Published in final edited form as:

Thromb Haemost. 2008 August ; 100(2): 224–228.

Elevated plasma fibronectin levels associated with venous thromboembolism

Natalie M Pecheniuk¹, Darlene J Elias^{1,2}, Hiroshi Deguchi¹, Patricia M Averell², and John H Griffin¹

¹ Department of Molecular and Experimental Medicine, The Scripps Research Institute, Scripps Clinic, La Jolla, CA, 92037, USA

² Division of Chest and Critical Care Medicine, Department of Medicine, Scripps Clinic, La Jolla, CA, 92037, USA

Summary

Elevated plasma fibronectin levels occur in various clinical states including arterial disease. Increasing evidence suggests that atherothrombosis and venous thromboembolism (VTE) share common risk factors. To assess the hypothesis that high plasma fibronectin levels are associated with VTE, we compared plasma fibronectin levels in the Scripps Venous Thrombosis Registry for 113 VTE cases vs age and sex matched controls. VTE cases had significantly higher mean fibronectin concentration compared to controls (127% vs 103%, $p < 0.0001$); the difference was greater for idiopathic VTE cases compared to secondary VTE cases (133% vs 120%, respectively). Using a quartile cut-off of $>90\%$ of the control values, the OR for association of VTE for fibronectin plasma levels above the 90th percentile were 9.37 (95% CI 2.73–32.2; $p < 0.001$) and this OR remained significant after adjustment for sex, age, BMI, factor V Leiden and prothrombin nt20210A (OR 7.60, 95% CI 2.14–27.0; $p = 0.002$). Additionally, the OR's were statistically significant for both idiopathic and secondary VTE before and after these statistical adjustments. In summary, our results suggest that elevated plasma fibronectin levels are associated with VTE and extend the potential association between biomarkers and risk factors for arterial atherothrombosis and VTE.

Keywords

fibronectin; venous thromboembolism; thrombosis

Introduction

Fibronectin is a glycoprotein that exists as a dimer of two ~250kDa monomers and is present in two forms, soluble plasma fibronectin and the less-soluble cellular fibronectin. Fibronectin plays an important role in many cellular processes involving the extracellular matrix (ECM), eg., cell adhesion, cell migration and cell differentiation.¹ Besides its important cell adhesive activities that are mediated through integrins, fibronectin also has important interactions with various other molecules including heparin, collagen and fibrin indicating possible importance after trauma or inflammation.² The concentration of plasma fibronectin which is synthesized predominantly by hepatocytes is approximately 300 µg/mL.

Corresponding Author: John H Griffin, PhD., Department of Molecular and Experimental Medicine (MEM-180), The Scripps Research Institute, 10550 North Torrey Pines Rd, La Jolla CA 92037 USA, Phone (858) 784 8220; Fax (858) 784 2243, jgriffin@scripps.edu.

¹ Due to the wide range of roles played by fibronectin, the association of fibronectin plasma levels with various disease states, such as certain cancers, coronary artery diseases and sepsis, has been implicated.

Plasma fibronectin may have a role in arterial disease in the development of atherosclerotic plaques as it contributes to foam cell formation due to lipoprotein uptake by phagocytic cells.³ Several studies reported an increase in plasma fibronectin concentrations associated with coronary artery disease (CAD)^{4,5} and that plasma fibronectin levels were positively correlated with other arterial disease markers such as serum lipids, hypertension and body mass index (BMI).^{5,6} Fibronectin also plays an integral role in blood coagulation firstly as a substrate for FXIIIa which crosslinks fibronectin with fibrin, thereby enhancing the fibrin clot structure,^{7,8} and secondly as an integrin-binding protein that promotes platelet adhesion via eg. α Ib β 3.⁹ Fibronectin is essential for normal platelet thrombus initiation, growth and stabilization.⁹

Increasing evidence suggests that arterial and venous thrombotic disease share common risk factors.^{10,11} For example, dyslipidemia and dyslipoproteinemia marked by a decrease in high density lipoproteins (HDL) are associated with VTE in younger men¹² and elevated levels of apolipoprotein AI levels, the major protein of HDL, protect against the risk of VTE recurrence.¹³ Due to these common factors shared by both arterial atherothrombosis and deep venous thrombosis, we hypothesized that elevated plasma fibronectin levels are associated with VTE in a well defined cohort of VTE patients. The results presented here provide clear support for this hypothesis and extend the growing list of biomarkers and risk factors shared by arterial atherothrombosis and venous thrombotic disease.

Materials and Methods

Study Population

Patients with objectively documented deep venous thrombosis with or without pulmonary embolism were recruited from the Scripps Anticoagulation Service and the community as part of an ongoing case-control study (The Scripps Venous Thrombosis Registry). Information regarding the cohort has been described previously for male subjects.¹² Briefly, the inclusion criteria for the current study included females as well as males, age at thrombosis < 55 years old, > 3 months since diagnosis of acute thrombosis, a life expectancy of > 3 years and no known malignancies or use of lipid-lowering therapy. Age and sex matched healthy controls were recruited through the Scripps General Clinical Research Center's (GCRC) blood donation program. Participants in the blood donation program had normal complete blood count and negative HIV, hepatitis B and C testing. Controls were from the community but most were employees or former employees of Scripps. Clinical data collection included detailed medical history and the presence of risk factors for venous thrombosis. All subjects provided written informed consent and the protocol was approved by the Scripps Clinic Institutional Review Board. Fasting blood samples were collected from all individuals, and serum and EDTA plasma were prepared and stored at -70°C for further analysis.

In this study, 113 VTE patients (males $n=49$; females=64) and 113 age-sex matched controls were analyzed; the study cohort demographics are shown in Table 1. Idiopathic VTE was defined as an event not occurring within 90 days after surgery, trauma or major immobilization in both genders and in females without pregnancy or oral contraceptive use, was observed in half of our VTE cohort (49.6%). Significant differences in body mass index, factor V Leiden, family history of thrombosis, total cholesterol and LDL cholesterol were observed. Smoking status between VTE patients and controls was similar (data not shown) and diabetes was present in three patients.

Fibronectin Analysis

Plasma fibronectin concentrations were determined by ELISA using a rabbit polyclonal antibody to fibronectin. Briefly, 96-well maxisorp plates (Nunc, Rochester, NY) were coated with the rabbit anti-human fibronectin antibodies (AbCam, Cambridge, MA) at pH9.6 and were further blocked for non-specific binding with 1% casein, tris-buffered saline (TBS) (0.05M Tris, 0.1M NaCl, pH7.5)/0.1% Tween. Following a wash step (TBS/Tween), EDTA anticoagulated plasma samples and standards diluted in TBS/0.2% bovine serum albumin were added to the plates and incubated at room temperature for one hr. Biotinylated anti-human fibronectin antibody (AbCam, Cambridge, MA) was then added to the samples and allowed to incubate for one hr at room temperature followed by washing. Streptavidin-labeled horseradish peroxidase (Pierce, Rockford, IL) was added and allowed to incubate for one hr at room temperature prior to addition of o-phenylenediamine dihydrochloride (Sigma, St Louis, MO) for color development. The change in absorbance was determined on an Optimax microplate reader (Molecular Devices, Sunnyvale, CA) and the reaction was stopped with 0.5 M sulfuric acid and the endpoint absorbance was read at 490 nm after 5 minutes. All samples were tested in duplicate and at two different dilutions. The test sample concentrations were expressed as percentage when compared to a standard curve of pooled normal plasma (George King, Overland Park, KS). The pooled normal plasma fibronectin concentration had been previously determined to be 330 µg/mL using purified plasma fibronectin (kind gift from Dr. Deane Mosher, University of Wisconsin, Madison, WI) as a standard and also by using the described extinction co-efficient. Specificity of both the primary and secondary antibodies was verified using Western blotting analysis of various dilutions of normal pooled plasma.

Statistical Analysis

Contingency table analysis (using chi-squared) was used for categorical data. Continuous data (presented as mean $\pm$ standard deviation) was checked for normality distribution and analyzed using unpaired t-tests or Mann-Whitney tests as appropriate (Prism 4.0, GraphPad Prism, San Diego, CA). Logistic regression was used to determine the odd ratios for VTE at a plasma fibronectin level of greater than 90th percentile of the control group values. Adjustments for well-known VTE risk factors, age, BMI, factor V Leiden and prothrombin 20210A were also performed using logistic regression (MiniTab 15, State College, PA). Linear trend across percentiles were performed using chi-squared test for trend (Prism) to observe the risk of increasing fibronectin concentration for VTE. Correlations between parameters were performed using Pearson or Spearman analysis as appropriate (Prism).

Results

When VTE cases were compared to matched controls, cases had significantly higher mean fibronectin concentrations (126% vs 103%; $p<0.0001$) (Figure 1 and Table 2). Compared to controls with 103% fibronectin levels, the elevated fibronectin concentrations were more apparent for VTE patients with idiopathic thrombosis compared to secondary VTE cases that had a predisposing risk such as surgery, immobilization or oral contraceptive use (133% vs. 120%, respectively).

When analysed by sex, males had higher levels of fibronectin compared to females (control group 109% vs 98.7%, $p=0.038$ and VTE group 141% vs 116%, $p=0.0005$). However, both male and female VTE patients had significantly higher fibronectin concentrations compared to male and female controls, respectively (Table 2). Fibronectin levels also showed a positive correlation with age and BMI (controls $r=0.214$ and $p=0.02$, $r=0.294$ and $p=0.002$, respectively). No significant differences in fibronectin levels were observed with prior or current smoking status (data not shown).

The OR for the association of VTE with elevated fibronectin levels was calculated using a cut-off value of >90% of the controls values (Table 3 and figure 1). Fibronectin levels greater than the 90th percentile of the control cohort were associated with an increased VTE risk with an OR of 9.37 (95% CI 2.73–32.2). After adjustment for established VTE risk factors of age, BMI, factor V Leiden, prothrombin 20210A, the clear statistical significance remained (OR = 7.60 (95%CI 2.14–27.0), $p=0.002$) (Table 3). The OR values for patients with idiopathic VTE were also significant before and after adjustment for these variables (Table 3), however the OR values for secondary VTE patients was not. When the total cohort was separated by sex and analysed, the OR values based on the 90%-ile of controls before and after adjustment for the employed established VTE risk factors in males was significant (Table 3), however not in females. In spite of significant correlations of fibronectin levels with plasma lipid levels (data not shown), adjustment for total cholesterol and triglycerides in addition to adjustments for age, BMI, Factor V Leiden and prothrombin 20210A, did not significantly change the results (total cohort OR 8.67 95%CI 2.33–32.2; male cohort OR 9.60 95%CI 1.94–47.7; female cohort OR 2.15 95%CI 0.34–13.5). We note that there were few females in our cohort that had idiopathic VTE (25%) as compared to 80% of male VTE cases being idiopathic; therefore, the significance of the OR for the whole group is driven by the males and not the females. To evaluate the linear association of VTE risk with fibronectin concentration, the probability for trend was calculated using pentile analysis and was found to be significant ($p<0.0001$) for increasing fibronectin concentration in the total cohort.

Discussion

Investigations of plasma fibronectin levels have previously focused on arterial disease and arterial thrombosis and have usually found significantly higher levels associated with ischemic heart disease and coronary artery disease severity.⁴ Because growing evidence suggests that arterial and venous thrombotic disease share common biomarkers or risk factors,^{10–15} we hypothesized that elevated plasma fibronectin levels are associated with VTE risk. To test this hypothesis, we used the Scripps VTE Registry and a fibronectin ELISA to show that fibronectin plasma levels are increased in VTE when compared to controls. Furthermore, patients with idiopathic VTE have higher levels of plasma fibronectin (133% $p<0.001$; OR 3.17, 95% CI 2.86–60.7) compared to patients with VTE in the presence of known risk factors such as surgery, immobilisation or oral contraceptive use (120%, $p=0.003$).

The risk of thrombosis as determined as an OR for a patient's fibronectin level greater than the 90th percentile of the control group was approximately 9 times higher than for those with fibronectin levels below the 90th percentile of the control group (OR 9.37 (95%CI 2.73–32.2)). Our study confirms previous reports that suggested plasma fibronectin levels are significantly influenced by age and sex and that males have higher levels than females.¹⁶ Statistical significance remained after adjustments for age and sex in addition to the known VTE risk factors of BMI, factor V Leiden and prothrombin 20210A (OR 7.60 (95%CI 2.14–26.7)) and also in addition to lipid parameters (OR OR 8.67 95%CI 2.33–32.2). Additionally, the OR's were significant for both idiopathic and secondary VTE before and after adjustments for sex, age and the other known thrombotic risk factors. In addition, increasing fibronectin levels as assessed by pentile analysis was significant for increased risk of VTE ($p<0.0001$). We found a significant positive correlation of plasma fibronectin levels with systolic and diastolic blood pressure (data not shown) as previously reported.⁶ Overall, our study clearly suggests that elevated plasma fibronectin may be an important biomarker or risk factor for VTE.

A significant role for plasma fibronectin in animal models of arterial thrombosis is well described and has been viewed as due to its strong interactions with platelet integrins such as $\alpha_{IIb}\beta_3$ and $\alpha_5\beta_1$.^{17–19} Platelet thrombus formation in ex vivo experiments is enhanced by increasing fibronectin concentrations and appears dependent on fibrin cross-linked to fibronectin by factor XIIIa.⁹ When ferric chloride-induced arterial thrombosis is studied in genetically altered mice, mice heterozygous for fibronectin (<50% of normal) showed delayed platelet thrombus initiation and growth in the mesenteric arterioles.²⁰ Thrombi formed in mice with severe deficiency of plasma fibronectin show unstable thrombi and continuous platelet shedding that impedes the growth of the thrombus.²¹ The use of these animal models further confirms the role of fibronectin for enhancing thrombus growth, stability and adherence to the endothelium.²² In addition, in vitro perfusion experiments at both high and low shear rates, possibly representing arterial and venous flow conditions, suggest that plasma fibronectin stimulates binding of platelets to collagen and the subendothelium.²²

Animal models for mechanistic studies of venous thrombosis are much less well developed than models for arterial thrombosis. Based on observed pathologic analysis of venous clots and some animal injury studies, the prevailing views generally emphasize that whereas platelets play key roles for development of arterial thrombosis, venous blood clots usually comprise fibrin and erythrocytes (red thrombi) with minimal to modest platelet contributions.^{23–25} Nonetheless, VTE and arterial thrombosis increasingly share common biomarkers and/or risk factors, and the basis for this fact is not clear. Given the apparent causative role for plasma fibronectin in arterial thrombosis in murine injury models, it may be speculated that elevated plasma fibronectin, or elevated isoforms of fibronectin²⁶ in humans might be a causative risk factor and not just a biomarker. However, new mechanistic studies are needed to support this speculation.

The limitations of this study include the modest numbers of VTE cases and controls and the mixture of unprovoked VTE cases with provoked VTE cases in the Scripps Venous Thrombosis Registry. Further, our control cohort consisting of predominantly healthy medical or science employees may not be truly representative of the general population. Both prospective and retrospective studies that would replicate our findings, preferably with larger numbers of VTE cases and controls that are well stratified for unprovoked vs provoked thrombosis, would help increase confidence in our conclusion that elevated plasma fibronectin levels are linked to VTE.

In summary, we show here in our small pilot study that elevated plasma fibronectin levels are associated with VTE. Whether the elevated levels of plasma fibronectin directly contribute to risk or simply serve as a biomarker for VTE merits further investigation. Nonetheless our study raises the novel possibility that there may be a significant role for plasma fibronectin in the pathogenesis of venous thrombosis.

Acknowledgments

We would like to thank Drs. Jose Fernandez for helpful discussions and Dr. Deane Mosher for providing a sample of purified plasma fibronectin and helpful discussions.

Support: This work was supported by grants from the National Institutes of Health (HL-21544) and the Skaggs Medical Scholar Program.

References

1. Mosher, DF. Fibronectin. San Diego, CA: Academic Press; 1989.

2. Pankov R, Yamada KM. Fibronectin at a glance. *Journal of Cell Science* 2002;115:3861–3863. [PubMed: 12244123]
3. Falcone DJ, Mated N, Shio H, Minick R, Fowler SD. Lipoprotein-heparin-fibronectin-denatured collagen complexes enhance cholesteryl ester accumulation in macrophages. *The Journal of Cell Biology* 1984;99:1266–1274. [PubMed: 6480690]
4. Ekmekci H, Ekmekci OB, Sonmez H, Ozturk Z, Domanic N, Kokoglu E. Evaluation of fibronectin, vitronectin, and leptin levels in coronary artery disease: Impacts on thrombosis and thrombolysis. *Clin Appl Thromb Hemost* 2005;11:63–70. [PubMed: 15678274]
5. Orem C, Durmus I, Kilinc D, et al. Plasma fibronectin level and its association with coronary artery disease and carotid intima-media thickness. *Coron Artery Dis* 2003;14:219–224. [PubMed: 12702925]
6. Song KS, Kim HK, Shim W, Jee SH. Plasma fibronectin levels in ischemic heart disease. *Atherosclerosis* 2001;154:449–453. [PubMed: 11166778]
7. Mosher DF. Cross-linking of cold-insoluble globulin by fibrin-stabilizing factor. *J Biol Chem* 1975;250:6614–6621. [PubMed: 1158872]
8. Mosher DF. Action of fibrin-stabilizing factor on cold-insoluble globulin and alpha2-macroglobulin in clotting plasma. *J Biol Chem* 1976;251:1639–1645. [PubMed: 56335]
9. Cho J, Mosher DF. Role of fibronectin assembly in platelet thrombus formation. *J Thromb Haemost* 2006;4:2533–2541. [PubMed: 17002656]
10. Prandoni P, Bilora F, Marchiori A, Bernardi E, Petrobelli F, Lensing AW, Prins MH, Girolami A. An association between atherosclerosis and venous thrombosis. *N Engl J Med* 2003;348:1435–1441. [PubMed: 12686699]
11. Kyrle PA, Eichinger S. Deep vein thrombosis. *Lancet* 2005;365:1163–1174. [PubMed: 15794972]
12. Deguchi H, Pecheniuk NM, Elias DJ, Averell PM, Griffin JH. High-density lipoprotein deficiency and dyslipoproteinemia associated with venous thrombosis in men. *Circulation* 2005;112:893–899. [PubMed: 16087810]
13. Eichinger S, Pecheniuk NM, Hron G, et al. High-density lipoprotein and the risk of recurrent venous thromboembolism. *Circulation* 2007;115:1609–1614. [PubMed: 17372172]
14. Vavalle JP, Wu SS, Hughey R, Madamanchi NR, Stouffer GA. Plasma fibronectin levels and coronary artery disease. *J Thromb Haemost* 2007;5:864–866. [PubMed: 17266699]
15. Prandoni P. Venous thromboembolism and atherosclerosis: is there a link? *J Thromb Haemost* 2007;5 (Suppl 1):270–275. [PubMed: 17635736]
16. Stathakis N, Fountas A, Tsianos E. Plasma fibronectin in normal subjects and in various disease states. *J Clin Pathol* 1981;34:504–508. [PubMed: 7251892]
17. Plow EF, McEver RP, Collier BS, Woods VL Jr, Marguerie GA, Ginsberg MH. Related binding mechanisms for fibrinogen, fibronectin, von Willebrand factor, and thrombospondin on thrombin-stimulated human platelets. *Blood* 1985;66:724–727. [PubMed: 3875376]
18. Gardner JM, Hynes RO. Interaction of fibronectin with its receptor on platelets. *Cell* 1985;42:439–448. [PubMed: 2411420]
19. Hynes RO. Integrins: a family of cell surface receptors. *Cell* 1987;48:549–554. [PubMed: 3028640]
20. Matuskova J, Chauhan AK, Cambien B, et al. Decreased plasma fibronectin leads to delayed thrombus growth in injured arterioles. *Arterioscler Thromb Vascular Biol* 2006;26:1391–1396.
21. Sakai T, Johnson KJ, Murozono M, et al. Plasma fibronectin support neuronal survival and reduces brain injury following transient focal cerebral ischemia but is not essential for skin-wound healing and hemostasis. *Nature Med* 2001;7:324–330. [PubMed: 11231631]
22. Ni H, Yuen PST, Papalia JM, et al. Plasma fibronectin promotes thrombus growth and stability in injured arterioles. *Proc Nat Acad Sci USA* 2003;100:2415–2419. [PubMed: 12606706]
23. Bastida E, Escolar G, Ordinas A, Sixma JJ. Fibronectin is required for platelet adhesion and for thrombus formation on subendothelium and collagen surfaces. *Blood* 1987;70:1437–1442. [PubMed: 3663940]
24. Sevtitt S. The structure and growth of valve-pocket thrombi in femoral veins. *J Clin Path* 1974;27:517–528. [PubMed: 4138834]

25. Lopez JA, Kearon C, Lee AY. Deep venous thrombosis. Hematology Am Soc Hematol Educ Program 2004;439–456. [PubMed: 15561697]
26. Chauhan AK, Kisucka J, Cozzi M, et al. Prothrombotic effects of fibronectin isoforms containing the EDA domain. Arterioscler Thromb Vasc Biol 2008;28:296–301. [PubMed: 17991876]

Figure 1. Fibronectin Levels in VTE Patients and Controls

Fibronectin levels are expressed as a percentage compared to pooled normal plasma. Solid thick lines indicate mean values and dotted lines indicate the value for the 90th percentile of the control values. Odds Ratios (95% CI) are shown **A.** total cohort of VTE cases and controls (n=113 each); **B.** VTE cohort expressed as either idiopathic or secondary (those having confounding VTE risk such as surgery, see methods for more details).

Table 1

Study Population

Variables	Controls (M=49;F=64)	VTE (M=49;F=64)	p-value
Age, yr (SD)	45.3 (9.3)	45.0 (9.8)	0.85
Body-mass Index, kg m ⁻² (SD)	27.1 (5.3)	29.3 (6.6)	0.005 [†]
Ethnic Group, %			
Non-Hispanic White	92.5	92.5	1.0
Risk factors, number of subjects (%)			
Factor V Leiden	6 (5.3)	28 (24.8)	<0.001 [‡]
Prothrombin 20210A	4 (3.5)	9 (8.0)	0.15
Family History of VTE	7 (6.2)	25 (22.1)	0.001 [†]
Idiopathic VTE		56 (49.6)	
Clinical Lipids, mean (SD)			
Total Cholesterol, mg/dL	197 (39.9)	207 (41.1)	0.04 [*]
Triglyceride, mg/dL	124 (72.3)	131 (84.6)	0.77
HDL-C, mg/dL	57.2 (16.5)	54.3 (18.5)	0.09
LDL-C, mg/dL	114 (33)	125 (37)	0.047 [*]
LDL-C/HDL-C ratio	2.17 (0.87)	2.57 (1.18)	0.021 [*]

* 0.01 ≤ P < 0.05;

[†] 0.001 ≤ P < 0.01;

[‡] P < 0.001

Table 2

Fibronectin Levels in VTE Patients and Controls

Cohort	Controls		VTE		p-value
	n	Mean (SD)	n	Mean (SD)	
Total	113	103.2 (25.10)	113	126.6 (39.04)	<0.001 [‡]
Idiopathic VTE			56	133.0 (41.96)	<0.001 [‡]
Secondary VTE			57	120.4 (35.21)	0.003 [‡]
Males	49	109.0 (25.06)	49	141.0 (41.52)	<0.001 [‡]
Females	64	98.66 (24.37)	64	115.6 (33.36)	0.003 [‡]

Results are shown as mean values (standard deviation) expressed as percentage compared to normal pooled plasma.

* 0.01 ≤ P < 0.05;

[‡] 0.001 ≤ P < 0.01;

[‡] P < 0.001

Table 3

Quartile-based ORs for VTE with levels of Fibronectin above the 90th percentile of controls.

Cohort	Unadjusted Values		Adjusted Values	
	OR (95% CI)	P	OR (95% CI)	P
Total	9.37 (2.73–32.2)	<0.001 [‡]	7.60 (2.14–26.7)	0.002 [‡]
Idiopathic	13.2 (2.86–60.7)	0.001 [‡]	12.4 (2.59–59.4)	0.002 [‡]
Secondary	5.81 (0.7–48.2)	0.103	3.35 (0.39–32.2)	0.264
Males	7.43 (2.00–27.6)	0.003 [‡]	7.66 (1.93–30.5)	0.004 [‡]
Females	4.43 (0.90–21.7)	0.067	2.69 (0.45–16.2)	0.279

OR (95%CI) values adjusted for age, BMI, factor V Leiden and prothrombin 20210A are also shown.

* 0.01 ≤ *P* < 0.05;

[‡] 0.001 ≤ *P* < 0.01;

[‡] *P* < 0.001