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ORIGINAL ARTICLE

Endothelial dysfunction is associated with 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 (CVD) 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 IFN in SLE.

Methods We enrolled 148 patients with SLE and 79 sex-matched and age-matched healthy controls (HCs). 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, soluble vascular cell adhesion molecule-1 (sVCAM-1) and endothelial microparticles (EMPs), and finger plethysmograph to determine Reactive Hyperaemia Index (RHI).

Results In patients with SLE, type I IFN activity was associated with endothelial activation, measured by high sVCAM-1 (OR 1.68, p<0.01) and elevated EMPs (OR 1.40, p=0.03). Patients with SLE with high type I IFN activity had lower RHI than HCs (OR 2.61, p=0.04), indicating endothelial dysfunction. Deposition of complement factors on platelets, a measure of platelet activation, was seen in patients with endothelial dysfunction. High levels of sVCAM-1 were 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 EMPs 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 CVD in SLE.

Key messages

What is already known about this subject?

- Patients with systemic lupus erythematosus (SLE) have increased risk for cardiovascular disease (CVD) and both activation of type I interferons (IFNs) 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 soluble vascular cell adhesion molecule-1 and endothelial microparticles.

What does this study add?

- Patients with SLE may have elevated type I IFN activation leading to impaired endothelial function including patients with low disease activity.
- Patients with SLE and endothelial dysfunction have activated platelets, which may contribute to an elevated risk of CVD.

How might this impact on clinical practice?

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

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. To some extent this increase may 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] In addition to their role in CVD and SLE pathogenesis,[6 7] platelets have an impact on endothelial function.[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 proinflammatory state with decreased endothelial anticoagulant ability.[9 10] 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 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 gold 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] and have been suggested to mediate increased endothelial progenitor cell (EPC) apoptosis and the differentiation of circulating angiogenic cells (CACs) to non-angiogenic cells. This imbalance between vascular damage and repair may cause endothelial dysfunction in SLE. In addition, type I IFNs affect the capacity of EPC/CAC to produce proangiogenic molecules which can 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 interleukin (IL)−1 signalling pathways and by affecting the inflammasome and promoting IL-18 activation as well as IL-1β 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 patients with SLE has been described, suggesting a role of type I IFNs in atherosclerosis development.[23]

Previous studies have established that platelets are of importance in the development of CVD.[6] 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.[7 24] Platelets also play a role in endothelial activation and function, as they attract and promote EPCs' adhesion to the injured vascular wall.[8 25] Therefore, the aim of this study was to investigate platelet activation in patients with SLE in relation to endothelial activation and type I IFN activity, since this has not been thoroughly investigated.

In brief, this study found that patients with SLE 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-matched and sex-matched healthy controls (HCs, n=79) were recruited at the Department of Rheumatology, Skåne University Hospital, Lund, Sweden. An overview of the clinical characteristics of the 148 patients with SLE and 79 healthy volunteers is presented in tables 1 and 2. Median disease duration of the patients with SLE was 11 years (range 0–46). Disease activity in the patients with SLE was assessed using Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K).[26] Median SLEDAI-2K Score in the patients with SLE was 1.5 (range 0–18) and SLEDAI-2K Scores are demonstrated in table 2. All but two patients with SLE 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/ACR Damage Index (SLICC/ACR-DI) Score of the patients with SLE 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 SLICC/ACR-DI[28] regardless of the 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.

Written informed consent was obtained from all participants.

IFN activity assay

Type I IFN activity was measured in three different ways:

1. Serum type I IFN activity was measured in patients with SLE as previously described.[29 30] Briefly, WISH

Table 1 Demographics and distribution of traditional cardiovascular risk factors in patients with systemic lupus erythematosus (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* (%)	43	18	<0.01
P-LDL (mmol/L), mean and SD	3.06±0.95	3.16±0.87	0.34
P-EMP (EMP/mL), median (25,75)	2.71×10^5 ($1.54, 7.70\times10^5$)	3.66×10^5 ($1.89, 7.67\times10^5$)	0.46
P-sVCAM-1 (ng/mL), median (25,75)	783 (623, 965)	517 (444, 592)	<0.01
RHI, median (25,75)	2.09 (1.70, 2.54)	2.16 (1.76, 2.64)	0.42
Platelet C1q dep (MFI ratio), median (25,75)	1.82 (1.59, 2.04)	1.61 (1.45, 1.79)	<0.01
Platelet C4d dep (MFI ratio), median (25,75)	1.93 (1.56, 3.33)	1.44 (1.31, 1.65)	<0.01
Serum IFN score (AU), median (25,75)	1.29 (1.05, 1.92)	ND	-
Acute myocardial infarction† (n)	10	0	-
Cerebrovascular insult† (n)	15	0	-
Deep venous thrombosis† (n)	24	0	-
Current medication			
Glucocorticoids‡ (n)	98	0	-
Hydroxychloroquine (n)	105	0	-
Azathioprine (n)	32	0	-
Mycophenolate mofetil (n)	20	0	-
Methotrexate (n)	13	0	-
Intravenous immunoglobulins (n)	2	0	-
Non-steroidal anti-inflammatory drugs (n)	12	0	-
Acetylsalicylic acid (n)	44	0	-
Warfarin (n)	23	0	-

*Hypertension treatment due to high blood pressure or systolic blood pressure ≥140 mm Hg at the time point of blood sampling.
†Medical history.
‡Median daily dose=5 mg, range 1–30 mg.
(25,75), 25th and 75th centiles; AU, arbitrary unit; BMI, body mass index; P-EMP, plama endothelial microparticle; IFN, interferon; P-LDL, plasma low density lipoprotein; MFI, median fluorescence intensity; ND, not determined; Platelet C1q-C4d dep, deposition; RHI, Reactive Hyperaemia Index; P-sVCAM-1, plasma soluble vascular cell adhesion molecule-1.

cells (CCL-25; American Type Culture Collection, Manassas, Virginia, USA) were cultured for 6 hours with patient serum after which lysis mixture (Panomics, Fremont, California, USA) was added. Cell lysates were analysed on a Luminex 100 (Luminex Corporation, Austin, Texas, USA) for mRNA expression of three housekeeping 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). 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) was measured in patients with SLE: 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 in the section above. PBMC type I IFN score was calculated as the mean expression of six type I IFN-regulated genes (LY6E, MX1, OAS1, ISG15, IFIT1 and EIF2AK2) normalised to three housekeeping genes (GAPDH, PPIB and B2M).
3. Quantification of the IFN- inducible protein galectin-3-binding protein (G3BP) was performed using the

Table 2 Clinical characteristics of the 148 patients with systemic lupus erythematosus (SLE) included in the study according to the 1982 ACR classification criteria and disease activity measured by SLEDAI-2K Score at the 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
Leucopenia (%)	37
Lymphopenia (%)	24
Thrombocytopenia (%)	14
Haemolytic anaemia (%)	2
Immunology* (%)	69
Anti-dsDNA antibodies (%)	59
Anti-Smith antibodies (%)	10
ANA (%)	98
SLEDAI-2K 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) *(urinary cast, haematuria, proteinuria or pyuria)*	16
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
Leucopenia (n)	9

*anti-dsDNA, anti-sm, lupus erythematosus cells, false-positive Wasserman test.
ACR, American College of Rheumatology; ANA, antinuclear antibody.

Human 90K/Mac-2BP Platinum ELISA kit (BMS234, Bender MedSystem, Vienna, Austria).[31] EDTA plasma samples, diluted 1:100 in sample diluent, were analysed in duplicate according to manufacturer's instructions.

Biomarkers of endothelial dysfunction/activation

Plasma sVCAM-1 was analysed by ELISA according to the manufacturer's protocol (R&D Systems Quantikine). The 95th centile of the 79 healthy individuals determined the cut-off for the upper limit of normal sVCAM-1 and was set to 764 ng/mL. For detection of EMPs, flow cytometry was performed directly on heparinised platelet-poor plasma.[32] EMPs were labelled with murine monoclonal anti-CD146-fluorescein isothiocyanate (FITC) or the relevant isotype-matched control antibody, as previously described.[32 33] The cut-off for upper limit of normal EMP was determined by the 95th centile of the healthy individuals and was set to 1.98×10^6 EMP/mL.

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 centile of HCs and was set to 2.40 and 2.09 median fluorescence intensity ratio, respectively.

Assessment of endothelial function

Endothelial function was determined using an EndoPAT 2000 (Itamar Medical, Caesarea, Israel), which has been validated and used in previous studies.[36–38] Subjects were examined according to the manufacturer's protocol and as previously described.[36] Changes in PWA in the finger artery during reactive hyperaemia were 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 computerised automated algorithm (Itamar Medical, 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 V.22 (IBM Corporation, Armonk, New York, USA) was used for all statistical analyses. For calculation of ORs and 95% 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 χ^2 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 patients with SLE and 79 HCs were included in the study and the clinical characteristics are demonstrated in table 1.

Table 3 Correlations between endothelial activation and serum type I IFN activity in systemic lupus erythematosus (SLE)

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

OR for indicators of endothelial activation; high sVCAM-1 and high EMP levels were calculated in patients with SLE in relation to serum type I IFN activity, adjusted for CVD risk factors.
*adjusted for age, gender, smoking, hypertension, p-LDL concentration. Serum type I IFN activity was measured by a reporter gene expression assay (serum IFN score).
CVD, cardiovascular disease; EMP, endothelial microparticles; IFN, interferon; LDL, low density lipoprotein; sVCAM, soluble vascular cell adhesion molecule-1.

Patients with SLE with activation of the type I IFN system have activated endothelium

Activation of the type I IFN system has been suggested to contribute to endothelial dysfunction. This study therefore set out to investigate if patients with SLE with type I IFN activity have signs of endothelial dysfunction. Three different assays to measure type I IFN activity were used, serum type I IFN activity, IFN signature in PBMCs and plasma levels of G3BP. Using Spearman's rank correlation test, the data showed 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). Serum type I IFN scores in the patients with SLE are presented in table 1. Median value for serum type I IFN score was 1.29 arbitrary units (AU) in patients with SLE. Patients with SLE had significantly higher plasma levels of G3BP compared with HCs (median 3345 ng/mL vs 2738 ng/mL, p<0.01). The median PBMC score in patients with SLE was 0.10 AU. As similar results were found for these three different assays, the serum type I IFN activity was used for future analyses.

In the study cohort of patients with SLE, activation of the type I IFN system was demonstrated and was associated with endothelial activation. The endothelial activation was measured as high sVCAM-1 and high EMPs. This association remained after adjusting for CVD risk factors (table 3).

Patients with SLE with endothelial activation have increased platelet activation

As platelets have been implicated in the development of CVD in patients with SLE, the association between platelet activation and endothelial activation in patients with SLE was examined. Both high serum levels of sVCAM-1 and EMPs, reflecting 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 three instead of five CVD risk factors was made. In order to investigate the independent effect of platelet activation on endothelial dysfunction, the data were also adjusted for serum IFN activity (table 4). After adjusting for IFN activation, the association between sVCAM-1, but not EMP, and platelet activation remained, possibly due to a relatively small sample size. No association between platelet activation and serum type I IFN activity was seen.

Patients with SLE with type I IFN activity have signs of vascular dysfunction measured by EndopPAT

As we had seen that patients with SLE with type I IFN activity had signs of endothelial activation, the patients were tested to determine if these patients had detectable signs of vascular dysfunction measured by EndoPAT.

Patients with SLE with high serum type I IFN activity, more often had a pathological RHI (<1.67) compared

Table 4 Activated platelets in patients with systemic lupus erythematosus (SLE) with endothelial activation

Endothelial activation	Platelet/C4d high			Platelet/C1q high		
	OR	95% CI	p Value	OR	95% CI	p Value
sVCAM-1 high*	4.57	2.14 to 9.79	<0.01	4.10	1.09 to 15.38	0.04
sVCAM-1 high†	4.17	1.90 to 9.15	<0.01	4.15	1.07 to 16.18	0.04
EMP high*	3.64	1.16 to 11.38	0.03	2.16	0.51 to 9.16	0.30
EMP high†	3.11	0.97 to 9.98	0.056	2.05	0.47 to 8.91	0.34

OR for markers of platelet activation; platelet-C4d and platelet C1q deposition was calculated in patients with SLE with and without high sVCAM-1 and high EMP levels. Endothelial activation and platelet activation values are dichotomous.
*Adjusted for age, gender, p-LDL concentration.
†Adjusted for age, gender, p-LDL and IFN activity.
EMP, endothelial microparticles; IFN, interferon; LDL, low density lipoprotein; platelet-C4d/platelet-C1q, platelet-C4d/C1q deposition; sVCAM-1, soluble vascular cell adhesion molecule-1.

Table 5 Endothelial dysfunction in patients with systemic lupus erythematosus (SLE) with increased type I IFN activity

	RHI		
Tested groups	OR	95% CI	p Value
SLE (n=142) versus HCs (n=77)	1.30	0.66 to 2.58	0.45
SLE (n=142) versus HCs (n=77)*	1.31	0.61 to 2.80	0.49
SLE IFN+ (n=31) versus HCs (n=77)	2.61	1.04 to 6.53	0.04
SLE IFN+ (n=31) versus HCs (n=77)*	3.33	1.003 to 11.06	<0.05

OR for Reactive Hyperaemia Index (RHI) were calculated in patients with SLE and HCs, unadjusted and adjusted for CVD risk factors. Subgroup analysis in patients with SLE with (IFN+) and without ongoing serum type I IFN activity were performed.
*adjusted for age, gender, hypertension, smoking, p-LDL concentration.
CVD, cardiovascular disease; HCs, healthy controls; IFN, interferon; LDL, low density lipoprotein.

with HCs (OR 2.61, 95% CI 1.04 to 6.53, p=0.04) and this association remained statistically significant after adjusting for CVD risk factors (age, gender, plasma LDL (p-LDL) concentration, smoking and hypertension) (table 5). No difference in RHI was seen when comparing all patients with SLE and HCs (table 5).

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

High levels of sVCAM-1 are associated with previous CVD comorbidity

In total, 43 patients with SLE had a history of CVD events (CVI, n=15), (AMI, n=10) or (DVT, n=24). High sVCAM-1 levels were associated with previous CVI in patients with SLE (OR 3.77, 95% CI 1.02 to 13.96, p<0.05) also after adjusting for CVD risk factors (OR 4.32, 95% CI 1.05 to 17.86, p=0.04).

None of the other tested parameters, type I IFN activity, levels of EMP, nor RHI, were associated with a history of CVD.

No correlation identified between the different markers of endothelial dysfunction

The data were interrogated to determine if RHI, sVCAM-1 and EMP were related. No correlation was found between RHI, sVCAM-1 and EMP in either patients with SLE or HCs. Furthermore, no direct correlation between sVCAM-1 and EMP in SLE or HCs could be found. Thus, the data derived from measurements of these variables may represent different aspects of endothelial dysfunction and endothelial activation.

DISCUSSION

In this study, a connection between the type I IFN system and endothelial function could be demonstrated since endothelial activation, measured as high sVCAM-1 and elevated EMPs was seen in patients with SLE with activated type I IFN system. In addition, we found impaired endothelial function, assessed with EndoPAT, in patients with SLE with high type I IFN activity. Furthermore, we show increased platelet activation in patients with SLE with endothelial activation. These observations support the theory that increased type I IFN activity affects endothelial function.

Type I IFNs have modulatory effects on the immune system and promote autoimmunity in SLE.[19] Furthermore, type I IFNs are suggested to negatively affect endothelial function and to increase prevalence of atherosclerosis.[5 40] This suggests that activation of type I IFN may have direct impact on the cardiovascular risk for patients with SLE. In our study, it was demonstrated that patients with SLE with type I IFN activity have altered vascular homoeostasis, assessed by decreased RHI and increased levels of sVCAM-1 and EMPs. Thus, our data, in concordance with previous findings, suggest that patients with SLE with type I IFN activity are at highest risk to develop CVD.

Perturbation of vascular homoeostasis 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] In this 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 concurrently. 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, *for* example, atherosclerosis.

There is growing evidence that platelets may play a part in the pathogenesis of SLE,[7] in addition to their role in development of atherosclerosis.[6 8] In vitro studies suggest that platelets can affect EPCs to differentiate either to endothelial cells or to macrophages or foam cells, and thereby contribute either to vascular repair or injury.[8 25] We have previously shown increased platelet activation in SLE and demonstrated platelets with an IFN signature in patients with SLE with CVD.[24 34] In SLE, platelets have also been shown to stimulate plasmacytoid dendritic cells to produce IFN, through CD40–CD154 interactions, with possible effects on the endothelium.[7] In a recent study, it was demonstrated that activated platelets in SLE can promote endothelial cell activation by an IL-1β-dependent pathway.[45] Injured endothelium, on the other hand, could affect the platelets and activate them,[8] 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 patients with SLE. Platelet activation was associated with both elevated serum sVCAM-1 concentration and high EMP levels. This is consistent with the hypothesis that interactions between activated platelets and activated endothelium occurs and that platelet activation might contribute to endothelial dysfunction. No direct correlation between platelet activation and type I IFN activity was established, 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.

As mentioned above, there are some limitations of our study. The patients in our study are treated with immunosuppressive medication, including steroids, at the time point of blood sampling and investigation and this may affect the results. It is well known that type I IFN signalling is affected by glucocorticoid treatment.[47] In addition, the relatively few numbers of patients in the different subgroups may result in lack of significance when calculating associations.

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 including in patients with SLE also with low disease activity.

In conclusion, patients with SLE with an activated type I IFN system have impaired endothelial function, connecting central pathogenic processes in SLE with endothelial dysfunction and CVD. We hypothesise that type I IFN-injured endothelium leading to platelet activation may play a role in the development of CVD 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.

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