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Elevated factor XIII level and the risk of peripheral artery disease

The prothrombotic state resulting from the elevation of certain clotting factors and suppression of fibrinolysis contribute to the risk of atherothrombotic diseases. Peripheral artery disease (PAD) has been associated with elevated plasma fibrinogen concentration, increased plasminogen activator inhibitor (PAI-1) level and decreased tissue plasminogen activator (tPA) activity.[1-3] Coagulation factor XIII (FXIII) circulates in association with fibrinogen and plays a central role in fibrin stabilization and fibrinolysis.[4] We have recently shown that in women with coronary stenosis and history of myocardial infarction adjusted plasma FXIII levels are elevated as compared to clinical controls, and in women, but not in men, elevated FXIII level represents a 2.5-3.0-fold increased risk of myocardial infarction.[5] In this study we investigated FXIII levels in patients with PAD and determined the risk of PAD conferred to patients by elevated FXIII level.

The study included 302 consecutive patients with PAD recruited over a 2-year period from the 3rd Department of Medicine, University of Debrecen. Patients with history of myocardial infarction, stroke, symptoms of angina and renal insufficiency were excluded. The patients were exempt of acute inflammation during the last two months before blood drawing and did not have any chronic inflammatory state, other than PAD. Finally, 278 patients (173 males and 105 females) with ankle brachial pressure index (ABPI) ≤0.9 remained in the study group (173 with Fontaine classification II and 105 with Fontaine classification III). Patients were compared to sex-matched clinical controls (n=278) who also presented at the hospital, but no significant health problem, other than diabetes mellitus in some of the patients, was diagnosed and ABPI was in the range of 0.91-1.3. The mean age±SD was 59.0±9.5 in the control and 64.3±12.2 in the patient group. The occurrence of diabetes mellitus was 20% and 25% among controls and patients respectively. Thirty percent of controls and 36% of patients were smokers. The differences were not statistically significant. Two hundred and seventeen PAD patients received aspirin and 33 patients received statins; since in the FXIII levels there was only a non-significant difference (<1%) between PAD patients on treatment and the rest of the patients, these groups were not analyzed separately. Eighty-eight percent of women were menopausal; none of them on hormonal replacement therapy. Ethical approval was obtained from the Ethics Committee of the University of Debrecen, and subjects gave their informed consent.

Plasma FXIII activity and antigen were measured by established methods[6,7] using commercially available reagent kits (REA-chrom FXIII assay and R-ELISA FXIII, Reanal-ker, Budapest, Hungary). In the measuring range, the CV for both assays was below 3%. Serum total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride, apo AI, apo B, lipoprotein (a) and high sensitivity C-reactive protein (hsCRP), plasma fibrinogen, homocysteine, folic acid and vitamin B12 were determined by routine laboratory methods and used for the determination of parameters independently associated with FXIII levels.

Neither FXIII activity nor FXIII antigen levels of clinical controls differed significantly from the reference interval established in our laboratory.[6,7] Mean FXIII activity and antigen levels, both non-adjusted and adjusted for the respective independently associated parameters, were moderately, but significantly higher in patients than in the clinical control group (Table 1). In a small (n=50) early study elevation of non-adjusted FXIII levels has also been observed in PAD patients.[8] However, in this case differences according to gender and severity of the disease were not analyzed. In our study PAD was associated with a statistically significant elevation of FXIII activity and antigen levels in women. In men with PAD, elevations were somewhat more moderate and in the case of FXIII antigen concentrations were not statistically significant. No difference in adjusted FXIII levels were found in PAD patients with Fontaine II and Fontaine III stage disease (*data not shown*). As determined by Pearson's method, ABPI values showed no significant correlation with FXIII activity or antigen level. One may conclude from these results that the existence of PAD and not its severity is associated with the elevation in FXIII levels. As opposed to controls, in the patient group there was a statistically significant correlation between fibrinogen and FXIII activity (r=0.273, $p<0.001$) or FXIII antigen (r=0.148, $p=0.014$) level. However, the calculated coefficients of determination (r^2) indicate that changes in fibrinogen level contributed to

only 7% and 2% to the changes in FXIII activity and antigen levels. Gender had no effect in this respect.

FXIII activity in the upper tertile (>120%) conferred a more than two-fold risk of PAD on females (Table 2). FXIII antigen in the upper tertile (>25.5 mg/L) also represented a two-fold risk of PAD in women, however in this case the *p* value was somewhat above the limit of statistical significance. In males elevated FXIII activity or antigen level did not increase the risk of PAD to a significant extent, although in the case of adjusted FXIII activity a tendency of increased risk was observed.

The reason for the elevation of FXIII levels in PAD is not known. Plasma FXIII consists of two potentially active A and two inhibitor/carrier B subunits (FXIII-A and FXIII-B).[4] FXIII-B is in excess to FXIII-A, the actual amount of FXIII A_2B_2 complex is determined by the amount of FXIII-A. Megakaryocytes are a major source of FXIII-A synthesis and circulating platelets contain a huge amount of FXIII-A.[4] Platelets may become damaged when pressed through occluded arteries and release their FXIII-A content into the circulation.

Impaired fibrinolysis caused by elevated PAI-1 level and decreased tPA activity increases the risk of PAD.[1,9-11] FXIII is a major regulator of fibrinolysis. Activated FXIII (FXIIIa) mechanically strengthens fibrin clot and makes it more resistant to shear forces and to fibrinolysis by cross-linking fibrin α-β and γ-chains and α2-plasmin inhibitor to fibrin. At elevated FXIII levels this mechanism could be more forceful and in PAD, could participate in impairing the fibrinolytic potential. Although it remains to be seen why the effect of elevated FXIII level is more prominent in women, the results support the suggestion that in atherothrombotic diseases the clotting/fibrinolytic system plays a more prominent role in females than in males.[12]

Table 1. Non-adjusted and adjusted factor XIII levels in male and female patients with peripheral artery disease.

	Clinical controls Mean (95% CI)	*PAD patients Mean (95% CI)*	*p value*
FXIII activity (%) NA			
total	108 (105-110)	115 (113-118)	<0.001
female	109 (105-112)	118 (114-122)	0.001
male	107 (104-111)	114 (111-117)	0.008
FXIII antigen (mg/L) NA			
total	23.4 (22.8-23.9)	24.5 (23.9-25.1)	0.007
female	23.4 (22.5-24.3)	25.2 (24.2-26.2)	0.009
male	23.3 (22.5-24.1)	24.1 (23.4-24.8)	0.16
FXIII activity (%) A			
total	107 (105-110)	116 (113-118)	<0.001
female	108 (105-113)	118 (114-122)	0.001
male	106 (103-110)	114 (111-118)	0.002
FXIII antigen (mg/L) A			
total	23.3 (22.8-23.9)	24.4 (23.8-25.0)	0.012
female	23.4 (22.4-24.4)	25.2 (24.3-26.0)	0.012
male	23.2 (22.5-24.0)	24.0 (23.2-24.7)	0.18

Values represent non-adjusted (NA) and adjusted (A) mean plasma FXIII activity or antigen (95% confidence interval). A multiple linear regression analysis was performed for FXIII activity and antigen to determine the parameters independently associated with FXIII levels and these parameters were used for adjustment. FXIII activity was adjusted for age, smoking, cholesterol, apo B, HDL-C and vitamin B12, and FXIII antigen values were adjusted for age, smoking, cholesterol, apo B, HDL-C and diabetes mellitus. The significance of differences in mean FXIII values between the clinical control and patient groups were tested by analysis of variance (ANOVA). Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS 11.5).

Table 2. The effect of FXIII levels in the upper tertile on the risk of peripheral artery disease in males and females.

	PAD patients versus clinical controls			
	Non-adjusted		*Adjusted*	
	*OR (95% CI)**	*p value*	*OR (95% CI)**	*p value*
FXIII activity				
female	2.341 (1.286-4.261)	0.005	2.316 (1.157-4.635)	0.02
male	1.300 (0.829-2.038)	0.25	1.646 (0.936-2.893)	0.08
FXIII antigen				
female	1.863 (0.947-3.668)	0.07	2.000 (0.943-4.240)	0.07
male	0.871 (0.516-1.470)	0.60	0.743 (0.389-1.418)	0.37

**The risk represented by non-adjusted and adjusted FXIII activity and antigen levels being in the upper tertile, as compared to the rest of the patients, was expressed as the odds ratio (OR) and 95 percent confidence interval. ORs were computed from the corresponding regression coefficient in the logistic regression model. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS 11.5).*

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Recurrent thromboembolism and major bleeding during oral anticoagulant therapy in patients with solid cancer: findings from the RIETE registry

Following an episode of venous thromboembolism (VTE), the risk of recurrent VTE and major bleeding complications during oral anticoagulant therapy with vitamin K antagonists (VKA) in cancer patients exceeds that observed in patients free from malignancy.[1-3] According to the results of several randomized clinical trials,[4-7] international guidelines recommend the use of sub-therapeutic doses of low-molecular-weight heparins (LMWH) for the long-term prevention of recurrent VTE in all cancer patients.[8-10] However, in clinical practice many clinicians still administer VKA to their cancer patients, especially to those with limited disease and longer life expectancy.

We determined the risk of recurrent VTE and major bleeding in a wide number of patients with and without cancer who were recruited in the international RIETE registry, had an initial treatment with (LMW)heparin overlapped by VKA (targeting an International Normalized Ratio [INR] between 2.0 and 3.0), and then had a 3-month follow-up.

Between March 2001 and May 2007, 18,883 consecutive patients with symptomatic, acute DVT or PE, as confirmed by objective tests were enrolled in the RIETE registry, and were, therefore, eligible for our investigation. Of these patients, 6,139 were excluded: 4,999 (of whom 1,496-29.9% - affected by cancer) because of treatment with antithrombotic drugs other than VKA, 838 because of lack of long-term antithrombotic treatment, 223 because of the development of manifest cancer during the 3-month follow-up period, and 79 because of hematologic malignancies. Of the remaining 12,744 patients, 11,365 were free from malignancy, 407 had cancer with distant metastases, and 972 had a more limited cancer disease. The incidence of recurrent VTE and major bleeding was calculated for patients with and without cancer, and then separately for cancer patients with and without distant metastases. The diagnosis of recurrent VTE and major bleeding was carried out according to widely accepted methods and criteria that have been extensively described elsewhere.[11,12] Odds ratios (OR) and their 95% confidence intervals (CI) were calculated for the clinical characteristics and the 3-month outcome for patients without malignancy in comparison to the whole group of cancer patients, and separately for those with and without distant metastases. Dichotomous variables were tested with χ^2 test and continuous variables with Student's t-test. In order to measure predictors of 3-month recurrent VTE and major bleeding, a multivariate analysis was carried out using a Cox proportional hazard analysis after adjusting for age, sex, and modality of clinical presentation (symptomatic DVT alone or symptomatic PE with or without DVT). Statistical analyses were conducted with SPSS for Windows Release 13.0 and Epidat 3.1.

Table 1 shows the main demographic and clinical characteristics of the study patients, separately for those without malignancy, cancer patients with distant metastases, and cancer patients with more limited disease. During the study period, 333 patients died: 189 (1.7%) belonging to the first group, 103 (25.3%) to the second group, and 41 (4.2%) to the third. During the first three months of VKA treatment, recurrent VTE developed in 154 patients without malignancy (1.4%, fatal in 16), in 27 cancer patients with distant metastases (6.6%, fatal in 3), and in 31 cancer patients with more limited disease (3.2%, fatal in 4). In comparison to patients without malignancy, the OR of recurrent VTE in the whole group of cancer patients was 3.2 (95% CI, 2.4-4.3), and in cancer patients with and without distant metastases was 5.2

Table 1. Main demographic and clinical characteristics of the study patients.

	No cancer	*Cancer without metastases*	*Cancer with metastases*	*OR (95% CI) cancer without metastases vs. no cancer*	*OR (95% CI) cancer with metastases vs. no cancer*
Patients, N	11365	972	407		
Clinical characteristics					
Gender (males)	5698 (50%)	553 (57%)	223 (55%)	1.3 (1.2-1.5)†	1.2 (1.0-1.5)
Body weight <65 kg	2500 (22%)	242 (25%)	127 (31%)	1.2 (1.0-1.4)†	1.6 (1.3-2.0)†
Age (mean, SD)	64.7 (17.3)	71.0 (11.6)†	67.7 (12.2)†	–	–
Risk factors for VTE					
Surgery	1292 (11%)	210 (22%)	42 (10%)	2.1 (1.8-2.5)†	0.9 (0.6-1.2)
Immobility ≥4 days	2629 (23%)	158 (16%)	61 (15%)	0.6 (0.5-0.8)†	0.6 (0.4-0.8)
Prior VTE	1950 (17%)	174 (18%)	73 (18%)	1.1 (0.9-1.2)	1.1 (0.8-1.4)
VTE characteristics					
Clinically overt PE	5217 (46%)	473 (49%)	165 (41%)	1.1 (1.0-1.3)	0.8 (0.7-0.9)†
Cancer characteristics					
Cancer >3 months earlier	-	702 (72%)	254 (62%)	–	–
Site of cancer,	–				
Lung		48 (4.9%)	59 (15%)	–	–
Breast		189 (19%)	56 (14%)	–	–
Gastrointestinal		188 (19%)	96 (24%)	–	–
Pancreas		4 (0.4%)	20 (4.9%)	–	–
Genitourinary		392 (40%)	125 (31%)	–	–
Cerebral		57 (5.9%)	6 (1.5%)	–	–
Other		94 (9.7%)	45 (11%)	–	–
Treatment					
Initial therapy, LMWH	10144 (89%)	875 (90%)	361 (89%)	1.1 (0.9-1.4)	0.9 (0.7-1.3)
Initial therapy, UFH	1053 (9.3%)	80 (8.2%)	39 (9.6%)	0.9 (0.7-1.1)	1.0 (0.7-1.5)
Inferior vena cava filter	117 (1.0%)	26 (2.7%)	14 (3.4%)	2.6 (1.7-4.1)†	3.4 (1.9-6.0)†

†*statistically significant.*