Factor XIII in Primary Antiphospholipid Syndrome

PAUL R.J. AMES, LUIGI IANNACCONE, JOSE DELGADO ALVES, ANNAMARIA MARGARITA, LUIS R. LOPEZ, and VINCENZO BRANCACCIO

ABSTRACT. ***Objective.*** To evaluate the clinical significance of factor XIII (FXIII) in primary antiphospholipid syndrome (APS).

Methods. A cross-sectional study including patients with primary APS (n = 29), persistent carriers of idiopathic antiphospholipid antibodies (aPL) with no history of thrombosis (n = 14), thrombotic patients with inherited thrombophilia (n = 24), healthy controls (n = 28), and patients with mitral and aortic valve prosthesis (n = 32, as controls for FXIII only). FXIII and fibrinogen were measured by functional assays: IgG anticardiolipin antibody (aCL), IgG anti-ß$_2$-glycoprotein I (anti-ß$_2$-GPI), and plasminogen activator inhibitor (PAI) by immunoassay; and paraoxonase activity by paranitrophenol formation. Intima-media thickness (IMT) of carotid arteries was determined by high resolution sonography.

Results. FXIII activity (FXIIIa) was highest in primary APS (p = 0.001), particularly in patients with multiple occlusions (n = 12) versus those with single occlusion (158 ± 45% vs 118 ± 38%; p = 0.02). In primary APS, FXIII positively correlated with PAI (p = 0.003) and fibrinogen (p = 0.005). Similarly in the thrombotic control group, FXIIIa correlated with PAI (p = 0.05) and fibrinogen (0.007). In primary APS, FXIIIa was related to the IMT of all carotid artery segments (p always < 0.01). In thrombotic controls FXIIIa correlated only to the IMT of the common carotid (p = 0.01). In primary APS, FXIIIa was strongly associated with IgG aCL and IgG anti-ß$_2$-GPI (p = 0.005 for both). These associations were weaker in the aPL group (FXIIIa with IgG aCL, p = 0.02, with IgG anti-ß$_2$-GPI, p = 0.04).

Conclusion. Enhanced FXIII activity may contribute to atherothrombosis in primary APS via increased fibrin/fibrinogen cross-linking. This pathway is not exclusive to primary APS, being present also in thrombotic controls, but the presence of IgG aPL may favor a higher degree of FXIIIa activation in the primary APS group. (J Rheumatol 2005;32:1058–62)

Key Indexing Terms:
ANTIPHOSPHOLIPID ANTIBODY FACTOR XIII ATHEROSCLEROSIS THROMBOSIS

The antiphospholipid antibody syndrome (APS) is characterized by arterial and venous thrombosis associated with the persistence of antiphospholipid antibodies (aPL)[1]. Animal models suggest that aPL can be associated with atherosclerosis[2]. This relationship has been investigated in systemic lupus erythematosus with controversial results[3-5]. In primary APS, one study did not report differences in the number of carotid plaques between patients with primary APS and controls with thrombosis[6], whereas a recent report shows a thicker intima media in elderly patients with primary APS compared to healthy subjects[7].

From the Academic Department of Rheumatology, Leeds General Infirmary, Leeds, UK; Pharmacology Department, Faculty of Medical Sciences, New University of Lisbon, Lisbon, Portugal; Corgenix, Westminster, Colorado, USA; and Angiology and Coagulation Units, Cardarelli Hospital, Naples, Italy.

Supported by Praxis Grant 436.00/27699, Foundation for Science and Technology, Portugal (J. Delgado Alves).

P.R.J. Ames, MD, Academic Department of Rheumatology, Leeds General Infirmary; J. Delgado Alves, MD, PhD, Pharmacology Department, New University of Lisbon; L.R. Lopez, MD, Corgenix; A. Margarita, MD; L. Iannaccone, BSc; V. Brancaccio, MD, Angiology and Coagulation Units, Cardarelli Hospital.

Address reprint requests to Dr. P.R.J. Ames, Academic Department of Rheumatology, Leeds General Infirmary, Great George Street, Leeds LS1 3EX, UK. E-mail: paxmes@aol.com

Accepted for publication January 17, 2005.

Nevertheless, IgG aCL titer was found to independently predict intima-media thickness (IMT) of carotid arteries, supporting the atherosclerosis hypothesis[8]. In the latter study the lipid profile bore no relevance to the IMT of carotid arteries; rather, elevated plasma fibrinogen and plasminogen activator inhibitor (PAI) were correlates of IMT. Because coagulation activation and depressed fibrinolysis contribute to increased fibrin turnover in primary APS[9], coagulation anomalies may be as relevant or more relevant to APS-related vascular damage than traditional atherosclerotic risk factors.

Factor XIII (FXIII) is a transglutaminase of endothelial origin that cross-links fibrin with itself and with the endothelial matrix; it is also implicated in thrombosis and atherosclerosis[10]. We explored whether FXIII was related to thrombosis and the IMT of carotid arteries in primary APS.

MATERIALS AND METHODS

Patients. The study included 99 consecutive patients attending the coagulation unit of the Cardarelli Hospital in Naples, Italy. Of these, 29 patients with primary thrombotic APS (22 women, 7 men, mean age 35 ± 10 yrs), 14 patients (11 women, 3 men, mean age 34 ± 10 yrs) who were persistent carriers of idiopathic aPL (in the absence of any underlying disorder except one case of hereditary spherocytosis), and 24 patients (16 women, 8 men, mean age 36 ± 8 yrs) with thrombosis not due to aPL (factor V Leiden, n = 12; prothrombin, n = 10; protein C deficiency, n = 2). All primary APS

patients met Sapporo revised criteria[1]. As many as 22 patients with APS had had venous events (2 events in 5 patients, 3 events in 1 patient), 4 had arterial events (2 events in 3 patients), and 3 had both arterial and venous events. In the inherited thrombophilia group, 20 underwent venous thrombosis (5 patients with 2 events) and 4 arterial thrombosis. All vascular occlusions had been diagnosed as required by Doppler ultrasound magnetic resonance imaging scan, ventilation/perfusion scan, angiogram, electrocardiogram, and echocardiogram. Patients with idiopathic aPL were detected because of the presence of lupus anticoagulant (LAC) on routine clotting screen for minor surgical procedures and/or for health checks; clinically they never showed symptoms of APS. At the time of study, 25 primary APS patients were taking warfarin with an international normalized ratio (INR) of 2.0–3.0, and 4 had INR of 3.0–4.0. In the aPL group 3 were taking aspirin, whereas all patients in the inherited thrombophilia group were taking warfarin with INR of 2.0–3.0. To control for a possible effect of warfarin on FXIII activity (FXIIIa) in a nonthrombotic group, FXIIIa was also measured in 32 patients (18 women, 14 men, mean age 49 ± 15 yrs) taking warfarin for valve replacements (mitral n = 16, aortic n = 16). Mitral valve replacement patients had an INR of 3.0–4.0, and those with aortic valve replacement of 2.0–3.0. Healthy hospital personnel (n = 28, 18 women, 10 men, mean age 34 ± 9 yrs) served as a control group. No participant was taking lipid-lowering agents or drugs with antioxidant properties at the time of study. Plasma samples from patients and controls were kept at –80˚C until the laboratory tests were performed. The study was carried out according to the Declaration of Helsinki and patients gave informed consent before entering the study.

aPL and coagulation assays. All APS and aPL patients had been screened for LAC by activated partial thromboplastin time (aPTT), dilute Russell viper venom time (DRVVT), and kaolin clotting time. Once an inhibitor was detected by mixing studies, the platelet neutralization procedure in the aPTT and DRVVT was used to confirm its phospholipid nature[11]. IgG anticardiolipin (aCL) were measured by ELISA (Melisa, Cambridge Life Sciences, Ely, UK), standardized with sera calibrated against the appropriate international reference material. IgG anti-ß$_2$-GPI was measured by ELISA (Corgenix, Westminster, CO, USA). As many as 120 serum samples from healthy blood donors were tested for IgG anti-ß$_2$-GPI antibodies to establish the cutoff of the assay at 20 units. Intra- and inter-assay coefficients of variability were 4.1% and 3%, respectively. In a preliminary analysis IgM aCL and IgM anti-ß$_2$-GPI were not found to be related to variables in primary APS (data not shown).

Factor XIII was measured by photometric assay (Behring, Germany) according to manufacturer's recommendations. Briefly, exogenous thrombin activates FXIII present in a sample, then FXIIIa links a specific peptide substrate with glycine ethyl ester that releases ammonia in the reaction with NADH to give NAD. The variable measured is the decrease in NADH monitored at 340 nm. Pooled normal plasma from 30 healthy blood donors served as control plasma for FXIIIa. Results are expressed as percentage from the reference curve established using serial dilutions of pooled plasma. The interassay coefficient of variation (n = 15) was 3.6% in our laboratory. Plasma fibrinogen was measured by Clauss assay, and PAI by ELISA (Behring).

Paraoxonase (PON) activity. PON activity was measured as described by Eckerson and La Du with modifications[12]. Briefly, PON (1.0 mM) freshly prepared in 300 µl of 50 mM glycine buffer containing 1 mM calcium chloride (pH 10.5) was incubated at 37ºC with 5 µl of serum for 15 min in 96 well plates (Polysorp, Nunc, Life Technologies, Paisley, UK). p-Nitrophenol formation was monitored at 412 nm, and activity was expressed as nmol p-nitrophenol per ml plasma per min. The cutoff point for normal limits had been previously determined as mean ± 3 SD of values obtained in a population of 30 healthy adults.

Measurement of IMT of carotid arteries. Carotid artery IMT was assessed by high resolution ultrasound with an Aloka 2000 sonograph equipped with a 5–10 MHz linear transducer. The carotid image acquisition protocol was derived from the Atherosclerosis Risk in Communities Study[13] with minor modifications. In brief, images were obtained with the patient supine, with the neck mildly extended. Longitudinal and lateral views of the distal 10 mm of the right and left common carotid arteries, carotid bifurcation, and internal carotid artery were taken. Scans were performed on 28 patients with primary APS, 14 aPL subjects, 24 thrombotic controls, and 24 healthy controls. Intra-reader reproducibility was assessed measuring the same IMT twice in 12 patients (yielding correlation coefficients superior to 97%) and by repeating the same carotid IMT measurement 4 times in a healthy person (male, 44 years of age) over 12 weeks (yielding a coefficient variability of 3–4% according to the carotid segment under study).

Statistical analysis. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA). Nonparametric tests were employed throughout: Kruskal-Wallis analysis of variance (with Dunn's post hoc as appropriate) for multiple comparisons, Mann-Whitney t test for comparison between 2 groups, and Spearman's rank test for associations between variables.

RESULTS

Factor XIIIa and other variables across groups. To evaluate persistence of activation, FXIII was remeasured after 3 months in 15 primary APS patients, yielding a coefficient of correlation r = 0.91 ($p \leq 0.0001$) and in 15 thrombotic controls yielding a coefficient of correlation r = 0.89 ($p < 0.0001$). The proportion of patients with FXIIIa above the mean + 2 SD of healthy controls was 38% (11/29) in primary APS, 7% (1/14) in aPL, 16.6% (4/24) in thrombotic controls, 12.5% (4/32) in valvular controls, and 3.5% (1/28) in healthy controls (p = 0.006). Because FXIIIa may be influenced by intake of warfarin[14], FXIIIa was also measured in nonthrombotic valvular controls at different INR intensities, although no differences were detected, as FXIIIa was 106 ± 24% in those with INR 2.0–3.0 (n = 16) and 110 ± 22% in those with INR 3.0–4.0 (n = 16). Thus the valvular control group consisted of all 32 FXIIIa values. Median FXIIIa increased more in primary APS than in other groups (Figure 1). In addition, primary APS presented with higher fibrinogen and PAI, and lower PON activity than the other groups (Table 1).

Factor XIII in antiphospholipid positive groups. Although FXIII was not significantly different between patients with arterial and venous occlusions (145 ± 54% vs 125 ± 38%) in APS, patients with multiple events (n = 12) showed a higher FXIIIa than those who had suffered one event only (158 ± 45% vs 118 ± 38%; p = 0.02). FXIIIa correlated with IgG aCL in both the APS and aPL groups (Figure 2A and 2B). Similar results were observed between FXIII and IgG anti-ß$_2$-GPI (Figure 2C and 2D). In addition, FXIIIa was inversely related to PON activity in both the APS and aPL groups (Figure 2E and 2F).

Further, in APS patients FXIIIa correlated to PAI (r = 0.54, p = 0.003) and fibrinogen (r = 0.51, p = 0.005), whereas fibrinogen inversely correlated with PON activity (r = –0.49, p = 0.009). A trend to correlation was seen between FXIIIa and PAI aPL patients (r = 0.51, p = 0.06). Finally, in APS patients FXIIIa positively correlated to IMT of carotid bifurcation (Figure 3), common carotid (r = 0.49, p = 0.006), and internal carotid (r = 0.47, p = 0.01). Significance was

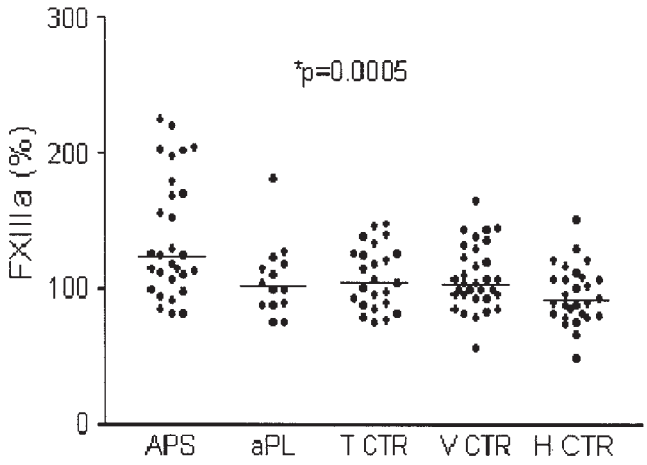

Figure 1. Median levels of factor XIII activity (FXIIIa) in patients with primary thrombotic antiphospholipid syndrome (APS), idiopathic antiphospholipid antibodies (aPL), thrombotic controls (T CTR), valvular controls (V CTR), and healthy controls (H CTR). *Analysis of variance (Kruskal-Wallis). APS versus H CTR, $p < 0.001$, APS vs aPL, $p < 0.05$ (Dunn's multiple comparison test).

slightly weaker after correction for both age and sex (bifurcation: p = 0.0017; common carotid: p = 0.008; internal carotid: p = 0.02). FXIII correlated to the internal carotid only (r = 0.66, p = 0.01) in the aPL group, where effect of age and sex was not determined due to small sample size.

Factor XIII in control groups. In the thrombotic control group, FXIIIa positively correlated with fibrinogen (r = 0.53, p = 0.007) and PAI (r = 0.04, p = 0.05) and negatively with PON activity (r = –0.54, p = 0.005). Fibrinogen and PON activity were negatively correlated (r = –0.42, p = 0.03). Also in this group, FXIIIa correlated with the intima media of the common carotid (r = 0.47, p = 0.01, p = 0.018 after correction for age and sex) and a trend was seen for the carotid bifurcation (r = 0.38, p = 0.06). We did not detect any of the above correlations in the healthy control group.

DISCUSSION

An enhanced risk of thrombosis characterizes primary APS[15]. Elevated plasma fibrinogen and depressed fibrinolysis are among the factors contributing to thrombosis and atherosclerosis in the general population[16,17]; they are linked to the thrombotic tendency of primary APS[9], where they may play an atherogenic role as they relate to the intima-media thickening of carotid arteries[8]. In the search for other coagulation pathways that could explain the atherothrombotic tendency of primary APS, we focused on factor XIII, whose role in APS had never been explored. Factor XIII is a transglutaminase that promotes primarily fibrin-fibrin and fibrin-fibronectin cross-linking, followed by cross-linking with other hemostatic molecules in a hierarchical order, stabilizing the forming clot on the endothelial surface and contributing to cell adhesion[10].

FXIIIa was particularly elevated in a proportion of our primary APS patients, contributing to the higher level of this group compared to thrombotic and nonthrombotic controls. This would be in keeping with the concept that only thrombotic subjects underwent activation of the coagulation system and clot stabilization, followed eventually by plasmin digestion. Indeed, FXIIIa positively correlated to plasma fibrinogen; also positively correlated were fibrin, a substrate of FXIIIa, and PAI antigen, a surrogate of depressed fibrinolysis, in both primary APS and thrombotic controls. Given that fibrinogen and PAI were higher in APS, the overall picture is one of increased FXIII activity contributing to fibrin cross-linking in a state of heightened fibrin turnover in primary APS[8].

As a result of enhanced cross-linking, fibrin is less amenable to plasmin digestion, and conceivably a fibrin film remains adherent and maintains the endothelial surface in persistent activation[18]. Indeed, when reassessed, FXIIIa correlated well to baseline measurements in a subset of our APS patients and thrombotic controls. These arguments pro-

Table 1. Antiphospholipid antibodies, fibrinogen, plasminogen activator inhibitor, and paraoxonase activity in patients and controls.

	APS	aPL	T CTR	H CTR	p*
IgG aCL (GPL)	159 ± 135 (22–573)	80 ± 87 (16–309)	5 ± 2.6 (1.6–12)	6 ± 3.7 (1.2–14)	< 0.0001
IgG ß$_2$GPI (U)	172 ± 46 (76–226)	119 ± 61 (40–216)	4.8 ± 3.5 (1.3–16)	5.2 ± 2.8 (2.1–12)	< 0.0001
FNG (mg/dl)	342 ± 53 (209–464)	283 ± 64 (176–364)	279 ± 62 (198–405)	251 ± 56 (185–364)	< 0.0001
PAI (ng/ml)	43 ± 18* (14–87)	35 ± 19 (12–76)	38 ± 15 (20–80)	30 ± 9 (12–44)	0.06
PONa (nm/ml/min)	0.902 ± 0.345 (0.478–1.405)	1.057 ± 0.237 (0.633–1.335)	0.918 ± 0.222 (0.620–1.300)	1.097 ± 0.225 (0.660–1.400)	0.03

APS: thrombotic antiphospholipid syndrome; aPL: idiopathic carriers of antiphospholipid antibodies; T CTR: thrombotic controls; H CTR: healthy controls; IgG aCL: anticardiolipin antibody; FNG: fibrinogen; PAI: plasminogen activator inhibitor; PONa: paraoxonase activity. * Kruskal-Wallis. APS vs H CTR $p < 0.05$ (Dunn's post hoc).

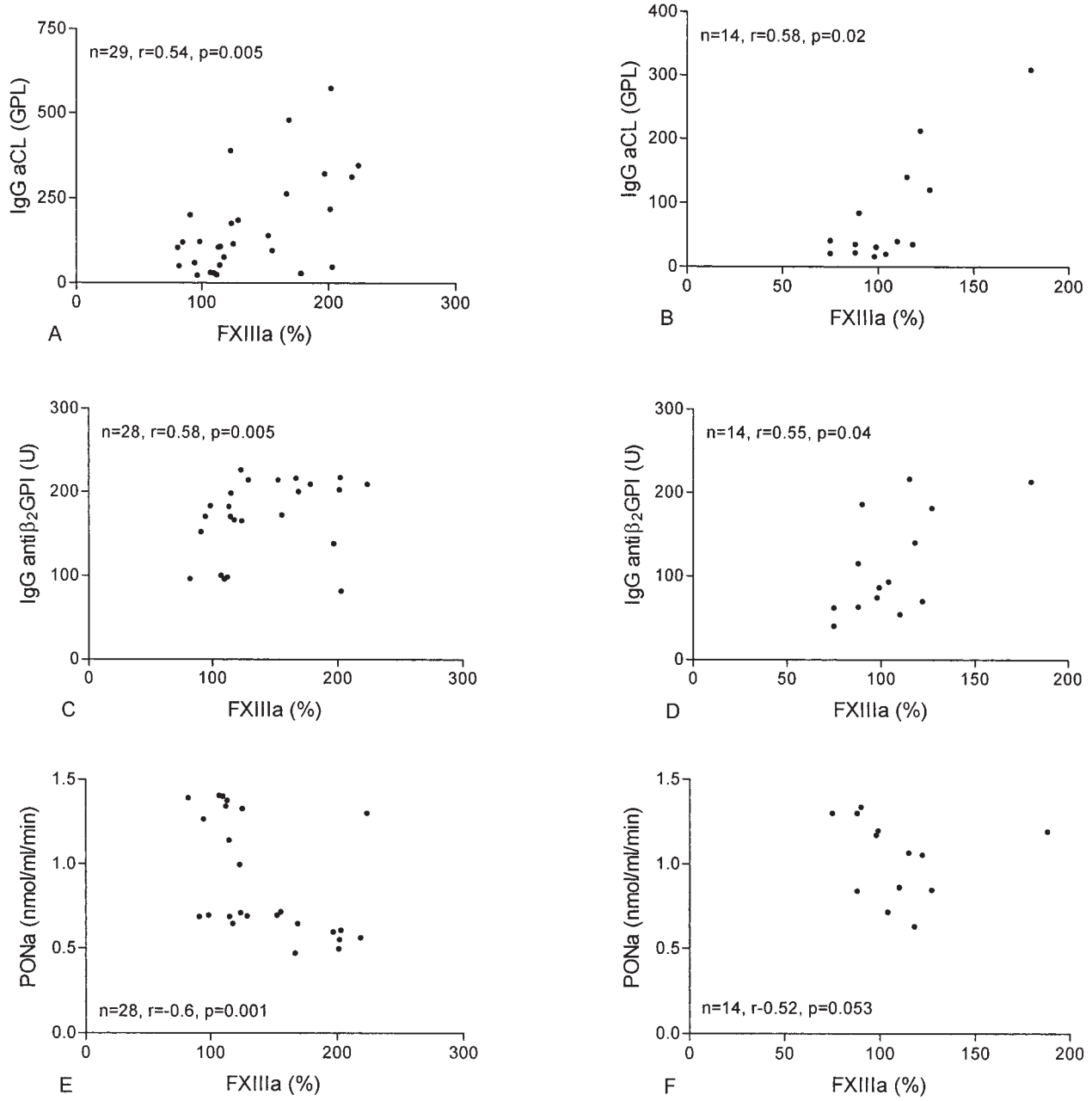

Figure 2. Spearman rank correlations between factor XIII activity (FXIIIa) and IgG anticardiolipin antibodies (aCL) in primary antiphospholipid syndrome (A) and in idiopathic carriers of antiphospholipid antibodies (B); FXIIIa and IgG anti-ß$_2$-glycoprotein I (anti-ß$_2$-GPI) in primary antiphospholipid syndrome (C) and aPL (D); FXIIIa and paraoxonase activity (PONa) in primary antiphospholipid syndrome (E) and aPL (F).

vide a rationale for the relationship we found between FXIIIa and IMT of carotid arteries in our primary APS group and to a lesser extent in thrombotic controls.

Nothing is known regarding FXIII regulation in primary APS, but the inverse relationship between FXIIIa and PON suggests that an imbalance in the antioxidant/oxidant ratio[19] as found in primary APS[20,21] could modulate FXIII activation. There is evidence that nitric oxide donors may inhibit FXIII activation in an antioxidant fashion[22], thus making oxidant activation of FXIII a possibility.

Activation of FXIII requires the detachment of its B subunit under the action of thrombin[10], the generation of which is heightened in primary APS[8]. Interestingly, the B subunit contains a number of Sushi domains[10], the same domains present on ß$_2$-GPI, target of aPL[23]. The relations between FXIIIa and IgG anti-ß$_2$-GPI in our antiphospholipid-positive groups may indicate a regulatory role for IgG anti-ß$_2$-GPI in FXIII activation.

As fibrinogen exhibits antioxidant properties[24], the negative relationship between PON and fibrinogen in primary

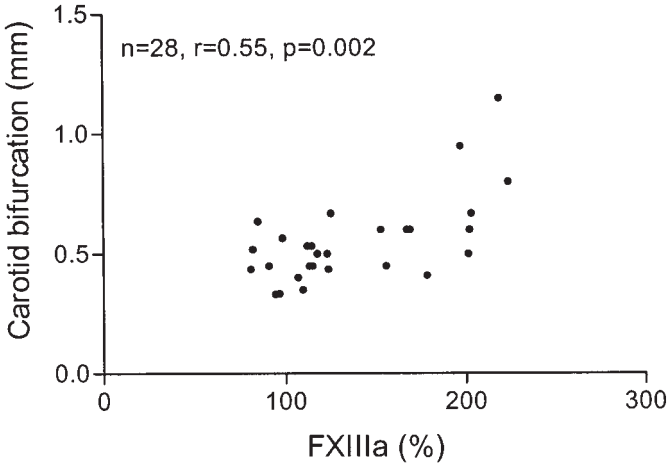

Figure 3. Spearman rank correlation between factor XIII activity (FXIIIa) and intima-media thickness (IMT) of carotid bifurcation in primary antiphospholipid syndrome.

APS may represent an adaptive response to hamper excess oxidation. Plasma fibrinogen was related to the number of arterial occlusions in APS[25], and is a determinant of carotid IMT in apparently healthy people[26] as well as in primary APS[8].

In conclusion, yet another coagulation step may be involved in the atherothrombotic tendency of APS. Although it does not seem exclusive to primary APS, the activity may operate at a higher set point because of the presence of aPL. Further investigation is required to determine whether anti-$ß_2$-GPI have direct and/or indirect effects on FXIII activation, and whether this is via cross-reactivity with the Sushi domains on FXIII B subunit or via oxidant stress associated with aPL.

REFERENCES

1. Wilson WA, Gharavi AE, Koike T, et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. Arthritis Rheum 1999;42:1309-11.
2. George J, Afek A, Gilburd B, et al. Atherosclerosis in LDL-receptor knockout mice is accelerated by immunization with anticardiolipin antibodies. Lupus 1997;6:723-9.
3. Manzi S, Selzer F, Sutton-Tyrrell K, et al. Prevalence and risk factors of carotid plaque in women with systemic lupus erythematosus. Arthritis Rheum 1999;42:51-60.
4. Roman MJ, Salmon JE, Sobel R, et al. Prevalence and relation to risk factors of carotid atherosclerosis and left ventricular hypertrophy in systemic lupus erythematosus and antiphospholipid antibody syndrome. Am J Cardiol 2001;87:663-6.
5. Svenungsson E, Jensen-Urstad K, Heimburger M, et al. Risk factors for cardiovascular disease in systemic lupus erythematosus. Circulation 2001;104:1887-93.
6. Bilora F, Boccioletti V, Girolami B, et al. Are antiphospholipid antibodies an independent risk factor for atherosclerosis? Clin Appl Thromb Hemost 2002;8:103-13.
7. Ames PRJ, Margarita A, Sokoll K, Weston M, Iannaccone L, Brancaccio V. Atherosclerosis in primary antiphospholipid syndrome [letter]. Ann Rheum Dis 2004;63:610-1.
8. Ames PRJ, Margarita A, Delgado Alves J, Tommasino C, Iannaccone L, Brancaccio V. Anticardiolipin antibody titre and plasma homocysteine level independently predict intima-media thickness of carotid arteries in subjects with idiopathic antiphospholipid antibodies. Lupus 2002;11:208-14.
9. Ames PRJ, Iannaccone L, Tommasino C, Brillante M, Cimino R, Brancaccio V. Coagulation activation and fibrinolytic imbalance in subjects with idiopathic antiphospholipid antibodies. A crucial role for acquired free protein S deficiency. Thromb Haemost 1996;76:190-4.
10. Ariens RA, Lai TS, Weisel JW, Greenberg CS, Grant PJ. Role of factor XIII in fibrin clot formation and effects of genetic polymorphisms. Blood 2002;100:743-54.
11. Greaves M, Cohen H, Machin SJ, Mackie I. Guidelines on the investigation and management of the antiphospholipid syndrome. Br J Haematol 2000;109:704-15.
12. Eckerson HW, La Du BN. A mathematical model for evaluating the reaction of paraoxon with human serum cholinesterase and with polymorphic forms of paraoxonase. Drug Metab Dispos 1984;12:57-62.
13. Heiss G, Sharrett AR, Barnes R, Chambless LE, Szklo M, Alzola C, for the ARIC investigators. Carotid atherosclerosis measured by B-mode ultrasound in populations: associations with cardiovascular risk factor in the ARIC study. Am J Epidemiol 1991;134:250-6.
14. van Wersch JW. Non-vitamin K-dependent clotting factors during oral anticoagulant treatment. Blood Coagul Fibrinolysis 1992;3:727-30.
15. 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;100:530-6.
16. Haverkate F. Levels of haemostatic factors, arteriosclerosis and cardiovascular disease. Vascul Pharmacol 2002;39:109-12.
17. Maresca G, Di Blasio A, Marchioli R, Di Minno G. Measuring plasma fibrinogen to predict stroke and myocardial infarction: an update. Arterioscler Thromb Vasc Biol 1999;19:1368-77.
18. Smith EB. Haemostatic factors and atherogenesis. Atherosclerosis 1996;124:137-43.
19. Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. Arterioscler Thromb Vasc Biol 2001;21:473-80.
20. Delgado Alves J, Ames PRJ, Donohue S, Norouz-Zadeh J, Ravirajan C, Isenberg DA. Antibodies to high-density lipoprotein and beta 2-glycoprotein I are inversely correlated to paraoxonase activity in systemic lupus erythematosus and primary antiphospholipid syndrome. Arthritis Rheum 2002;46:2686-94.
21. Ames PRJ, Nourooz-Zadeh J, Tommasino C, Alves J, Brancaccio V, Anggard EE. Oxidative stress in primary antiphospholipid syndrome. Thromb Haemost 1998;79:447-9.
22. Catani MV, Bernassola F, Rossi A, Melino G. Inhibition of clotting factor XIII activity by nitric oxide. Biochem Biophys Res Commun 1998;249:275-8.
23. Matsuura E, Igarashi M, Igarashi Y, et al. Molecular definition of human beta 2-glycoprotein I (beta 2-GPI) by cDNA cloning and inter-species differences of beta 2-GPI in alternation of anticardiolipin binding. Int Immunol 1991;3:1217-21.
24. Kaplan IV, Attaelmannan M, Levinson SS. Fibrinogen is an antioxidant that protects beta-lipoproteins at physiological concentrations in a cell free system. Atherosclerosis 2001; 158:455-63.
25. Ames PRJ, Pyke S, Iannaccone L, Brancaccio V. Antiphospholipid antibodies, haemostatic variables and thrombosis. A survey on 144 patients. Thromb Haemost 1995;73:768-75.
26. Lee AJ, Mowbray PI, Lowe GD, Rumley A, Fowkes FG, Allan PL. Blood viscosity and elevated carotid intima-media thickness in men and women: the Edinburgh Artery Study. Circulation 1998;97:1467-73.