Endothelium-dependent and independent dilation capability of peripheral arteries in patients with systemic lupus erythematosus and antiphospholipid syndrome

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Abstract

Objective

The study evaluated the systemic inflammatory response and endothelium-dependent and independent function of the brachial artery (BA) in systemic lupus erythematosus (SLE) patients with and without antiphospholipid syndrome (APS).

Methods

The study group consisted of 42 women with SLE (21 without APS; mean age 36.1±9.1, and 21 with APS; mean age 43.9±13.1) and 22 healthy controls (mean age 43.5±10.3). Endothelium-dependent functional response was evaluated using the flow-mediated vasodilatation (FMD) of brachial artery and endothelium-independent vasodilatation by application of glyceryl trinitrate (GTN). Using biochemical methods, circulating inflammatory markers were determined.

Results

In comparison to controls, in both groups of patients endothelium-dependent dilation of BA was significantly reduced, and there were no differences in FMD between patients with or without APS: SLE - 7.7% (11.9–12.1), SLE+APS 7.8% (2.4–12.8), controls - 14.6% (11.2–21.1), p<0.001. However, endothelium-independent dilation of the brachial artery was significantly lower in SLE-APS patients than in controls and also lower than in the SLE group: SLE - 24.3% (15.0–28.6), SLE+APS-17.4% (13.1–22.6), controls - 23.0% (17.8–30.1), p=0.015 vs. p=0.027. Patients with SLE had significantly higher values of VCAM-1, hs-CRP, and fibrinogen than controls. In patients with SLE+APS, an additional significant increase of inflammatory markers was registered.

Conclusion

The results of our study indicate that patients with SLE have deteriorated endothelium-dependent and those with APS also independent vascular function which could be, together with increased inflammatory response, involved in vascular complications in these patients. The presence of APS aggravates systemic inflammatory response.

Key words

antiphospholipid syndrome, systemic lupus erythematosus, vascular endothelium, inflammation, thrombosis

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Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune connective tissue disease associated with an increased risk of atherosclerosis (1-3). It is commonly related to antiphospholipid syndrome (APS) (4), that is characterised by recurrent arterial and/or venous thrombosis and/or foetal loss in the presence of circulating antiphospholipid antibodies (aPL) (5). A wide spectrum of mechanisms has been proposed to account for vascular damage in patients with SLE.

In patients with SLE, beside classical risk factors for atherosclerosis and prolonged treatment with steroids, aPL mediated endothelial cell (EC) injury has been identified as a factor potentially involved in the pathogenesis of thrombosis (6, 7).

Endothelial dysfunction is an early event in the history of atherosclerosis and defined as loss of the vasodilatory, antithrombotic and anti-proliferative capacities of the endothelium (8). It s a consequence of the presence of risk factors and represents the key underlying mechanism in the atherosclerotic process, preceding formation of atherosclerotic plaques (9). Endothelial dysfunction can be assessed by determination of circulating markers of endothelial damage and/or activation. However, the most frequently used method for determination of endothelial function is monitoring the vasodilatory response of the peripheral arteries to shear stress evoked by increased blood-flow (flow mediated vasodilatation – FMD) (10).

There is evidence that SLE patients have deteriorated endothelial function in comparison to healthy controls (11-14) and consequently premature vascular disease (15). However, most studies have not differentiated SLE patients with and without APS (11, 16). Therefore, there is no information on the influence of APS on endothelial function in patients with SLE.

Further, vascular function depends on functional capability of smooth muscle cells (SMC) of vessel wall. It was shown that endothelium-independent (SMC related) dilation is deteriorated in patients with risk factors of atherosclerosis (10) as well as in patients with venous thrombosis (17). According to these findings, it is expected that in patients with SLE, with or without APS, also endothelium-independent functional capability of peripheral vessels is deteriorated and as such involved in the thrombogenesis.

As vascular wall dysfunction (endothelium-dependent or independent) represents an indicator of vessel wall damage, it is expected that it could be involved in the development of vascular (arterial and venous) complications in SLE and the presence of APS could additionally promote thrombus formation through endothelial dysfunction.

Therefore, the aim of our study was to investigate the endothelium-dependent and independent dilation capability of the brachial artery, and systemic inflammatory response in SLE patients with or without APS and to compare it with healthy subjects.

Patients and methods

Forty-two women with SLE and low disease activity and 22 healthy women were included in the study. All patients fulfilled the diagnostic criteria of the American College of Rheumatology (18). Most of the patients with SLE (37 out of 42) were recruited during the complete remission of the disease. Twenty-one were diagnosed as having secondary APS (mean age 36.1±9.1 years) according to the Sapporo criteria (19). Fourteen patients had moderate-to-high positive IgG aCL and 4 IgM aCL antibody titers. Lpus anticoagulants were found in blood from thirteen patients and moderate-to high levels of IgG anti-beta 2 GPI were detected in thirteen patients and IGM in nine patients. The other 21 SLE patients (mean age 43.9±13.1 years) had never been positive for anticardiolipin (aCL), lupus anticoagulant (LA) or anti beta2 glycoprotein I (anti-β2GPI) antibodies. Clinical manifestations of secondary APS in our patients were stroke in 10, venous thrombosis in 11, peripheral arterial disease and myocardial infarction in 1 patient. The mean disease duration in patients with SLE plus APS was 14.6±11.1 years and 6.8±4.5 years in SLE patients without APS (p<0.01). 17 patients with APS

were treated with oral anticoagulants, one with acetylsalicylic acid, 14 patients (11 with APS) were treated with angiotensin-converting enzyme (ACE) inhibitors and 4 APS patients with statins. In the group of patients with SLE without APS seventeen patients were treated with glucocorticosteroids, whereas twelve patients from the group of SLE and APS.

On inclusion in the study 16 secondary APS patients and 12 non-APS patients were treated regularly with methylprednisolone (p=NS). As large majority of patients was in the phase of the disease remission, only 5 patients were treated with immunosuppressive drugs: 2 with cyclosporine in the SLE group, 2 with hydrochloroquine and 1 with cyclophosphamide in the group of SLE plus APS.

The control group consisted of 22 healthy women without SLE or APS and without atherosclerotic disease or thromboembolic events (mean age 43.5±10.3 years). The distribution of risk factors is shown in Table I.

All participants underwent a full medical history and physical examination. Cardiovascular risk factors such as arterial hypertension, diabetes mellitus, smoking, obesity and hyperlipidemia were registered. All participants gave written consent for all procedures. The study was approved by the State Ethical Committee of Slovenia.

Table I. The distribution of risk factors of atherosclerosis in patients with systemic lupus erythematosus only (SLE), with SLE and antiphospholipid syndrome (SLE+APS) and controls.

Risk factor	The frequency and percentage of individual risk factors			Statistical differences between groups expressed as p-value		
	SLE (n=21)	SLE+APS (n=21)	controls (n=22)	SLE/ controls	SLE+APS/ controls	SLE+ APS/SLE
Age (years, mean ± SD)	36.2±9.1	43.9±13.1	43.5±10.3	0.027*	0.850	0.046*
Hypertension (n, %)	7 (31.8%)	13 (61.9%)	3 (14.3%)	0.179	0.004*	0.069
Hyperlipidemia (n, %)	1 (4.5%)	10 (47.6%)	2 (9.5%)	0.527	0.015*	0.002*
Diabetes mellitus (n, %)	0 (0%)	0 (0%)	1 (4.8%)	0.306	0.317	1.00
Smoking (n, %)	3 (13.6%)	2 (9.5%)	2 (9.5%)	0.678	1.00	0.678
BMI >27 (n, %)	0 (0%)	2 (9.50%)	1 (4.8%)	0.306	0.527	0.143

Abbreviations: SLE: systemic lupus erythematosus; APS: antiphospholipid syndrome; BMI: body mass index; SD: standard deviation; *- significant difference.

Biochemical analysis

Blood was sampled from all subjects in the morning after an overnight fast for the following laboratory tests: cholesterol (total and fractions), triglycerides, glucose, anti-β2GPI, vascular cell adhesion molecule-1 (VCAM-1), fibrinogen, D-dimer, plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) antigens and activities.

Concentrations of serum glucose, total cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides were determined by standard colorimetric assays (Ektachem 250 Analyzer, Eastman Kodak Company, Rochester, USA). Low density lipoprotein cholesterol (LDL) was calculated from Friedewald's formula.

Anti-β2GPI levels were measured by ELISA (enzyme-linked immunosorbent assay) (Costar, Cambridge, MA, USA). The level of anti-β2GPI in each sample was derived from a standard curve according to the defined dilutions of monoclonal antibodies according to previous report (20).

Lupus anticoagulant assay was performed using an LA test kit (Gradipore, North Ryde, Australia), which is based on a simplified dilute Russell viper venom time described by Exner *et al*. Ratios equal to or larger than 1.3 were considered positive for LA (21).

Soluble VCAM-1 were measured by ELISA (R&D Systems, Abingdon, UK). Fibrinogen was determined by a modified Clauss method using Multifibren U (Dade Behring) on an automated coagulation analyser (BCT, Dade Behring, Marburg, Germany).

Tissue plasminogen activator activity was detected with an immunoactivity assay, Chromolize® tPA and PAI-1 activity with Chromolize® PAI-1 (both Trinity Biotech Plc, Co Wicklow, Ireland).

Tissue plasminogen activator and PAI-1 antigens were detected by ELISA (Imulyse® t-PA and Imulyse® PAI-1, respectively, both Biopool, Umeå, Sweden).

D-dimer was determined by ELISA (Asserachrom® D-dimer, Diagnostica Stago, Asnières, France).

Determination of endothelium-dependent and independent dilation of brachial artery

Endothelium-dependent (FMD) and glyceryl trinitrate-induced endothelium-independent (NMD) dilation of the brachial artery were studied using a high resolution B mode ATL 5000 ultrasound system with a 7 MHz linear array transducer, as described by Celermajer (22). The subjects rested in the supine position for ten minutes before haemodynamic measurements were performed. The right brachial artery was scanned in the longitudinal section 2 to 15 cm above the elbow to find the clearest images of the anterior and posterior wall layers. The mean arterial diameter was measured at the end of diastole, which was determined by simultaneous monitoring of the electrocardiogram. At least three cardiac cycles were analysed for each scan and the measurements were averaged. The flow velocity was measured at a fixed incident angle of 60° to the vessel with the range gate of 1.5 mm located in the centre of the artery. The baseline (resting) blood flow was estimated by multiplying the velocity time integral of the Doppler flow signal (corrected for incident angle) by the vessel cross-sectional area. Hyperaemic flow increase was induced by inflation of a blood pressure tourniquet, placed around the forearm, to a pressure of 300 mmHg

for 4.5 minutes. Hyperaemic flow (with increased flow producing an endothelium-dependent stimulus for vasodilatation) was recorded for the first 15 seconds and diameter measurements were taken 45-60 seconds after cuff deflation. The endothelium-dependent dilation was expressed as the percentage change of the diameter after reactive hyperaemia relative to the baseline scan. Ten minutes were allowed for vessel recovery, after which a further resting scan was taken. Endothelium-independent dilation was provoked by sublingual administration of 400 μg of glyceryl trinitrate (GTN). The final scan was performed 4.5 minutes later. Endothelium-independent dilation was expressed as the percentage change in the diameter after GTN administration relative to the baseline scan.

To assess the reproducibility of measurements, 20 subjects were selected at random for repeated vascular studies. The correlation coefficient between the absolute differences and mean values of paired measurements was 0.92, $p<0.05$.

Statistical analysis

Data are presented as means (±SD) or medians (min-max). Student's *t*-test was performed to compare parametric variables between cases and controls. The Mann-Whitney U-test was performed for non-parametric parameters. Pearson's correlation coefficient was used to examine the relation between brachial FMD and several study variables. Levels of $p<0.05$ were considered significant. All data analyses were performed using SPSS for Windows version 13.0 (SPSS, Inc., Chicago IL, 2004).

Results

Clinical characteristics and risk factors

Clinical characteristics of SLE patients with and without APS and of the healthy controls, and the distribution of risk factors of all investigated groups are presented in Table I. Patients with SLE and without APS were younger. Hyperlipidemia was less frequently observed in SLE patients than in controls and in patients with SLE plus APS. Hypertension in both groups of patients was more frequently present than in the control subjects.

Circulating markers of inflammation and endothelial dysfunction

Table II shows levels of measured circulating markers of endothelial activation/damage and indicators of the activity of the endogenic fibrinolytic system in SLE patients with or without APS and healthy controls. Patients with SLE had significantly higher values of VCAM-1, hs-CRP and fibrinogen than controls. In patients with SLE+APS an additional significant increase of inflammatory markers in comparison to SLE patients without APS was registered. Of the fibrinolytic markers only tPA-antigen was significantly higher in SLE+APS patients than in controls and PAI-activity was reduced in both groups of patients in comparison to healthy subjects (Table II).

Endothelium-dependent and independent dilation of the brachial artery

Results for the endothelium-dependent and independent dilatation capability of brachial artery of all three investigated groups are shown in Table III and Figure 1.

In comparison to the controls, in both groups of patients (SLE, SLE+APS) the endothelium-dependent dilatation capability of the brachial artery (provoked by reactive hyperaemia) was significantly reduced and there were no differences in FMD between the

Table II. Concentrations of plasma makers of inflammation and values of haemostatic markers in patients with systemic lupus erythematosus only (SLE), systemic lupus erythematosus and secondary antiphospholipid syndrome (SLE+APS) and healthy subjects (controls) (values are expressed as medians and interquartile range).

Parameter	SLE (n=21)	P (SLE/ controls)	Controls (n=22)	P (SLE+APS/ controls)	SLE+APS (n=21)	P (SLE / SLE+APS)	P (SLE / SLE+ APS/controls)
VCAM-1 (ng/L)	683.00 529.25–795. 00	<0.001*	464.0 427.50–544.00	< 0.001*	865.00 650.00–1006.00	0.029*	<0.001*
hs CRP (mg/L)	0.60 0.32–3.93	0.528	1.10 0.43–3.51	0.003*	3.46 1.42–14.95	0.001*	0.001*
Fibrinogen (g/L)	2.98 2.48–3.70	0.193	2.61 2.33–3.23	0.003*	3.60 2.95–4.70	0.027*	0.006*
D-dimer (μg/L)	400.50 267.00–770.50	<0.001*	139.00 65.00–231.00	0.005*	284.00 135.00–602.00	0.152	<0.001*
tPA Ag	6.50 3.40–9.48	0.543	6.30 4.30–10.00	0.02*	9.00 7.50–14.15	0.005*	0.01*
tPA Activity	1.00 0.70–1.33	0.724	0.90 0.62–1.45	0.676	0.80 0.55–1.40	0.442	0.74
PAI-1 Ag	8.60 5.78–15.30	0.520	10.60 5.30–17.55	0.252	13.50 9.75–24.55	0.032*	0.119
PAI-1 Activity	6.75 3.28–12.28	0.04*	2.20 0.00–8.42	0.002*	12.90 7.20–19.80	0.052	0.003*

VCAM-1: vascular cell adhesion molecule-1; hs CRP: high sensitive C-reactive protein; tPA: Tissue plasminogen activator antigen; PAI-1: plasminogen activator inhibitor-1; Ag: antigen ; *= significant difference.

Table III. Haemodynamic characteristics of brachial arteries in patients with systemic lupus erythematosus only (SLE), systemic lupus erythematosus with accompanying antiphospholipid syndrome (SLE+APS) and healthy controls at rest, during reactive hyperaemia or application of glyceryl-trinitrate.

Parameter	SLE (n=21)	P (SLE/ controls)	Controls (n=22)	P (SLE+APS/ controls)	SLE+APS (n=21)	P (SLE / SLE+APS)	P (SLE / SLE+ APS/ controls)
D-BA at rest (mm)	3.5 (3.2–3.9)	0.20	3.7 (3.4–4.2)	0.70	3.8 (3.4–3.9)	0.29	0.37
D-BA during reactive hyperaemia (mm)	3.7 (3.5–4.2)	0.009*	4.2 (3.9–4.9)	0.11	4.0 (3.6–4.3)	0.26	0.03*
D-BA after GTN (mm)	4.3 (4.0–4.7)	0.103	4.6 (4.1–5.2)	0.113	4.3 (4.1–4.7)	0.95	0.18
FMD (%)	7.7 (1.9–12.1)	<0.001*	14.6 (11.2–21.1)	0.001*	7.8 (2.4–12.8)	0.688	<0.001*
NMD (%)	24.3 (15.0–28.6)	0.789	23.0 (17.8–30.1)	0.015*	17.4 (13.1–22.6)	0.027*	0.027*

D-BA: diameter of the brachial artery; FMD: flow mediated dilation; NMD: glyceryl trinitrate mediated dilation; GTN: glyceryl trinitrate; *= significant difference.

groups of patients in spite of longer disease duration in SLE plus APS patients group than in SLE group, FMD was not related to the duration of the disease (r=0.23, p=0.590). However, endothelium-independent (GTN induced) dilation of the brachial artery was significantly lower in SLE+APS patients than in the controls and also lower than in the SLE group (Fig. 2). The GTN-induced dilation capability of the brachial artery was significantly lower in APS patients with arterial thrombosis in comparison to patients with venous thrombosis: 15.4% (13.2%–18.4%) *vs*. 21.0% (17.4%–24.7%), p<0.05.

Correlations

In all investigated groups flow mediated endothelium-dependent dilation of the brachial artery was significantly and inversely related to circulating VCAM-1 adhesion molecules (r=-0.38, p=0.002) and endothelium-independent dilation was significantly interrelated with tPA-antigen concentration (r=-0.26, p=0.041) and with PAI-1 activity (r=-0.27, p=0.034). In both groups of patients (SLE, SLE+APS) VCAM-1 was positively related with hs-CRP (r=0.34, p=0.025) and with D-dimer (r=0.33, p=0.033). In the group of healthy subjects both FMD and NMD were inversely related to tPA-antigen: FMD r=-0.59, p=0.005, NMD r=-0.49, p=0.025 and to PAI-1 activity r=-0.64, p=0.002. In each individually investigated group and in all groups together a very close interrelationship between FMD and NMD was found.

Discussion

Endothelial dysfunction, characterised by an imbalance between anticoagulant and procoagulant mediators, pro- and anti-inflammatory markers and a decreased vasodilatory response of the peripheral vessels, most probably represents the common denominator of harmful effects of risk factors and may lead to atherosclerotic or thrombotic vessel disease. Using ultrasound, it is possible to follow endothelium-dependent as well as independent functional capability of peripheral arteries *in vivo*. It was shown that particularly flow-mediated dilatory response is closely related to endothelial activation/damage (10).

Recent studies showed that endothelial function is significantly impaired in patients with SLE (11-13). In patients with SLE various factors can cause endothelial damage and dysfunction, including immune complexes, chronic inflammation and hypertension, which are frequently present in these patients (23, 24). The contribution of secondary APS to endothelial dysfunction in patients with SLE has not been elucidated. Therefore we investigated several parameters of endothelial dysfunction in SLE patients with or without APS and in healthy controls.

The principal finding of this study provides evidence of endothelial functional deterioration in SLE patients both with and without APS. One of the most frequently a used and standardised technique for endothelial function is monitoring the vasodilatory response of peripheral arteries to increased blood flow during reactive hyperaemia (10). In previous studies an impaired vasodilatory response was found in SLE patients compared to healthy subjects (11, 12). However, in these studies the influence of the presence of APS on FMD was not investigated. In our study FMD was significantly reduced in both groups of patients in comparison to healthy subjects and there were no differences between patients with or without APS. Our results are in agreement with other studies which found no differences in endothelium-dependent FMD between APS positive and APS negative SLE patients (11, 12). This could mean that the presence of SLE itself affects the maximal endothelium-dependent vasodilatory response and that the presence of APS has no additional deteriorating effects on endothelial function. Furthermore, in our study more patients with APS were treated with angiotensin-converting enzyme inhibitors than patients without APS. These drugs improve FMD and might have masked the possible difference in endothelial function between the groups. Further anti-inflammatory therapy utilised in SLE patients may also have influenced nitrogen oxide bioavailability. Further, the duration of the dis-

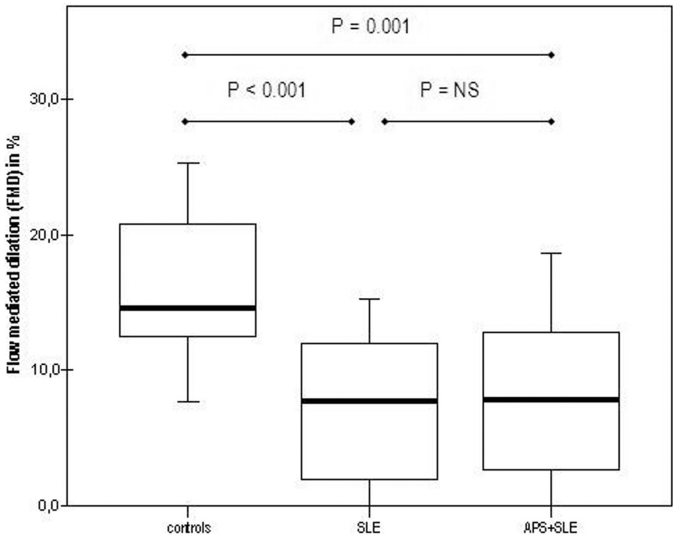

Fig 1. Endothelium-dependent flow-mediated dilation of the brachial artery (FMD) in systemic lupus erythematosus patients with (SLE + APS) and without antiphospholipid syndrome (SLE) and in healthy controls.

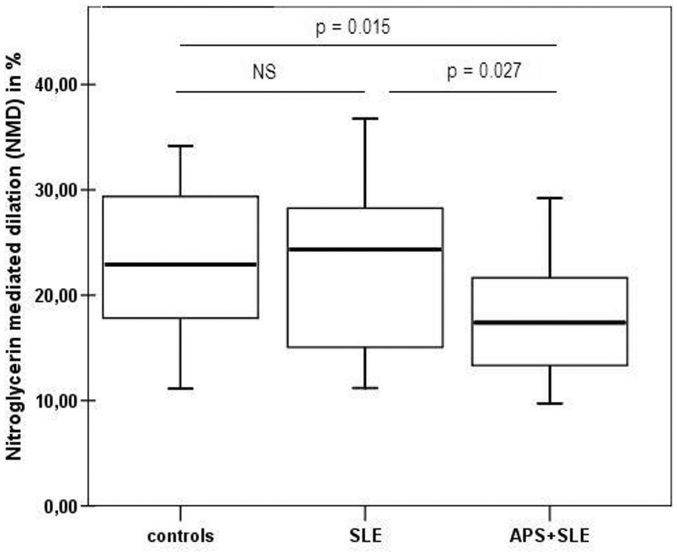

Fig 2. Endothelium-independent (GTN-induced), vasodilatory response (NMD) in patients with systemic lupus erythematosus (SLE) with and without antiphospholipid syndrome (APS) and controls.

ease may influence FMD. In our study the duration of the disease was longer in SLE plus APS patients group than in the SLE group. However, we couldn't demonstrate any effect of duration of the disease on the FMD. Therefore, the determination of endothelium-dependent FMD is possibly not the most suitable method for detection of subtle differences in endothelial dysfunction between the subgroup of SLE patients with or without APS. However, most findings confirm that all SLE patients have deteriorated endothelium-dependent FMD which could influence thromboembolic complications and cardiovascular events in these patients and is probably responsible for accelerated atherosclerosis in SLE patients (25). In our study values of FMD in controls as well as in patients were higher in comparison to some previous findings (26, 27). In spite of numerous studies and consensus documents on FMD evaluation there are no data on standards of normal values of FMD. In an overview of the FMD determination, Corretti *et al*. reported that FMD greater than 11% is generally accepted as a normal range for this test (28). The result of measurement of FMD depends on different factors like probe position, cuff occlusion time and environmental factors such as room temperature, ingestion of food of caffeine and women in the phase of menstrual cycle (29). Furthermore, it known that a larger baseline diameter of the artery yields a smaller percentage of an increase of the diameter during reactive hyperaemia and smaller arteries appear to dilate more than larger ones (22). Our study included only females and it is known that the diameter of peripheral arteries is significantly lower than in females, therefore a higher increase in the diameter of the investigated brachial arteries in our study in comparison to the study of Cugno *et al*. in which males were also included, could be the reason for the different findings of FMD in these two studies.

Our study showed for the first time, a reduced GTN-induced vasodilatory response in SLE patients with APS in comparison to healthy controls and SLE patients without APS. This indicates that patients with APS have a deteriorated functional capability of the smooth muscle cells and probably vascular fibrosis and remodelling of the peripheral arteries which all influence dilatory capability. In contrast to our findings Cugno *et al*. did not find any difference in the endothelium-independent response (GTN-induced dilation of brachial artery) between APS patients and controls (26). It could be caused by different patient characteristics in these two studies. In our study all patients had SLE and in half of them also APS was present. But in the study of Cugno most of the patients (31/40) had primary APS and a few (9) had SLE. Furthermore, the duration of the disease that could influence endothelium-independent vascular reactivity was much longer in our study (patients with SLE 6.8 years and in SLE plus APS 14.6 years) than in before mentioned study (3.5 years). In our study it was also shown that GTN induced dilation capability of the brachial artery progresses with the duration of APS. Further, it was shown that endothelium-independent GTN-induced vasodilatation was significantly more decreased in APS patients with arterial events than in those with venous thrombosis. These findings imply that the presence of APS in patients with SLE affects the smooth muscle cells much more than the endothelial cell function. The relation between reduced GTN-induced vasodilatation and arterial thrombosis indicates that dysfunction of the arterial wall may promote thrombus for-

mation and vascular complications in APS-positive SLE patients. These findings may also help to explain the higher prevalence of atherosclerotic disease in APS patients. Our positive APS patients had a higher prevalence of classical risk factors (hypertension, dyslipidaemia), which additionally increase the risk for cardiovascular events through damage of the vessel wall and inflammation. All these factors may cause deterioration of the vessel wall function (reduced FMD and/or NMD) and finally morphological damage with atherosclerotic plaque or thrombus formation.

In patients with SLE higher levels of systemic markers of endothelial activation/damage, of inflammation and of deterioration of the endogenic fibrinolytic system were found than in the healthy subjects. Patients with SLE plus APS had much higher levels of the investigated markers than SLE patients without APS. Plasma levels of soluble adhesion molecules including VCAM-1 have been reported to be increased in different disorders characterised by the presence of endothelial dysfunction (30, 31). Soluble forms of adhesion molecules enzymatically cleaved from the endothelial cells surface have been detected in sera of patients with SLE and in some studies their levels correlate with disease activity (32-36). The increased levels of VCAM-1 molecules in our study are in agreement with these findings. However, influence of the presence of APS on VCAM-1 levels was not confirmed in other studies. Frijns *et al*. found no significant difference between APS-negative and positive SLE patients with a history of thrombosis regarding VCAM-1 (37). In the present study, VCAM-1 levels correlated with disease duration. As our APS patients had longer disease durations and were older than the SLE patients without APS, this might influence the high VCAM-1 levels in the APS patients and could be the reason for the different results in these two studies. The higher frequency of multiple thrombotic events in our study group may also influence the concentration of VCAM-1 molecules, as it was also shown in the study of Kaplanski *et al*. (16). Therefore, the results of our study confirm that patients with SLE have increased levels of VCAM-1 and indicate that APS contributes to an additional increase in the level of adhesion molecules in SLE patients. This is in agreement with the findings of Zaccagni *et al*. (38). As in our study, plasma levels of VCAM-1 strongly correlated with FMD, which indicates that VCAM-1 level might be the useful parameter in monitoring endothelial cell function and activation in SLE patients with low disease activity.

Inflammation most probably represents one of the basic mechanisms of progression and thromboembolic complication in SLE patients. It is also an indicator of the activity of the disease. Therefore, increased levels of inflammatory markers (fibrinogen, hs-CRP) in our SLE patients are in agreement with expectations. Higher levels of inflammatory markers in SLE patients with APS favour the hypothesis that the presence of antiphospholipid antibodies aggravates the inflammatory response and consequently increases the risk of thromboembolic events in these patients. The data indicate that inflammation might initiate thrombus formation and that inflammation and the coagulation system are coupled with a common activation pathway. During inflammation, the haemostatic balance may be disturbed resulting in an increased production of procoagulant factors and in the down-regulation of anticoagulant mechanisms (39). Inflammation probably also triggers a prothrombotic state through deterioration of endothelial function.

Endothelial function also includes control over coagulation and thrombolysis. In the case of endothelial dysfunction, activated endothelial cells overproduce tPA and PAI-1 resulting in an imbalance of the fibrinolytic system (40). In patients with SLE, deterioration of the fibrinolytic system with a decrease of tPA activity and elevated levels of PAI-1 were reported and were associated with an increased tendency to thrombosis (41). Impaired fibrinolysis has also been reported in patients with APS (42), but the results are controversial (43). In our study, tPA antigen and PAI-1 activity levels were higher in SLE patients with APS than in SLE patients without APS. However, SLE patients without APS also had higher levels of tPA antigen and PAI-1 activity in comparison to healthy controls. These findings support the hypothesis that among patients with SLE the endothelium is more activated than in healthy subjects and that APS additionally affects endothelial activation/damage. The results of our study are in agreement with those of Violo *et al*. who found markedly increased levels of tPA and PAI-1 levels in SLE patients without thrombosis (44). In our study, most APS patients (60%) had venous thrombosis but no correlation between venous thrombosis and tPA or PAI-1 levels was found.

In conclusion, the results of our study show that in patients with SLE some inflammatory and circulating markers of endothelial damage are increased. In SLE patients with APS, an additional increase in the investigated markers is present which indicates advanced damage to endothelial cells. In comparison to healthy subjects, patients with SLE also had deteriorated endothelium-dependent flow-mediated dilation capability of the brachial artery which represents the most valuable indicator of endothelial dysfunction, but the presence of APS does not cause additional reduction of the endothelium-dependent dilatory response. However, the presence of APS is related to a significant decrease of endothelium-independent smooth muscle cell related dilatory response of the peripheral arteries.

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