

Endothelial dysfunction is associated with ongoing activation of the type I interferon system and platelets in patients with systemic lupus erythematosus

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ABSTRACT

Objectives

Endothelial dysfunction may be connected to cardiovascular disease in systemic lupus erythematosus (SLE). Type I interferons (IFNs) are central in SLE pathogenesis and are suggested to induce both endothelial dysfunction and platelet activation. In this study, we investigated the interplay between endothelial dysfunction, platelets and type I IFNs in SLE.

Methods

We enrolled 148 SLE patients and 79 sex- and age-matched healthy controls. Type I IFN activity was assessed with a reporter cell assay and platelet activation by flow cytometry. Endothelial dysfunction was assessed using surrogate markers of endothelial activation; sVCAM-1 and endothelial microparticles and finger plethysmograph to determine reactive hyperaemia index (RHI).

Results

In SLE patients, type I IFN activity was associated with endothelial activation, measured by high sVCAM-1 (OR 1.68 $p < 0.01$) and elevated endothelial microparticles (OR 1.40 $p = 0.03$). SLE patients with high type I IFN activity had lower RHI, indicating endothelial dysfunction, as compared to healthy controls (OR 2.61 $p = 0.04$).

Deposition of complement factors on platelets, a measure of platelet activation, was seen in patients with endothelial dysfunction. High levels of sVCAM-1 were shown to be associated with increased deposition of C4d (OR 4.57 $p < 0.01$) and C1q (OR 4.10 $p = 0.04$) on platelets. High levels of endothelial microparticles were associated with C4d deposition on platelets (OR 3.64 $p = 0.03$).

Conclusions

Endothelial dysfunction was associated with activation of platelets and the type I IFN system. We suggest that an interplay between the type I IFN system, injured endothelium and activated platelets may contribute to development of cardiovascular disease in SLE.

Keywords:

Systemic Lupus Erythematosus, Cardiovascular disease, Disease Activity, Inflammation

INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that predominately affects women of reproductive age. An increased prevalence of cardiovascular disease (CVD) in SLE is well described. This increase may only to some extent be explained by traditional CVD risk factors, such as smoking, dyslipidaemia and diabetes,¹⁻³ and SLE disease-related risk factors, including steroid treatment, renal impairment and presence of antiphospholipid antibodies.⁴ Type I Interferons (IFNs) have been suggested to be linked to CVD in SLE as those cytokines may mediate imbalance between endothelial destruction and repair leading to endothelial dysfunction, an early stage in atherosclerosis development.⁵ Platelets have an impact on endothelial function⁶ in addition to their role in CVD and SLE pathogenesis.^{7,8} The aim of this study, was to take both type I IFN activity and platelet activation into account when investigating endothelial dysfunction in SLE.

Endothelial dysfunction is a state of impaired endothelial dependent vasodilatation that also consists of endothelial activation; a pro-inflammatory state with decreased endothelial anticoagulant ability.⁹¹⁰ Several methods may be used to assess endothelial dysfunction. Serum levels of endothelial derived markers, such as soluble vascular cell adhesion molecule-1 (sVCAM-1), is elevated as a consequence of endothelial activation and dysfunction.^{11, 12} Furthermore, endothelial microparticles (EMPs), subcellular vesicular fragments that shed from endothelial cells (ECs) in response to certain stimuli,¹³ have been reported to correlate with endothelial dysfunction^{14, 15} and endothelial damage.¹³ Non-invasive techniques to assess endothelial dysfunction have been developed, with flow mediated dilatation (FMD) measurement of the brachial artery considered as the golden standard.⁹ However, the method is complex and operator-dependent. Abnormal pulse wave amplitude (PWA) in peripheral arteries as a marker of atherosclerosis and predictor of cardiovascular events may be used as an alternative.^{16,17} Peripheral artery tonometry (PAT) using a device called Endopat has been developed to measure PWA in finger arteries and is an easy, investigator independent, method to assess

endothelial dysfunction. A linear relationship between endothelial dysfunction measured with FMD and EndoPAT has been demonstrated previously.¹⁸

Type I IFNs are key cytokines in the pathogenesis of SLE with a number of regulatory effects on both innate and adaptive immunity.¹⁹ They have been suggested to contribute to the imbalance between vascular damage and repair seen in SLE through increased endothelial progenitor cell (EPC) apoptosis and by affecting circulating angiogenic cells (CACs) to differentiate to nonangiogenic cells. EPC/CAC function and capacity to produce proangiogenic molecules also seem to be impaired by type I IFNs, properties that could be restored by blocking type I IFNs *in vitro*.⁵ In SLE, type I IFNs have been demonstrated to exert their effects on EPC/CACs through downregulation of the proangiogenic IL-1 signalling pathways and by affecting the inflammasome and promoting IL-18 activation as well as IL-1 β repression.^{20 21} Elevated levels of type I IFNs correlate with endothelial dysfunction and EPC decrease in SLE.²² In line with this observation, an association between increased serum type I IFN activity and markers of subclinical atherosclerosis in SLE patients has been described, suggesting a role of type I IFNs in atherosclerosis development.²³

It is well established that platelets are of importance in the development of cardiovascular disease.⁷ In recent years, the role of platelets in the pathogenesis of SLE with a possible link between the type I IFN system and platelet function has been investigated.^{8 24} Platelets also play a role in endothelial activation and function, as they attract and promote EPCs adhesion to the injured vascular wall.^{6 25} Therefore, we set out to investigate platelet activation in SLE patients in relation to endothelial activation and type I IFN activity, since, to our knowledge, this has not been thoroughly investigated.

In brief, we found that SLE patients with an activated type I IFN system had impaired endothelial function and the patients with endothelial activation had increased platelet activation. Thus, we suggest that activation of platelets and platelet-endothelium interactions may contribute to impaired endothelial function and the development of CVD in SLE.

MATERIALS AND METHODS

Patients and controls

Patients with SLE (n=148) as well as age- and sex-matched healthy controls (HC, n=79) were recruited to participate in studies related to cardiovascular disease at the Department of Rheumatology, Skåne University Hospital, Lund, Sweden. An overview of the clinical characteristics of the 148 SLE patients and 79 healthy volunteers is presented in Table 1 and 2. Median disease duration of the SLE patients was 11 years (range 0-46). Disease activity in the SLE patients was assessed using SLEDAI-2K.²⁶ Median SLEDAI-2K score in the SLE patients was 1.5 (range 0-18) and SLEDAI-2K scores are demonstrated in Table 2. All but two SLE patients fulfilled at least four American College of Rheumatology (ACR) 1982 classification criteria for SLE.²⁷ The last two patients fulfilled three ACR criteria, had a clinical SLE diagnosis with at least two organ manifestations characteristic of SLE, autoimmune phenomena, and no other diagnosis that could better explain the symptoms. The median Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SLICC/ACR-DI) score of the SLE patients was 0 (range 0-8). The participants completed questionnaires concerning smoking, general health and medication. All subjects were examined by a rheumatologist at inclusion into the study. Traditional cardiovascular risk factors; age, gender, hypertension (systolic blood pressure equal or higher than 140 mm Hg at the time point of blood sampling or hypertensive treatment due to high blood pressure) and plasma low density lipoprotein (LDL) cholesterol levels, were analysed. History of cerebrovascular incident (CVI), acute myocardial infarction (AMI) and deep venous thrombosis (DVT) or pulmonary embolism were verified in medical records and defined by the SLICC/ACR Damage Index (DI)²⁸ regardless of time point of SLE diagnosis. Overnight fasting blood samples, were drawn according to standard procedures at Skåne University Hospital, Lund for determination of plasma lipids. Sera and EDTA plasma samples were stored at -80°C. Complement and autoantibodies were measured by routine analyses at the Division of Clinical Immunology and Transfusion Medicine, Skåne University Hospital, Lund, Sweden.

The study was approved by the Lund University regional ethics board (LU-06014520) and written informed consent was obtained from all participants.

Interferon activity assay

Type I IFN activity was measured in three different ways:

1. Serum type I IFN activity was measured as previously described.^{29 30} Briefly, WISH cells (CCL-25; American Type Culture Collection, Manassas, VA, USA) were cultured for 6 hours with patient serum after which lysis mixture (Panomics Inc., Fremont, CA, USA) was added. Cell lysates were analysed on a Luminex 100 (Luminex Corporation, Austin, TX, USA) for mRNA expression of three house-keeping genes (GAPDH, PPIB, B2M) and six type I IFN-regulated genes (LY6E, MX1, OAS1, ISG15, IFIT1 and EIF2AK2) using the Quantigene Plex 2.0 assay as described by the manufacturer (Panomics Inc.). The IFN score (indicating serum type I IFN activity) was calculated as the relative type I IFN-regulated genes expression in WISH cells exposed to SLE serum as compared with unstimulated WISH cells. The limit for a high serum type I IFN score was set to >2.0 as described earlier.²⁹
2. Type I IFN signature in peripheral blood mononuclear cells (PBMCs): PBMCs were isolated using Lymphoprep (Axis-Shield) according to manufacturer's protocol and type I IFN signature analysed with the Quantigene Plex 2.0 assay as described above. The PBMC IFN signature was only measured in SLE patients.
3. Quantification of the IFN- inducible protein galectin-3-binding protein (G3BP) was performed using an ELISA.³¹ EDTA plasma samples, diluted 1:100 in Sample Diluent, were analysed in duplicate according to manufacturer's instructions using the Human 90K/Mac-2BP Platinum ELISA kit (BMS234, Bender MedSystem, Vienna, Austria).

Biomarkers of endothelial dysfunction/activation

Serum sVCAM-1 was analysed by ELISA according to the manufacturer's protocol (R&D Systems Quantikine). The 95th percentile of the 79 healthy individuals determined the cut-off for the upper limit of normal sVCAM-1. For detection of EMPs, flow cytometry was performed directly on heparinized platelet-poor plasma (PPP).³² MPs were labelled with murine monoclonal anti-CD146-FITC or the relevant isotype-matched control antibodies, as previously described. The cut-off for upper limit of normal EMP was determined by the 95th percentile of the healthy individuals.^{32 33}

Platelet activation

Platelet C1q and C4d deposition were analysed by flow cytometry as described previously.^{24 34 35} The upper limits of normal C1q and C4d deposition on platelets was determined by the 95th percentile of healthy controls.

Assessment of Endothelial Function

Endothelial function was determined using EndoPAT 2000 (Itamar Medical, Caesarea, Israel), which has been validated and used in previous studies³⁶⁻³⁸ Subjects were examined according to the manufacturer's protocol and as previously described.³⁶ Changes in PWA in the finger artery during reactive hyperaemia was detected with a finger plethysmograph. A finger probe was placed on the index finger of the right hand and PWA was recorded with PAT at baseline, during suprasystolic cuff occlusion and during reactive hyperaemia. PWA was also recorded from the contralateral, left index finger not undergoing reactive hyperaemia testing as a control. PAT measurements were analysed with a computerized automated algorithm (Itamar Medical Ltd, Caesarea, Israel). The cut-off for reactive hyperaemia index (RHI) was set to 1.67 according to manufacturer's instructions (Itamar Medical).³⁹

Statistics

SPSS Statistics version 22 (IBM Corporation Armonk, NY, USA) was used for all statistical analyses. For calculation of odds ratios (OR) and 95% confidence interval (CI), logistic regression analysis was applied. Results are presented unadjusted and after adjusting for CVD risk factors (age, gender, LDL plasma concentration, current smoking and hypertension) in all groups larger than n=30. In smaller groups (n=17) adjustment for age, gender and LDL plasma concentrations were made. Spearman's rank correlation test was used to analyse correlations. Mann -Whitney U -test and Chi squared test were used when comparing values between groups shown in table 1. A p-value <0.05 was considered statistically significant.

RESULTS

Patient characteristics

In total 148 SLE patients and 79 healthy controls were included in the study and the clinical characteristics are demonstrated in table 1.

SLE patients with activation of the type I IFN system have ongoing endothelial activation

Activation of the type I IFN system is important in the pathogenesis of SLE and has been suggested to contribute to endothelial dysfunction. We therefore set out to investigate if SLE patients with ongoing type I IFN activity have signs of endothelial dysfunction. Three different assays to measure type I IFN activity was used; serum type I IFN activity, IFN signature in PBMCs and plasma levels of G3BP. Using Spearman's rank correlation test, we found that there was a good correlation between all assays (serum type I IFN activity and IFN signature in PBMCs ($r=0.65$, $p<0.01$), serum type I IFN activity and plasma levels of G3BP ($r=0.54$, $p<0.01$) and IFN signature in PBMCs and plasma G3BP protein levels ($r=0.48$, $p<0.01$). As similar results were found for these three different assays, we only used serum type I IFN activity in our future analyses.

In our cohort of patients with SLE, we could indeed demonstrate that ongoing activation of the type I IFN system, was associated with endothelial activation, measured as high sVCAM-1 and high EMPs. This association remained after adjusting for CVD risk factors (Table 3).

SLE patients with endothelial activation have increased platelet activation

As platelets have been implicated in the development of CVD in patients with SLE, we analysed if there was an association between platelet activation and endothelial activation in patients with SLE. Both high serum levels of sVCAM-1 and EMPs, reflecting ongoing endothelial activation, were associated

with activated platelets measured as high platelet C4d deposition (table 4). High levels of sVCAM-1, but not high EMP, was associated with platelet activation measured as high platelet C1q deposition (table 4). Since the smallest group consisted of 17 individuals, adjustment for 3 instead of 5 CVD risk factors were made. No association between platelet activation and serum type I IFN activity was seen.

SLE patients with ongoing type I IFN activity have signs of vascular dysfunction measured by Endopat

As we had seen that SLE patients with ongoing type I IFN activity had signs of endothelial activation, we analysed if these patients had detectable signs of vascular dysfunction measured by Endopat.

SLE patients with ongoing serum type I IFN activity, more often had a pathological RHI (<1.67) compared with healthy controls (OR 2.61 95% CI 1.04-6.53 $p=0.04$) and this association remained statistically significant after adjusting for CVD risk factors (age, gender, p-LDL concentration, smoking and hypertension) (Figure 1). No difference in reactive hyperaemia index (RHI) was seen when comparing all SLE patients and healthy controls (Figure 1).

These data indicate that vascular dysfunction could be detected by Endopat in SLE patients with high type I IFN activity.

Only high levels of sVCAM-1 is associated with previous CVD comorbidity

In total, 43 SLE patients had a history of CVD events (CVI, $n=15$), (AMI, $n=10$) or (DVT, $n=24$). SLE patients with previous CVI had higher OR for having a high sVCAM-1 value than patients without CVI (OR 3.77 95%CI 1.02-13.96 $p<0.05$) also after adjusting for CVD risk factors (OR 4.32 95% CI 1.05-17.86 $p=0.04$).

Neither ongoing type I IFN activity, levels of EMP, nor RHI were associated with a history of CVD.

No correlation between the different markers of endothelial dysfunction

We wanted to investigate if RHI, sVCAM-1 and EMP were related. No correlation was found between RHI, sVCAM-1 and EMP in either SLE or HCs. Nor did we find a direct correlation between sVCAM-1 and EMP in SLE or healthy controls. Thus, the data derived from measurements of these variables may represent different aspects of endothelial dysfunction and endothelial activation.

DISCUSSION

In this study, we demonstrate that SLE patients with activation of the type I IFN system have ongoing endothelial activation measured as high sVCAM-1 and elevated EMPs. Furthermore, we show increased platelet activation in SLE patients with ongoing endothelial activation. In addition to biomarkers, vascular function was assessed with Endopat, and we found impaired endothelial function in SLE patients with high type I IFN activity, supporting the theory that increased type I IFN activity affects endothelial function.

Type I IFNs drive important parts of the autoimmune and inflammatory process in SLE, such as monocyte maturation to antigen presenting dendritic cells, prolonged survival for activated T lymphocytes, decreased threshold for activation of B cells and increased proliferation, as well as enhanced differentiation of B cells to antibody-secreting plasma cells.¹⁹ Furthermore, type I IFNs are suggested to affect endothelial function through downregulation of proangiogenic IL-1 signalling pathways, leading to effects on EPC/CACs. Together with myelomonocytic CACs, EPCs are necessary for endothelial repair after vascular injury as they can differentiate into mature ECs.⁵ Moreover, EPCs/CACs have the capacity to produce proangiogenic molecules, including vascular endothelial growth factor (VEGF) that enhance the release of EPCs from bone marrow and help migrate and incorporate ECs in the injured blood vessel wall. Increased EC apoptosis, thought to contribute to endothelial dysfunction, has been reported in SLE as a proposed contributor to the increased prevalence of atherosclerosis.⁴⁰ Under normal conditions, endothelial damage leads to recruitment of bone marrow-derived EPCs. In contrast, however, SLE patients have a decreased number of circulating EPCs, likely due to effects mediated by type I IFNs. Thus, theoretically activation of type I IFN may have direct impact on the cardiovascular risk for patients with SLE.

In our study, we demonstrated that SLE patients with ongoing type I IFN activity have affected vascular homeostasis, assessed by decreased RHI and increased levels of sVCAM-1 and EMPs. Thus, our data,

in concordance with previous findings, suggest that SLE patients with ongoing type I IFN activity are at highest risk to develop CVD.

Affected vascular homeostasis can be measured with biomarkers, including sVCAM-1 and EMPs. Furthermore, endothelial function of the peripheral circulation can be assessed by non-invasive methods, such as FMD and by a finger plethysmograph, Endopat. EMPs and sVCAM-1 have both been described as markers of endothelial dysfunction and endothelial activation^{10 11 13-15 41} and are potential surrogate markers for FMD and RHI. However, reports of correlations between EMP, sVCAM-1 and RHI or FMD have been contradictory,^{13 42-44} with results dependent on study population and disease severity. Our study is cross-sectional and thus our cohort consists of patients in different disease stages and with different disease activity. In our study, no correlation was seen between the sVCAM-1, EMP and RHI values. Although all these markers are related to endothelial dysfunction, they may represent different parts of disease processes occurring in sequence, rather than all together at a given time-point. The different markers may be partly induced by different stimuli, including type I IFNs, and this may explain the lack of correlation. Further studies are required to understand the relations between the different markers of endothelial dysfunction and activation and their corresponding contribution to end-term damage, *e.g.* atherosclerosis.

There is growing evidence that platelets may play a part in the pathogenesis of SLE⁸ in addition to their role in development of atherosclerosis.^{6 7} *In vitro* studies suggest that platelets can affect EPCs to differentiate either to ECs or to macrophages or foam cells and thereby contribute, either to vascular repair or injury.^{6 25} We have previously shown increased platelet activation in SLE and demonstrated platelets with an IFN signature in SLE patients with CVD.^{24 34} In SLE, platelets have also been shown to stimulate pDCs to produce IFN, through CD40-CD154 interactions, with possible effects on the endothelium.⁸ In a recent study, it was demonstrated that activated platelets in SLE can promote endothelial cell activation by an interleukin (IL) -1 β -dependent pathway.⁴⁵ Injured endothelium, on the

other hand, could affect the platelets and activate them,⁶ leading to a vicious circle of endothelium-platelet interaction with increased cardiovascular risk.

In the current study, we made the novel observation that platelet activation was related to endothelial activation in SLE patients. Platelet activation was associated with both elevated serum sVCAM-1 concentration and high EMP levels, consistent with the hypothesis that interactions between activated platelets and activated endothelium occurs and that platelet activation might contribute to endothelial dysfunction. We did not find any direct correlation between platelet activation and type I IFN activity, suggesting that platelet activation might be a result of the activated or dysfunctional endothelium in the patients. Indeed, in patients with stable coronary heart disease platelet activation correlates to endothelial dysfunction.⁴⁶ Nevertheless, further studies are needed to understand the mechanistic relation between type I IFN activity and platelet activation.

Although most of the patients in our cross sectionally studied cohort had relatively low disease activity, they still had signs of endothelial and platelet activation that could contribute to increased CVD risk. Therefore, we believe it is important to further investigate the mechanisms behind endothelial and platelet activation in SLE patients also with low disease activity.

In conclusion, SLE patients with activated type I IFN system have impaired endothelial function, connecting central pathogenic processes in SLE with endothelial dysfunction and cardiovascular disease. We hypothesise that type I IFN-injured endothelium leading to platelet activation, may play a role in the development of cardiovascular disease in SLE. Our results suggest that assessing RHI, type I IFN signature and markers of platelet activation, in addition to traditional CVD risk factors, may be important when evaluating CVD risk in the individual patient.

KEY MESSAGES

What is already known about this subject?

Patients with Systemic Lupus Erythematosus (SLE) have increased risk for cardiovascular disease and both activation of type I interferons and platelets have been implicated in this process.

Endothelial dysfunction, an early step in the development of atherosclerosis, can be assessed by non-invasive techniques as well as by surrogate markers, such as sVCAM-1 and endothelial microparticles.

What does this study add?

Patients with SLE, may have ongoing type I IFN activation leading to impaired endothelial function also in patients with low disease activity.

Patients with SLE and endothelial dysfunction have activated platelets, that may contribute to elevated risk of cardiovascular disease.

How might this impact on clinical practice?

Analysing type IFN signature, endothelial function and platelet activation may add valuable information when evaluating cardiovascular risk in the individual SLE patient.

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CONTRIBUTORS

Design of the study: HT, CL, AJ, AAB. Performed the statistical analyses: HT. Analysed the data: HT, CL, BG, AJ, RK, ABB. Assessment of reactive hyperaemia index: HT. Performed the experiments: CL, BG, CTN, NHH. Wrote the paper: HT, RK, AAB. All authors critically revised the manuscript.

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COMPETING INTERESTS

The authors have declared no conflicts of interest

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Table 1: **Demographics and distribution of traditional cardiovascular risk factors in SLE and healthy controls**

Characteristics	SLE	Healthy controls	p-value
Number	148	79	
Female (%)	87	85	0.62
Age (years) median (range)	48 (20-82)	47 (18-81)	0.95
Current smoker (%)	21	9	0.02
BMI, mean and SD	25.48 ± 4.94	23.45 ± 3.05	<0.01
Hypertension ^a (%)	43	18	<0.01
S-LDL (mmol/L), mean and SD	3.06 ± 0.95	3.16 ± 0.87	0.34
Acute myocardial infarction ^b (n)	10	0	-
Cerebrovascular insult ^b (n)	15	0	-
Deep venous thrombosis ^b (n)	24	0	-
Current medication			
Glucocorticoids ^c (n)	98	0	-
Hydroxychloroquine (n)	105	0	-
Azathioprine (n)	32	0	-
Mycophenolatmofetil (n)	20	0	-
Methotrexate (n)	13	0	-
Intravenous immunoglobulins (n)	2	0	-
Non-steroidal antiinflammatory drugs (n)			
Acetylsalicylic acid (n)	44	0	-
Warfarin (n)	23	0	-

^a Hypertension treatment due to high blood pressure or systolic blood pressure ≥140 mm Hg at time point of blood sampling. ^b medical history of. ^c median daily dose =5 mg, range 1–30 mg.

Table 2: **Clinical characteristics of the 148 SLE patients included in the study** according to the 1982 ACR classification criteria and disease activity measured by SLEDAI-2K score at time- point of investigation

Characteristics	SLE (n=148)
ACR criteria, median (range)	5 (3-10)
Malar rash (%)	52
Discoid rash (%)	20
Photosensitivity (%)	56
Oral ulcers (%)	24
Arthritis (%)	78
Serositis (%)	39
Renal disease (%)	33
Neurological disorders (%)	6
Haematological manifestations (%)	55
Leukopenia (%)	37
Lymphopenia (%)	24
Thrombocytopenia (%)	14
Haemolytic anaemia (%)	2
Immunology ^a (%)	69
Anti-dsDNA antibodies (%)	59
Anti-smith antibodies (%)	10
ANA (%)	98
SLEDAI-2 K score, median (range)	1.5 (0-18)
Active disease SLEDAI≥4	29
Organic brain syndrome (n)	1
Lupus headache (n)	3
Vasculitis (n)	1
Arthritis (n)	15
Kidney involvement (n)	16
(urinary cast, haematuria, proteinuria or pyuria)	
Rash (n)	18
Alopecia (n)	4
Oral or nasal ulcers (n)	4
Pleurisy (n)	2
Low complement (C3 or C4) (n)	36
Anti-dsDNA antibodies (n)	21
Thrombocytopenia (n)	2
Leukopenia (n)	9

^aanti-dsDNA, anti-sm, LE cells, false positive Wasserman test

Table 3: **Correlations between endothelial activation and type I IFN activity in SLE.**

Endothelial activation	sVCAM-1 high			EMP high		
	OR	CI 95%	p	OR	CI 95%	p
IFN activity ^a	1.68	1.15-2.47	<0.01	1.40	1.04-1.88	0.03

OR for indicators of endothelial activation; high sVCAM-1 and high EMP levels were calculated in SLE patients in relation to serum type I IFN activity, adjusted for CVD risk factors. EMP (endothelial microparticles). ^a adjusted for age, gender, smoking, hypertension, p-LDL concentration

Table 4: Activated platelets in SLE patients with endothelial activation.

Endothelial activation	Platelet/C4d high			Platelet/C1q high		
	OR	95%CI	p	OR	95% CI	p
sVCAM-1 high ^a	4.57	2.14-9.79	<0.01	4.10	1.09-15.38	0.04
EMP high ^a	3.64	1.16-11.38	0.03	2.16	0.51-9.16	0.30

OR for markers of platelet activation; platelet-C4d and platelet C1q deposition was calculated in SLE patients with and without high sVCAM-1 and high EMP levels. Endothelial activation- and platelet activation values are dichotomous. EMP (endothelial microparticles), platelet-C4d/platelet-C1q (platelet-C4d/C1q deposition). ^a adjusted for age, gender, p-LDL concentration

FIGURE LEGENDS

Figure 1

Endothelial dysfunction in SLE patients with increased type I IFN activity. OR for reactive hyperaemia index (RHI) were calculated in SLE patients and HC, unadjusted and adjusted for CVD risk factors. Subgroup analysis in SLE patients with and without ongoing serum type I IFN activity were performed. ^a adjusted for CVD risk factors.

RHI

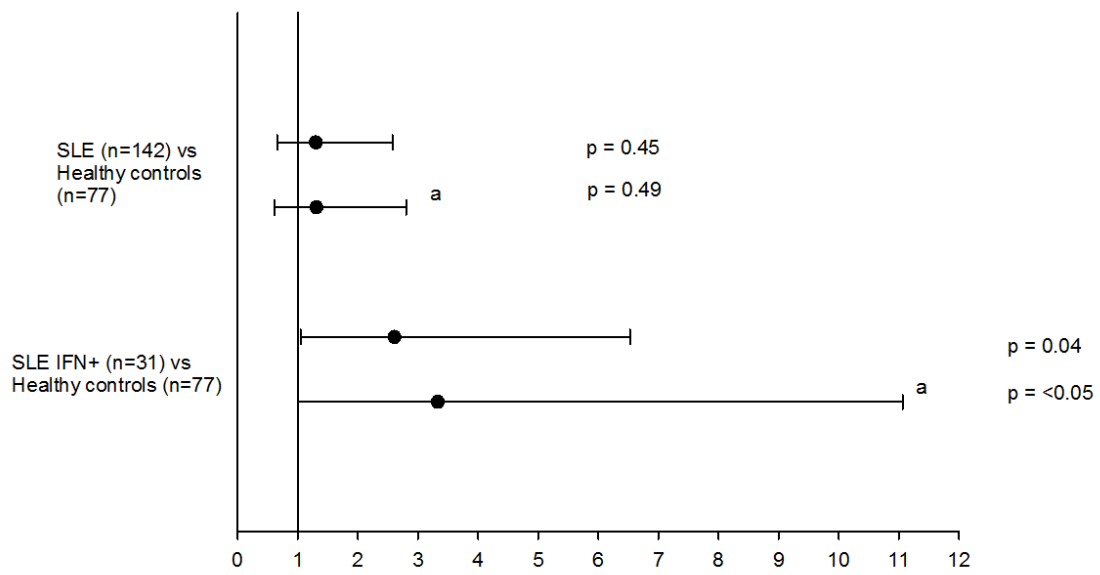


Figure 1

Odds ration (95% CI)
^a adjusted for CVD risk factors