The contribution of inherited and acquired thrombophilic defects, alone or combined with antiphospholipid antibodies, to venous and arterial thromboembolism in patients with systemic lupus erythematosus

Jan-Leendert P. Brouwer, Marc Bijl, Nic J. G. M. Veeger, Hanneke C. Kluin-Nelemans, and Jan van der Meer

Systemic lupus erythematosus (SLE) is associated with an increased risk of venous (VTE) and arterial thromboembolism (ATE). Lupus anticoagulant (LA) and anticardiolipin antibodies (ACAs) are established risk factors. We assessed the contribution of deficiencies of antithrombin, protein C, total protein S, factor V Leiden, the prothrombin G20210A mutation and APC resistance, either alone or in various combinations with LA and/or ACAs, to the thrombotic risk in a cohort of 144 consecutive patients with SLE. Median follow-up was 12.7 years. VTE had occurred in 10% and ATE in 11% of patients. LA, ACAs, factor V Leiden, and the prothrombin mutation were identified as risk factors for VTE. Annual incidences of VTE were 2.01 (0.74-4.37) in patients with one of these disorders and 3.05 (0.63-8.93) in patients with 2 disorders. The risk of VTE was 20- and 30-fold higher, respectively, compared with the normal population. In contrast with LA and ACAs, thrombophilic disorders did not influence the risk of ATE. In conclusion, factor V Leiden and the prothrombin mutation contribute to the risk of VTE in patients with SLE, and potentiate this risk when one of these thrombophilic defects are combined with LA and/or ACAs. (Blood. 2004; 104:143-148)

Introduction

Systemic lupus erythematosus (SLE) is associated with an increased risk of venous and arterial thromboembolism.[1,2] Antiphospholipid antibodies (APAs), like lupus anticoagulant (LA) and anticardiolipin antibodies (ACAs), have been recognized as important risk factors. LA was reported in 15% to 34% of patients with SLE and was associated with an approximately 6-fold increased risk of venous thromboembolism (VTE). ACAs, demonstrated in 12% to 30% of patients with SLE, increased this risk 2.5-fold.[3-5] Data about the relative risk of arterial thromboembolism (ATE) in patients with SLE with LA and/or ACAs are not available.

There is growing evidence that VTE is often due to the interaction of genetic defects and environmental factors that interfere with the normal hemostatic mechanism.[6] Genetic thrombophilic defects include deficiencies of antithrombin, protein C and protein S, factor V Leiden, and the prothrombin G20210A mutation. Oral contraception, pregnancy, surgery, trauma, and immobilization are known environmental factors. Although it is likely that thrombophilic defects increase the risk of VTE in patients with SLE with APA, only little is known about possible interactions.

We performed a study to assess the contribution of thrombophilic defects, either alone or in various combinations with APA, to the risk of thrombosis in patients with SLE.

Materials and methods

Study population

All 144 living adult patients with SLE who were registered in our hospital over the last 10 years were enrolled in the study between July 1999 and November 2000. They fulfilled at least 4 criteria for the classification of SLE, as defined by the American College of Rheumatology.[7] After SLE was diagnosed, all remained under control in our hospital. Five patients had died prior to enrollment. None of them had a history of venous or arterial thromboembolism. Two patients died from pneumonia, the others from emphysema, heart failure due to aortic valve stenosis, and cerebral bleeding. Actual disease activity and accumulated damage since the onset of SLE were estimated using the SLEDAI (SLE Disease Activity Index) and the SLICC/ACR-DI (Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index).[8,9] A questionnaire was used to collect detailed information about previous episodes of venous and arterial thromboembolism, and exposure to risk factors for venous thromboembolism. In women, the use of oral contraceptives and their obstetric history were documented.

After clinical data had been obtained, a blood sample was taken for extensive laboratory testing. In patients on prolonged oral anticoagulant treatment, the blood sample was collected after interruption of this treatment for at least 2 weeks; meanwhile, nadroparine was given subcutaneously. In this way, all laboratory tests could be done, including coagulation tests that are affected by oral anticoagulants, but not by (low-molecular-weight) heparins. Venous and arterial thromboembolism were considered to be established if objectively verified by venography, ultrasonography, or impedance plethysmography (deep vein thrombosis); ventilation-perfusion lung scanning or pulmonary angiography (pulmonary embolism); funduscopy or fluorescence angiography (retinal vein thrombosis); electrocardiography, elevated plasma levels of myocardial enzymes, and/or coronary angiography (myocardial infarction); computed tomographic scanning or magnetic resonance imaging (ischemic stroke, transient ischemic attack [TIA]). Venous thromboembolism was defined as spontaneous if it had not occurred during or less than 3 months after exposure to one or more exogenous risk factors, including surgery, trauma, immobilization

From the Division of Haemostasis, Thrombosis and Rheology, Department of Hematology; the Department of Immunology; and the Trial Coordination Centre, University Hospital Groningen, the Netherlands.

Submitted December 1, 2003; accepted February 28, 2004. Prepublished online as *Blood* First Edition Paper, March 16, 2004; DOI 10.1182/blood-2003-11-4085.

Reprints: Jan-Leendert Pouwel Brouwer, Division of Haemostasis, Thrombosis and Rheology, Department of Hematology, University Hospital Groningen (AZG), Hanzeplein 1, 9713 GZ Groningen, the Netherlands; e-mail: j.l.p.brouwer@int.azg.nl.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

for more than 7 days, pregnancy, use of oral contraceptives or hormonal replacement therapy, and malignancy. It was considered secondary in the presence of any of these risk factors. The study was approved by the institutional review board of our hospital and all participants gave their informed consent.

Laboratory studies

Blood samples for coagulation studies were collected by venipuncture on one-tenth volume of 0.109 M trisodium citrate. Platelet-poor plasma was prepared by centrifugation at 200*g* for 10 minutes and for tests on LA followed by centrifugation at 10 000*g* for 5 minutes. Plasma samples were stored at −80°C until testing.

Screening for LA was performed by 3 different phospholipid-dependent coagulation tests: DRVVT (dilute Russell viper venom time), APTT (activated partial thromboplastin time) and TTI (tissue thromboplastin inhibition).[10] If a test was abnormal, all tests were repeated on a 1:1 mixture of patient's and normal plasma to exclude deficiencies of coagulation factors. If the test remained abnormal, phospholipid dependency was demonstrated by a phospholipid neutralization test; phospholipids added to the patient's plasma bind to antiphospholipid antibodies (ie, LA), thereby normalizing the test result. DRVVT was performed using reagents (LA-screen and LA-confirm) from Gradipore (North Ryde, Australia). LA-screen contains venom, phospholipids, and calcium, required to induce clot formation. LA-confirm has a high content of phospholipids and is used to neutralize antiphospholipid antibodies as the cause of an abnormal LA-screen test. APTT was performed using actin FSL (Dade Behring, Marburg, Germany) as screening and by addition of lysed platelets (ie, phospholipids) for confirmation.TTI was performed using Thromboplastin IS (Dade Behring) in 2 dilutions (1:50 and 1:500).

ACAs were measured by enzyme-linked immunosorbent assay (ELISA) in samples diluted 1:100 in phosphate-buffered saline (PBS)/10% fetal calf serum (FCS). Nine calibrators for immunoglobulin G (IgG) and IgM anticardiolipin antibodies (Louisville APL Diagnostics, Louisville, KY) were used in duplicate to prepare a calibration curve according to the manufacturer's instructions. Results were expressed in IgG phospholipid (GPL) and IgM phospholipid (MPL) units as derived from the calibration curve. Levels more than or equal to 40 GPL or MPL were considered positive. Samples with levels more than or equal to 100 U were retested after dilution in plasma.

Factor V Leiden and the prothrombin G20210A mutation were demonstrated by polymerase chain reaction, as described previously.[11,12] Protein C and protein S antigen levels were measured by ELISA (with reagents obtained from Dako, Glostrup, Denmark), activity of protein C (Berichrom Protein C; Behring), antithrombin (Coatest; Chromogenix AB, Mölndal, Sweden), and plasminogen (S2251; Chromogenix AB) by chromogenic substrate assays. Free protein S antigen was measured after precipitation of protein S complexed with C4b-binding protein with 3.75% polyethylene glycol 6000.[13] Sensitivity to APC, expressed as normalized APC sensitivity ratio (nAPC-SR) was measured by the Coatest APC resistance kit (Chromogenix AB).

Antithrombin, protein C, total protein S, free protein S, and plasminogen plasma levels were expressed as percentage of the levels measured in pooled normal plasma set at 100%. Normal ranges (mean ± 2 SD) were determined in healthy volunteers who had no (family) history of venous thromboembolism and were neither pregnant nor had used oral contraceptives during the last 3 months. Normal ranges were adjusted for age and gender.

Statistical analysis

Continuous variables were expressed as median values and ranges and categorical variables as counts and percentages. Differences between groups were evaluated by the Student *t* test or Mann-Whitney *U* test, depending on the normality of data for continuous data and by Fisher exact test for categorical data. Freedom from either venous or arterial thromboembolism was estimated using the Kaplan-Meier method. The effects of deficiencies of antithrombin, protein C, total protein S, free protein S or plasminogen, factor V Leiden, the prothrombin G20210A mutation, LA, or ACAs on the adverse outcome were evaluated by calculating the incidence rates of either venous or arterial thromboembolism for each of these variables. In these calculations, the observation time was defined as the period from onset of SLE until the end of the observation period, or the first episode of VTE or ATE. In the calculation of specific incidence rates, the concomitance of other disorders was not considered. Calculating the incidence rates of VTE, the occurrence of ATE was ignored, and vice versa. Therefore, the person-time used in the denominator was different for each calculation. The 95% confidence intervals (95% CIs) around the incidence rates were calculated under the Poisson distribution assumption. To evaluate the effects of clustering of abnormalities, relative risks of VTE and ATE were estimated for single and multiple risk factors, as compared with the absence of these risk factors. In this analysis, a Cox proportional hazards regression model was used, adjusting for relevant covariates (clinically relevant and significant at a level of $P < .15$ from univariate analysis). A 2-tailed P value of less than .05 was considered to indicate statistical significance. Statistical analyses were performed using SAS software, version 6.12 (SAS Institute, Cary, NC).

Results

Clinical data

Clinical characteristics of 144 patients who were enrolled are summarized in Table 1. Ninety percent were women. There were no differences between women and men. Median age at study entry was 41 years (range, 19-76 years) and at onset of SLE 26 years (range, 8-70 years). Median follow-up was 12.7 years (range, 0.9-40.6 years). VTE had occurred in 15 patients (10%) and ATE in 16 patients (11%). Only 2 patients (1.4%) had experienced an episode of VTE and an episode of ATE. Median age at time of the first episode was 32 years (range, 17-61 years) for VTE and 41 years (range, 22-74 years) for ATE. A history of fetal loss was reported in 18% of women. Twelve patients (8%) were on oral anticoagulant treatment at the time of enrollment for the following reasons: recurrent VTE (3 patients), recurrent ATE (3 patients), an arterial event succeeding a prior episode of VTE (2 patients), ischemic stroke (2 patients), atrial fibrillation (1 patient), and peripheral arterial occlusive disease (1 patient). Of 15 venous

Table 1. Clinical characteristics of 144 patients with SLE

	Total
No. of patients	144
Women	130 (90)
Age at study entry, y	41 (19-76)
Age at diagnosis of SLE, y	30 (9-73)
VTE	15 (10)
ATE	16 (11)
Fetal loss (% women)	24 (18)
Both VTE and ATE	2 (1)
Both VTE and fetal loss (% women)	1 (1)
Both ATE and fetal loss (% women)	5 (4)
Actual platelet number, $\times 10^9$/L	232 (44-576)
Thrombocytopenia, $< 100 \times 10^9$/L	2 (1)
Actual drug therapy	
Oral contraceptives (% women)	23 (18)
Immunosuppressive drugs	78 (54)
Oral anticoagulants	12 (8)
SLEDAI score	2 (0-25)
SLICC/ACR-DI score	0 (0-5)

Continuous variables denoted as median (range), categorical variables as number (%).

VTE indicates venous thromboembolism; ATE, arterial thromboembolism; SLEDAI, SLE Disease Activity Index; SLICC/ACR-DI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

thromboembolic events, 11 (72%) were deep vein thrombosis and/or pulmonary embolism and 8 (53%) were classified as spontaneous (Table 2). Of 16 arterial thromboembolic events, 13 (81%) were myocardial infarction or cerebral ischemic event. Venous recurrences were reported in 5 patients (33%) and arterial recurrences in 4 patients (25%). In another 2 patients, an arterial event succeeded a prior episode of VTE.

Thromboembolic event-free survival curves showed that half of VTE episodes occurred within the first 2.5 years and half of ATE episodes within the first 8.5 years after onset of SLE (Figure 1).

Laboratory data

Laboratory findings are presented in Table 3. LA was demonstrated in 8% of patients, ACAs in 9% (IgG, 3% and IgM, 6%), and both LA and ACAs in 2%. Median levels of protein C antigen were slightly increased in women (112%; range, 53%-198%) and men (110%; range, 91%-150%), considering that these were expected to be 100%. Median levels of total protein S antigen were decreased in women (91%; range, 49%-174%), but increased in men (112%; range, 91%-132%). The difference in total protein S antigen levels between women and men remained when women who used oral contraceptives were excluded (92%; range, 50%-174%). Deficiencies of either antithrombin, protein C (type I or II), protein S (type I), or plasminogen were observed in 7% of patients. Type III protein S deficiency (only tested in the first 67 consecutive patients) and APC resistance were the most frequently observed thrombophilic defects, mainly in women and regardless of the use of oral contraceptives. All carriers of factor V Leiden (9%) or the prothrombin G20210A mutation (2%) were heterozygotes.

The prevalences of LA ($P = .017$), ACAs ($P = .13$), factor V Leiden ($P = .097$), and the prothrombin G20210A mutation ($P = .01$) were higher in patients with VTE than in asymptomatic patients (Table 4). There was a higher frequency of LA ($P = .11$) and ACAs ($P = .03$) in patients with ATE than in asymptomatic patients.

Annual incidences of VTE and ATE associated with LA, ACAs, and each of the 7 tested thrombophilic defects, regardless of their concomitance, are summarized in Table 5.

The annual incidence of VTE was 2.01% in patients with one of the 4 disorders that were identified as risk factors by univariate analysis (LA, 4 patients; ACAs, 8 patients; factor V Leiden, 9 patients; prothrombin G20210A mutation, 1 patient), and 3.05% in patients with various combinations of 2 of these disorders (LA and ACAs, 4 patients; LA and factor V Leiden, 2 patients; LA and prothrombin G20210A mutation, 1 patient) (Table 6).

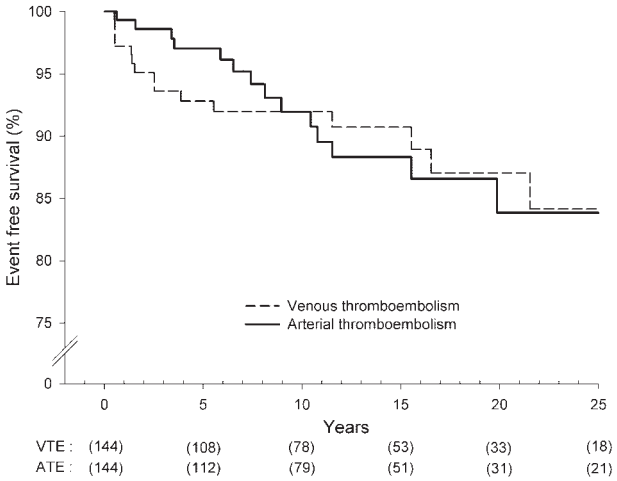

Figure 1. Kaplan-Meier event-free survival comparing patients with venous thromboembolism and patients with arterial thromboembolism. The number of exposed patients is shown between brackets.

Discussion

Twenty percent of the here-reported cohort of 144 consecutive patients with SLE experienced a thromboembolic event during a median follow-up of 12.7 years. These events were either venous or arterial, each in half of the cases. Venous events occurred 0.5 to 21.5 years after the onset of SLE and 50% occurred within the first 2.5 years. Arterial events occurred after 0.6 to 33.0 years, of which 50% occurred within the first 8.5 years. Recurrence rates were 33% and 25% in patients with a first episode of venous and arterial thromboembolism, respectively.

Although LA and ACAs were shown to be risk factors, particularly LA for VTE and ACAs for ATE, only half of these events could be attributed to the presence of APA. LA was

Table 2. Classification of thromboembolic events in 144 patients with SLE

Event	n	Spontaneous	Recurrence
Venous thromboembolism			
Deep vein thrombosis	6	3	3*
Pulmonary embolism	3	1	1
Deep venous thrombosis and pulmonary embolism	2	2	0
Retinal vein thrombosis	2	2	1†
Subclavian vein thrombosis	2	0	0
Any venous thrombosis	15	8	5
Arterial thromboembolism			
Myocardial infarction	5	—	1‡
Ischemic stroke	5	—	2§
TIA	3	—	0
Peripheral arterial occlusion	3	—	1
Any arterial event	16	—	4

— indicates not applicable.
*Two patients had a preceding TIA (transient ischemic attack).
†Caval vein thrombosis.
‡Ischemic stroke.
§TIA.

Table 3. Laboratory characteristics of 144 patients with SLE

Variable	Median (range)	Defect, n (%)
Lupus anticoagulant	—	11 (7.7)
Anticardiolipin antibodies	0 (0-104)	12 (8.7)
IgG, ≥ 40 GPL	0 (0-243)	4 (2.9)
IgM, ≥ 40 MPL	0 (0-279)	9 (6.5)
Antithrombin, %	99 (67-140)	5 (3.5)
Protein C activity, %	106 (51-207)	3 (2.1)
Protein C antigen, %	112 (53-198)	1 (0.7)
Total protein S antigen, %	92 (49-174)	4 (2.8)
Excluding women on oral contraceptives	95 (50-174)	1 (0.8)
Free protein S antigen, %	22 (6-39)	47 (70)
Excluding women on oral contraceptives	22 (6-39)	41 (75)
Plasminogen, %	104 (64-183)	1 (0.7)
nAPC-SR	0.88 (0.53-1.12)	49 (35)
Factor V Leiden	—	11 (7.9)
Prothrombin G20210A	—	2 (1.4)

nAPC-SR indicates normalized activated protein C sensitivity ratio; —, not applicable.

Table 4. Comparison of patients with SLE with thromboembolism

	Venous thromboembolism			Arterial thromboembolism		
	+	−	*P*	+	−	*P*
No.	15	129	—	16	128	—
Age at study entry, y	40 (19-73)	41 (21-76)	.83	48 (28-75)	41 (19-76)	.038
Age at diagnosis SLE, y	29 (17-62)	30 (9-73)	.81	37 (15-73)	30 (9-71)	.25
SLEDAI score	2 (0-25)	2 (0-12)	.59	2 (0-8)	2 (0-25)	.35
SLICC/ACR-DI score	1 (0-3)	0 (0-5)	.39	2 (0-5)	0 (0-4)	< .001
Lupus anticoagulant	4 (27)	7 (5.5)	.017	3 (19)	8 (6.3)	.11
Anticardiolipin antibodies	3 (20)	9 (7.0)	.13	4 (27)	8 (7.0)	.03
IgG ≥ 40 GPL	1 (6.7)	3 (2.4)	.37	2 (13)	2 (1.6)	.058
IgM ≥ 40 MPL	2 (13)	7 (5.7)	.25	2 (13)	7 (5.7)	.25
Antithrombin deficiency	1 (6.7)	4 (3.1)	.43	1 (6.3)	4 (3.1)	.45
Protein C deficiency	0	3 (2.3)	1.00	0	3 (2.3)	.75
Protein S deficiency						
Type I	0	4 (3.1)	1.00	0	4 (3.2)	.99
Type III*	5 (71)	42 (70)	1.00	9 (82)	38 (68)	.48
Plasminogen deficiency	0	1 (0.8)	1.00	0	1 (0.8)	.99
APC resistance	6 (40)	43 (35)	.78	5 (36)	44 (35)	.99
Factor V Leiden	3 (20)	8 (6.4)	.097	0	11 (8.8)	.61
Prothrombin G20210A	2 (13)	0	.011	0	2 (1.6)	.99

Continuous variables denoted as median (range), categorical variables as number (%). — indicates not applicable.
*Measured in the first 67 enrolled patients.

demonstrated in 8% of patients, ACAs in 9%, and their combination in 2%. Higher prevalences of LA (15%-34%) and ACAs (12%-30%) were reported from previous studies on SLE.[3-5] These differences may be explained by variations in applied criteria for LA and in the sensitivitiy of laboratory tests for the detection of ACAs, and by possible selection bias. We classified patients as LA-positive if the results of 3 different laboratory tests were consistent, according to International Society on Thrombosis and Haemostasis (ISTH) guidelines.[10] The cut-off level of ACAs in our study was 40 GPL units, because we found, as did Finazzi et al previously,[14] that higher levels were predictive for a vascular event. By including consecutive patients, we avoided selection bias. A few patients (3%) could not be enrolled because they had died before enrollment. However, they had no history of thrombosis and death was not due to thrombosis. As a result of the cross-sectional design of our study, assessment at time of enrollment might not represent disease activity and LA and/or ACA levels at time of the thrombotic events, considering a plausible relationship between thrombosis and disease activity. For example, actual immunosuppressive drug therapy in approximately 50% of our patients may have decreased plasma levels of LA and ACAs.[15] Despite a possibly underestimated prevalence of LA and/or ACAs, these antibodies were obviously related with previous thrombotic events in our risk estimation.

Thrombophilic defects were observed much more frequently (61% of patients) than were LA and ACAs. These defects included factor V Leiden, the prothrombin G20210A mutation, deficiencies of antithrombin, protein C, total protein S, plasminogen, and APC resistance. However, only factor V Leiden and the prothrombin G20210A mutation were associated with an increased risk of VTE, whereas none of these thrombophilic defects was associated with ATE. This finding supports the assumption that thrombophilic defects mainly contribute to venous rather than arterial thrombotic events.[16]

The annual incidence of VTE in patients with SLE who were heterozygous carriers of factor V Leiden was 2.12%. Hence it was approximately 3.5-fold higher than in patients without this mutation, any other thrombophilic defect, and LA or ACAs. This finding is in agreement with the results of 2 previous studies that showed a 2- to 5-fold increased risk in patients with SLE who harbored this mutation.[17,18]

The high annual incidence of VTE in heterozygous carriers of the prothrombin G20210A mutation should be interpreted cautiously, considering their small number. Only one other study addressed the same topic, but did not show a relationship, maybe due to the small size of the study cohort.[19]

Table 5. Risk of venous and arterial thromboembolism associated with thrombophilic defects and antiphospholipid antibodies

		Venous thromboembolism			Arterial thromboembolism		
Abnormality	Patients, n	Events, n	Observation period, total y	Annual incidence, % (95% CI)	Events, n	Observation period, total y	Annual incidence (95% CI)
No abnormality	80	6	1030	0.58 (0.21-1.27)	8	1006	0.80 (0.34-1.57)
Lupus anticoagulant	11	4	164	2.43 (0.66-6.23)	3	203	1.48 (0.30-4.32)
Anticardiolipin antibodies	12	3	186	1.62 (0.33-4.72)	4	208	1.93 (0.53-4.94)
Antithrombin deficiency	5	1	79	1.27 (0.03-7.09)	1	92	1.09 (0.03-6.1)
Protein C deficiency (type I or II)	1	0	22	0 (0-16.59)	0	22	0 (0-16.59)
Protein S deficiency (type I)	4	0	58	0 (0-6.40)	0	58	0 (0-6.40)
Plasminogen deficiency	1	0	22	0 (0-16.59)	0	22	0 (0-16.59)
APC resistance	49	6	652	0.92 (0.34-2.00)	5	692	0.72 (0.23-1.69)
Factor V Leiden	11	3	142	2.12 (0.44-6.20)	0	161	0 (0-2.29)
Prothrombin G20210A	2	2	4	51.3 (6.21-185.3)	0	39	0 (0-9.54)

Protein S deficiency type III was excluded because it was not tested in all patients.

Table 6. Risk of venous thromboembolism associated with lupus anticoagulant, anticardiolipin antibodies, factor V Leiden and the prothrombin G20210A mutation, either alone or in combinations

	No defects, n = 115	Single defect, n = 22	Two defects, n = 7
Patients with VTE, n	6	6	3
Observation period, total y	1479	299	98
Annual incidence, % (95% CI)	0.41 (0.15-0.88)	2.01 (0.74-4.37)	3.05 (0.63-8.93)
Relative risk (95% CI)	1	5.10 (1.64-15.86)	8.20 (2.04-32.9)
P	—	.005*	.003*

— indicates not applicable.
*Compared with no defects.

Remarkably, the annual incidence of VTE in patients with SLE without LA, ACAs, or any of 7 tested thrombophilic defects was 0.58%, and thus approximately 6-fold higher than that reported in the healthy population (0.1%).[20,21] Apparently, SLE is a risk factor as such, or these patients had one or more other thrombophilic disorders for which they were not tested, like increased levels of factors VIII, IX, or XI or hyperhomocysteinemia. The increased annual incidence of VTE in the absence of LA, ACAs, and any thrombophilic defect can also be explained by a previous and transient thrombophilic state which had recovered at time of enrollment.

Analysis of the contribution of factor V Leiden and the prothrombin G20210A mutation to the risk of VTE in patients with LA and/or ACAs showed an annual incidence of VTE that amounted to 2.01% in patients who had a single disorder and 3.05% in patients with 2 disorders. Compared with patients with SLE without any of these 4 disorders, the risk of VTE was 5- and 8-fold higher, respectively. However, compared with the healthy population (annual incidence 0.1%), patients with SLE with one disorder had a 20-fold higher risk, while it was even 30-fold higher in patients with 2 abnormalities.[20,21] LA and ACAs are thus not the only determinants of the thrombotic risk in patients with SLE, whereas interactions between these antibodies and thrombophilic defects are likely.

The numbers of patients with a deficiency of either antithrombin, protein C, or total protein S were too small to allow an accurate assessment of the associated risk of VTE. By contrast, lowered levels of free protein S deficiency were demonstrated in 70% of tested patients, suggesting an acquired origin, like high levels of C4 binding protein in patients with SLE.[22] Although a type III protein S deficiency was more prevalent than a type I protein S deficiency, an association of the former with an increased risk of thrombosis was not observed.

APC resistance was also not identified as a risk factor. It was observed in 34% of patients and could be explained only in a part of them by either factor V Leiden, LA, or the use of oral contraceptives. In 2 previous studies, APC resistance was associated with VTE in children and with ATE in adults, respectively.[23,24] This discrepancy might be due to differences in the applied laboratory tests.

The incidence of thrombophilic defects other than LA and ACAs appeared to be increased compared with the healthy population. However, cosegregation of inherited defects with LA and/or ACAs rather than clustering of inherited defects in patients with SLE contributed to the risk of VTE. This was demonstrated for factor V Leiden and the prothrombin G20210A mutation, the only defects with a proven genetic origin and incidences that were comparable to the general population. Inheritance of other defects was not established by testing relatives, whereas they did not contribute to the risk of VTE. It is likely that a majority of the observed abnormalities was acquired, maybe secondary to chronic endothelial injury or inflammation.

The clinical implications of our findings are important. Primary thromboprophylaxis should be considered, particularly in patients with SLE with a combination of LA and/or ACAs and factor V Leiden or the prothrombin G20210A mutation. The high absolute risk of VTE in this subgroup certainly will exceed the reported risk of major bleeding due to oral anticoagulant treatment.[25,26] Although our study did not enable us to estimate the additional risk of oral contraceptives or hormonal replacement therapy in postmenopausal women, both should be discouraged for the same reason (ie, the high absolute risk of VTE) in female patients with SLE with one or more of these disorders. Screening of symptomatic or asymptomatic patients with SLE on thrombophilic defects other than factor V Leiden and the prothrombin G20210A mutation seems unnecessary, unless another inherited thrombophilic defect has been demonstrated already in one or more relatives. The need for standardization of laboratory tests of LA and ACAs shall be clear, given the aforementioned possible impact of a positive test.

In conclusion, acquired or inherited thrombophilic defects were frequently demonstrated in patients with SLE, apart from LA and ACAs. Of these, only factor V Leiden and the prothrombin G20210A mutation were identified as independent risk factors for VTE. Alone or in combination with LA and/or ACAs, these factors increased the risk of VTE 20- and 30-fold, respectively, compared with the general population. Thrombophilic defects, in contrast with LA and ACAs, did not influence the risk of ATE.

Acknowledgment

We thank J. Swart for data entry.

References

1. Cervera R, Khamashta MA, Font J, et al. Morbidity and mortality in systemic lupus erythematosus during a 5-year period: a multicenter prospective study of 1,000 patients. European Working Party on Systemic Lupus Erythematosus. Medicine (Baltimore). 1999;78:167-175.
2. Petri M, Rheinschmidt M, Whiting-O'Keefe Q, Hellman D, Corash L. The frequency of lupus anticoagulant in systemic lupus erythematosus: a study of sixty consecutive patients by activated partial thromboplastin time, Russell viper venom time, and anticardiolipin antibody level. Ann Intern Med. 1987;106:524-531.
3. Merkel PA, Chang Y, Pierangeli SS, Convery K, Harris EN, Polisson RP. The prevalence and clinical associations of anticardiolipin antibodies in a large inception cohort of patients with connective tissue diseases. Am J Med. 1996; 101:576-583.
4. Love PE, Santoro SA. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders: prevalence and clinical significance. Ann Intern Med. 1990;112:682-698.
5. Cervera R, Khamashta MA, Font J, et al. Systemic Lupus erythematosus: clinical and immunologic patterns of disease expression in a cohort of 1,000 patients. The European Working Party on Systemic Lupus Erythematosus. Medicine (Baltimore). 1993;72:113-124.
6. Rosendaal, FR. Venous thrombosis: a multicausal disease. Lancet. 1999;353:1167-1173.

7. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1982;25: 1271-1277.
8. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI: a disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum. 1992;35:630-640.
9. Gladman D, Ginzler E, Goldsmith C, et al. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. Arthritis Rheum. 1996;39:363-369.
10. Exner T, Triplett DA, Taberner D, Machin SJ. Guidelines for testing and revised criteria for lupus anticoagulants. SSC Subcommittee for the Standardization of Lupus Anticoagulants. Thromb Haemost. 1991;65:320-322.
11. Bertina RM, Koeleman BPC, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature. 1994;369:64-67.
12. Danneberg J, Abbes AP, Bruggeman BJM, Engel H, Gerrits J, Martens A. Reliable genotyping of the G-20210-A mutation of coagulation factor II (prothrombin). Clin Chem. 1998;44:349-351.
13. Comp PC, Thurnau GR, Welsh J, Esmon CT. Functional and immunologic protein S levels are decreased during pregnancy. Blood. 1986;68: 881-885.
14. Finazzi G, Brancaccio V, Moia M, et al. Natural history and risk factors for thrombosis in 360 patients with antiphospholipid antibodies: a four-year prospective study from the Italian Registry. Am J Med. 1996;100:530-536.
15. Out HJ, van Vliet M, de Groot PG, Derksen RH. Prospective study of fluctuations of lupus anticoagulant activity and anticardiolipin antibody titre in patients with systemic lupus erythematosus. Ann Rheum Dis. 1992;51:353-357.
16. Rosenberg RD, Aird WC. Vascular-bed-specific hemostasis and hypercoagulable states. N Engl J Med. 1999;340:1555-1564.
17. Fijnheer R, Horbach DA, Donders RC, et al. Factor V Leiden, antiphospholipid antibodies and thrombosis in systemic lupus erythematosus. Thromb Haemost. 1996;76:514-517.
18. Regeczy N, Lakos G, Balogh I, Ajzner E, Kiss E, Szegedi G. The Leiden mutation of coagulation factor V in Hungarian SLE patients. Clin Appl Thromb Hemost. 2000;6:41-45.
19. Topaloglu R, Akierli C, Bakkaloglu A, et al. Survey of factor V Leiden and prothrombin gene mutations in systemic lupus erythematosus. Clin Rheumatol. 2001;20:259-261.
20. Nordstrom M, Lindblad B, Bergqvist D, Kjellstrom T. A prospective study of the incidence of deep-vein thrombosis within a defined urban population. J Intern Med. 1992;232:155-160.
21. Anderson FA, Wheeler HB, Goldberg RJ, et al. A population-based perspective of the hospital incidence and case-fatality rates of deep vein thrombosis and pulmonary embolism. The Worcester DVT Study. Arch Intern Med. 1991;151:933-938.
22. Dahlback B, Stenflo J. High molecular weight complex in human plasma between vitamin K-dependent protein S and complement component C4b-binding protein. Proc Natl Acad Sci U S A. 1981;78:2512-2516.
23. Muñoz-Rodríguez FJ, Reverter JC, Font J, et al. Clinical significance of acquired activated protein C resistance in patients with systemic lupus erythematosus. Lupus. 2002;11:730-735.
24. Male C, Mitchell L, Julian J, et al. Acquired activated protein C resistance is associated with lupus anticoagulants and thrombotic events in pedriatric patients with systemic lupus erythematosus. Blood. 2001;897:844-849.
25. Beyth RJ, Quinn LM, Landefeld CS. Prospective evaluation of an index for predicting the risk of major bleeding in outpatients treated with warfarin. Am J Med. 1998;105:91-99.
26. Palareti G, Leali N, Coccheri S, et al. Bleeding complications of oral anticoagulant treatment: an inception-cohort, prospective collaborative study (ISOCAT). Italian Study on Complications of Oral Anticoagulant Therapy. Lancet. 1996;17:423-428.