Increased Levels of Factor VIII and Fibrinogen in Patients with Venous Thrombosis Are not Caused by Acute Phase Reactions

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Summary

Factor VIII activity (factor VIII:C) levels ≥150 IU/dl are associated with a 5- to 6-fold increased risk of venous thrombosis compared to levels <100 IU/dl, and fibrinogen levels ≥5.0 g/l increase the thrombosis risk 4-fold. These high levels are present in 25% resp. 3% of the patients with a first episode of venous thrombosis. These findings were based on measurements after the thrombotic event, so the factor VIII and fibrinogen levels in thrombosis patients may have been influenced by acute phase reactions or ongoing inflammatory responses. In the present study we measured plasma C-reactive protein (CRP) as a sensitive marker of an acute phase reaction in 474 thrombosis patients and 474 age- and sex-matched healthy controls, that were part of the Leiden Thrombophilia Study (LETS). Mean and median CRP levels were higher in thrombosis patients than in the controls, suggesting inflammation in some patients. CRP affected both factor VIII and fibrinogen levels, in patients and controls alike. After adjustment for the effect of CRP, high factor VIII:C levels still increased the thrombosis risk 6-fold and high fibrinogen levels 4-fold, which is for both very similar to the risk before correction for CRP levels. These results show that although systemic inflammation may be present in some of the patients, elevated levels of factor VIII:C and fibrinogen were in general not caused by acute phase reactions. This further supports a causal relationship between both high factor VIII:C and fibrinogen levels and venous thrombosis.

Introduction

Factor VIII procoagulant activity (factor VIII:C) levels ≥150 IU/dl are associated with a 5- to 6-fold increased risk of venous thrombosis when compared to levels below 100 IU/dl (1). The prevalence of elevated factor VIII levels is high: 25% of the patients with a first episode of venous thrombosis and 11% of the healthy population (1). Furthermore, we and others have shown that elevated factor VIII:C levels, as measured with a one-stage assay, are highly correlated with factor VIII antigen (factor VIII:Ag) levels. This suggests that the observed elevation of factor VIII:C levels is not the result of activation of the coagulation system during the blood collection procedure (2, 3). Indeed, also factor VIII:Ag levels ≥150 IU/dl were found to be associated with a 5-fold increased risk of venous thrombosis (2).

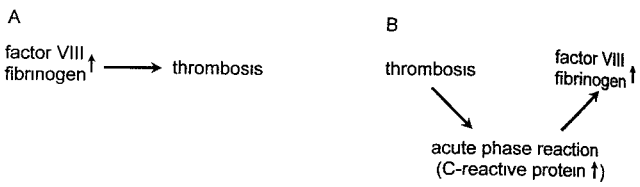

Fig. 1 Hypothetical pathways for the relation between elevated factor VIII levels and venous thrombosis

Fibrinogen has also been associated with an increased risk for thrombosis (4). We previously reported that fibrinogen levels ≥5.0 g/l increase the risk 3- to 4-fold, compared to the reference category (<3.0 g/l). Such high levels were present in 3% of the thrombosis patients and 1% of the controls.

The conclusions that elevated factor VIII and fibrinogen levels are associated with an increased thrombosis risk, are based on measurements in patients after the thrombotic event; it can therefore not be excluded that these measurements were influenced by ongoing inflammatory processes triggered by the thrombosis itself or its sequelae. So, elevated factor VIII and fibrinogen levels may either be the cause of thrombosis (Fig. 1A), or the consequence of chronic inflammatory reactions that follow the thrombosis (Fig. 1B).

In the present study we investigated whether factor VIII:C and fibrinogen levels in thrombosis patients are associated with C-reactive protein levels, which is a sensitive marker for acute phase processes underlying inflammation (5). We measured these levels in 474 thrombosis patients and 474 healthy controls, that were part of the Leiden Thrombophilia Study (LETS).

Patients and Methods

Study Population

The patients and controls included in this study came from a population-based case-control study on venous thrombosis, the Leiden Thrombophilia Study (LETS) (1). This study includes 474 unselected consecutive out-patients, younger than 70 years who were referred for anticoagulant treatment after a first, objectively confirmed episode of deep-vein thrombosis and who did not have an underlying malignancy. The median time between the occurrence of the deep-vein thrombosis and blood collection was 18 months (range,

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Table 1 Mean and median plasma C-reactive protein levels in thrombosis patients and healthy controls

	C-reactive Protein (mg/l)		
	Mean	95% CI	Median
Patients (474)	1 49	1 32-1 68	1 42
Controls (474)	1 12	1 00-1 25	1 09

6-48 months). Each thrombosis patient provided his or her own sex- and age-matched healthy control subject according to predefined criteria (6). The mean age for patients and controls was 47 years (range 16 to 70 years for patients, 16 to 73 for controls).

Blood Collection and Plasma Assays

Blood was collected in tubes containing 0.106 mmol/l trisodium citrate. Plasma was prepared by centrifugation for 10 min at 2000 × g at room temperature and stored at -70° C. Factor VIII:C levels were measured by a one-stage clotting assay (1). The fibrinogen concentration was determined according to the von Clauss method using Dade® thrombin (Baxter, Miami, Fl). C-reactive protein was measured by a sandwich enzyme immunoassay (Kordia, Leiden, The Netherlands), based on two polyclonal rabbit antibodies against CRP. The lower detection level of this test is 0.05 mg/l, so it is sufficiently sensitive to measure CRP levels within the normal range (0.2-6.0 mg/l). Pooled normal plasma was used as a reference.

Statistical Analysis

For calculations of the mean CRP level, C-reactive protein values were logarithmically transformed, because the distribution of the values was skewed. Geometric mean concentrations were calculated for both patients and controls. The non-transformed CRP levels were entered in the regression model, because logarithmic transformation led to the same results. Factor VIII:C levels were stratified into quartiles based on patient and control values (1), fibrinogen into four groups, based on an increase of 1 g/l in the plasma fibrinogen level (4), to facilitate comparisons with our earlier reports. We calculated risks relative to the group with the lowest level. As covariates we included smoking and body mass index. Multivariate analysis was performed with a conditional logistic model, that calculated matched odds ratios (OR) and 95% confidence intervals (95% CI) as a measure for the relative risk.

Results

Among the controls, mean CRP level was 1.12 mg/l, with a range between 0.06 and 58 mg/l. Smoking, age, and body mass index (BMI) were associated with the CRP level: smokers (n = 241) had higher levels than nonsmokers (n = 607) (1.30 vs. 1.03 mg/l). An increase in BMI of 1 kg/m^2 increased the CRP level by 0.18 mg/l. For every successive 10 years of age, the mean CRP level increased 0.7 mg/l. Plasma CRP levels were the same in men (1.31 mg/l, n = 404) and women (1.28 mg/l, n = 544).

We compared the CRP values in thrombosis patients and controls. Table 1 shows that both the mean and median level of C-reactive protein were higher in patients (1.49 mg/l, 95% CI, 1.32-1.68) than in controls (1.12 mg/l, 95% CI, 1.00-1.25). This difference was not affected by body mass index or smoking status. Fifty-three patients (11%) had plasma CRP levels above the 95th percentile of the control values (9.75 mg/dl). When the analysis was restricted to CRP levels below 9.75 mg/dl, the difference in CRP levels between patients and controls disappeared.

Next, we analyzed the thrombosis risk of both factor VIII:C and fibrinogen after adjusting for CRP. We adjusted for CRP first by entering factor VIII, fibrinogen and CRP as continuous variable in the logistic regression model, and second as categorized variables. As unadjusted continuous variable, the odds ratio for a 10 IU/dl increase in factor VIII:C level was 1.19 (95% CI, 1.14-1.23) and this risk remained the same after adjustment for CRP levels (OR 1.19, 95% CI, 1.14-1.23). Table 2 shows that after categorization, factor VIII:C levels ≥150 IU/dl gave a nearly sevenfold increased risk of venous thrombosis as compared with the reference category (factor VIII:C <100 IU/dl). After adjustment for categorized CRP levels, the risk of elevated factor VIII:C levels remained virtually unaffected. The adjusted odds ratio of factor VIII:C levels ≥150 IU/dl was 6.7 (95% CI, 4.0-11.2). The thrombosis risk of fibrinogen increased by 1.38 (95% CI, 1.16-1.64) for every g/l increase. Adjustment for CRP levels did not change this risk estimate materially (OR 1.28, 95% CI, 1.05-1.56). Also the thrombosis risk of elevated fibrinogen levels remained the same after adjustment for CRP levels: subjects with a fibrinogen level above 5 g/l had a fourfold higher risk than those in the reference category (<3.0 g/l) (Table 2).

We investigated the influence of CRP on factor VIII:C and fibrinogen levels by linear regression. The influence of CRP (mg/l) on factor VIII:C levels (IU/dl) was weak, without a clear difference between thrombosis patients and controls (regression coefficient patients 1.49, 95% CI, 0.98-2.00; regression coefficient controls 1.22, 95% CI, 0.67 to 1.77). For fibrinogen, no differences in the influence of CRP between patients and controls were found. The regression coefficient was 0.05 (95% CI, 0.04-0.06) in patients and 0.07 (95% CI, 0.05-0.07) in controls.

Because in this study all samples were drawn after the thrombotic event, we investigated whether elevated CRP levels in the patients might have been caused by the thrombotic event. If a relation exists between the thrombosis and a subsequent inflammatory response, one

Table 2 Unadjusted and adjusted thrombosis risk for factor VIII:C and fibrinogen levels

Variable	cases (474)	controls (474)	univariate OR	multivariate OR#	95% CI
Factor VIII C (IU/dl)					
<100	85	187	1*	1*	
100-125	146	147	2 7	2 8	1 8-4 1
125-150	130	91	3 6	3 6	2 4-5 5
≥150	113	49	6 7	6 7	4 0-11 2
Fibrinogen (g/l)					
<3 0	150	174	1*	1*	
3 0-3 9	225	243	1 1	1 1	0 8-1 4
4 0-4 9	72	50	1 7	1 6	1 0-2 6
≥5 0	27	7	4 4	4 3	1 7-10 5

*Reference category #Factor VIII C and fibrinogen levels adjusted for categorized C-reactive protein levels

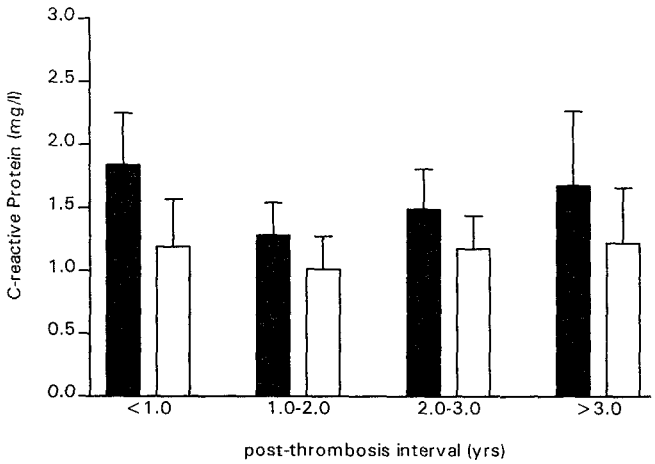

Fig. 2 Mean C-reactive protein levels (± SE) in thrombosis patients (black bars) and in controls (white bars) at different time-intervals between the thrombotic event and the time of venepuncture

would expect an inverse relationship between CRP levels and the time between the thrombotic event and the venepuncture. Fig. 2 shows the mean CRP levels for four different post-thrombosis intervals, here defined as the time between the occurrence of the thrombosis and the collection of the plasma samples. We found no association between CRP levels and post-thrombosis intervals, although the first interval, <1 year after the thrombosis, seemed to have a higher CRP level (1.82 mg/l) than those collected in the other intervals. The mean CRP levels were always higher in patients than in controls.

Discussion

Elevated factor VIII levels substantially increase the risk of venous thrombosis (1). Blood group and von Willebrand factor (vWF) are important determinants of the factor VIII level in plasma, and factor VIII appears to be the final effector in promoting deep-vein thrombosis. High levels of fibrinogen are also associated with an increased risk of thrombosis (4). We measured an established acute phase marker, C-reactive protein (5), to assess if these associations might be the result of acute phase reactions. Although the mean CRP levels were higher in thrombosis patients than in controls, the effect of CRP levels on factor VIII and fibrinogen levels did not differ between patients and controls. The thrombosis risk of both elevated factor VIII:C and fibrinogen remained virtually unchanged after adjustment for CRP levels. We therefore conclude that the previously reported associations between high levels of factor VIII and fibrinogen are not caused by post-thrombotic inflammatory responses.

In our patient population, most CRP values fell within the normal range, indicating that overall an acute-phase response was absent (7, 8). The minimum interval between the thrombotic event and the time of the venepuncture was six months. Comparing four different time-intervals, we found no clear relation between the average CRP level and the post-thrombotic time-interval. Mean CRP levels were higher in thrombosis patients than in controls, independent of the time elapsed since the thrombosis. This difference was the result of the higher number of patients with elevated CRP levels: 11% of the patients had CRP levels above the 95th percentile of the control value (9.75 mg/l). This finding suggests that systemic inflammation, although infrequent, was more prominent among the patients than among the controls. CRP levels may have been affected by the post-thrombotic syndrome, a disease that occurs in about 30-50% of the thrombosis patients (9, 10). In addition, elevated CRP levels may be a risk factor itself for venous thrombosis. In the latter case, elevated CRP levels were already present before the occurrence of the thrombosis. This possibility is supported by the effect of CRP on tissue factor expression in monocytes (11), which at least in vitro might result in a procoagulant state. The only prospective study that studied elevated CRP levels and venous thrombosis, the Physicians Health Study (12), found no relation. It is unclear whether the elevated CRP levels in our patients are persistent, and what the clinical consequences of these elevations are.

O'Donnell et al. found no clear relation between high factor VIII:C levels and C-reactive protein in thrombosis patients (3). Our results agree with, and extend these observations, because we analyzed the influence of CRP on factor VIII and fibrinogen comparing patients and controls.

Theoretically, there are two approaches to assess the relation between high factor VIII and fibrinogen levels and the risk of venous thrombosis, when there is concern that the thrombosis itself affected the clotting factor levels, e.g., by chronic inflammatory responses. The first is to measure and to adjust for systemic inflammation, in this case by CRP. When high factor VIII and fibrinogen levels in thrombosis patients are the result of inflammation, no effect will be visible conditional on the CRP levels and correction will lead to attenuation of the observed thrombosis risk. We showed that this is not the case. A second approach is to assess the effect of genetic determinants of clotting factor levels, since they, obviously, cannot be affected by the occurrence of thrombosis (13). Our previous observation that blood group, which acts via vWF, affects the factor VIII level (1), can be viewed as such an analysis, i.e., showing an unconfounded effect of factor VIII levels which is not brought about by post-thrombotic phenomena. However, to what extent high factor VIII levels are genetically determined is still unclear. Factor VIII:C levels show a familial clustering, which remains after correction for blood group and vWF (14), but a molecular basis of elevated factor VIII levels within the factor VIII gene has not been found (15, 16). The next step will be to analyze whether there is a relationship between elevated factor VIII levels and familial thrombophilia, in order to investigate the heritability of high factor VIII levels and its influence on the thrombosis risk in families.

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