CONCISE REPORT

Factor XII autoantibodies as a novel marker for thrombosis and adverse obstetric history in patients with systemic lupus erythematosus

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Aim: To investigate the clinical significance of anti-factor XII (FXII) in a large cohort of patients with systemic lupus erythematosus (SLE).

Patients and methods: This study comprised 127 patients with SLE. IgG and IgM anti-FXII were tested by an in-house ELISA. 123 healthy donors comprised the control group.

Results: 51 (40%) patients with SLE and 9 (7%) healthy controls were positive for anti-FXII. IgG and IgM anti-FXII were frequently found in patients with thrombosis (28% and 13%, respectively). Levels of IgG and IgM anti-FXII were higher in patients with thrombosis than in the control group ($p < 0.001$ and $p = 0.005$, respectively). Anti-FXII was more frequent in patients with arterial thrombosis (31% vs 4% for IgG and 14% vs 3% for IgM, respectively) and venous thrombosis than in controls (37% vs 4% for IgG). IgG anti-FXII were more frequent in patients with miscarriages and fetal death (35% and 40% vs 4% for IgM). The prevalence of IgM anti-FXII was not different between groups.

Conclusion: Anti-FXII are frequent in patients with SLE. Their presence is associated with thrombosis and adverse obstetric history, making these antibodies a novel marker for the antiphospholipid syndrome.

The antiphospholipid syndrome is characterised by thrombosis and/or pregnancy morbidity in the presence of antiphospholipid antibodies (aPL).¹ In clinical practice, anticardiolipin antibodies (aCL) and lupus anticoagulant (LA) are the most used and standardised tests for the detection of aPL. However, a variety of plasma proteins, known as phospholipid binding proteins, have also been implicated as targets for aPL.

Factor XII (FXII), originally identified in 1955,² is an 80 kDa protein containing 596 amino acids. It has six major structural domains including a kringle and two growth factor-like domains, with a concentration of 35 µg/ml in human plasma.³

FXII has an important role in fibrinolysis and in the inhibition of thrombin-induced platelet activation. Its deficiency, although resulting in a prolonged activated partial thromboplastin time, is associated with thrombotic and not bleeding incidents.⁴

Autoantibodies to FXII (anti-FXII) have been associated with pregnancy complications,⁵ but their association with thrombosis remains obscure.³ We designed this study to investigate the clinical significance of anti-FXII in a large cohort of patients with SLE.

PATIENTS AND METHODS

Patients

We included 127 patients, all fulfilling at least 4 of the 1982 criteria for SLE⁶ (123 women, with a mean (SD) age of 42

(12.3) years and a mean (SD) disease duration of 12.6 (8.5) years). Sapporo criteria for antiphospholipid syndrome was fulfilled by 22 patients.⁷

A total of 46 patients had a history of thrombotic events. Of these, 22 (48%) had arterial, 11 (24%) had venous, and 13 (28%) had both arterial and venous events. A total of 83 women had obstetric history available. Of these, 18 (21%) fulfilled Sapporo criteria for pregnancy morbidity characterised by ≥ 3 miscarriages (< 10 th week of gestation) and/or fetal death (death of a morphologically normal fetus beyond the 10th week of gestation). In all, 17 (20%) patients had one or more miscarriages and 48 (57%) women had normal pregnancies. The control group included 123 healthy donors, all of whom had no history of thrombosis or adverse obstetric history.

Ethical approval was obtained from St Thomas' ethics committee, and all patients gave their written consent.

METHODS

ELISA for anti-FXII antibody

Microtitre plates (Nunc Maxisorp, Roskilde, Denmark) coated with 2.5 µg/ml of human FXII (Enzyme Research Lab, Indiana, USA) in borate-buffered saline (BBS; pH 8.4) were blocked with 0.5% bovine serum albumin–0.4% Tween 80 in BBS. FXII was $> 95\%$ pure as judged on a 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis gel, appearing as a single band showing no reduction on incubation with 2-mercaptoethanol (data supplied by the manufacturer). After washing with BBS, serum samples diluted 1:50 with BBT were added in duplicate, followed by alkaline phosphatase conjugation (Sigma). *p*-nitrophenylphosphate disodium in 1 M diethanolamine buffer (pH 9.8) was added, and optical density was measured at 405/620 nm and converted to arbitrary units (AU), with a sample showing a high binding used as a standard. The cut-off points for IgG and IgM anti-FXII assay were established at ≥ 18 AU for IgG and at ≥ 2 AU for IgM (mean + 3 SD of 123 controls).

ELISA for aCL and anti-β2 glycoprotein I

The aCL ELISA was performed by a standardised technique.⁸ Antibodies to β2 glycoprotein I were detected as described previously.⁹

Antiprothrombin antibodies detection

Antiprothrombin antibodies (aPT) were detected using irradiated plates or in complex with phosphatidylserine (aPS–PT), as reported previously.^{10 11}

Abbreviations: aCL, anticardiolipin antibodies; aPL, antiphospholipid antibodies; aPS–PT, antibodies to phosphatidylserine–prothrombin complex; aPT, antiprothrombin antibodies; BBS, borate-buffered saline; FXII, factor XII; LA, lupus anticoagulant; SLE, systemic lupus erythematosus

Statistical analysis

Comparisons were expressed as odds ratio (OR) with its 95% CI, where a lower limit ≥ 1 was considered significant. The degree of linear association between anti-FXII and other aPL was quantitated by the Spearman correlation method. Differences between means were analysed by the Mann-Whitney U test. All p values were determined by Fisher's exact test.

RESULTS

Prevalence of anti-FXII and other aPL

Anti-FXII were present in 51 of 127 patients with SLE (40%). In all, 36 (28%) patients were positive for IgG, 8 (6%) for IgM and 7 (6%) for both IgG and IgM. Out of 123 healthy control subjects, 9 (7%) were positive for anti-FXII: 5 (4%) were positive for IgG anti-FXII and 4 (3%) for IgM anti-FXII. IgG anti-FXII were more frequently found in patients with SLE than in control subjects (40% vs 7%, OR 8.5 (95% CI 3.9 to 18.3)), and the difference was statistically significant ($p < 0.001$). Prevalence of other aPL is summarised in table 1.

Correlation of anti-FXII with other aPL

There was no correlation between IgG anti-FXII and other IgG aPL. No correlation was found between IgM anti-FXII and IgM antibodies to $\beta 2$ glycoprotein I and aPT. Although there was a significant correlation between IgM anti-FXII and IgM aCL and IgM aPS-PT ($p < 0.001$ and $p = 0.035$, respectively), many patients with discrepant results (eg, positive for one antibody but negative for the other antibody) were seen (fig 1).

Relationship of anti-FXII with thrombosis

Patients with thrombosis had IgG and IgM anti-FXII more frequently than controls (28% vs 4% and 13% vs 3%, respectively). Levels of IgG and IgM anti-FXII were also higher in patients with thrombosis than in controls (13.4 (12.7) AU vs 7.6 (3.3) AU, $p < 0.001$ and 2 (1.8) AU vs 1.5 (0.01) AU, $p = 0.005$, respectively).

Table 1 Prevalence of antiphospholipid antibodies in systemic lupus erythematosus

	SLE, n = 127	%
Anti-FXII	51	40
IgG	36	28
IgM	8	6
IgG+IgM	7	5
aCL	71	56
IgG	41	32
IgM	12	9
IgG+IgM	18	14
$\alpha\beta 2$ GPI	21	16
IgG	13	10
IgM	7	6
IgG+IgM	1	1
aPT	20	16
IgG	10	8
IgM	8	6
IgG+IgM	2	2
aPS-PT	29	23
IgG	17	13
IgM	8	6
IgG+IgM	4	3
LA	34	27

Anti-FXII, antibodies to factor XII; aCL, anticardiolipin antibodies; $\alpha\beta 2$ GPI, antibodies to $\beta 2$ glycoprotein I; aPT, antibodies to solid-phase prothrombin; aPS-PT, antibodies to phosphatidylserine-prothrombin complex; LA, lupus anticoagulant; SLE, systemic lupus erythematosus.

Patients were divided into subgroups (venous and arterial thrombosis) for further analysis. IgG and IgM anti-FXII were more frequently found in patients with arterial thrombosis than in controls (31% vs 4% and 14% vs 3%, respectively). Levels of IgG and IgM anti-FXII were also higher in patients with arterial thrombosis than in controls (13.8 (13) AU vs 7.6 (3.3) AU, $p < 0.001$ and 2.1 (2) AU vs 1.5 (0.01) AU, $p = 0.001$, respectively).

IgG but not IgM anti-FXII were also more frequent in patients with venous thrombosis than in controls (37% vs 4% and 12% vs 3%, respectively). Levels of IgG and IgM anti-FXII were also higher in patients with venous thrombosis than in controls (17.2 (13.5) AU vs 7.6 (3.3) AU, $p < 0.001$ and 1.8 (1.4) AU vs 1.5 (0.01) AU, $p = 0.02$, respectively).

Relationship of anti-FXII with adverse obstetric history

Patients with adverse obstetric history had IgG but not IgM anti-FXII more frequently than controls (37% vs 4% and 9% vs 3%, respectively). IgG anti-FXII were also more frequent in patients with miscarriages and fetal death (35% and 40% vs 4%, respectively). When considering only patients who fulfil Sapporo criteria, IgG but not IgM anti-FXII were more frequently found in patients with pregnancy morbidity than in controls (39% vs 4% and 11% vs 3%, respectively).

Anti-FXII was the sole aPL in three patients. Two patients had a history of arterial thrombosis and one had adverse obstetric history in the absence of all other tested aPL. Table 2 summarises all clinical associations.

Relationship of anti-FXII with the lupus anticoagulant

Out of 34 patients with lupus anticoagulant, 15 (44%) had anti-FXII. Interestingly, 6 of 15 (40%) patients had a history of thrombosis. From the obstetric side, one male patient and three female patients who had never been pregnant were excluded from analysis. Of the remaining 11 patients, 6 (54.5%) had adverse obstetric history, although only 3 (28%) fulfilled Sapporo criteria for pregnancy morbidity (≥ 3 miscarriages (< 10 th week of gestation) and/or fetal death (death of morphologically normal fetus beyond the 10th week of gestation)).

The prevalence of vascular events in patients with anti-FXII and lupus anticoagulant was not different from that in patients with LA alone (11/19 had thrombotic events and 6/11 women who had been pregnant had adverse obstetric history).

DISCUSSION

In this study, we evaluated the prevalence and clinical significance of anti-FXII detected by ELISA in a large cohort of patients with SLE and found that these antibodies are present in 40% of the patients.

We found an association between the presence of anti-FXII and the occurrence of vascular events and adverse obstetric history. However, when cases of thrombosis were subdivided into arterial and venous events, only the correlation between anti-FXII and arterial thrombosis remained significant.

Most of the studies available in the literature suggest an association between the presence of anti-FXII and obstetric complications such as recurrent fetal loss.³⁻⁵ In our study, we found an association between anti-FXII and adverse obstetric history in patients with SLE.

We found no correlation between anti-FXII and most aPL tested in this study. Although there was a significant correlation between IgM anti-FXII and IgM aCL and IgM aPS-PT, many patients were found to have discrepant results (eg, positive for one antibody but negative for the other antibody). Overall, these data suggest that anti-FXII are an independent population of autoantibodies, and that the

Figure 1 Correlation between anti-factor XII (FXII) and other antiphospholipid antibodies (aPL). (A) Correlation between anti-FXII and anticardiolipin antibodies (aCL). (B) Correlation between anti-FXII and anti- β_2 glycoprotein I (β_2 GPI). (C) Correlation between anti-FXII and antibodies to prothrombin (aPT). (D) Correlation between anti-FXII and antibodies to phosphatidylserine-prothrombin complex (aPS-PT).

association between anti-FXII and thrombosis is not due to the cross-reaction with other aPL.

It has previously been demonstrated that anti-FXII lead to a reduction in the levels of FXII,¹² and patients with FXII deficiency have been shown to have impaired fibrinolytic activity.¹³ Perhaps, this mechanism could be responsible for

the increased risk of thrombosis seen in patients with anti-FXII.

Low levels of FXII and a high prevalence of anti-FXII have also been reported in patients with lupus anticoagulant.¹⁴ Although in our study we did not consider the levels of FXII, we did find a high prevalence of anti-FXII in patients with

Table 2 Antiphospholipid syndrome-related clinical associations of anti-factor XII in patients with systemic lupus erythematosus

	IgG		IgM	
	OR (95% CI)	p Value	OR (95% CI)	p Value
Thrombosis	9.3 (4.3 to 28)	<0.001	4.5 (1.2 to 17)	0.03
Arterial	11 (3.4 to 34)	<0.001	5 (1.2 to 20)	0.03
Venous	14 (4.2 to 48)	<0.001	4.2 (0.9 to 20)	0.09
Adverse obstetric history*	14 (4.5 to 43)	<0.001	2.8 (0.6 to 13)	0.2
Miscarriages	12 (3.7 to 42)	<0.001	3.9 (0.8 to 18)	0.1
Fetal death	16 (4 to 62)	0.002	4.6 (0.8 to 27)	0.13
Pregnancy morbidity†	15 (4.1 to 55)	<0.001	3.7 (0.6 to 22)	0.2

All data are analysed against controls.

*Adverse obstetric history includes patients with any number of miscarriages and/or fetal death who do not necessarily fulfil Sapporo criteria for antiphospholipid syndrome.

†Pregnancy morbidity includes exclusively those patients fulfilling 1998 Sapporo criteria for pregnancy morbidity.

lupus anticoagulant. However, the prevalence of vascular events was not different between patients with anti-FXII and lupus anticoagulant and those with lupus anticoagulant alone. This may be explained by the fact that low FXII levels are also seen in patients with lupus anticoagulant in the absence of anti-FXII.¹⁵

In summary, anti-FXII are frequently seen in SLE, and their presence is associated with thrombosis and adverse obstetric history. As functional properties of these antibodies are still obscure, further research into the immunological characteristics of anti-FXII is warranted.

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