J, Frostegård J:
Risk Factors for Cardiovascular Disease in Systemic Lupus Erythematosus.
Circulation 2001;104:1887-1893

II Jensen-Urstad K, **Svenungsson E**, de Faire U, Hamsten A, Frostegård J:
Cardiac valvular abnormalities are frequent in SLE patients with, but not without, manifest arterial disease.
Lupus 2002 11(11):744-52

III **Svenungsson E**, Guo-Zhong F, Jensen-Urstad K, Silveira A, de Faire U, Hamsten U, Frostegård J:
TNF-α - a link between inflammation and dyslipoproteinemia in SLE patients with cardiovascular disease.
Accepted for publication in *Lupus*

IV **Svenungsson E**, Gunnarsson I, Guo-Zhong F, Lundberg I E, Klareskog L, Frostegård J:
High triglycerides and low HDL are markers of Disease Activity and closely related to an upregulation of theTNF-α/TNFR system in Systemic Lupus Erythematosus.
Submitted

V Frostegård J, **Svenungsson E**, Wu R, Gunnarsson I, Lundberg I E, Klareskog, L, Hörkkö S, Witztum J:
Lipid-peroxidation is enhanced in SLE patients and associated with arterial and renal disease manifestations.
Manuscript

ABSTRACT

Premature cardiovascular disease (CVD) is a major cause of morbidity and mortality in systemic lupus erythematosus (SLE). This is an important clinical problem and a main objective of this study was to identify risk factors and underlying mechanisms. Since both atherosclerosis and SLE are chronic inflammatory conditions, SLE can also be regarded as a "human model" for studies of inflammatory components in CVD, not least in women, who make up 90% of SLE cases.

A cohort comprising 208 SLE patients is the base of these studies. From this cohort a nested case control study was designed. Twenty-six women (52±8.2 yrs) with SLE and a history of CVD (SLE cases) were compared to 26 age-matched women with SLE but without manifest CVD (SLE controls) and 26 age-matched population-based control women (population controls). Common carotid intima-media thickness (IMT) was measured by B-mode ultrasound as a surrogate measure of atherosclerosis. Echocardiography was performed to assess valvular abnormalities. A monoclonal antibody (E06) to oxidized low density lipoprotein (oxLDL) was used to determine oxidation epitopes on LDL.

This is the first study to show that SLE cases had enhanced atherosclerosis, as measured by increased IMT, in comparison to population controls while the IMT of SLE controls did not differ from that of population controls. SLE cases were, as compared to both control groups, distinguished by higher degree of inflammation: raised levels of acute phase reactants (α-1-antitrypsin, C-reactive protein, orosomucoid and erythrocyte sedimentation rate) and soluble TNF-α; dyslipidemia with raised triglycerides (TG) and lipoprotein (a), and decreased high density cholesterol (HDL); raised plasma concentrations of circulating oxLDL, and autoantibodies to epitopes of oxLDL (aoxLDL); lupus anticoagulant (LAC) and hyper-homocysteinemia. The cumulative prednisolone dose was higher in the SLE case group. Disease duration, smoking, BMI or diabetes mellitus did not differ between the groups.

Valvular abnormalities were essentially confined to the SLE case group with a strong correlation to hyperhomocysteinemia and high TG, two risk factors with known potential to induce endothelial dysfunction and damage.

Levels of TNF-α and soluble TNF-α receptors correlated with high TG/low HDL. This was first noticed among SLE cases and later confirmed in the large cohort. In other settings, TNF-α is known to cause similar patterns of dyslipoproteinemia through known mechanisms.

Dyslipoproteinemia, especially high TG, and activity in the TNF-α system also correlated to higher disease activity and cumulative disease damage. Elevations of TG and TNF-α activity were more pronounced in a subgroup of patients with CVD and renal manifestations. Thus, they were markers for more severe SLE.

High levels of LAC were also associated with CVD. AoxLDL are closely related to aPL, and were elevated in SLE in general. We also measured oxidation epitopes per LDL particle and found that they were enhanced in SLE in general, but in particular in SLE patients with CVD and renal manifestations. These data support a role for immune responses to "neo-self" antigens, generated during oxidation, in SLE related CVD.

In conclusion, we here demonstrate that atherosclerosis is enhanced in SLE patients with CVD. We have identified a set of traditional and non-traditional risk factors, which are important in SLE-related CVD. Several of these risk factors, like raised TNF-α levels and closely associated dyslipoproteinemia, correlate with disease activity. TNF-α may be a major factor in SLE-related CVD acting both by causing dyslipidemia and by promoting atherosclerosis-related inflammation. Enhanced expression of oxidatively modified "neo-antigens" on LDL may also be of importance for premature CVD and a trigger for autoimmunity to phospholipids (aPL). Thus, the majority of CVD risk factors identified in these studies are more or less strongly associated with SLE per se.

ABBREVIATIONS

ACL	anticardiolipin
ACR	American College of Rheumatology
ANA	antinuclear antibodies
anti ds-DNA	antibodies to double stranded DNA
aoxLDL	antibodies to oxidized LDL
aPL	anti-phospholipid antibodies
apo-A	apolipoprotein A
apo-B	apolipoprotein B
APS	antiphospholipid syndrome
ARA	American Rheumatism Association
aRNP	anti ribonucleoprotein
ß2-GP1	ß2-glycoprotein 1
BILAG	British Isles Lupus Assessment Group index
BMI	body mass index
CuoxLDL	Copper oxidized low density lipoprotein
CRP	C-reactive protein
CVD	cardiovascular disease
ECLAM	European Consensus Lupus Activity Measurement
ELISA	enzyme linked immunosorbent assay
HDL	high density lipoprotein
HTG	hypertriglyceridemia
IDL	intermediate density lipoprotein
IFN-λ	interferon-λ
IL	interleukin
IMT	intima media thickness
LAC	lupus anticoagulant
LDL	low density lipoprotein
LO	lipooxygenase
Lp(a)	lipoprotein (a)
LPC	lysophosphatidycholin
LPL	lipoprotein lipase
MDA	malondealdehyde
MI	myocardial infarction
MTHFR	methylenetetrahydrofolate reductase
NO	nitric oxide
oxCL	oxidized cardiolipin
oxLDL	oxidized low density lipoprotein
PC	phosphorylcholin
PLA2	phospholipas A2
RA	rheumatoid arthritis
Rf	rheumatoid factor
SLAM	Systemic Lupus Activity Measure
SLE	Systemic Lupus Erythematosus
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index
SLICC/ACR	Systemic Lupus International Collaborating Clinics/American College of Rheumatology
SSA	Sjögren´s syndrome A antibodies
SSB	Sjögren´s syndrome B antibodies
sTNFRs	soluble TNF-α receptors
TG	triglycerides
TNF-α	tumor necrosis factor-α
VLDL	very low density lipoprotein

TABLE OF CONTENTS

SYSTEMIC LUPUS ERYTHEMATOSUS

Introduction and historical background

Systemic lupus erythematosus (SLE) is a multisystemic autoimmune disease characterized by the production of autoantibodies and immune complexes in association with typical clinical symptoms. During childbearing ages 90 % of SLE patients are women, the female preponderance in prepubertal and postmenopausal ages being somewhat lower[1].

Historically, the systemic nature of SLE was gradually recognized. The first clinical descriptions of SLE were focused on skin manifestations at a time when the term lupus, Latin for wolf, was used to describe all kinds of facial skin lesions resembling a wolf´s bite. In 1845 von Hebra described skin eruptions affecting "the face and nose in a distribution not dissimilar to a butterfly"[2]. A few years later, in 1851, a more reliable description of SLE skin lesions was made by Cazanave, who was also the first to use the term lupus erythematosus (red skin)[3]. In 1872 Kaposi recognized the systemic nature of lupus especially the joint involvement[4]. In the late 1890´s Sir William Osler further distinguished cutaneous and systemic aspects of the disease introducing nephritis and vasculitis as manifestations of Lupus[5].

Diagnosis of SLE has further improved by the development of immunological testing. The first of these tests was the lupus erythematosus cell test by Hargraves in 1949[6]. The introduction of the anti-DNA antibody test in 1957 further sharpened diagnostic procedures in SLE[7,8].

Clinical aspects and definitions

SLE is a chronic inflammatory disorder though the clinical course is varied. In most SLE patients the disease is mild and only a limited number of organs are involved, in others involvement includes many organs, and severe SLE can be life threatening, especially when internal organs are affected. The organs most often involved are the joints (arthralgias, arthritis), skin (malar rash, photosensitivity, discoid rash), blood (leukopenia, trombocytopenia, anemia), serous membranes (pleurisy, pericarditis), kidneys (glomerulonephritis), central nervous system (cognitive dysfunction, psychiatric dysfunction and other non focal and focal lesions), blood vessels (thrombocclusive manifestations and

vasculitis) and lungs. General symptoms, such as muscle ache and fever and especially fatigue are also very common in SLE.

Onset in SLE may be either insidious over several years or more acute with multiorgan involvement. A recent longitudinal study by Barr et al defined three patterns of SLE disease activity: chronic active, relapsing-remitting (periods of disease flares or exacerbations) alternating with periods of fewer symptoms, or in some cases clinical remission and long quiscence[9]. During follow up time the chronic active pattern was most common, followed by the relapsing-remitting pattern. This implies that persistent disease activity with a chronic inflammation prevails, a finding, which may be of importance for the subsequent risk for SLE patients to develop premature cardiovascular disease (CVD).

In addition to clinical symptoms SLE is characterized by the presence of autoantibodies, among them antinuclear antibodies (ANA), anti double stranded DNA antibodies (anti ds-DNA), Sjögren´s syndrome A antibodies (SSA), Sjögren´s syndrome B antibodies (SSB), rheumatoid factor (Rf), anti-ribonucleoprotein (aRNP) antibodies, anti-Sm antibodies (Sm), antiphospholipid antibodies (aPL) and many more not studied in clinical practice[10].

Due to the varied clinical picture with multiorgan involvement it has not been possible to make a uniform definition of SLE. In clinical practice the best definition may be an autoimmune disease with involvement of at least two organ systems together with the presence of typical autoantibodies. For research purposes however, the need for a more precise definition led to the publication of the ARA (American Rheumatism Association, former American College of Rheumatology) preliminary classification criteria for SLE first published in 1971[11] and revised by Tan in 1982[12]. They have since been widely used in most studies of SLE patients. These criteria include 9 clinical and 2 immunological criteria and according to them, definite SLE is present if a patient fulfills four or more of eleven specified criteria (table 1). These criteria were modified in 1997 the most important change being inclusion of antiphospholipid antibodies in the 10th criterion[13]. Implementation of this modification would substantially change the population of SLE patients, but most studies still use the unmodified 1982 revised criteria.

Table 1

1982 Revised criteria for SLE and their frequency

Criteria	*Definition*	*Frequency % Tan*[12]	*Frequency % KS*
Malar rash	Fixed erythema over the malar eminence	57	57
Discoid rash	Erythematous raised patches	18	20
Photosensitivity	Rash caused by unusual reaction to sunlight	43	70
Oral ulcers	Oral or nasopharyngeal ulceration	27	28
Arthritis	Nonerosive arthritis involving two or more peripheral joints	86	85
Serositis	Pleuritis or pericarditis	56	45
Renal disorder	Persistant proteinuria > 0.5 g/day, or cellular casts	51	35
Neurologic disorder	Seizures or psychosis	20	15
Hematologic disorder	Hemolytic anemia, leukopenia, lymphopenia or thrombocytopenia	59	67
Immunologic disorder	Positive LE-cell preparation, anti ds-DNA, anti-Sm or false positive test for syphilis	85	60
Antinuclear antibody	Abnormal titer of ANA	99	99

Definition of the 11 revised criteria used for classification of SLE [12]. Frequency of manifestations in the original publication and in our cohort comprising 208 SLE patients at the Karolinska Hospital (KS).

Incidence, prevalence and mortality in SLE

SLE is a worldwide disease, but prevalence and incidence figures vary considerably and are difficult to compare due to varying epidemiological methods both with respect to retrieval and case definition. Several studies indicate that SLE is more common in certain ethnic groups. In the Unitited States, a three- to fourfold increased prevalence and an earlier disease onset was seen among African American women as compared to Caucasians[14,15]. In

epidemiological studies from the Nordic countries, United Kingdom and the United States prevalence figures vary between 12 - 68/100 000 and annual incidence figures between 1.8-7.6/100 000[1,16,17]. According to the most recent study from a defined region in southern Sweden, prevalence was 68/100 000 with an annual incidence of 4.8/100 000. Consecutive studies from the same region show an increasing prevalence but constant incidence figures in accordance with longer survival and better prognosis of Swedish SLE patients.

The first epidemiologic study on SLE patients is from the 1955 and report a 5 year survival of 51 %[18]. It was carried out at a time when only severe cases of SLE were diagnosed and modern treatment with corticosteroids and immunosuppression was unheard of. Since, diagnostic procedures and treatment have improved considerably resulting in better prognosis and survival. According to the latest longitudinal studies, 5 year survival in Sweden is 93% and 10 year survival 83%.

Inflammatory markers in SLE

Different inflammatory states are characterized by different patterns of inflammatory markers. Most bacterial infections and rheumatoid arthritis (RA) have a strong acute phase response, which correlate with present disease activity. In SLE the chronic inflammation is different and comparatively modest elevations of C-reactive protein (CRP) and other acute phase reactants are seen in active disease[19-21]. This is especially true for SLE patients with active renal disease[22]. The reason for this is not clear. Cytokines, mainly interleukin-6 (IL-6), tumor necrosis factor α (TNF-α) and interleukin-1 (IL-1) are believed to be crucial in eliciting the acute phase response[23]. Despite modest or absent acute phase response, circulating levels of these cytokines are comparable to or even higher in SLE than in RA[24,25].

Instead, other laboratory markers of disease activity have been used including antibodies to ds-DNA. They usually correlate with disease activity but are detected only in approximately 50% of SLE patients most of whom have nephritis[26,27]. Other traditional markers which also are useful in subgroups of SLE patients are related to complement consumption and include complement levels and complement split products[28-30]. Lately the search for biological markers of disease activity has been focused on cytokines and their soluble receptors. Proinflammatory cytokines (IL-6 and TNF-α) and also cytokines and cytokine receptors thought to have mainly anti inflammatory effects (interleukin-10 (IL-10), soluble TNF-α receptors and soluble interleukin-2 (IL-2) receptors) have been claimed, though with some inconsistence, to correlate with SLE disease activity[31-35].

Estimations of disease activity and disease damage

The varied clinical picture seen in SLE makes comparative studies of disease activity difficult. Over the years a number of indices, combining clinical and laboratory information, for measurement of disease activity have been developed. Best validated and most commonly used are the Systemic Lupus Activity Measure (SLAM)[36], SLE Disease Activity Index (SLEDAI)[37], the British Isles Lupus assessment Group index (BILAG)[38] and European Consensus Lupus Activity Measurement (ECLAM)[39]. Comparative studies indicate that all these instruments are valid measures of SLE activity and have similar correlations to physicians' global assessment of disease activity. SLEDAI was somewhat less sensitive to change and SLAM correlated closer to patients own assessment of disease activity[40,41].

For the purpose of calculating cumulative disease damage over time there is essentially only one widely used index, Systemic Lupus International Collaborating Clinics /American College of Rheumatology (SLICC/ACR) damage index[42]. The SLICC/ACR index registers longstanding damage caused both by disease activity per se as well as damage caused by side effects of medication.

In activity indices as well as in the SLICC/ACR index clinical data are registered for specific organ systems. The points from the different organ systems are, when applicable, added to points from other variables (laboratory measures etc.) making a total sum, which is used for comparisons longitudinally or between patients.

IMMUNE SYSTEM

General overview

The principal task of the immune system is to protect us from invading infectious agents. To do this there are several lines of defense. A first line consists of physical barriers like the skin and the mucous membranes lining the gastrointestinal, respiratory and reproductive tracts. Also within the body there are barriers such as the endothelial lining, which separates the blood compartment from other tissues.

A second line of defense is the innate immune system. Here, macrophages (big eaters) are pivotal. They express a limited number of highly conserved pattern recognition receptors (PRRs) such as scavenger receptors and Toll like receptors. Macrophages use these receptors

when patrolling the body in search for foreign material, which they immediately ingest on encounter. The complement system and acute phase proteins bind to (opsonize) bacteria and other invaders as well as to debris from the own body´s dying cells. When doing so they signal to macrophages to attack and engulf what needs to be taken care of. Thus innate immunity is a quick but not very specific way to dispose of external invaders and internal garbage.

Vertebrates have a third line of defense, which is often referred to as the specific or adaptive immune system. It is made up of B- and T-lymphocytes and it is characterized by ability to continuously change and adapt in response to invasion. It also has the ability to remember old enemies and fight them more effectively in case of return (immunity). Both B- and T-cells have receptors with an almost infinite diversity, which recognize every possible invader, though they have been taught to discriminate between self and non-self and not to attack self-antigens. Each B- and T-cell only expresses one particular type of receptor. Thus, there are relatively few cells with each receptor before these cells have been specifically activated. On encounter with its specific antigen each cell has the capacity to multiply and amplify the immunologic response to suit the particular antigen (clonal expansion), but this amplification takes some time. Thus, adaptive immunity is specific but much slower than innate immunity.

The humoral immune response is made up of B-cells, which make a large number of highly specific antibodies (immunoglobulins), i.e. soluble B-cell receptors that recognize foreign material and tag it in order to facilitate uptake and destruction by macrophages or kill microbes by means of complement system.

The cellular immune response consists of T-cells, which also have highly specific and diverse receptors (T-cell receptors) that recognize an antigen when it is presented to them by other cells, usually by a specialized cell for this purpose, the dendritic cell, a macrophage or a B-cell. T-cells are of either T-helper or T-killer (cytotoxic cells) type. T-killer cells recognize surface structures and kill potentially harmful cells, which have been infected by viruses or otherwise transformed. Activated T-helper cells produce large amounts of cytokines as a signal to other cells, especially, macrophages, to become activated or to recruit other cells like neutrophils for help. In this way T-helper cells initiate a local inflammatory response. [43,44]. T-helper cells also provide signals that are essential for the differentiation and activation of B-cells.

Cytokines

Cytokines are low molecular weight proteins, which are messengers in cell-cell communication. Their action is thought to be mainly local in tissues either on neighboring cells (paracrine action) or in a feedback fashion on the cells that produced them (autocrine action). However, they can also act at a distance in an endocrine fashion. They convey their information via specific high affinity cell-surface receptors. These are usually made up of three domains, the extracellular ligand binding domain, the transmembrane domain and the intracellular signal transducing domain [43].

T-helper cells are often subdivided into differenat subpopulations, based on the production of functionally distinct cytokine profiles The major subpopulations are denoted Th-1 and Th-2 cells. Th-1 cells produce interferon-γ (IFN-γ), IL-2 and interleukin 12 (IL-12), TNF-α, though mainly derived from macrophages, is also secreted by Th-1 cells. Th-1 cells activate macrophages and promote a local inflammatory response. Th-2 cells secrete interleukin 4 (IL-4), interleukin 5 (IL-5) and IL-10 and are essential in the activation of B-cells promoting proliferation into plasma cells which produce large quantities of antibodies.
IFN-γ and cell-cell contact with Th-1 cells is pivotal in the activation of macrophages. Activated macrophages secrete TNF-α, which in an autocrine fashion amplifies macrophage activation and also has the potential to activate other immune cells and kill virus infected cells[43].

ATHEROSCLEROSIS, AN INFLAMMATORY DISEASE

The Vessel Wall

Endothelial cells form a continuous monolayer that lines the inner surface of all blood and lymph vessels in the body. Their principal tasks are to separate the blood compartment from the body's interstitium and to facilitate transport of molecules in both directions between these compartments. Healthy endothelium has anti-adhesive and anti-thrombotic properties and serves as a barrier that prevents harmful agents from entering the vessel wall.

In arteries the vessel wall consists of three distinct layers, the intima, the media and the adventitia. The composition of these layers varies with type and size of vessel. The *tunica intima* is thin and consists of the endothelial lining, a thin sub-endothelial layer of connective tissue and a thin internal elastic membrane. The *tunica media* is composed of smoth muscle cells and collagen fibres, its principal task is regulation of vessel volume and blood pressure. The outer layer, *tunica adventitia*, is a loose connective tissue made up of fibroblasts and elastic fibers. In larger vessels nerves and small blood vessels supply the smooth muscle cells of the *tunica media* via the adventitia [45](fig 1).

The vessel wall

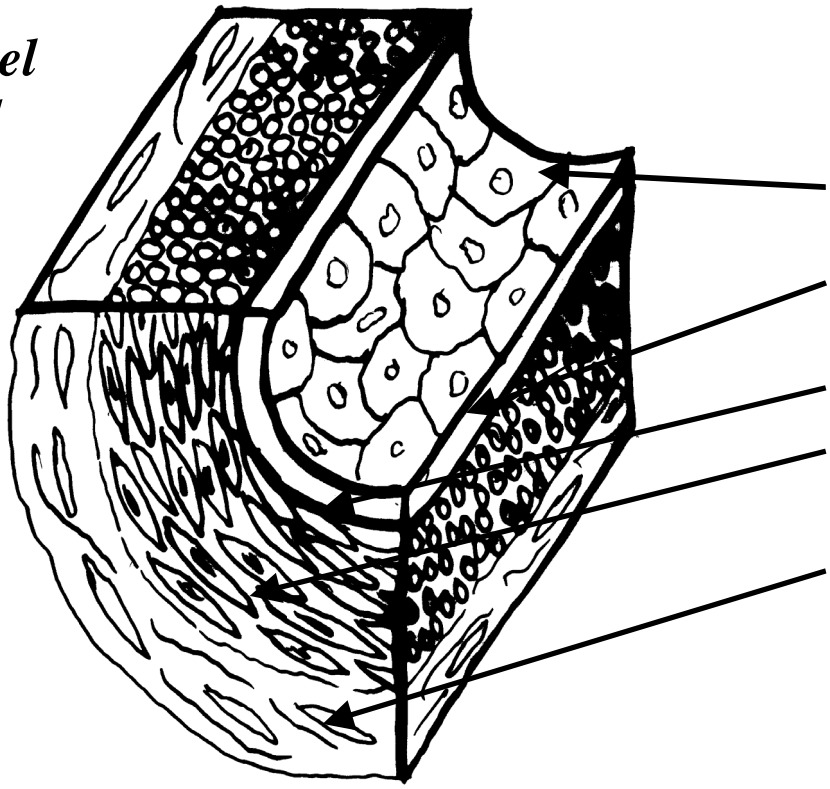
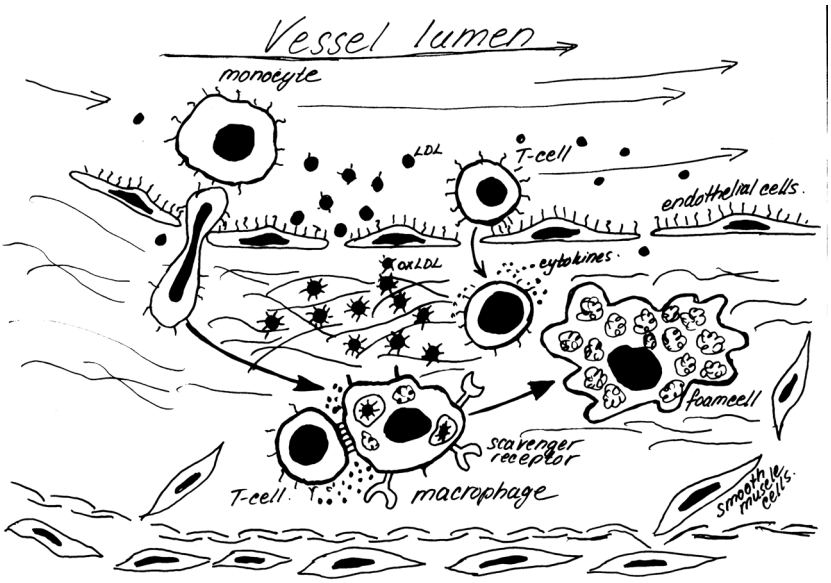


Fig 1
The vessel wall with internal lining of endothelium

Atherosclerosis - a general introduction

The formation of atherosclerotic plaques in our blood vessels is a lifelong process affecting all humans to a varying degree. Initially it is asymptomatic but gradually advanced atherosclerotic lesions are formed which eventually may obstruct blood flow, become unstable and rupture. Complications to this process including myocardial infarction, stroke and peripheral vascular disease, are the most common causes of mortality in developed countries[46]. Men are affected more often and at an earlier age than women[47]. According to

The life of a plaque



Fi 2 a

Fatty streak

Early atherosclerotic lesion, with gathering of monocytes/macrophages and T cells.
First foamcells develop.
Smooth muscle cells migrate from the media.

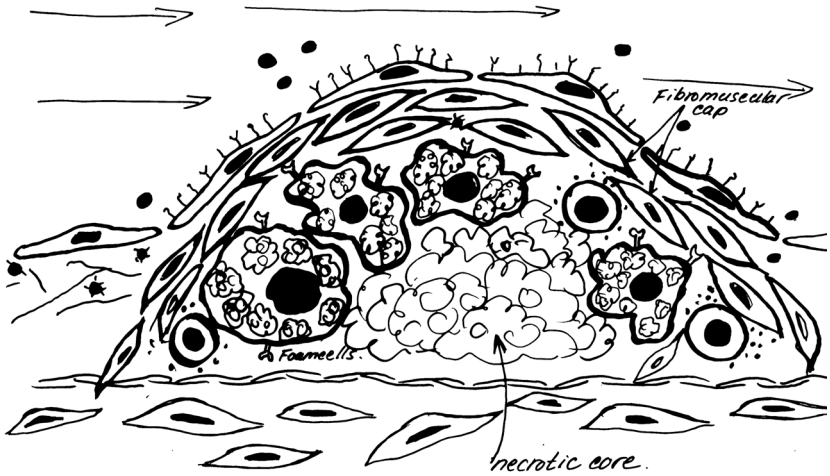


Fig 2b

Fibromuscular plaque

Smooth muscle cells migrate into the plaque through the internal elastic lamina. They group around the fat filled core of the plaque, forming a stabilizing fibromuscular cap.

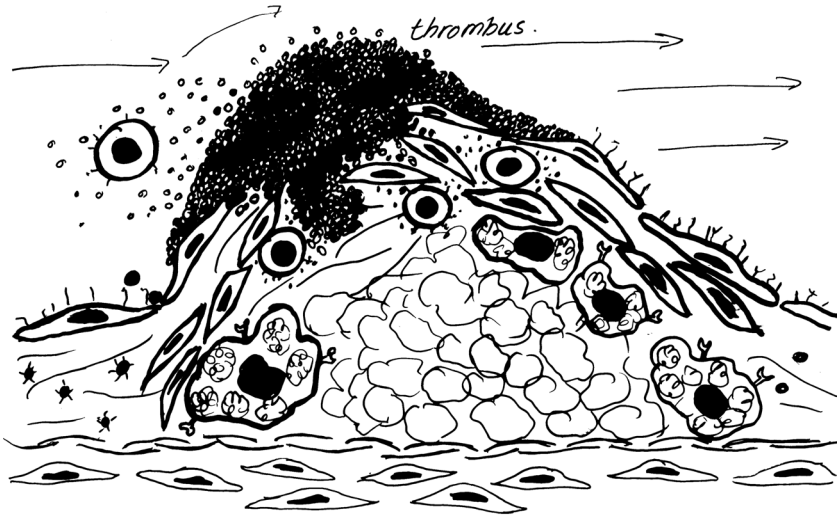


Fig 2 c

Ruptured plaque

T-cells and their proinflammatory cytokines have weakened the fibromuscular cap. Rupture exposes tissue factor to the coagulation cascade.
A thrombus is formed to cover the ruptured site.

recent Swedish national statistics, 46% of men and 47% of women died of cardiovascular disease (CVD) in 2000[48].

Epidemiological studies have identified several risk factors for CVD. Age, male gender, family history of CVD, smoking, hypertension, diabetes mellitus and hypercholesterolemia are generally accepted as risk factors for CVD. A number of additional items are being discussed but due to conflicting data they are less established as risk factors. Hypertriglyceridemia (HTG), low levels of high density lipoproteins (HDL), obesity, physical inactivity, impaired coagulation/fibrinolysis, hyperhomocysteinemia, psychosocial stress, and infections are such non traditional risk factors, some of which may be more important in subgroups of CVD patients[49].

The atherosclerotic plaque

The atherosclerotic lesions are confined to the intima just beneath the thin endothelial lining but above the internal elastic membrane and the underlying muscular layer. Atherosclerosis starts in early childhood with the development of fatty streaks (fig 2a). Monocytes enter the intima, mature into macrophages, engulf excess amount of lipids and turn into large lipid filled foam cells which are joined by infiltrating activated T-cells. In the teens and early adulthood fatty streaks transform into fibromuscular plaques. These are elevated focal smooth surfaced lesions with sharp borders, they are predominately distributed in medium and large size arteries, often gathering at sites of turbulent blood flow such as vessel bifurcations. Smooth muscle cells from the underlying *tunica media* have infiltrated the intima and at this point formed a fibromuscular cap, which surrounds and stabilizes the atherosclerotic lesion (fig 2 b). With time, the plaque with its necrotic lipid filled core grows and causes narrowing of the vessel lumen and this process may eventually obstruct blood flow. When the coronary arteries are affected, partial obstruction results in angina pectoris and when peripheral arteries are narrowed clinical symptoms of intermittent claudication arise. If the lipid filled plaque ruptures, pro-thrombotic substances like tissue factor are exposed to the coagulation system, which initiates local thrombus formation. The thrombus may occlude the vessel and stop blood flow (fig 2c). This is the pathophysiology behind most acute cardiovascular events like myocardial and other infarctions[46,50].

Lipids and atherosclerosis

For many years atherosclerosis was regarded as a passive accumulation of lipids in the vessel wall related to disorders of lipid metabolism. This view was supported by a now well established positive relationship between high levels of total and low density cholesterol (LDL) and an increased risk for CVD[51]. Numerous studies also identify high triglycerides (TG)[52] and low high density lipoprotein cholesterol (HDL)[53] as risk factors for CVD. Since high TG/low HDL often occur together as part of the metabolic syndrome, which also comprises high blood pressure, central obesity and insulin resistance[54], it has been difficult to sort out their independent impact on CVD risk. Hence the controversy on their role as risk factors.

Pathogenetically, lipid disorders can be divided into primary and secondary hyperlipidemias. Primary hyperlipidemias are genetically determined, an example is the LDL receptor deficiency seen in familial hyper cholesterolemia. Secondary hyperlipidemias arise as a consequence of diet, drugs or other disorders such as diabetes mellitus, hypothyroidism or kidney disorders. Though usually not mentioned in this context, rheumatic diseases like SLE and RA are causes of lipid disturbances, which consequently should be referred to as secondary lipid disorders.

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Lipoproteins and their metabolism

In the circulation both cholesterol and TG are transported as part of circulating lipoproteins. These are spherical in shape, consisting of a lipophobic core containing cholesterol esters and TG surrounded by a coat made up of apolipoproteins, phospholipids and unesterified cholesterol (fig 3). Depending on apolipoprotein content, size, and density, lipoproteins are divided into subclasses comprising chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) (fig 2). Chylomicrons, VLDL, IDL, and LDL all contain apolipoprotein-B (apo-B) and share metabolic pathways. HDL contains apolipoprotein A1 and has a different route of metabolism[55].

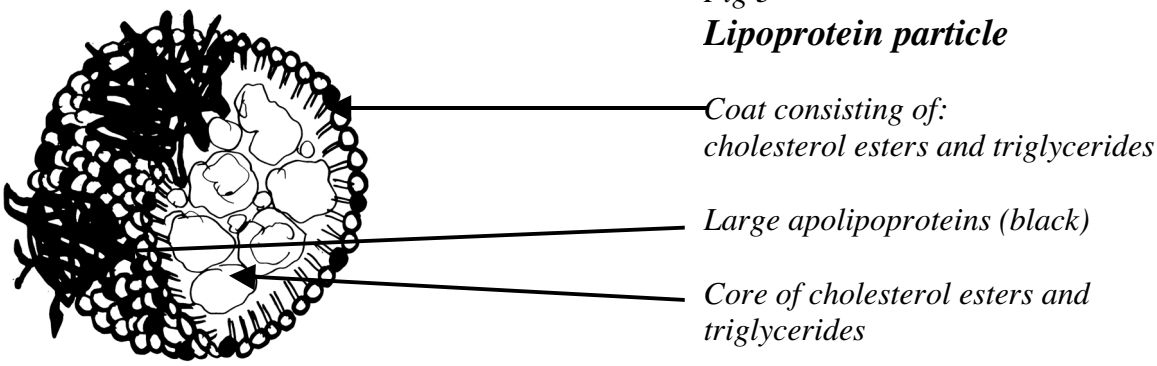


Fig 3
Lipoprotein particle

Exogenous lipid metabolism starts with dietary lipids, mainly consisting of triglycerides, which are absorbed by the enterocytes, packaged into chylomicrons and transported via the portal vein to the liver.

The origin of the endogenous lipid metabolism is the synthesis of TG rich VLDL and nascent HDL by the liver. VLDL synthesis is regulated by how much and what kinds of fatty acids are available. It has been demonstrated that "fish fatty acids" (eicosapentaenoic acid, EPA, and docosahexaenoic acid, DHA) decrease VLDL secretion while proinflammatory cytokines like TNF-α, IL-1 and interferon-α (INF-α) have been shown to stimulate VLDL synthesis[56,57]. As VLDL is degraded IDL and LDL are formed (fig 4).

The main enzyme responsible for degradation of apo-B containing lipoproteins (chylomicrons, VLDL, IDL and LDL) in the circulation is lipoprotein lipase (LPL) located on the luminal side of the endothelium. It binds to circulating triglyceride rich lipoproteins and causes release of free fatty acids, facilitating uptake by neighboring tissues. LPL is more abundant in tissues with greater energy demands like skeletal muscle, heart muscle and adipose tissue. Endothelial LPL thus has a lipid lowering effect in the circulation. In recent studies, LPL has been identified in atherosclerotic lesions in the artery wall. This LPL is synthesized by local macrophages and is thought to have mainly pro-atherogenic effects, promoting LDL binding to proteoglycans and their subsequent oxidation and uptake by macrophages/foam cells. Activity of LPL is regulated by hormones, among them insulin, which causes postprandial increase in LPL activity. Proinflammatory cytokines like TNF-α inhibit LPL activity[56,58]. An overview of lipid metabolism is presented in fig 4.

Lipoprotein (a) (Lp(a)) is an LDL like lipoprotein carrying an extra glycoprotein, apo(a) which is covalently bound to apo-B-100. Lp(a) has a thrombotic effect since it because of structural similarity, competes with plasminogen, for the plasminogen binding site on the endothelium, resulting in decreased conversion to plasmin and a decreased fibrinolysis [59].

Lipoprotein metabolism

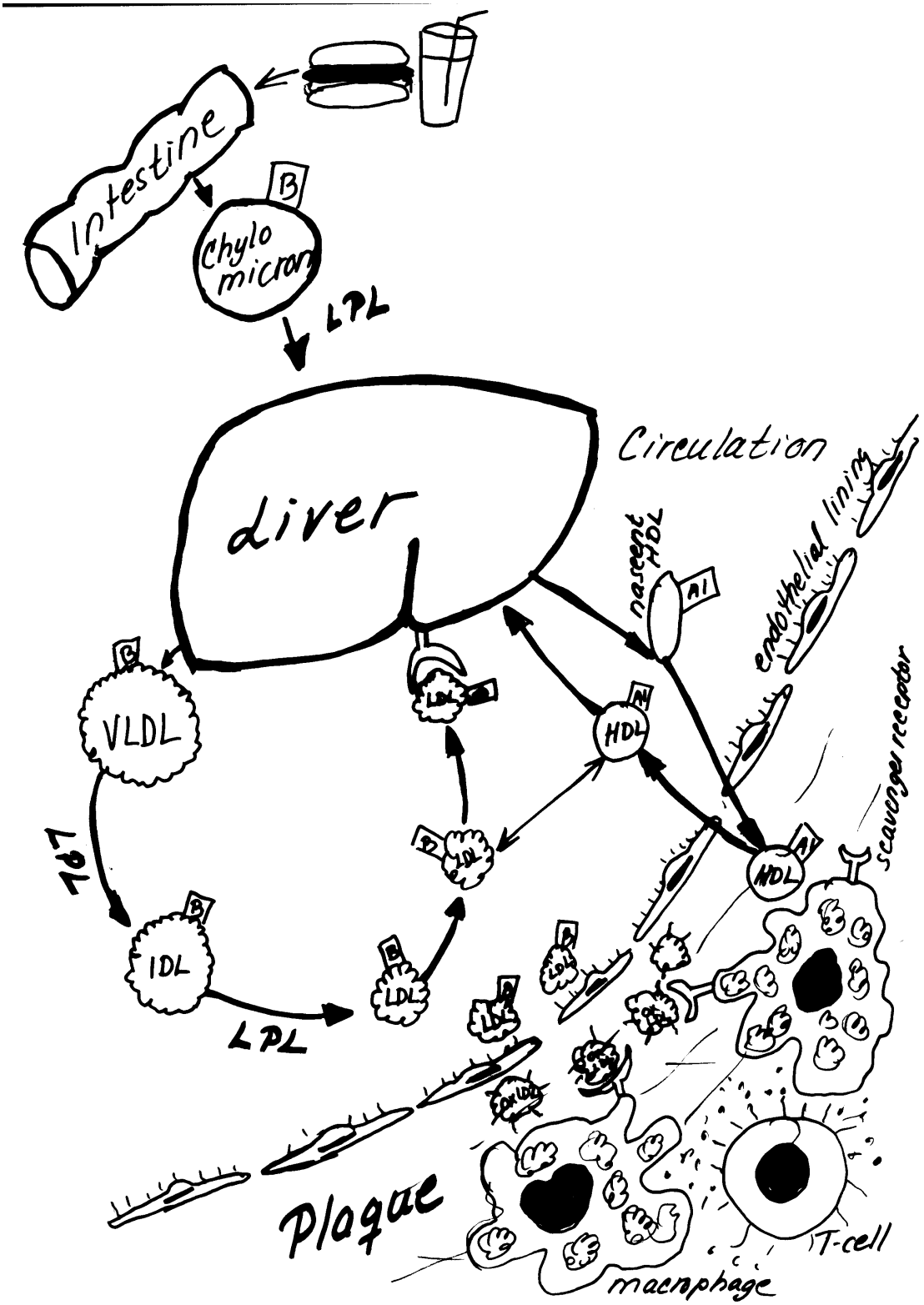


Fig 4

Overview of lipoprotein metabolism.

Exogenous lipids are carried from the intestine to the liver by chylomicrons. The liver synthesizes VLDL which circulates back to the liver via IDL and LDL (apo-B containing lipoproteins). On the way apo-B containing lipoproteins release free fatty acids to the tissues by help of LPL. Some LDL enters the endothelium where it is oxidized and incorporated into atherosclerotic lesions. HDL is also synthesized in the liver. It transports cholesterol from LDL and tissue back to the liver.

Lp(a) can in similarity to LDL be oxidized and taken up by macrophages and it has been isolated from atherosclerotic plaques[55,60]. High levels of Lp(a) have in the general population been linked to CVD[61].

Atherosclerosis is a chronic inflammatory disease

During the last decade evidence has mounted that atherosclerosis is an inflammatory disorder. Presence of inflammation has been demonstrated both in clinical and histopathological studies[46,62]. Systemic inflammation, as measured by modestly but definitely enhanced levels of CRP[63,64], IL-6 and TNF-α[65] or other pro inflammatory substances, have convincingly been associated with an enhanced risk of developing atherosclerotic complications and are also associated with adverse outcome once such complications have occurred[65].

From a histopathological perspective, activated immunocompetent cells and pro inflammatory substances are present in the atherosclerotic plaques as signs of an ongoing inflammatory process[66]. This process is thought to start by patch wise activation of the endothelium, resulting in expression of selectins, integrins and adhesion molecules. Risk factors for CVD like hypertension[67], hyperlipidemia[68], hyperglycemia[69] and hyper-homocysteinemia[70] all have the potential to activate the endothelium. Circulating monocytes and T-cells are halted, start rolling, adhere and penetrate the endothelial lining, which also increases its permeability for passing macromolecules like LDL. In the intima, monocytes transform into macrophages, express scavenger receptors, start to ingest modified LDL and develop into foam cells, which gather in the lipid filled center of the atherosclerotic plaque (fig 2a). T-cells are activated and further enhance the local inflammation by secretion of proinflammatory substances. At a later stage, smooth muscle cells migrate from the *tunica muscularis* and form the musculofibrous cap, which surrounds and stabilizes the growing lipid filled core of the plaque (fig 2b). Infiltrating inflammatory cells like macrophages and T-cells synthesize proinflammatory cytokines and enzymes, which cause the fibromuscular cap to thin and weaken, in some cases leading to plaque rupture[71](fig 2 c).

In summary, inflammatory processes play an important role for both growth and degradation of the atherosclerotic plaques[46,50,62].

Oxidative changes and immune reactions to modified LDL in atherosclerosis

According to the modified LDL hypothesis, alterations of LDL and to a lesser extent of other lipoproteins, are essential for the initiation of the atherosclerotic process[72]. Modifications as a result of oxidation are believed to be most important, and the probable site for these modifications is in the intima of the artery wall. After entering through the endothelial lining, lipoproteins are trapped in a network of proteoglycans which facilitate their exposure to oxidants, hydrolyzing enzymes and other modifying substances[73]. Though, not understood in detail, exposure to oxygen free radicals, nitric oxide (NO) or enzymes like phopholipase A2 (PLA2) or 15-lipooxygenase (15-LO) are believed to cause LDL oxidation in the intima[74]. As a response to oxidation, LDL changes its configuration both with respect to its protein (apo B-100) and lipid moieties. New antigens are exposed (neo-self antigens, oxidatively or otherwise modified) on or bind to the surface of the oxLDL particle and as a consequence oxLDL is no longer recognized by the LDL receptor. Phosphorylcholin (PC), lysophosphatidylcholin (LPC) and β2-glycoprotein-1 (β2-GP1) are such epitopes, which are antigenic on oxLDL[75,76]. PC is also present on the capsular polysaccharide of many bacteria, e.g. *Streptococcus pneumoniae*[77], and it has recently been shown to be present also on apoptotic cells[78]. These "neo-self " antigens are recognized by the scavenger receptors of macrophages, a function of the innate immune system[79]. Through this route, macrophages ingest modified LDL in an unregulated fashion, they fill with lipid droplets and transform into the large foam cells, characteristic of the atherosclerotic plaques[50] (fig 2 b and 2 c). Furthermore, oxLDL has pro inflammatory properties, it activates the endothelium, attracts monocytes and induces local production of pro inflammatory cytokines[80-82] (fig5). Other modifications like glycosylation, which takes place in diabetes may also render LDL atherogenic in a similar fashion.

OxLDL is an antigen also for the humoral immune system. Antibodies to oxidized LDL (aoxLDL) are present in atherosclerotic lesions[83] and in the circulation of normal healthy individuals. Levels are enhanced in patients with documented late atherosclerosis and in these they seem to correlate to degree of atherosclerosis[84-86]. On the other hand, in a group of patients with early lesions, as exemplified by borderline hypertension, aoxLDL levels were depressed[87]. Another observation is that in young mice immunization with oxLDL was protective for the development of subsequent atherosclerois[88,89]. A speculative but possible interpretation of these data may be that an early and adequate immune response to oxLDL by

the adaptive immune system (B- and T-cells) creates an immunologic memory which is helpful later in life when a growing burden of proatherogenic substances like oxLDL has to be taken care of. Or this may be a matter of balance, so that at some stage later in life very high levels of aoxLDL may become pathogenic.

LDL oxidation

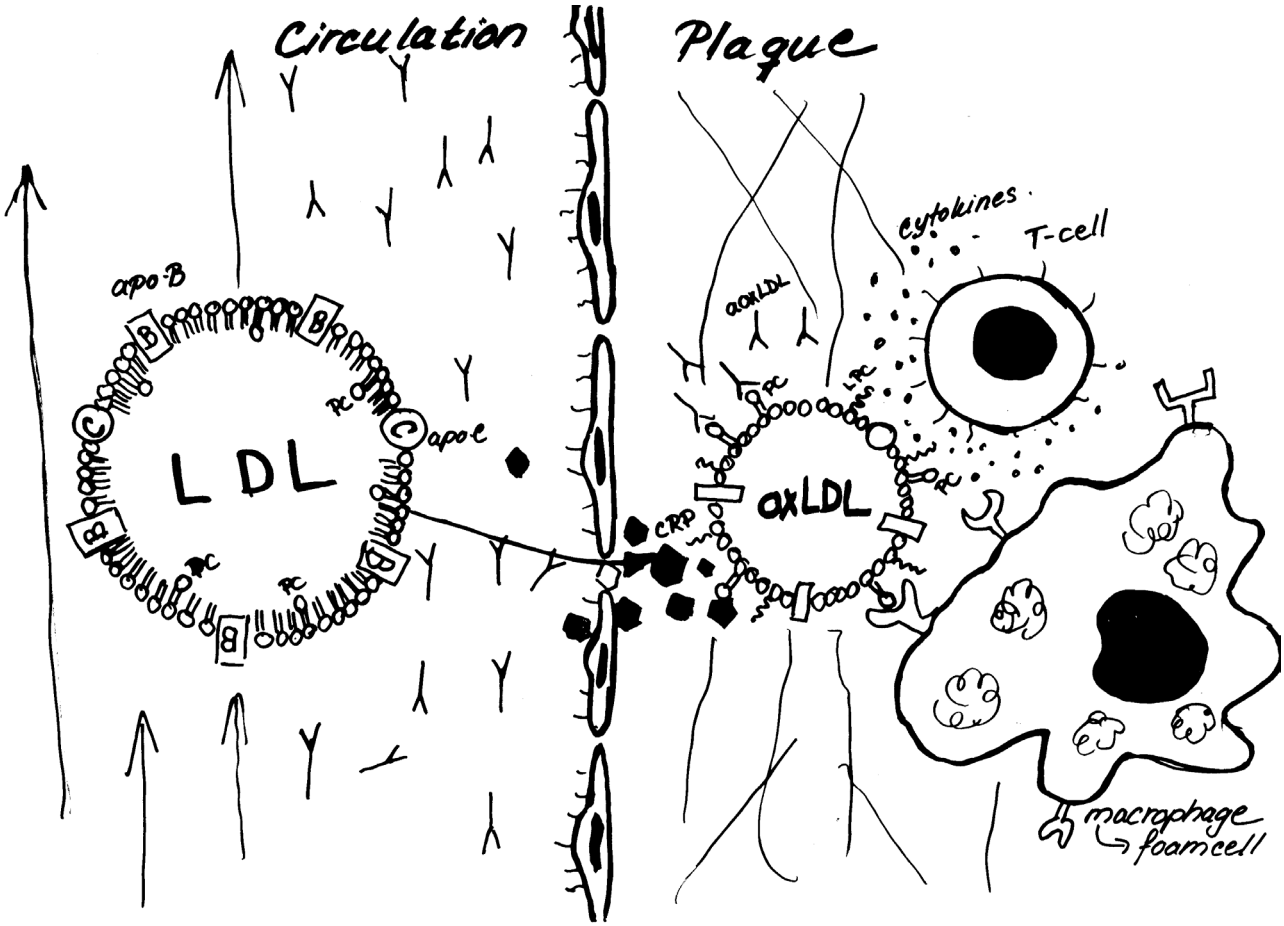


Fig 5
When LDL i s oxidized, it exposes new epitopes, "neo-antigens", like PC and LPC. oxLDL thus becomes immunogenic and elicits innate, humoral and cellular immune responses. Scavenger receptors and CRP are part of the innate immune response. Antibodies to PC and LPC are features of humoral immunity while T cells contribute with activating proinflammatory cytokines like IFN-γ (schematic presentation).

Women and cardiovascular disease

In affluent countries, CVD is the most important cause of death both for women and men. However, CVD has been much less studied in women, higher age at presentation may be one reason behind this. Women lag at least 10 years behind men in their presentation of CVD but since they live longer they "catch up" and eventually 46-47% of both sexes die from CVD[47,48,90]. Thus, arterial vascular disease is rare in women before the age of 55 but at menopause there is a steep three to fourfold increased risk of CVD[91]. Postmenopausal status is thus the single most important risk factor for CVD in women. With menopause comes a negative shift in lipid profile with increasing levels of total and LDL cholesterol and decreasing HDL[90]. Otherwise, most common risk factors are shared between men and women, though diabetes and dyslipoproteinemia with high TG/low HDL has been shown to be especially atherogenic in women[47,52,92,93].

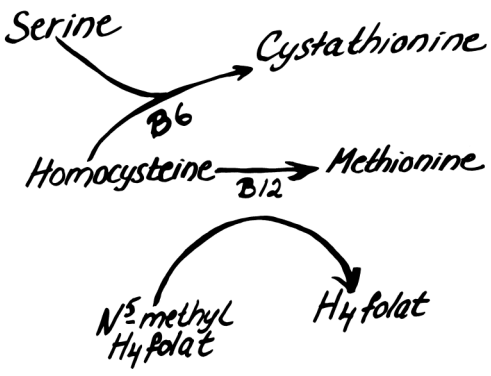
Homocysteine as a riskfactor for atherosclerosis

Homocysteine is a nonessential sulphur-containing amino acid. In normal metabolism homocysteine is essentially metabolized in two directions, either methylated to form methionine, a reaction dependent on the enzyme methylenetetrahydrofolate reductase (MTHFR) and vitamin B12, or condensed with serine to form cystathionine, catalyzed by cystathionine synthetase, which has vitamin B6 as an essential cofactor (fig6).

In 1969 the first case report of a child with hyperhomocysteinemia and atherosclerosis was reported[94]. Since then it has been demonstrated that moderately elevated levels of homocysteine are an independent risk factor for coronary, cerebrovascular and peripheral arterial disease[95-97].

Hyperhomocysteinemia may have multiple causes. It may be due to genetic defects such as polymorphisms in the above mentioned enzymes or lack of vitamin B6 or B12, all of which contribute to retarded metabolism and accumulation of plasma homocysteine. In renal insufficiency, impaired secretion is a cause of hyperhomocysteinemia. Increasing age, nutritional status, smoking, male gender, certain medicines (methotrexate, nitrous oxide) and inflammatory states are also factors which are associated with an elevation of plasma homocysteine[98].

Fig 6



Numerous studies have demonstrated that both chronic and acute elevations of plasma homocysteine are associated with impaired endothelial function as measured by flow mediated endothelium dependent vaso-dilatation[70,99-101]. Though the exact mechanism by which homocysteine impairs endothelial function is not known it has been shown that hyperhomocysteinemia induces oxidative stress in the endothelium and it is capable of promoting LDL oxidation[102,103]. It can also alter platelet function and coagulation factors[104]. In animal studies, high levels of homocysteine causes endothelial damage[105,106]. Lowering homocysteine levels with vitamin B supplementation has been reported to restore endothelial function[100] and is further support for the negative effects of hyperhomocysteinemia on the endothelium.

Phospholipid antibodies

Antiphospholipid antibodies are a family of antibodies with specificity for negatively charged phospholipids alone or in complex with binding proteins. It was first observed in SLE patients that these antibodies were associated with false positive tests for syphilis and also with an increased risk of both arterial and venous thrombotic complications, miscarriages and trombocytopenia[107]. In the early 1980´s the antiphospholipid syndrome (APS) was described in more detail both as a primary disorder or secondary to SLE, or more rarely to other autoimmune diseases[108-110]. At the same time a sensitive solid-phase immunoassay for detection of cardiolipin was developed[111] and was used together with the lupus anticoagulant (LAC) test for diagnosis of APS. There are several LAC tests, all of which are coagulation assays in which, when positive, coagulation is retarded due to the presence of LAC (immunoglobulins of mixed specificity, which interfere with the clot formation in vitro). Recent studies have shown that many are specific for prothrombin and some for β2-GP1[112].

In the early 1990´s it was discovered that many cardiolipin antibodies require the presence of a co-factor protein, β2-GP1, in order to bind to cardiolipin[113,114]. Several other protein co-factors like prothrombin, protein S, protein C, and annexin V have been identified

thereafter[115]. Some aPL have been shown to have specificity only for the co-factors. Thus an ELISA for detection of β2-GP1 has been added as a routine investigation for APS in many hospitals[116]. Further studies have demonstrated that aPLs preferably recognize oxidized phospholipds such as oxidized cardiolipin (oxCL)[117]. This may be one reason for difficulty in standardizing tests for aCL.

Animal studies support a causal roll for aPL in the development of thrombosis and pregnancy loss. Immunization of mice with aPL, β2-GP1 or antibodies to β2-GP1 result in fetal wastage and formation of larger thrombi following experimental injury[118-120]. Though a matter of intensive research the mechanisms by which aPL are thrombogenic are not known. They have the capacity to activate endothelial cells as assessed by enhanced expression of adhesion molecules and cytokine secretion[121]. It has been suggested that they interfere with natural anticoagulants such as protein C and protein S. Another possible mechanism was recently demonstrated by Rand et al who showed that aPL can disrupt the crystalline shield of annexin V which protects phospholipid membranes in placenta and endothelial cells from clotting[122]. Another study by Holers et al demonstrate the necessity of a functional complement system for induction of aPL related experimental fetal wastage[123]. Infectious origin of APS is another possibility supported by Blank et al who could elicit APS in mice via transfer of infectiously induced antibodies with cross reactivity to β2-GP1[119].

For a long time there was no consensus on the definition of APS. However in 1999 an international consensus on preliminary classification criteria for definite APS was reached according to which definite APS is present if a patient fulfills one clinical criteria (arterial or venous thrombosis or pregnancy morbidity) and one laboratory criteria (medium or high titer aCL antibodies of IgG or IgM isotype or positive LAC test on two or more occasions, 6 weeks apart)[124].

In a recent study of 1000 patients with definite APS, 53 % had primary APS whereas 41% were associated with SLE or lupus like syndromes. Both groups had similar clinical presentation. The most common symptoms at onset in this study was venous thrombosis/pulmonary embolism together accounting for 41%, stroke was seen in 13%, fetal loss was reported in 8%, and MI in 3%[125].

Oral anticoagulation with warfarin is the treatment of choice for venous and arterial thrombosis. Existing studies indicate that increased risk of recurrence motivates that longer therapy should be given to patients with APS[126].

TNF-α and its receptors TNFR1 and TNFR2

TNF-α was first described in 1975 as a macrophage derived product which caused hemorrhagic necrosis of tumors[127,128]. Concurrently, chachectin was identified as a metabolic hormone, which among other effects suppressed lipoprotein lipase. As chachectin was sequenced it was shown to be identical to TNF-α[129]. It is now known that TNF-α is a cytokine, which is first synthesized as membrane bound pro-TNF-α, a 26 kDa protein which expresses TNF-α activity in cell to cell contact. It is cleaved from the membrane by TNF-α converting enzyme (TACE). After cleavage, TNF-α is an 157 amino acid, 17 kDa, secreted protein which readily homotrimerizes to form active homotrimeric TNF-α[130].

Monocytes and macrophages are the main sources of TNF-α, at least during inflammatory responses. However, numerous other cell types are capable of producing TNF-α, among them are adipocytes, B-cells, T-cells, eosinophiles, dendritic cells, neutrophils and mast cells.

TNF-α is a key regulator in innate immunity and inflammation. Among its many effects should be mentioned that 1) it induces inflammation, 2) initiates apoptosis (programmed cell death) in transformed cells, virally infected cells, T-lymphocytes, epithelial and endothelial cells, 3) produces cachexia and dyslipoproteinemia through inhibition of LPL and stimulation of lipogenesis in the liver, 4) induces insulin resistance, and 5) has important impact on growth and differentiation in various ways[130,131].

TNF-α exerts its action through two structurally distinct receptors, TNFR1 (p55) and TNFR2 (p75). Both receptors can be found on all cell surfaces except erythrocytes. They are transmembrane glycoproteins which present as preformed dimers on the cell surface. Activation is dependent on binding of TNF-α or its sister cytokine lymfotoxin-β (LT-β, also referred to as TNF-β) to the receptors (fig7).

TNFR1 is constitutively expressed, its binding to TNF-α has high affinity and is almost irreversible, it carries the intracellular death domain, and is the main route through which TNF-α induces apoptosis. TNFR2 is more common on endothelial and hematopoetically derived cells, it is easily induced, and kinetic studies indicate that binding is highly reversible. This renders sTNFR2 the main candidate to function as a ligand passer i.e. keeping excess amounts of TNF-α in a circulating reservoir, thus protecting from degradation and over time possibly enhancing the effects of TNF-α[132].

Both receptors are shed into the circulation as a response to inflammatory signals. During chronic and acute inflammation soluble levels of these receptors (sTNFR1 and sTNFR2) usually parallel circulating TNF-α. They are more easily measured since they occur in the circulation at levels which are 100-1000 times higher than circulating TNF-α. Due to low sensitivity of available kits, studies of circulating TNF-α have previously been difficult to carry out. However, with the new high sensitivity kits for TNF-α, levels down to about 0.5 pg/ml can be detected which is sufficient for most human situations.

Despite their abundance in inflammatory states, the role of soluble TNFRs (sTNFRs) is not clear. It has been suggested that they are natural antagonists neutralizing the effects of TNF-α, or that they prolong half life by acting as ligand passers protecting from degradation[132].

The importance of TNF-α in chronic inflammatory diseases has recently been demonstrated in clinical practice as TNF-α blocking medication has been shown to be very effective in the treatment of RA, Crohn´s disease, psoriatic arthritis and spondarthritis. To date two principles have been used, TNF-α specific antibodies and synthetic TNFR2 analogues. Both have similar efficacy in clinical studies[133,134].

TNF-α and its soluble receptors

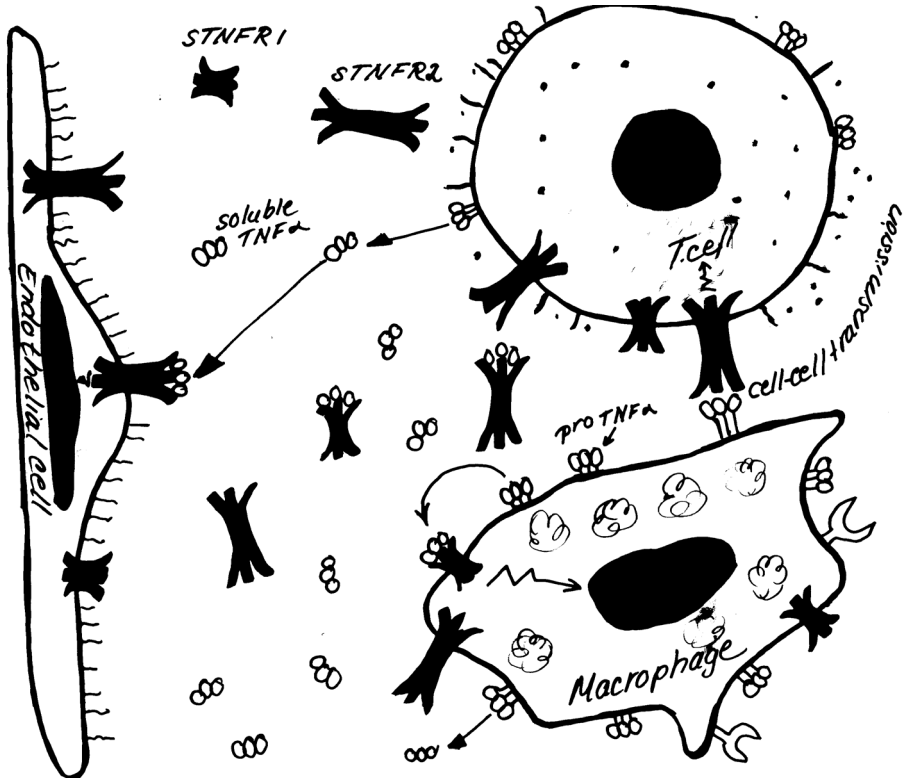


Fig 7
TNF-α is synthesized as a cellbound procytokine. It is released into the circulation where it circulates as trimeric TNF-α, either free or bound to either of its receptors TNFR1 or TNFR2. The receptors can be membrane bound where they transduce signals of TNF-α to the cell. They are also common as soluble TNFRs in the circulation, especially in inflammatory states (schematic presentation).

CARDIOVASCULAR DISEASE IN SLE

Epidemiology

Comparing studies on causes of death in SLE reveals a changing pattern over time where in earlier studies mortality in SLE was usually due to active SLE manifestations or infection. Urowitz described for the first time in 1976 that mortality in SLE has a bimodal distribution, in which early deaths, the first two years after diagnosis, were usually attributable to SLE itself or side effects of its treatment (infections) whereas late deaths, more than two years after disease onset, were predominantly caused by CVD[135]. In subsequent studies this pattern has been confirmed[136,137]. Probably because of better treatment and longer survival, cardiovascular mortality has gradually become relatively more important in SLE patients. According to two recent studies CVD is the most common cause of death among Swedish SLE patients. In the study by Stahl-Hallengren from a defined region in southern Sweden 76% of deaths were attributed to CVD [1]. This very high figure is probably influenced by the old age of this particular cohort. Björnådal et al studied a large population-based cohort of 3866 patients discharged from Swedish hospitals with a diagnosis of SLE during the period of 1964-94. Among them CVD was the major cause of mortality responsible for 42% of deaths[138]. In a recent European multi center study comprising 1000 patients CVD, SLE per se, and infections contributed equally to mortality[139]. For a summary of larger cohorts during the past 50 years, see table 2. Though figures vary considerably, CVD is today a major cause of mortality among SLE patients.

The increased risk for CVD in SLE has also been demonstrated in epidemiological studies. Manzi et al compared incidence rates for coronary heart disease of SLE patients with historical controls from the Framingham study. She reported an overall increased risk which was most evident for women in the age group 35-44, they were 50 times more likely to have a MI as compared to age matched controls[140], an intriguing figure since women of this age are normally protected from CVD. In another study by Esdaile et al, the overall relative risk for coronary heart disease was 7.5 and the risk for stroke was 7.9, after controlling for traditional Framingham risk factors[141]. Other explanations than traditional risk factors obviously have to be investigated in order to understand why SLE patients get premature CVD.

Table 2

Causes of mortality in cohort studies of SLE patients

Author	*Study period*	*Cohort size*	*No of deaths*	*CVD %*	*SLE %*	*Infection %*	*Malignancy %*	*CVD late*
Dubois[142]	50-63	491	249	9	57	15	4	
Rosner [143]	65-78	1103	222	11	33	33	?	
Wallace [144]	50-80	609	128	18	34	21	3	
Ward[145]	69-83	408	144	6	34	22	6	
Urowitz [135]	70-74	84	11	45	18	36		+
Reveille[146]	75-84	389	74	11	12	47	7	
Gudmunsson[147]	75-88	76	17	29	12	6		
Abu-Shakra [137]	70-93	665	124	25	18	32	6	+
Stahl-Hallengren[1]	81-91	81	17	76	24	6	6	+
Cervera[139]	90-95	1000	45	27	29	29	6	

Causes of mortality in a selection of studies on SLE patients during the last 50 years. CVD late indicates if a study noted CVD as a cause of mortality after several years disease.

Atherosclerosis in SLE

The increased risk of CVD in SLE has often been attributed to accelerated atherosclerosis. Indeed, severe atherosclerosis has been demonstrated in several autopsy studies of single or series of cases[148-151], many of these patients died at a young age. In a study of juvenile onset SLE, children with SLE had increased intima media thickness (IMT) as a sign of early onset atherosclerosis[152]. Prior to these studies there were no controlled studies on occurrence or quantity of atherosclerosis in adult SLE patients.

Lipids in SLE

Two distinct types of dyslipoproteinemia are present in SLE. High levels of VLDL triglycerides and VLDL cholesterol and low levels of HDL characterize the first pattern. This lipid profile is present in inactive, untreated SLE but is more pronounced among patients with active disease[153,154]. Little is known about the mechanisms behind this lipid disorder though decreased activity of endothelial LPL and impaired chylomicron removal from plasma[155] are

two factors, present in SLE patients, which may contribute to elevations of TGs. Antibodies to apo-A1 are also detected in some SLE patients and may contribute to low levels of HDL[156,157].

The second pattern of lipid disturbance often seen in SLE arise as a complication to steroid treatment[158-161]. Steroid treatment induces an increase in total cholesterol (including both LDL and HDL) and a more modest elevation of TGs[154,162]. Patients with renal disease also have increased levels of total and LDL cholesterol together with high TG and low HDL.

High Lp(a) levels have also been detected in SLE patients[163,164], in one study these findings were confined to a group of SLE patients with proteinuria.

Thus dyslipoproteinemia in SLE is a mixture of different patterns depending on SLE per se, steroid treatment or renal disease.

Antiphospholipid antibodies in SLE

30-50 % of SLE patients have aPL and about one third to half of these patients develop symptoms of APS[165,166]. Though the clinical profile is similar in primary and secondary APS, a recent comparative study reported that trombocytopenia was more common among SLE patients[125]. Positive LAC and high titer of the IgG aCL have been shown to be best predictors of risk for venous thrombosis whereas LAC seems to have a stronger association with risk for arterial occlusion[165]. In a large study of SLE patients, presence of secondary APS has been shown to be associated with increased mortality[167].

Heart valves in SLE

In 1924 Libman Sacks described the noninfectious verrucous valvular lesions that have since been referred to as Libman-Sacks endocarditis[168]. The prevalence of these valvular abnormalities vary considerably in different studies18-54%[169-174]. Different patient selection criteria and echocardiographic techniques are reasons behind this, but despite more sensitive techniques, verrucous lesions have become less common in recent studies. Whether this is due to better treatment is not clear. More abundant is diffuse thickening of valvular leaflets, occurring in 14-40%[169,172-174]. In some cases valve involvement leads to regurgitation and less frequently to stenosis of heart valves. An association between aPL and valvular lesions have been reported in several studies and this combination has been demonstrated to constitute a special risk for cerebrovascular lesions[169,174-176].

THE STUDIES OF THIS THESIS

Aims of the studies

From a rheumatological point of view it is a clinical challenge to better understand and treat the factors associated with premature CVD, which now is major cause of mortality among SLE patients in developed countries.

In cardiovascular research, SLE can be regarded as a human model of premature CVD from which it should be possible to extract general information on how chronic inflammation affects the development of CVD.

In the present studies we have tried to combine these two perspectives when addressing the following questions.

- ➔ ***Is atherosclerosis enhanced in SLE?***
- ➔ ***Which are the most important risk factors for CVD in SLE?***
- ➔ ***How do risk factors for CVD in SLE relate to each other and to disease activity and organ manifestations?***
- ➔ ***What are the underlying mechanisms causing CVD in SLE?***
- ➔ ***What is the extent of lipoprotein oxidation in SLE patients, and how does it relate to disease activity and organ manifestations?***
- ➔ ***Is presence of valvular disease associated with CVD in SLE?***

PATIENTS AND METHODS

Patients and study design

All SLE patients at the Department of Rheumatology, Karolinska Hospital, who fulfilled four or more of the 1982 revised American College of Rheumatology Criteria for classification of SLE[12] were asked to participate, 208 agreed and were included in our cohort during the period July 1995 – December 1999. The initial investigation included an interview and a physical examination by a rheumatologist and blood sampling after overnight fasting. This cohort is the basis for paper IV. In paper V, in which some analyzes were done before the cohort was completed, 147 consecutive women were included. 60 age matched women (blood donors) were used as controls.

In study I, II and III a nested case control design was applied. All women in the large cohort, except one who declined participation, with a history of arterial disease were included. Three additional women with SLE and CVD were added from Huddinge University Hospital. Thus a total of 26 women with SLE surviving one or more manifestations of CVD, defined as a history of MI (confirmed by electrocardiography and a rise in creatine kinase, n=7), angina (coronary insufficiency confirmed by excercise stress test, n=9), cerebral infarction (thromboembolic and not hemorrhagic or vasculitic stroke, confirmed by computed tomography or magnetic resonance imaging, n=15) or claudication (peripheral atherosclerosis confirmed by angiogram, n=4).

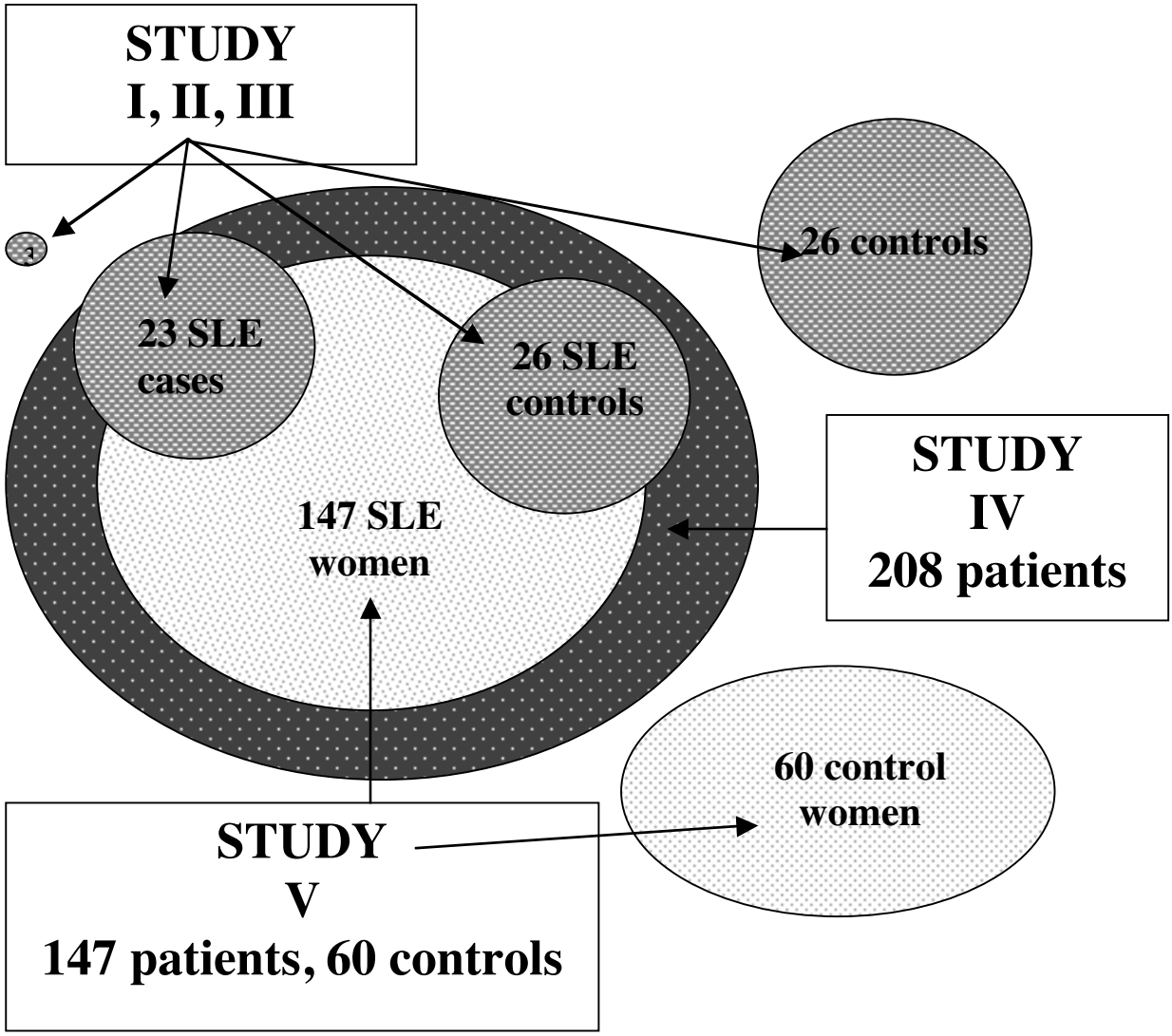
For every woman with CVD, the woman closest in age in the large SLE cohort was asked to participate as an SLE control. Population controls were selected from the population registry. We asked women to participate who were living in the same area as our patients and who were born on the day in the middle between SLE cases and their respective SLE controls.

This procedure resulted in three groups, SLE cases, SLE controls and population controls, each comprising 26 individually age matched women. These women were investigated in random order by a rheumatologist and a clinical physiologist. Blood samples were drawn after overnight fasting.

The studies were approved by the local Ethics Committee of the Karolinska Hospital, Stockholm, Sweden. All subjects gave informed consent before entering the studies. Selection of patients and controls is depicted in (fig 8).

Fig 8

STUDY DESIGN



Disease activity and damage

SLE disease activity was determined with SLAM[36] and organ damage was determined with the SLICC/ACR damage index[42]. This was done at the initial inclusion in the large cohort and at inclusion in the cardiovascular studies.

Body mass index and Waist Hip ratio (I, II, III)

Body mass index (BMI) was calculated using the formula weight/height$^{2.}$ Waist-Hip ratio was calculated as the waistline divided by the hip circumference.

Routine laboratory measures (I-V)

Plasma electrophoresis was analyzed on agarose gels (Sebia-systems). Plasma homocysteine was determined by fluorescence-polarization immunoassay (Abbot).

Autoantibodies (I-V)

Anti-dsDNA antibodies were determined by immunofluorescence using *Crithidia lucillae* kinetoplast assay. Anticardiolipin antibodies (aCL) were measured by enzyme-linked immunosorbent assay (ELISA) using ethanol fixed cardiolipin (Sigma) and HRP-conjugated fractionated rabbit immunoglobulins against human IgG and IgM respectively (Dako). Lupus anticoagulant was determined using a modified Dilute Russel Viper Venom method, using Bioclot lupus anticoagulant (Biopool, Umea, Sweden). ß2-GPI antibodies were determined using ELISA (R&D Systems). ANA, SSB and Rf were determined using routine laboratory techniques.

Lipoproteins (I-V)

All plasma lipoprotein determinations were done on venous blood samples taken after overnight fasting. In study I, II, and III plasma lipoprotein concentrations were determined by a combination of preparative ultracentrifugation followed by lipid analyses of the lipoprotein fractions as described[177]. Lp(a) was determined by use of ELISA (TintELIZE Lp (a), Biopool Int., Umeå, Sweden). In study IV and V total cholesterol, LDL, HDL and triglycerides were determined at the Department of Clinical Chemistry, Karolinska Hospital, using routine techniques.

TNF-α and sTNFRs (III and IV)

Commercially available kits were used for the measurement of serum and plasma cytokine levels by ELISA according to the manufacturer's instruction. The detection range for TNF-α was 0.5 to 32 pg/ml with a sensitivity of 0.18 pg/ml (high sensitivity human TNF-α kits, catalog HSTA50, R&D, UK). Serum with a dilution of 1:2 was used for TNF-α. The detection range for TNFR1 was 7.8 to 500 pg/ml with a sensitivity of 3 pg/ml (human sTNF R1/TNFRSF1A kits, catolog DRT100, R&D, UK). The detection range for TNFR2 was 7.8 to 500 pg/ml with a sensitivity of 1 pg/ml (human sTNF RII/TNFRSF1B kits, catolog DRT200,

R&D, UK). EDTA plasma with a dilution of 1:10 was used for measuring TNFR1 and TNFR2 in the circulation.

Oxidation related measures (I and V)

LDL was isolated from pooled plasma of healthy donors by sequential preparative ultracentrifugation under conditions to minimize oxidation and proteolysis and subsequently oxidized by copper or modified by malonealdehyde (MDA) as described[178]. The chemiluminescent assay for autoantibodies binding to oxLDL was performed with modifications as described on plasma dilutions of 1:250[87]. Data are expressed as relative light units per millisecond (RLU/msec). Each determination was done in triplicate and all samples were measured in a single assay. The coefficients of variation for low and high standards were 6-8 %.

The EO6 epitope concentration on apo-B-100 containing particles was measured by a chemiluminescent modification of a previously described assay[87;179]. This sandwich assay utilizes an antihuman apo B-100 monoclonal antibody, MB47, to capture apo-B containing lipoproteins, and a biotin-labeled anti-oxLDL antibody, E06, to measure the amount of the E06-epitope present on the apo-B containing lipoproteins captured. The number of apo-B containing particles should saturate the binding capacity of the plated MB47. To verify this, in parallell wells we determined the binding of biotinylated MB24, another apo-B-specific monoclonal antibody that binds to a distinct apo-B epitope on apo-B apart from that recognized by MB47, as described[179].

Data were expressed as a ratio of the amount of EO6 bound per well normalized by the number of apo B lipoproteins per well, e.g. the ratio of EO6/MB24[179]. All samples were measured in a single assay and the intra-assay coefficients of variation of low and high standards were 6-8%.

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Insulin and insulin resistance (III)

Plasma insulin was measured by an ELISA based on two monoclonal antibodies (DAKO Diagnostics, Cambridgeshire, UK). Insulin sensitivity was estimated by homeostasis model assessment (HOMA)[180].

Carotid ultrasound (I, II, III)

The right and left carotid arteries were examined using an Acuson Sequoia with a 8 MHz linear array transducer. Subjects were investigated in the supine position. The IMT of the far wall of the common carotid artery just proximal to the bulb was measured[181]. Plaque was defined as a local intimal/medial thickening, with a thickness greater than 1 mm and a 100% increase in wall thickness compared with adjacent wall segments. It was scored as being present or absent.

Echocardiography (II)

Echocardiography was performed with an Acuson Sequoia (Mountain View, California, USA) with a 2.5 or 3.5 MHz transducer. The echocardiographer was unaware of the clinical details of patients and population controls who were investigated in random order. Two dimensional measures were taken as recommended by the American Society of Echocardiography[182]. Measures of wall thickness, left ventricular (LV) diameter and mitral doppler are mean of two measurements. Global LV function was assessed with visual estimation of ejection fraction[183] and by measuring atrioventricular plane displacement[184].

The valves were studied for valve thickening and other malformations. Doppler and color doppler was used to assess valvular stenoses and/ or leakage. Regurgitation was graded from 1- 4 where 1 is mild and 4 is severe and considered present if it was grade 1 or more. Valvular abnormalities were classified as either abnormal localized echodensity adjacent to valve leaflets or valve thickening. Increased echodensity (sclerosis) in the posterior mitral ring is a not uncommon finding in elderly and was reported but not regarded as a valvular abnormality.

Statistics

Skewed continuous variables were logarithmically or reciprocally transformed. For variables that did not attain a normal distribution non-parametric tests were used. For comparisons between groups, ANOVA/students t-test was used for normally distributed variables and Mann Whitney U-test/Friedmans test for variables not normally distributed. In study I and II paired t-test was used as post hoc analysis, and Mc Nemars test was used for comparisons of nominal variables. In study III reviewers requested slightly different calculations with unpaired ANOVA/Chi square tests for comparasions between groups and

Fischer´s PLSD as post hoc test. In study IV ordinal multiple logistic regression was used to evaluate independence of risk factors. The significance level was put at $p<0.05$.

RESULTS

Paper I

Risk factors for cardiovascular disease in systemic lupus erythematosus

Though many case reports and epidemiological studies have documented the presence of premature CVD in patients with SLE there were no properly controlled studies measuring the extent of atherosclerosis in adult SLE patients prior to this study. The main objective was thus to quantify and compare measures of atherosclerosis in age matched SLE patients and controls. A second objective was to investigate the presence of other risk factors for CVD in SLE patients and controls. A nested case control design as described above was used. Two groups of 26 SLE patients, one with (SLE cases) and one without CVD (SLE controls) were compared to population controls. As a surrogate measure of atherosclerosis, B-mode ultrasound was used to measure IMT and plaque occurrence.

Results

Atherosclerosis

SLE cases had increased IMT and more atherosclerotic plaques as compared to both control groups. The SLE control group had more plaques but similar IMT in comparasion to population controls.

Traditional risk factors

Dyslipoproteinemia was present exclusively in the SLE case group. These patients had lower levels of HDL-C and higher levels of TGs in plasma, and in their LDL and VLDL fractions. Also the Lp(a) concentration was significantly higher in this group but total or LDL cholesterol did not differ between groups.

Blood pressure, smoking habits, BMI, and prevalence of diabetes were other traditional risk factors which did not differ between the groups.

Nontraditional and SLE related risk factors

Disease duration did not differ between SLE groups. Many measures of inflammation such as erythrocyte sedimentation rate and plasma concentration of acute phase reactants

(orosomucoid, α_1-antitrypsine and C-rective protein) were significantly higher in the SLE case group. Cumulative, but not present prednisone dose was higher in the SLE case group. Also homocysteine levels were higher in this group. Phospholipid antibodies were measured and we found that among them only the lupus anticoagulant ratio was significantly higher in SLE cases, β2GP-I was of borderline significance, and aCL did not differ with respect to CVD.

IgG antibodies to oxLDL as determined by two methods (Cu and MDA oxidation) discriminated between SLE cases and SLE controls. In the SLE case group apo-B containing lipoproteins (mainly LDL) expressed higher levels of oxidized phospholipids as determined by the specific antibody EO6.

Conclusion

This study demonstrates that premature atherosclerosis is present in SLE cases. The situation in SLE controls is more difficult to interpret. They had similar IMT but more atherosclerotic plaques in comparison to age matched controls. This study also identifies a set of traditional and non traditional risk factors which were exclusively more predominant among SLE cases. These were dyslipidemia, inflammation, oxidation related factors, homocysteine levels and enhanced lupus anticoagulant activity. Blood pressure, smoking habits, BMI and prevalence of diabetes did not differ between the groups. Since many SLE cases were on antihypertensive therapy it is difficult to draw any conclusions about the role of hypertension in this study.

Many of the identified risk factors in this study are features of SLE or related to chronic inflammation. Since they were not present in SLE controls it is possible that only a subset of SLE patients are at increased risk for CVD.

Paper II

Cardiac valvular abnormalities are frequent in systemic lupus erythematosus patients with manifest arterial disease

Valvular abnormalities as well as pericarditis have long been known to be cardiac manifestations of SLE. It is much more recently that the association between SLE and premature CVD has been described. However it has not been studied how these cardiovascular manifestations relate to each other. In this study cardiac valve morphology and function and ventricular function were studied in the same setting as in paper I, i.e. in SLE cases, SLE controls and population controls. Echocardiography was performed to assess ventricular and valvular function and morphology.

Results

Valve morphology and function

13/26 SLE cases had valvular abnormalities. Three of these had had previous valvular replacement, one had major aortic insufficiency, one had vegetations of the mitral subvalvular apparatus and nine had mild thickening of mitral leaflets not affecting valve function. Only one patient had thickening of both aortic and mitral valves. 1/26 SLE controls had thickened mitral valves. Among population controls no valve thickening was found.

Left and right ventricular function

The seven patients with previous myocardial infarction all had wall motion abnormalities. Five had left ventricular ejection fraction < 50% and three had an enlarged left ventricle. One SLE case had severe pulmonary hypertension.

Biochemical and immunologic tests in relation to valvular involvement

Comparasion between SLE cases with and without affected heart valves showed that SLE cases with valve abnormalities had significantly higher concentrations of plasma TGs and plasma homocysteine than SLE cases with normal heart valves. Phospholipid antibodies, acute phase reactants, antibody titers to oxLDL and oxidation epitopes on LDL particles did not differ between SLE cases with or without valvular abnormalities.

Conclusion

Valvular abnormalities and CVD are strongly associated in SLE. Hypertriglyceridemia and hyperhomocysteinemia are common risk factors for CVD and valve abnormalities. In SLE patients without CVD, heart valves are usually not affected and left ventricular function is normal.

Paper III

TNF-α -a link between hypertriglyceridemia and inflammation in SLE patients with cardiovascular disease

and

Paper IV

High triglycerides and low HDL are markers of disease activity and closely related to an upregulation of the TNF-α/TNFR system in systemic lupus erythematosus

TNF-α is a potent proinflammatory cytokine but it is also central in metabolic regulation where it among other effects contributes to dyslipoproteinemia with a similar pattern as seen in SLE. It is present in atherosclerotic plaques and circulating levels of TNF-α are elevated both in SLE and in patients with atherosclerosis in the general population. However, the role of TNF-α had not been studied with respect to CVD and dyslipoproteinemia in SLE patients. These studies are focused on how circulating levels of TNF-α and its soluble receptors sTNFR1 and sTNFR2 relate to SLE patients with CVD (study III) and dyslipoproteinemia and disease activity in an unselected cohort of 208 SLE patients (study IV). In study III we used the same patients and controls as in study I and II.

Results

TNF-α/sTNFRs and dyslipoproteinemia

Among SLE cases we saw a strong correlation between TNF-α/sTNFRs and TGs. In the large cohort we could confirm this association and there was also a weaker but significant association between TNF-α and sTNFR2 and low levels of HDL.

TNF-α/sTNFRs in relation to SLAM and SLICC/ACR damage index

In the large cohort, disease activity correlated with high levels of TGs and sTNFRs and with low levels of HDL. In logistic regression models TNFR2 and TGs were independent determinants of active disease (SLAM > 7). There was also a correlation between TNF-α and both sTNFRs and anti DNA antibodies.

SLICC showed similar correlations as SLAM with the exception that there was no correlation with low HDL, instead SLICC correlated with total and LDL cholesterol. In multiple logistic regression models only cholesterol and TNFR1 were independent determinants of more severe damage (SLICC > 1).

TNF-α/sTNFRs and CVD in SLE patients

In study III, levels of TNF-α and sTNFRs were higher among SLE patients with CVD, intermediate among SLE controls and lowest among population controls. A similar association was seen in the large cohort, patients with previous arterial disease had higher levels of TNF-α/sTNFRs.

TNF-α/sTNFRs and renal disease manifestations

In the large cohort, high levels of TNF-α/sTNFRs were very strongly associated with present or previous renal disease.

Conclusion

Dyslipoproteinemia correlates with SLE disease activity. Furthermore activity in the TNF-α/sTNFR system seems to be an important factor for both disease activity and dyslipoproteinemia in SLE. It is present at elevated levels in SLE patients with CVD. It may thus be pivotal for the increased risk of premature CVD in these patients pushing them into a high risk profile for CVD with both enhanced inflammation and dyslipoproteinemia of kind that is known to be especially atherogenic in women.

Paper V

Lipid-peroxidation is enhanced in SLE patients and associated with arterial and renal disease manifestations

Oxidative modifications of LDL is important in atherogenesis as it makes LDL immunogenic and susceptible to uptake by the macrophage scavenger receptor which is crucial for the development of foam cells. Several studies have demonstrated that antibodies to oxLDL are raised in SLE patients and in study I we demonstrated that both antibodies to oxLDL as well as oxidation epitopes on LDL particles were present at enhanced levels in SLE patients with CVD. However, oxidation epitopes have not been studied in a larger unselected cohort of SLE patients before. In this study occurrence of oxidation epitopes and antibodies to oxLDL was investigated in 147 female SLE patients and 60 age matched women (blood donors). When measuring oxidation epitopes the same technique as in study I with EO6 antibodies was used. These antibodies have been shown to block epitopes on apo B containing lipoproteins (mainly LDL) so that they are no longer recognized and taken up by the scavenger receptors and they also recognize minimally modified LDL.

Results

Oxidation related epitopes

LDL from SLE patients expressed significantly higher levels of oxidized/EO6-specific epitopes as compared to controls.

Antibodies against CuoxLDL, MDA-LDL and CL

The levels of antibodies to CuoxLDL, MDA-LDL and CL were higher in SLE patients as compared to controls.

aCL antibodies and reduced vs. oxidized CL

In a separate experiment, high titer aCL sera from 20 patients was incubated for 3 hours with either oxidized or reduced CL. Thereafter immune complexes were pelleted by centrifugation and the supernatant was tested for IgG binding to CL using a standard procedure. The fraction incubated with oxCL was void of aCL whereas approximately the

original amounts of aCL was retained in the fraction which was incubated with reduced CL. This experiment shows that aCL in high titer SLE patients preferentially bind to oxidized CL

Clinical manifestations and LDL-oxidation related measures

SLE patients with arterial and renal disease had significantly higher levels of oxidation epitopes on their apo B containing lipoproteins (mainly LDL). IgM antibodies to CuoxLDL were also enhanced in SLE patients with arterial and renal disease.

Conclusion

Enhanced lipid peroxidation is present in this unselected cohort of SLE women, and it is more pronounced in subgroups of SLE patients with renal and cardiovascular disease. These two groups are partly overlapping and have been associated with worse prognosis as compared to SLE patients on the whole. Oxidative modification is also important with respect to phospholipid antibodies and we report that high titer aCL in these patients bind with higher affinity to oxCL as compared to reduced CL. Thus oxidative modification seems to be of importance in SLE and especially among SLE patients with more severe disease including patients with renal and cardiovascular disease and those with high titer IgG aCL.

GENERAL DISCUSSION

Role of atherosclerosis in SLE

In study I the presence of further advanced atherosclerosis was documented in a group of SLE women with CVD as compared to age and gender matched controls. This was an expected finding despite the fact that there are other contributing factors to occlusive arterial disease in SLE such as pro thrombotic antiphospholipid antibodies. Several early autopsy studies have also shown that severe early atherosclerosis is present in some young SLE patients. Bulkely et al described severe coronary atherosclerosis in 8/36 young patients with SLE all of whom had been subject to steroid treatment[148]. Since coronary atherosclerosis was "virtually never described" in SLE presteroid treatment, she concluded that accelerated atherosclerosis was an effect of steroids. However, in later autopsy studies this strong association with corticosteroid use has not been confirmed[151]. Indeed Fukumoto et al reported a negative correlation between coronary intima thickness and corticosteroid use[150].

A more surprising finding in study I is that the group of SLE controls did not have increased IMT as compared to age matched population controls. This group had also been subject to long term treatment with comparatively high doses of corticosteoids. Thus, it seems like steroid treatment per se is not associated with premature CVD. Haider et al reported similar findings in 22 autopsy studies of young women with SLE. Ten of them had severe coronary atherosclerosis while the others did not have more atherosclerosis than age matched controls[149]. In another study on patients with juvenile onset SLE, increased IMT was confined to six patients with nephrotic range proteinuria while the other 20 had comparable IMT to controls[152]. These results indicate that not only mortality but also atherosclerosis in SLE may show a bimodal pattern with a subgroup of patients developing severe atheroslerosis while others are unaffected. If this pattern can be confirmed in larger studies, the identification of this subgroup is an essential task for the future.

SLE controls did however have more localized atherosclerotic plaques than population controls. Roman et al also report an increased occurrence of carotid plaques while IMT did not differ between SLE patients and controls[185]. In an uncontrolled study of 175 SLE women, plaque occurrence but not IMT correlated to lupus related risk factors like prednisolone use[186]. One interpretation of these findings may be that atherosclerosis related to SLE favors local plaque formation, as compared to a general thickening of the intima media. The chronic

inflammation present in SLE may enhance inflammatory activity in these plaques making them more susceptible to destabilization and rupture. Thus, it is possible that clinical symptoms may complicate atherosclerosis at an "earlier stage" in SLE than in the general population. So far there are no comparative studies on CVD in SLE and the general population.

Dyslipoproteinemia

Previous studies by Ilowite and Borba have very elegantly shown that SLE per se causes a secondary dyslipoproteinemia with high TGs (including high levels of both VLDL, TGs and cholesterol), low HDL and essentially normal LDL/total cholesterol [153,154]. This is a lipoprotein profile which in the general population is often seen as part of the metabolic syndrome and it has also been shown to be especially atherogenic in women[54,187,188]. In study I we demonstrate that this pattern of dyslipoproteinemia constitutes a risk factor for CVD among SLE women. Other features of the metabolic syndrome were investigated in study III. Both SLE groups tended to have more central adiposity as measured by waist/hip ratio but we did not find that SLE cases had higher blood pressure, fasting glucose levels or more insulin resistance as compared to the control groups. Thus SLE patients share some features of the metabolic syndrome such as dyslipoproteinemia and central adiposity but other signs of this syndrome are not present.

Elevated cholesterol levels in SLE are also common and usually attributable to steroid treatment or renal disease[158,159,161]. Petri et al showed that a 10 mg increase in prednisolone dose is associated with a 7.5% increase in cholesterol[158]. Several previous studies have reported high levels of cholesterol to be an important risk factor for CVD in SLE[189,190] and we could also confirm such an association in study IV. Somewhat surprising there was no association between elevated LDL cholesterol and CVD in study I. A probable explanation for this is that in this study 9/26 SLE cases were taking cholesterol lowering statin therapy and this may have alleviated any difference with respect to cholesterol. Study IV on the other hand was carried out before statin therapy was generally used and in this study only negligible 3/208 SLE patients were on these drugs.

In study I we could also link high levels of Lp(a) to CVD in SLE patients, an observation also reported by Kawai[191].

Thus it seems like the lupus pattern of dyslipoproteinemia with high TG/low HDL levels together with steroid induced hypercholesterolemia and high levels of Lp(a) all contribute to an atherogenic lipid profile and to an increased risk for CVD in SLE patients.

Role of inflammation, TNF-α and disease activity for CVD in SLE

In study I we identified inflammation as a risk factor for CVD in SLE. This was a very consistent finding as measured by several acute phase reactants including α-1-antitrypsin, high sensitivity CRP, orosomucoid and also the erythrocyte sedimentation rate. Manzi et al has previously presented similar results identifying fibrinogen and CRP as risk factors for plaque occurrence among SLE patients[186]. These data definitely support systemic inflammation as a factor of importance for premature CVD in SLE patients. Since the risk of CVD is very high in SLE[140] and the inflammatory pattern differs from that of other chronic inflammatory disorders[24,192] it was natural to further analyze markers of the inflammatory process in relation to CVD risk and risk factors.

When doing so we have so far focused on TNF-α since it has been shown to be of importance for SLE disease activity, it is present in the atherosclerotique plaques[66] and also in the circulation of patients in the general population who are affected with CVD[65,193,194]. When given to rodents or humans TNF-α, can induce insulin resistance[195] and dyslipoproteinemia[57,196-198] of a similar kind as seen in SLE. Inhibition of LPL[128] and induction of de novo synthesis of VLDL[56,199] in the liver are two known mechanisms through which TNF-α promotes dyslipoproteinemia.

Both in study II and study IV we show that circulating levels of TNF-α/sTNFRs peak among SLE patients with CVD. Furthermore the levels of TNF-α/sTNFRs correlated with high levels of TG in SLE cases (study III) and with lupus dyslipoproteinemia (high TG/low HDL, study IV). Acute phase reactants (APR) did not correlate with dyslipoproteinemia among SLE cases in study III. In SLE controls, on the other hand, the pattern was different with strong correlations between TNF-α and acute phase reactants but not between TNF-α and dyslipoproteinemia. This is intriguing and we can only speculate on the reason for this discrepancy. It could be related to dose, and higher levels of TNF-α activity may be needed in order to induce dyslipoproteinemia. Other studies have shown that the strong correlation between pro inflammatory cytokines like IL-6, TNF-α and acute phase proteins, which is seen in most inflammatory conditions, is for unknown reasons absent in SLE [24,192]. In subgroups of SLE patients, e.g. patients with nephritis, the APR is minimal or absent[22]. Antibodies to CRP

and other acute phase proteins are common in SLE patients[200] and they have been suggested to contribute to lowering circulating levels of these proteins in SLE.

Since TNF-α activity, as measured by sTNFRs, has been shown to be one of the best markers of disease activity in SLE[24,32,33,35], it was logical to investigate how SLE disease activity relates to dyslipoproteinemia. In study IV we show for the first time that there is a very good correlation between disease activity, as measured by SLAM, and levels of TGs. In this cross sectional study TGs are together with sTNFRs the best marker of disease activity. If confirmed in longitudinal studies these findings may be of importance both for monitoring and treatment of these patients in the future.

Also in the general population there are numerous reports that moderately elevated levels of inflammatory substances, as exemplified by CRP and TNF-α, are risk factors for CVD[63-65], and some reports also show that they correlate to dyslipoproteinemia[193,194]. The combination of dyslipoproteinemia and subclinical but enhanced inflammation has also been shown to be especially atherogenic[201]. In studies in the general population levels of TNF-α are approximately 1/3 of those of SLE patients and also CRP levels are substantially lower than in our patients.

Thus, in SLE systemic inflammation and high TG/low HDL are two closely related risk factors for CVD, which both correlate to SLE disease activity. This suggests that a high level of TNF-α activity, and possibly also of other inflammatory mediators, may be capable of inducing both inflammation and dyslipoproteinemia and thus render SLE patients more susceptible to CVD.

Endothelial dysfunction, hyperhomocysteinemia and heart valve abnormalities

Endothelial dysfunction is an early manifestation of atherosclerosis, and it has been associated with many risk factors for CVD including hypertension, hyperlipidemia, hyperglycemia, and hyperhomocysteinemia[68,100,202-205]. In SLE patients endothelial dysfunction was recently shown to be present as measured by endothelium dependent brachial artery flow mediated dilatation[206]. Several studies have also documented enhanced levels of markers of endothelial activation such as soluble adhesion molecules, von Willebrand factor and thrombomodulin, which in some cases correlated to SLE disease activity[31,207-210]. Further support for widespread endothelial injury/activation in SLE is a recent study in which activated endothelial cells occurred in the circulation of SLE patients in numbers that

increased with active disease[208]. Thus endothelial activation/dysfunction is present in SLE as studied by different methods and according to some studies it seems to correlate with disease activity.

As stated before, valvular changes are frequent in SLE patients and we demonstrated in study III that they are strongly associated with CVD. It should be remembered in this context that heart valves are thin structures, lined by endothelium on both sides. They are exposed to noxious contents of the blood and located at a site of extraordinary turbulent blood flow. In study III we demonstrate for the first time that there is a very strong association between valvular abnormalities and in particular hyperhomocysteinemia but also with hypertriglyceridemia but not with the other risk factors for CVD which we identified in study I.

Hyperhomocysteinemia is well documented as an inductor of endothelial dysfunction in humans, experimental animals and cell cultures[99,106,211]. Men with CVD and chronic hyperhomocysteinemia had impaired endothelium dependent brachial artery flow mediated dilatation, which was reversed after lowering homocystein levels with vitamin B supplementation[100]. Hyperhomocysteinemia was identified as a risk factor for arterial disease in SLE patients in study I, an association also reported by others[162,212,213].

Hypertriglyceridemia was the other factor which constituted a common risk for both CVD (study I) and valvular abnormalities (study III). TGs have also been shown to have negative effects on endothelial function promoting endothelial cell activation and inflammation[214]. In fact both acute and chronic elevations of TGs have been associated with endothelial dysfunction [68,203,215].

Thus, in SLE valvular changes occur together with and share common risk factors for CVD. These two risk factors, homocysteine and TGs, are well documented as inducers of endothelial dysfunction and we propose that valvular abnormalities in SLE may be regarded as another manifestation of endothelial activation and damage and possibly as a marker of risk for subsequent CVD.

Enhanced oxidation, antiphospholipid antibodies and immune response to modified lipoproteins

High levels of LAC and trend wise also of aβ2-GP1 were associated with CVD in study I. Similar results have been reported by Petri who demonstrated an association between LAC and antibodies to β2-GP1 (aβ2-GP1) and arterial thrombosis in the Hopkins lupus

cohort[165] and by Romero et al, who reported an association between aβ2-GP1 and arterial disease among British SLE patients[216]. There is less evidence for an association between aCL and arterial disease in SLE. Several studies have addressed this issue with conflicting results[165]. However, in the general population aCL have been predictive of future MI and adverse outcome after infarction[86,217,218]. Though there is extensive overlap and cross reactivity between aPL, our data together with previous studies suggest that positive tests for LAC and aβ2-GP1 are better predictors of arterial disease while aCL is more closely related to venous occlusion. However these tests measure overlapping entities since aβ2-GP1 together with anti-prothrombin antibodies are responsible for a substantial part of the lupus inhibitor activity measured in the LAC test[112].

AoxLDL are closely related to aPL[219] and may also be regarded as aPL. They were in these studies measured by two methods with LDL modified either by incubation with malondialdehyde (MDA-LDL) or by copper induced oxidation (CuoxLDL) as substrate. Albeit different, both methods generate epitopes, which are recognized by human sera, but they are so far not well characterized. Recent studies have revealed other epitopes expressed on oxLDL like LPC[220], PC[75] and β2-GP1, the latter binds to the surface of oxLDL[76]. All these epitopes are believed to be of importance for the uptake of oxLDL by the scavenger receptors, or in case of β2-GP1 through initial binding by aβ2-GP1 and then uptake by the Fc-γ receptors of macrophages[76]. We have in a previous study shown that SLE patients have enhanced levels of antibodies to LPC and that these cross react with aoxLDL and aCL [221]. All these epitopes are presently under intense investigation.

In both study I and study V aoxLDL of both the IgM and IgG class were generally higher among SLE patients than among controls as also reported by Hayem et al[222]. Less consistent was the association between aoxLDL and arterial disease, which in study I was significant for the IgG class (both CuoxLDL and MDA-LDL) and in study V only for CuoxLDL of the IgM class. This issue has been investigated in two other studies of patients with SLE, neither of them found an association between aoxLDL and arterial disease[222,223]. In the general population, titers of aoxLDL are generally high in patients with advanced atherosclerosis and they are predictive of myocardial infarction and progression of carotid atherosclerosis[85,86,224], while in earlier phases of disease, like borderline hypertension, titers are low[87]. Thus methodological considerations, age, duration, and stage of disease may be reasons behind the diverging results concerning aoxLDL.

Oxidation epitopes were measured per LDL particle using E06 antibodies. These antibodies bind to oxLDL but not to native LDL, they block the binding of oxLDL to scavenger receptors and inhibit oxLDL uptake by macrophages[75,79]. Recent studies have shown that the E06 antibodies bind to the phosphorycholine headgroup (PC) which is normally cryptic but is expressed when phosphatidylcholines are oxidized on the surface of oxLDL[75]. In both study I and study V we demonstrate that these oxidation epitopes were enhanced in SLE in general and in particular in SLE patients with arterial and renal disease. This is a new finding which is supported by a recent report showing that antioxidant activity in the circulation of SLE patients is reduced as measured by low activity of the antioxidant enzyme paroxonas (PON)[225].

Apoptotic cells and the capsules of many bacteria also express PC[77,78]which is thus a common epitope on oxLDL, apoptotic cells and bacteria. PC binds to scavenger receptors and to E06 and very recently it was also shown to be a target for CRP binding[77,78]. If this has implications in SLE, where CRP levels are unexpectedly low during disease flares, needs to be investigated in future studies.

Taken together these findings indicate enhanced activity both in the innate and adaptive immune systems when handling abundance of “neo self “epitopes, which are generated as a result of oxidation or enhanced apoptosis in SLE[226-228]. Chronic inflammation may be a contributing factor. Oxidatively modified epitopes may thus be of importance both for premature CVD in these patients, but also for aPL and other autoimmune processes integral to SLE per se.

CONCLUDING REMARKS

➔ ***Atherosclerosis is enhanced in SLE patients with CVD.***
SLE patients, with no clinical signs of CVD, had similar IMT, but more localized atherosclerotic plaques than age matched population controls.

➔ ***Inflammation, dyslipoproteinemia, enhanced LDL oxidation, phospholipid antibodies, and hyperhomocysteinemia are risk factors for CVD in SLE***

➔ ***Dyslipoproteinemia is strongly associated with TNF-α activity.***
TNF-α may, in similarity to other settings, be partly responsible for inducing dyslipoproteinemia in SLE.

➔ ***Dyslipoproteinemia and inflammation, as measured by TNF-α activity, are markers of active SLE disease, and they are more elevated among SLE patients with CVD and renal manifestations.***

➔ ***Lipoprotein oxidation is enhanced in SLE in general, and is also related to CVD and renal manifestations.***

➔ ***Valvular abnormalities are essentially confined to the group of SLE patients with CVD.***

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