

Factor V Antigen Levels and Venous Thrombosis Risk Profile, Interaction With Factor V Leiden, and Relation With Factor VIII Antigen Levels

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Abstract—Clotting factor V has a dual function in coagulation: after activation, procoagulant factor V stimulates the formation of thrombin, whereas anticoagulant factor V acts as a cofactor for activated protein C (APC) in the degradation of factor VIII/VIIIa, thereby reducing thrombin formation. In the present study, we evaluated whether plasma factor V levels, either decreased or increased, are associated with venous thrombosis. High procoagulant factor V levels may enhance prothrombinase activity and increase the thrombosis risk. Low anticoagulant factor V levels could reduce APC-cofactor activity in the factor VIII inactivation (APC-resistant phenotype), which might also promote thrombosis. Low factor V levels in combination with factor V Leiden could lead to a more severe APC-resistant phenotype (pseudohomozygous APC resistance). To address these issues, we have measured factor V antigen (factor V:Ag) levels in 474 patients with thrombosis and 474 control subjects that were part of the Leiden Thrombophilia Study (LETS). Factor V:Ag levels increased by 7.6 U/dL for every successive 10 years of age. Mean factor V:Ag levels were 134 (range 41 to 305) U/dL in patients and 132 (range 47 to 302) U/dL in controls. Neither high nor low factor V:Ag levels were associated with venous thrombosis. We found that factor V:Ag and factor VIII antigen levels in plasma were correlated, but factor V did not modify the thrombotic risk of high factor VIII levels. The normalized APC ratio was not influenced by the factor V:Ag level in subjects with or without factor V Leiden. We conclude that neither low nor high factor V:Ag levels are associated with venous thrombosis and that factor V:Ag levels do not mediate the thrombotic risk associated with high factor VIII levels. (*Arterioscler Thromb Vasc Biol.* 2000;20:1382-1386.)

Key Words: factor V ■ venous thrombosis ■ factor V Leiden ■ factor VIII

Deep-vein thrombosis is a common disorder with an incidence in the general population of ≈ 1 in 1000 individuals per year.^{1,2} Despite growing insight into inherited as well as acquired risk factors for thrombosis, the cause of many thrombotic episodes remains unknown. A single point mutation in the factor V gene (G1691A, R506Q, factor V Leiden)³ results in a reduced sensitivity of plasma factor Va to inactivation by activated protein C (APC)⁴ and is present in $\approx 20\%$ of white patients with deep-vein thrombosis.⁵ Coagulation factor V has a central role in procoagulant and anticoagulant pathways: factor V is activated by factor Xa or thrombin⁶⁻⁸ and markedly accelerates the activation of prothrombin by factor Xa before factor Va is degraded by APC.^{9,10} In addition, factor V (but not factor Va) has been reported to act as a cofactor of APC in the proteolytic degradation of both factor VIII and factor VIIIa.^{4,11-13}

Whether the level of factor V in plasma affects the risk of thrombosis is unclear, although, hypothetically, high as well as low factor V levels may increase the thrombotic risk. Elevated factor VIII levels are associated with an increased

risk for thrombosis,¹⁴ and factor V is, like factor VIII, an important cofactor in one of the steps of the coagulation cascade. Therefore, high plasma levels of factor V might lead to an increased prothrombinase activity and increased risk of thrombosis. In 1966, Gaston¹⁵ reported a family with an association between elevated factor V levels and venous thrombosis.

Low factor V levels are associated with a reduced APC cofactor activity in the inactivation of factor VIII/VIIIa.¹¹⁻¹³ This results in an APC-resistant phenotype and therefore might be associated with an increased risk of thrombosis, especially when the factor V/factor VIII ratio is low. In that case, low factor V levels would increase the risk of thrombosis.

Finally, we have to consider the influence of factor V levels on the thrombotic risk of carriers of factor V Leiden. Reduced expression of factor V by the non-factor V Leiden allele (low factor V levels) might result in a more severe APC-resistant phenotype, as has been reported for patients pseudohomozygous for APC resistance (combined heterozy-

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gous factor V deficiency and heterozygous factor V Leiden mutation).^{16–19} Similarly, enhanced expression of factor V by the non-factor V Leiden allele (high factor V levels) might result in a less severe APC-resistant phenotype.

To address these issues, we measured the factor V antigen (factor V:Ag) level in 474 patients with thrombosis and 474 healthy control subjects that were part of the Leiden Thrombophilia Study (LETS).²⁰

Methods

Study Population

The patients and control subjects included in the present study came from a population-based case-control study of venous thrombosis (LETS).²⁰ The present study includes 474 unselected consecutive outpatients aged <70 years who were treated with anticoagulants after a first episode of objectively confirmed 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 6 to 48 months). Each thrombotic patient provided his or her own sex- and age-matched healthy control subject according to predefined criteria.¹⁴ The mean age for patients and controls was 47 years (range 16 to 70 years for patients and 16 to 73 years 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 minutes at 2000g at room temperature and stored at -70°C in 1.5 mL aliquots. Factor V:Ag was measured by an in-house-developed sandwich-type ELISA with 2 monoclonal antibodies, both with a high affinity for (activated) factor V. The monoclonal antibodies, denoted V-6 and V-9, were selected from a panel of antibodies isolated on the light chain of factor V, as demonstrated by Western blot analysis with the use of purified factor Va under reducing conditions. Ninety-six-well plates were coated with monoclonal antibody V-6 (at a concentration of 3 mg/mL in 0.1 mol/L NaHCO_3 and 0.5 mol/L NaCl, pH 9.0) and left overnight at 4°C . Plasma samples were diluted 1/100 to 1/400 in 0.05 mol/L triethanolamine, 0.1 mol/L NaCl, 0.01 mol/L EDTA, and 0.1% Tween 20, pH 7.5, and 100 μL per sample was incubated for 3 hours in the coated wells. Monoclonal antibody V-9, labeled with horseradish peroxidase (Zymed Laboratories Inc) and diluted to 2 mg/mL in 0.05 mol/L triethanolamine, 0.1 mol/L NaCl, 0.01 mol/L EDTA, and 0.1% Tween 20, pH 7.5, was used for the detection of immobilized factor V (2-hour incubation). In the final step, 0.42 mmol/L 3,3',5,5'-tetramethylbenzidine, 0.1 mol/L sodium acetate, and 1.1 mol/L H_2O_2 , pH 5.5, were added, and the reaction was stopped after 20 minutes with 100 μL H_2SO_4 . After every step, plates were washed 4 times with 0.05 mol/L triethanolamine, 0.1 mol/L NaCl, and 0.1% Tween 20, pH 7.5. Pooled normal plasma (PNP), prepared from the plasma of 60 healthy volunteers (mean age 40 years) and diluted 1/50 to 1/3200, was used as a reference and defined to contain 100 U/dL. In each analytical run, we included 2 samples of an in-house PNP and 2 samples of a commercial high factor V plasma (Mallinckrodt Baker) as controls. PNP contained, on average, 103 ± 8 U/dL factor V:Ag (interassay variation 8.2%, intra-assay variation 6.7%). High factor V plasma contained, on average, 136 ± 12 U/dL factor V:Ag (interassay variation 8.9%, intra-assay variation 5.2%). Results were the same for plasma and serum samples (after correction for the dilution with the anticoagulant) of the same individual and not substantially altered by repeated freezing and thawing.

Factor VIII antigen levels have been measured by a sandwich-type ELISA with two monoclonal antibodies directed against the light chain of factor VIII.²¹ CLB Cag 117 was used as a catching antibody, and CLB-A was used as a tagging antibody. Both antibodies were kindly provided by Dr J.A. van Mourik (Department of Blood Coagulation, CLB, Sanquin Blood Supply Foundation, Amsterdam, the Netherlands). PNP, calibrated against the World Health Organization standard (91/666 ratio) for factor VIII antigen (factor VII:Ag), was used as a reference. The sensitivity of plasma to APC was

TABLE 1. Risk of Venous Thrombosis and Factor V:Ag Levels

Factor V:Ag, U/dL	Patients, n (%)	Controls, n (%)	OR*	95% CI
<110	113 (23.8)	118 (24.9)	1†	
110–130	118 (24.9)	127 (26.8)	1.0	0.7–1.4
130–150	111 (23.4)	122 (25.7)	1.0	0.7–1.4
≥ 150	132 (27.9)	107 (22.6)	1.3	0.9–1.8

*Matched OR.

†Reference category.

determined as previously described²² and expressed in a normalized APC sensitivity ratio.²²

Statistical Analysis

An unconditional logistic model was used to calculate odds ratios (ORs) as a measure of relative risk with 95% CIs derived from the model. This OR estimates the risk of venous thrombosis in the presence of a risk factor relative to the absence of the particular risk factor, the reference category. Factor V and factor VIII levels were entered into the logistic model as categorized variables. The cutoff points were 100, 125, and 150 U/dL for factor VIII and 110, 130, and 150 U/dL for factor V. These cutoff points corresponded approximately to quartiles.

Results

Factor V:Ag Levels

The total mean factor V:Ag level was 132 U/dL, with a range between 41 to 305 U/dL. The factor V:Ag levels were normally distributed. In multiple regression, age and smoking were associated with the plasma factor V:Ag level. For every successive 10 years of age, the factor V:Ag level increased 7.6 (95% CI 6.1 to 9.1) U/dL. Smokers had 8.3 (95% CI 4.0 to 12.6) U/dL higher factor V:Ag levels than did nonsmokers.

Factor V:Ag Levels and Venous Thrombosis

The mean factor V:Ag level was 134 (range 41 to 305) U/dL for the patients and 132 (range 47 to 302) U/dL for the controls. Table 1 presents the ORs of patients and controls after stratification of the factor V:Ag levels into 4 groups. The relative risk of factor V:Ag levels ≥ 150 U/dL was 1.3 (95% CI 0.9 to 1.8) compared with the reference category (factor V:Ag level <110 U/dL). The data of Table 1 also show that reduced factor V levels are not associated with an increased risk of thrombosis. The influence on thrombosis of either low or high factor V:Ag levels was analyzed in more detail by stratification of the patients and controls into 10 groups; we calculated the relative risk for the patients with high factor V:Ag compared with the lowest category of factor V:Ag (<93 U/dL). Even the highest factor V:Ag level (>171 U/dL) was not associated with an increased risk of thrombosis compared with the reference category (Figure 1). The same was true for the lowest factor V:Ag category; even factor V levels <70 U/dL did not increase the risk of thrombosis.

Interaction Factor V:Ag Levels and Factor V Leiden

Factor V Leiden was determined in 945 of the 948 subjects. In 3 patients, DNA was unavailable. One hundred six subjects carried the factor V Leiden mutation, of whom 8 were homozygous. The factor V:Ag level (mean \pm SD) was 132 ± 34 U/dL for wild-type factor V, 137 ± 33 U/dL for heterozygous factor V Leiden, and 146 ± 42 U/dL for ho-

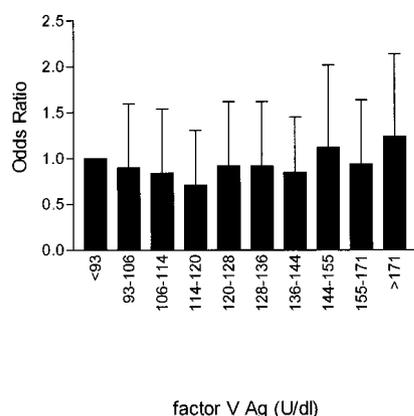


Figure 1. Risk of thrombosis (+95% CIs) for 10 categories of factor V:Ag levels. Reference category is factor V:Ag <93 U/dL.

mozygous carriers of factor V Leiden. These groups were entered in a regression model (0 represents wild-type factor V; 1, heterozygous factor V Leiden; and 2, homozygous factor V Leiden) with factor V:Ag as a dependent variable; the regression coefficient β was 5.1 (95% CI -1 to 11). Further analyses explored the association between factor V Leiden and venous thrombosis for different factor V:Ag levels. Because only 8 subjects were homozygous for factor V Leiden, calculations for factor V Leiden carriers were based on homozygous and heterozygous carriers as a single group. For carriers of factor V Leiden who had factor V:Ag levels ≥ 150 U/dL, the risk of venous thrombosis increased nearly 13-fold (95% CI 3.8 to 41) compared with carriers of wild-type factor V with factor V:Ag levels <110 U/dL (Table 2). (This risk is not very different from the risk of factor V Leiden carriers with factor V antigen levels <150 U/dL, especially when we realize that the calculations in Table 2 are based on relatively low numbers in the control group.

Relation Between Factor V:Ag and Factor VIII:Ag Levels

Factor V and factor VIII are related proteins and probably share common biosynthetic pathways, as reflected by the identification of patients with combined factor V and factor VIII deficiency.²³⁻²⁵ It was of interest to analyze to what extent factor V and VIII levels in plasma are correlated. We found that factor VIII levels and factor V levels are correlated

TABLE 3. Combined Effects of Factor V and Factor VIII Levels on Thrombotic Risk

Factor V:Ag, U/dL	Factor VIII:Ag, IU/dL	Patients, n	Controls, n	OR (95% CI)
<110	<100	34	54	1*
<110	>150	11	8	2.2 (0.8-6.0)
>150	<100	11	22	0.8 (0.3-1.8)
>150	>150	34	9	6.0 (2.6-12)

*Reference category.

($r=0.26$, $P<0.001$). For carriers of the wild-type factor V, factor VIII:Ag rose by 3.5 U/dL for every 10-U/dL increase in factor V:Ag, whereas for factor V Leiden carriers, the factor VIII:Ag level increased by 4.6 U/dL. Adjustment for age and separate analysis in thrombotic patients and controls yielded the same results.

Next, we investigated whether the effect of high factor VIII levels on thrombosis is conditional on the factor V level. Because factor V Leiden itself is a risk factor for thrombosis, analysis was restricted to carriers of the normal factor V genotype. For these calculations, we took the highest quartiles of factor V and factor VIII and compared them with the lowest quartiles. Table 3 shows that factor VIII levels >150 U/dL in combination with factor V levels <110 U/dL gave an OR of 2.2. The combination of factor V and VIII levels >150 U/dL increased the thrombotic risk 6.0 (95% CI 2.6 to 12) times compared with factor V levels <110 U/dL and factor VIII levels <100 U/dL (Table 3). This risk is similar to the previously reported 6-fold increase in the risk of thrombosis for unadjusted factor VIII:Ag levels >150 U/dL,²¹ indicating that high factor VIII:Ag does not interact with high factor V:Ag levels in promoting thrombosis.

Factor V:Ag Levels and APC Ratio

The association of factor V:Ag levels and the normalized APC ratio in subjects with normal factor V and factor V Leiden is illustrated in Figure 2. Patients using oral anticoagulants ($n=48$) or with a lupus anticoagulant ($n=4$) were excluded from this analysis because they have a prolonged activated partial thromboplastin time in the absence of APC, which gives unreliable results in the APC resistance test. In factor V Leiden and factor V wild-type subjects, factor V:Ag

TABLE 2. Thrombotic Risk of Factor V:Ag Levels With or Without Presence of Factor V Leiden

Factor V:Ag, U/dL	Factor V Genotype	Patients (N=471),* n (%)	Controls (N=474), n (%)	OR (95% CI)
<110	Wild-type	96 (20.4)	115 (24.3)	1†
	FV Leiden	17 (3.6)	3 (0.6)	7.9 (4.4-14.2)
110-130	Wild-type	91 (19.3)	122 (25.7)	0.9 (0.6-1.3)
	FV Leiden	27 (5.7)	5 (1.1)	6.3 (2.4-16.5)
130-150	Wild-type	94 (20)	119 (25.1)	0.9 (0.6-1.4)
	FV Leiden	16 (3.4)	3 (0.6)	6.2 (1.8-21.6)
≥ 150	Wild-type	98 (20.8)	104 (22)	1.2 (0.8-1.7)
	FV Leiden	32 (6.8)	3 (0.6)	12.8 (3.8-41)

*In 3 patients, DNA was unavailable.

†Reference categories are noncarriers with factor V:Ag levels <110 U/dL. Age-adjusted logistic regression led to similar results.

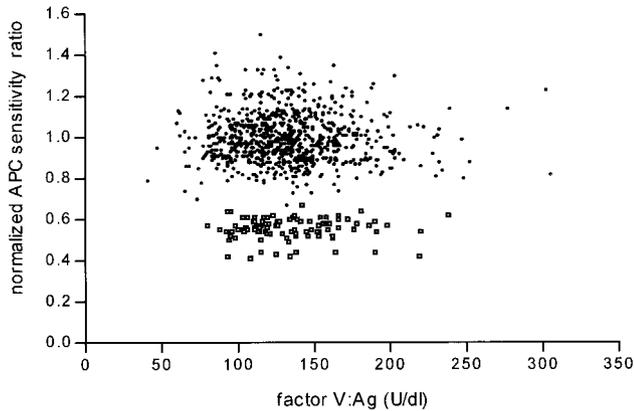


Figure 2. Distribution of normalized APC sensitivity ratios for wild-type factor V (●) and factor V Leiden (□) in relation to factor V:Ag levels.

levels had no effect on the APC ratio ($r=0.01$ for factor V Leiden, $r=-0.04$ for wild-type factor V).

Discussion

The present study evaluates the relation between factor V levels and venous thrombosis in a large population-based case-control study. Factor V:Ag levels were found to be influenced by age and smoking. The 7.6-U/dL increase in factor V that we found for every successive 10 years of age is in agreement with the findings of Brozovic et al,²⁶ who reported an increase in factor V activity of 0.6% per year (≈ 6 U/dL per decade).

Factor V:Ag levels were measured by ELISA with the use of 2 monoclonal antibodies against the light chain of factor V. The mean factor V:Ag level of the control group was 132 U/dL higher than we would have expected. Our PNP contained, on average, 103 U/dL factor V. The intra-assay and interassay variation were relatively low and very acceptable for this type of test.

Neither low nor high factor V:Ag levels in plasma were associated with venous thrombosis. Apparently, unlike factor VIII,¹⁴ high levels of plasma procoagulant factor V do not promote venous thrombosis, nor do reduced levels of anticoagulant factor V. It is possible that this is related to a balance between the procoagulant and anticoagulant functions of factor V; a high factor V level may enhance prothrombinase activity, as does factor V Leiden,²⁷ but at the same time, high anticoagulant factor V may result via its APC-cofactor activity in an increased degradation of factor VIII/VIIIa.¹¹⁻¹³ The present study provides no information on the role of platelet factor V in the pathogenesis of venous thrombosis. Platelet factor V has been implicated as an important regulator of hemostasis in vivo.²⁸ Recently, however, the origin of platelet factor V (biosynthesis in platelets or endocytosis of plasma factor V by platelets) has been questioned.^{29,30} More information is required to determine how platelet factor V concentrations relate to plasma factor V levels before we can discuss an independent role of platelet factor V in thrombosis.

The conclusion that low factor V levels are not associated with venous thrombosis is not a complete surprise because of the lack of reports on thrombotic events in heterozygous factor V-deficient family members of patients with severe factor V deficiency. Very recently, Redondo et al³¹ found that

high factor V activity levels are associated with arterial thrombosis. No mechanism for this relation was provided.

Carriers of factor V Leiden with factor V:Ag levels >150 U/dL had a 13-fold increased risk compared with those with wild-type factor V with factor V:Ag levels <110 U/dL. Because this result is based on only 3 controls, we do not believe that this indicates a higher risk for this group of factor V Leiden carriers.

Factors V and VIII are related proteins and share common biosynthetic pathways, as reflected by recent studies of Nichols and colleagues^{23,25} and Neerman-Arbez et al²⁴ in combined factor V and VIII deficiencies. In the present study, we found that factor V:Ag levels correlate with plasma factor VIII:Ag levels, suggesting that common posttranslational modifications²³ explain a small part of the large variation in plasma factor V levels and VIII levels. The thrombosis risk of high factor VIII:Ag levels was not affected by factor V levels. The combination of high factor V and high factor VIII levels did not result in a higher thrombotic risk than that of high factor VIII levels by themselves; thus, factor V does not modify the thrombotic risk of elevated factor VIII levels.

The normalized APC ratio was not influenced by the factor V:Ag level in subjects with or without factor V Leiden. Freyburger et al³² found slightly lower mean APC ratios for thrombotic patients with factor V:C levels $>100\%$ compared with those with levels $<100\%$. In healthy pregnant women, results are conflicting: Walker et al³³ found no relation between the APC ratio and factor V activity levels, whereas Clark et al³⁴ observed a relation ($r=-0.33$, $P=0.03$) after excluding subjects with factor VIII:C levels >120 U/dL. In our subjects, after adjustment for the influence of elevated factor VIII:Ag levels, no relation between factor V and the normalized APC sensitivity ratio could be observed. Our data suggest that the synthesis of factor V in subjects with factor V Leiden is normal and that the elevation of the factor V:Ag level does not affect the APC ratio in heterozygotes and therefore cannot be considered as a cause of acquired APC resistance. We also found no relation between low factor V levels and the APC sensitivity ratio, which is explained by the fact that a reduction of the APC ratio occurs only at very low factor V levels ($<25\%$).³⁵

In conclusion, factor V:Ag levels are not associated with a risk of venous thrombosis. Factor V levels and factor VIII levels are correlated in plasma, but factor V does not mediate the thrombotic risk of high factor VIII levels. There seems to be no clear relation between factor V:Ag levels and the normalized APC ratio in subjects with and without factor V Leiden.

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