Indications of vascular endothelial cell dysfunction in systemic lupus erythematosus

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SUMMARY Fibrinolytic and other factors have been measured in 73 patients with systemic lupus erythematosus or related conditions to determine whether clinical thrombosis, a common feature of these disorders, is associated with defective fibrinolysis. Twenty five of 72 (35%) patients, compared with two of 22 (9%) controls, showed a low level of plasminogen activator activity in response to venous occlusion, suggesting decreased fibrinolytic potential. In addition, mean plasma levels of von Willebrand factor antigen and fibronectin were markedly raised in the patients (mean (SD) 384.5 (277)% and 727 (436) mg/l respectively) compared with healthy controls (100 (50)% and 306 (65) mg/l). These data suggest a degree of endothelial cell dysfunction. No clear correlation was found between a history of thrombosis and any plasma factor measured, except for prolongation of clotting tests suggestive of the 'lupus anticoagulant'.

Key words: fibrinolysis, von Willebrand factor antigen, fibronectin.

Thrombosis is a well recognised feature of systemic lupus erythematosus (SLE). Both clinically detected arterial or venous thrombosis and low grade coagulation activation manifest by features such as glomerular thrombi and fibrin deposition in lupus nephritis have been described.^{1,2} One factor already identified in association with thrombosis is the presence of the lupus anticoagulant, an antiphospholipid antibody which interferes with *in vitro* coagulation tests.^{1,3} The mechanism whereby this antibody predisposes to thrombosis is unknown, though it may block conversion of arachidonic acid to prostacyclin (PGI₂), a potent inhibitor of platelet aggregation.⁴

Under normal physiological conditions activation of coagulation automatically stimulates the release of plasminogen activator (PA) from the vascular endothelium.⁵ Plasminogen activator is the major regulator of fibrinolysis and prevents unwanted fibrin deposition. The potential of the fibrinolytic system can be assessed by inducing venous occlusion in an arm and assaying plasminogen activator

activity, which normally increases six- to eightfold over resting levels. 'Poor responders' to venous occlusion may be identified, who frequently show a tendency towards fibrin deposition.⁶ Such a defect has been implicated in recurrent venous thrombosis, postphlebotic syndrome, coronary artery disease, and diabetic microangiopathy,⁷⁻⁹ and in some reports is associated with release of excessive amounts of PA inhibitor, which is also secreted by the endothelium.¹⁰ This present study was designed to assess the fibrinolytic system in patients with SLE.

Patients and methods

Seventy three patients with a clinical diagnosis of SLE or mixed connective tissue disease were identified from laboratory results and hospital records in several departments. Sixty eight patients (63 women, five men) had a diagnosis of SLE based on multisystem disease with serological features. Of these, 62 (91%) satisfied the revised criteria for the diagnosis of SLE, the other six fulfilling three criteria.¹¹ The other five patients (three women, two men) had a diagnosis of mixed connective tissue disease based on clinical features of an overlap syndrome, with the presence of antibodies to

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ribonucleoprotein. The mean (SD) age of the patients studied was 42.7 (14.5) years, with a mean (SD) disease duration of 9.2 (7.3) years. Each patient was examined and their notes reviewed.

Eleven patients were not receiving any therapy at the time of the study, 41 were taking corticosteroids, and 20 were taking cytotoxic drugs. Nineteen patients were receiving non-steroidal anti-inflammatory drugs, usually naproxen, and two were taking low dose aspirin (300 mg twice weekly). A variety of other drugs were used by some patients, most frequently diuretics (13 patients) and β blockers (eight patients).

In addition to assays of fibrinolytic proteins, haematological, biochemical, and serological tests were performed. Healthy subjects drawn from laboratory and secretarial staff were examined to obtain control data.

Plasminogen activator activity was assayed in venous blood samples taken after 20 minutes of supine rest and after 10 minutes of venous occlusion at 80 mmHg.¹² Blood was anticoagulated with one tenth volume 0.1 M trisodium citrate, then cooled on ice and centrifuged at 2000 g (4°C) for 10 minutes. Euglobulin fractions were obtained by addition of 9.5 ml of cold acetic acid (0.014%) to 0.5 ml plasma. After 15 minutes on ice the samples were spun at 2000 g for 10 minutes, the supernatants discarded, and the precipitates redissolved in 0.5 ml of 50 mM NaCl/27.6 mM sodium barbitone/HCl buffer, pH 7.4. Plasminogen activator activity was assayed by applying 50 μ l euglobulin fractions (in duplicate) to fibrin plates within 45 minutes of drawing the blood. Fibrin plates (9 cm diameter) were prepared using bovine fibrinogen (10 ml, 2

Table 2 Plasma levels of endothelial cell derived proteins in patients with SLE and in controls

	SLE mean (SD) (n)	Healthy controls mean (SD) (n)
Fibronectin (mg/l)	727 (436) (45)	306 (65) (32)
von Willebrand factor antigen (%)	384.5 (277) (71)	100 (50)* (32)
Plasminogen activator (CTA units/ml)		
baseline	0.17 (0.16)	0.15 (0.11)
after occlusion	1.1 (1.2) (72)†	0.95 (0.88) (22)

* $p < 0.001$ (Student's *t* test).

†One patient not tested.

mg/ml) in 0.15 M NaCl and clotted with 50 μ l of 50 units/ml thrombin. The plates were incubated at 37°C for 17 hours and the lysis areas converted to Committee for Thrombolytic Activity (CTA) units by reference to a urokinase dilution curve.¹³

Plasma samples obtained before venous occlusion were also stored at -20°C for the assay of fibrinogen, plasminogen, α_2 macroglobulin, and α_1 antiplasmin by radial immunodiffusion,¹⁴ α_1 and trypsin by nephelometry using a Beckman LGS analytical system, antithrombin III by chromogenic assay,¹⁵ fibronectin by radioimmunoassay (Allington and Govier, in preparation), and von Willebrand factor antigen by rocket immunoelectrophoresis.¹⁶ All antibodies used were prepared by the research group with the exception of anti-factor VIII antibody (Hoechst Pharmaceuticals, Hounslow, London, UK).

The kaolin clotting time (KCT) was used as a screening test for coagulation abnormalities. This was carried out by incubating plasma (100 μ l) with kaolin (100 μ l, 5 mg/ml) for three minutes at 37°C after which 0.15 M NaCl (100 μ l) and 0.025 M CaCl_2 (100 μ l) were added and the clotting time measured. Mixing experiments were not performed.

Statistical analysis was performed using Student's *t* test and χ^2 test with Yates's correction.

Results

LABORATORY RESULTS

Table 1 shows the mean plasma values for fibrinogen, plasminogen, α_2 macroglobulin, α_2 antiplasmin, antithrombin III, and α_1 antitrypsin in patients with SLE compared with healthy controls. The patients showed significantly higher values ($p < 0.001$) for all of these factors except α_2 antiplasmin. Plasma values of proteins secreted by the endothelium are shown in Table 2. Plasma fib

Table 1 Plasma proteins related to fibrinolysis in patients with SLE† and in healthy controls

Plasma proteins	SLE mean (SD) (n)	Healthy controls mean (SD) (n)
Fibrinogen (g/l)	3.7 (1.0) (66)	3.1 (0.5)* (32)
Plasminogen (g/l)	0.207 (0.066) (67)	0.148 (0.034)* (32)
α_2 Macroglobulin (g/l)	2.939 (1.014) (59)	2.23 (0.52)* (32)
α_2 Antiplasmin (g/l)	0.666 (0.226) (59)	0.70 (0.14) (32)
Antithrombin III (g/l)	0.349 (0.077) (68)	0.285 (0.046)* (32)
α_1 Antitrypsin (g/l)	2.117 (0.548) (39)	1.63 (0.26)* (30)

* $p < 0.001$ (Student's *t* test).

†Not all the patients underwent all the tests.

ronectin and von Willebrand factor antigen were both greatly raised in the patients ($p<0.001$), but the mean levels of plasminogen activator activity both before and after venous occlusion were not significantly different in patients and controls. Fig. 1 shows the frequency distribution of the response to venous occlusion. Poor response was here defined as failure to demonstrate PA activity >0.3 CTA U/ml in response to venous occlusion.⁹ Twenty five of 72 (35%) patients were poor responders, compared with two of 22 (9%) controls ($\chi^2=4.2$, $p<0.05$).

When the results for plasma factors in SLE responders were compared with those for poor responders (Table 3) no significant differences were found between mean values, though the poor responders showed a tendency towards greater

abnormality of the proteins secreted by the endothelium. Similar comparisons of plasma factors between patients with or without a history of thrombosis, or patients with or without prolonged coagulation, showed no differences.

The mean value (SD) of the KCT for controls was 62 (10) seconds and values of 83 seconds or longer were considered abnormal. Twenty five of 60 (42%) patients had a prolonged KCT. One additional patient was receiving warfarin therapy for thrombotic problems and a previously diagnosed lupus anticoagulant.

CLINICAL CORRELATIONS

Twenty four patients (33%) had a past history of venous or arterial thrombosis. Thirty eight per cent

Fig. 1 Response to venous occlusion in patients and controls. Responders show plasminogen activator (PA) activity >0.3 CTA U/ml after venous occlusion. Twenty five of 72 patients with SLE are poor responders, compared with two of 22 controls ($\chi^2=4.2$, $p<0.05$). (One patient could not be classified as responder or poor responder as the venous occlusion test was not carried out on her.)

Table 3 Results for patients with SLE: responders and poor responders to venous occlusion*

	Responders (47) mean (SD) (n)	Poor responders (25) mean (SD) (n)
Fibrinogen (g/l)	3.7 (1.1) (42)	3.78 (0.73) (23)
Plasminogen (g/l)	0.206 (0.063) (43)	0.207 (0.073) (24)
α_2 Macroglobulin (g/l)	2.88 (1.21) (37)	2.95 (0.82) (22)
α_2 Antiplasmin (g/l)	0.678 (0.260) (37)	0.645 (0.153) (22)
Antithrombin III (g/l)	0.349 (0.066) (44)	0.348 (0.095) (24)
α_1 Antitrypsin (g/l)	2.239 (0.544) (23)	1.943 (0.522) (16)
Fibronectin (g/l)	0.644 (0.278) (30)	0.892 (0.627) (15)
von Willebrand factor antigen (%)	365 (247) (46)	420 (328) (25)

*One patient could not be classified as responder or poor responder as the venous occlusion test was not carried out on her.

of poor responders to venous occlusion had a history of thrombosis compared with 32% of responders, whereas 33% of patients with a prolonged KCT had a history of thrombosis compared with 15% of those without ($p < 0.02$, χ^2 test). There was no correlation between response to venous occlusion and prolongation of KCT, age, disease duration, or drug therapy.

Discussion

The pathogenesis of thrombosis in SLE is multifactorial.¹ Vascular endothelium contributes to prevention of fibrin deposition and thrombosis by secretion of plasminogen activator, the major regulator of fibrinolysis and its specific inhibitor. In this study the mean plasma levels of the fibrinolytic proteins were generally raised in the patient group (Table 1), though remaining within the physiological ranges for these proteins. These modestly raised levels probably reflect an acute phase response. For plasminogen and antithrombin III, a deficiency rather than an excess is associated with thrombosis. The finding of normal plasma levels of the fibrinolytic inhibitor α_2 antiplasmin is in contrast with the study of Glas-Greenwalt *et al*, where high levels were found in patients with SLE.¹⁷

In a study of 28 patients with SLE Angles-Cano and coworkers found that fibrinolytic potential, as assessed by euglobulin lysis times before and after venous occlusion, was reduced in 16 (57%) patients.¹⁸ In our study plasminogen activator activ-

ity in response to venous occlusion was assessed more directly by applying euglobulin precipitates to bovine fibrin plates. No differences were found between mean plasma values for plasminogen activator activity before or after venous occlusion for either patients with SLE or controls. Analysis of the frequency of response to this stress test, however, allowed division of the patients into two groups—those with a poor response to venous occlusion (post PA activity < 0.3 CTA units/ml) and those with a good response. Thirty five per cent of the patients and 9% of the controls were poor responders. Poor response to venous occlusion has been found in patient groups where thrombosis or fibrin deposition are well recognised complications,^{7,8} and in some patients this finding is related to increased levels of plasminogen activator inhibitor rather than to diminished plasminogen activator release from the endothelium.¹⁰ In our patients poor response to venous occlusion did not correlate with a history of thrombosis, age, disease duration, or drug therapy, but this group did tend to show the more severe endothelial protein abnormalities (Table 3). No correlation was found between poor response to venous occlusion and prolonged coagulation.

Other proteins secreted by the endothelium were also found in abnormally high levels in our patient group (Table 2). Like Angles-Cano *et al*,¹⁸ we found raised levels of von Willebrand factor antigen. Although it is not clear whether these high levels have a procoagulant effect, raised levels are found in other conditions where angiopathy is seen.¹⁹ Similarly, plasma fibronectin levels were raised. Fibronectin, a glycoprotein present in plasma and over cell surfaces, is secreted by endothelium and rises in response to venous occlusion in diabetics.^{9,20} High levels may reflect endothelial damage as fibronectin is produced by blood vessels in response to injury,²¹ and may contribute to thrombosis by affecting adhesion of blood cells to the endothelial surface. Levels of von Willebrand factor antigen and fibronectin did not correlate with a past history of thrombosis, or prolonged coagulation in our patients.

An association between clinical thrombosis and the presence of the circulating lupus anticoagulant has been described by a number of researchers.¹ The lupus anticoagulant is an antibody directed against phospholipid determinants,²³ and interferes with in vitro coagulation tests. When the thromboplastin (the platelet phospholipid substitute) is removed from the clotting test the sensitivity of detection of the anticoagulant increases, though its presence should be confirmed by mixing experiments.²⁴ In this study, using the KCT as screening test, 25 patients showed prolonged

coagulation. It is likely that this abnormality was due to the lupus anticoagulant in most patients as other causes of prolonged clotting are much more uncommon.¹ It has been suggested that this antibody binds to the phospholipid of endothelial cell walls and disturbs arachidonic acid metabolism to prostacyclin (PGI₂), the potent inhibitor of platelet aggregation.⁴ Prolonged coagulation correlated with a past history of thrombosis in our patients but was unrelated to fibrinolytic potential, plasma von Willebrand factor antigen, and fibronectin, other indicators of endothelial function.

In conclusion, raised plasma levels of the fibrinolytic proteins are found in patients with SLE, but these are of little clinical significance. Evidence of endothelial dysfunction is suggested by markedly raised levels of von Willebrand factor antigen and fibronectin. A poor response in plasminogen activator activity to venous occlusion did not identify patients with clinical thrombotic problems. A past history of thrombosis in these patients did not correlate with any plasma factor measured, except prolongation of coagulation, suggesting the presence of the lupus anticoagulant. These findings do suggest endothelial cell dysfunction, which may contribute to microvascular disease in these patients.

The cause of this endothelial cell defect is at present unknown but it is possible that autoantibodies similar to the lupus anticoagulant may react with endothelial membrane determinants causing dysfunction that promotes low grade coagulopathy and fibrin deposition.

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