High plasma concentration of factor VIII coagulant is also a risk factor for venous thromboembolism in the elderly

EMMANUEL OGER, KARINE LACUT, PATRICK VAN DREDEN, LUC BRESSOLLETTE, JEAN-FRANÇOIS ABGRALL, MARIE-THÉRÈSE BLOUCH, PIERRE-YVES SCARABIN, DOMINIQUE MOTTIER

Background and Objectives. A high level of coagulant factor VIII is a well known risk factor for venous thromboembolism, but most studies have enrolled patients under 70 years old. This study aimed to test the hypothesis that an association also exists in the elderly.

Design and Methods. This hospital-based case-referent study took place at the Department of Internal Medicine and Chest Diseases, University Hospital, Brest, France. We enrolled 161 patients with a first episode of venous thrombosis and 239 subjects, referred for a clinical suspicion of venous thromboembolism which was subsequently ruled out. Factor VIII coagulant activity and plasma fibrinogen concentration were measured.

Results. High factor VIII coagulant activity was significantly associated with venous thromboembolism, irrespective of the age group. Patients over 70 years with factor VIII coagulant activity above 225% had a 2.4-fold increased risk of venous thromboembolism compared to those patients with levels below 130% (age- and fibrinogen-adjusted odds ratio: 2.6, 95% CI 1.1 to 6.1).

Interpretation and Conclusions. Our results show that high levels of factor VIII coagulant, a determinant of venous thrombosis in adulthood, is also a risk factor in the elderly.

Key words: factor VIII coagulant, venous thromboembolism, risk factor, elderly.

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From the G.E.T.B.O (Groupe d'Etude de la Thrombose de Bretagne Occidentale), Hôpital de la Cavale Blanche, Brest (EO, KL, LB, J-FA, M-TB, DM), Serbio (Diagnostica Stago), Gennevilliers (PVD) and INSERM Unit 258, Villejuif P-YS), France.

Correspondence: Emmanuel Oger, MD, PhD, Department of Internal Medicine and Chest Diseases, Hôpital de la Cavale Blanche, F-29609 Brest Cedex, France. E-mail: emmanuel.oger@chu-brest.fr

Recent literature has suggested that an elevated level of factor VIII coagulant activity is a risk factor for venous thromboembolism.[1-3] Koster *et al.*, in their analysis of the Leiden Thrombophilia Study,[1] reported a 5-fold increased risk of venous thrombosis associated with a factor VIII coagulant activity above 150 IU/dL compared to levels below 100 IU/dL. This population-based case-control study included patients under 70 years with a first episode of venous thrombosis; controls were age- and sex- matched healthy neighbors or partners of the patients. A dose-response relationship was suggested that supported a causal association. More recently, another age- and sex-matched case-control study 3, including 65 patients with a mean age of 55 years and 60 control subjects, confirmed both the association and the dose-response relationship. Once again, a 4-fold increased risk of venous thromboembolism was associated with a factor VIII coagulant activity above 150 IU/dL. Lastly, a prospective study[4] of 360 patients, with a mean age of 48 years, followed-up over an average period of 30 months after a first episode of venous thromboembolism showed that patients with a factor VIII coagulant activity greater than 230 IU/dL had a relative risk of recurrence of 6.7 (95 percent confidence interval, 3.0 to 14.8). In addition, studies with repeated blood sampling have reported that factor VIII levels are consistent over time in thromboembolic patients.[3,5] Moreover, high factor VIII coagulant activity was not related to an acute phase reaction, as assessed by C-reactive protein and fibrinogen plasma concentrations.[6,7]

As all these studies included patients mainly under 70 years old, it is unclear whether high factor VIII coagulant activity is implicated in venous thromboembolism in older patients. We previously estimated, using community-based data from a defined population of 342,000 inhabitants, that 53% of all venous thromboembolism events occurred in patients above 70 years.[8] Therefore, we set up the present case-referent study to investigate the association between plasma factor VIII coagulant levels and the risk of thrombosis in unselected consecutive patients, including patients above 70 years, with an objectively confirmed first episode of either deep vein thrombosis or pulmonary embolism, and we planned a stratified analysis according to age.

Design and Methods

Selection of patients and control subject

Since 1994, data from all patients admitted to our

medical unit with a suspected deep vein thrombosis and/or pulmonary embolism have been prospectively gathered in a register. All patients had objective tests in order to assess or to exclude the diagnosis of acute thromboembolism within 24 hours of admission. The diagnostic flow-chart has already been described elsewhere.[9] Briefly, venous thrombosis of the lower limbs was diagnosed either by compression ultrasonography and/or contrast venography whereas acute pulmonary embolism was diagnosed by: 1) positive pulmonary angiography or spiral CT scan, or 2) high probability ventilation-perfusion lung scanning according to the PIOPED criteria,[10] or 3) the presence of signs and symptoms that are suggestive of pulmonary embolism and a proven deep vein thrombosis. Several baseline characteristics were recorded in a register on admission. These characteristics included: date and type of event (deep vein thrombosis and/or pulmonary embolism), age, sex, acquired risk factors for thrombosis (including surgery in the past 3 months, active malignancy excluding non-melanoma skin cancer). Patients with one or more of the following triggering risk factors, such as recent surgery or delivery, pregnancy, stroke with hemiparesis, active malignancy, were categorized as having *secondary* thrombosis, whereas those with none of these risk factors were categorized as having *idiopathic* thrombosis.

The present study analyzed 161 consecutive patients with a first objectively diagnosed episode of venous thromboembolism, referred to our medical unit between March 1997 and December 1999, hereafter called *cases*, and 237 consecutive patients for which the diagnosis of venous thromboembolism was ruled out and who had no personal history of venous thromboembolism, hereafter called *referents*. The study protocol was approved by the Ethics Committee of our Institution, and written informed consent was obtained from all participants.

Blood collection and plasma assays

Blood samples were collected within 24 hours of admission irrespective of overnight fasting in 1/9 volume of 0.109M sodium citrate. Plasma was separated by centrifugation for 10 minutes at 2000g at room temperature and stored at –70°C, in 1.5 mL aliquoted volumes, until use. All samples were sent to the Laboratory of Hematology, Cavale Blanche University Hospital, Brest, France, at the end of the study. Factor VIII coagulant activity was measured by one-stage clotting assay with STA®- Deficient VIII and automated activated partial thromboplastin time (APTT) on an STA® automate (Diagnostica Stago, Asnières, France). Pooled normal plasma was used as a reference. The median values for factor VIII coagulant was 100 IU/dL (normal range: 60 to 120 IU/dL). All samples were analyzed with the same batch of reagents, using the same pooled normal plasma, within a two-month period, without knowing whether the sample was from a patient or a control subject. A duplicate analysis was performed on a random subset of 100 patients and the coefficient of variation was 1.3%. Fibrinogen was measured according to the method of Clauss.

Statistical analysis

The statistical analysis used procedures available in EPI-INFO version 6 and the Statistical Package for Social Sciences (SPSS) software, version 10. Two sets of analysis were planned. Firstly, the distribution of factor VIII coagulant activity was determined in the referent group (median, quartiles). Determinants of factor VIII coagulant activity were assessed using a linear regression model. Secondly, we checked for a dose-response relationship using factor VIII coagulant as an ordinal variable according to approximate quartiles estimated in each referent age group (less or equal to 70 years and over 70 years) as well as in the whole referent population. Age- and fibrinogen-adjusted odds ratios and their 95% confidence interval were calculated in terms of relative risks of venous thromboembolism for different quartiles of factor VIII coagulant activity compared to the first quartile, according to age groups. Linear trend and homogeneity among strata were assessed using a logistic regression model.

Results

Characteristics of patients with documented venous thrombosis and referents subjects

Table 1 shows the clinical characteristics of the 400 analyzed subjects. Mean age and sex ratio were not different between cases and controls. Among the 161 consecutive patients with a first objectively diagnosed episode of venous thromboembolism, 117 had a proven deep vein thrombosis (45 proximal and 13 distal isolated deep vein thrombosis and 59 deep vein thrombosis associated with a symptomatic pulmonary embolism) and 44 an isolated pulmonary embolism diagnosed either by positive angiography (n=2), a positive spiral CT scan (n=25, among whom 10 had a high probability lung scan) or a high probability lung scan (n=17). All the 239 referent subjects had normal venous compression ultrasonography of the lower limbs; in addition, 37 had a normal or near-normal lung scan, 72 a low probability lung scan and 41 a normal spiral CT scan.

Distribution and determinants of factor VIII coagulant levels in the referent group

Two variables positively and independently influenced the level of factor VIII coagulant activity: age and plasma concentration of fibrinogen. Table 2 shows the estimated regression coefficients. These variables explained almost 25% of factor VIII coagulant activity variance. Factor VIII coagulant activ-

ity was not different in patients with active malignancy or previous recent surgery procedures from that in the other patients.

Factor VIII coagulant activity as a risk factor for venous thromboembolism

In order to check for a dose-response relation between factor VIII coagulant activity and venous thrombosis, the variable was categorized according to the approximate quartiles estimated from the distribution of factor VIII coagulant activity determined in each referent age group (Table 3) as well as in the whole referent population (Table 4). Among patients over 70 years, the risk of venous thromboembolism increased linearly with increasing levels of factor VIII coagulant activity: the test for linear trend had a *p* value of 0.019 using quartiles estimated from the referent group aged over 70 years, and a *p* value of 0.017 using quartiles estimated from the whole referent group. The risk of venous thromboembolism was 2.4-fold higher in patients with a factor VIII coagulant activity above 225% than among patients with activity levels below 90%. Adjustment for age and fibrinogen level slightly modified the risk estimate in patients over 70 years.

Finally, we analyzed the data excluding those patients with a known cause of high factor VIII levels, such as surgery, pregnancy and malignancy (Table 5). This analysis showed a clear association between high factor VIII level and venous thromboembolism.

Discussion

This case-referent study, including consecutive patients referred for a clinical suspicion of venous thromboembolism, suggests that the association between high levels of factor VIII coagulant and venous thromboembolism observed in patients under 70 years, is also valid in older patients. Among patients under 70 years, factor VIII coagulant activity above 130 IU/dL was associated with an eight-fold higher risk of venous thromboembolism than the risk in patients with a value below 90 IU/dL; whereas, in patients over 70 years, there was an almost three-fold increased risk of venous thromboembolism for patients with factor VIII coagulant activity above 225 IU/dL, compared to the risk in those patients with a factor VIII coagulant activity below 130 IU/dL.

Considering our younger group (less than 70 years), our results are in keeping with previous reports[1,3] of a 5-fold and a 4-fold increased risk of venous thromboembolism associated with a factor VIII coagulant activity considering a cut-off level of 150 IU/dL.[1] A dose-response relationship was detected among patients under 70 years in accordance with Koster *et al.*[1] Such a dose-response relationship could point to high levels of factor VIII coagulant being a cause of thrombosis rather than a consequence.

As regards our group of patients over 70 years, the difference in the cut-off value may be explained by the well-established strong positive correlation between factor VIII coagulant activity and age. In addition, an increased risk of venous thromboembolism with an increasing level of factor VIII coagulant activity was also shown.

Our case-referent study has strengths and weaknesses and we focus here on two points: the study design, including the choice of the referent group, and the timing of the measurements.

The definition of the referent group may raise concerns in such studies. Patients with a clinical suspicion of venous thromboembolism which was ruled out may provide a comparison group that follows the basic principles of comparability.[11,12] First of all,

Table 1. Clinical characteristics of the study population.

Variables	*Cases (n = 161)*	*Referents (n = 239)*	*p value*
Female, n (%)	83 (51.6)	132 (55.5)	0.47
Age (years)	66.1±19.2	65.9±18.2	0.93
Age groups, n (%)	–	–	0.79
≤ 70	79 (49.1)	114 (47.7)	
> 70	82 (50.9)	125 (52.3)	
No risk factors, n (%)	117 (72.7)	189 (79.1)	
Risk factors present, n (%)	44 (27.3)	50 (20.9)	0.14
Surgery	11	14	0.18
Cancer surgery	5	1	
Cast for lower limb trauma	5	5	
Pregnancy or delivery	3	4	
Active malignancy	26	27	0.16
Stroke with hemiparesis	0	2	
Deep vein thrombosis, n (%)	58 (36)		
DVT with PE, n (%)	59 (37)		
Pulmonary embolism, n (%)	44 (27)		

Values are mean ± standard error or number (percent).

Table 2. Determinants of factor VIII coagulant activity estimated from a linear regression model in 239 controls.

Variables	*Regression coefficients*	*p value*
Age, years	1.06±0.25	< 0.001
Fibrinogen, g/L	19.27±2.77	< 0.001

Table 3. Unadjusted and age- and fibrinogen-adjusted odds ratios (95% confidence interval, CI) for venous thrombosis in patients in the second, third, and fourth quartiles of factor VIII coagulant (FVIIIc) activity compared with those in the first quartile of each age group.

Age group	*Levels of FVIIIc*	*Unadjusted odds ratios*	*95% CI*	*Adjusted odds ratios*	*95% CI*
Under 70 years	1st quartile	1.0		1.0	
	2nd quartile	2.9	1.1-8.0	4.3	1.4-13.4
	3rd quartile	4.1	1.6-10.9	7.9	2.5-24.7
	4th quartile	3.4	1.3-9.2	7.6	2.3-25.6
Over 70 years	1st quartile	1.0		1.0	
	2nd quartile	1.1	0.5-2.6	1.0	0.4-2.4
	3rd quartile	1.2	0.5-3.0	1.3	0.5-3.1
	4th quartile	2.4	1.1-5.3	2.6	1.1-6.1

Boundaries for quartiles are, for subjects under 70 years old: first quartile < 90%, second quartile 90 to 130%, third quartile 130 to 195% and fourth quartile > 195%; and for subjects over 70 years old: first quartile < 130%, second quartile 130 to 175%, third quartile 175 to 225% and fourth quartile > 225%. Values for fibrinogen were missing for 17 patients, 9 under 70 years old and 8 over 70 years old.

Table 4. Unadjusted and age- and fibrinogen-adjusted odds ratios (95% confidence interval, CI) for venous thrombosis in patients in the second, third, and fourth quartiles of factor VIII coagulant (FVIIIc) activity compared with those in the first quartile, as defined in the whole referent group.

Age group	*Levels of FVIIIc*	*Unadjusted odds ratios*	*95% CI*	*Adjusted odds ratios*	*95% CI*
Under 70 yrs.	1st quartile	1.0		1.0	
	2nd quartile	3.3	1.4-8.2	5.8	2.0-16.2
	3rd quartile	4.6	1.8-11.7	10.1	3.2-31.3
	4th quartile	3.1	1.2-7.8	7.7	2.4-24.9
Over 70 yrs.	1st quartile	1.0		1.0	
	2nd quartile	0.9	0.2-3.3	1.0	0.2-3.9
	3rd quartile	1.0	0.3-3.7	1.0	0.3-3.8
	4th quartile	2.0	0.6-7.1	2.5	0.7-9.7

Boundaries for quartiles are: first quartile < 100%, second quartile 100 to 150%, third quartile 150 to 200% and fourth quartile > 200%. Values for fibrinogen were missing for 17 patients, 9 under 70 years old and 8 over 70 years old.

Table 5. Unadjusted and age- and fibrinogen-adjusted odds ratios (95% confidence interval, CI) for venous thrombosis in 317 patients without cancer, surgery or pregnancy, in the second, third, and fourth quartiles of factor VIII coagulant (FVIIIc) activity compared with those in the first quartile, as defined in the whole referent group.

Levels of FVIIIc	*Unadjusted odds ratios*	*95% CI*	*Adjusted odds ratios*	*95% CI*
1st quartile	1.0		1.0	
2nd quartile	2.5	1.1-5.8	3.2	1.3-7.9
3rd quartile	2.2	0.9-5.0	2.9	1.2-7.4
4th quartile	2.8	1.2-6.1	4.7	1.8-12.3

Boundaries for quartiles are: first quartile < 100%, second quartile 100 to 150%, third quartile 150 to 200% and fourth quartile > 200%. Values for fibrinogen were missing for 13 patients.

controls should be selected from the same source population or study base. Our study can be viewed as nested in a cohort that was made up of all patients referred to our Department for a clinical suspicion of venous thromboembolism, independently of their past history of venous thromboembolism. Patients referred for a clinical suspicion which was ruled out are appropriate referents if the differential diagnosis is unrelated to the exposure of interest.[13] Indeed, most of our referents were discharged with the diagnosis of infectious disease and increased levels of factor VIII may be related to systemic inflammation. Consequently, the association may have been underestimated and we used fibrinogen in an attempt to control this bias. Unfortunately, C-reactive protein was measured in too few patients to adjust for.

As regards the timing of the measurements, venipuncture was performed on admission when the venous thrombotic event was diagnosed. Since factor VIII is an acute-phase reactant, we could debate whether the high factor VIII coagulant level is a risk factor *per se* or a post-thrombotic phenomenon. Factor VIII coagulant level strongly correlated with plasma concentration of fibrinogen. As high fibrinogen level is under debate as an independent risk factor for venous thromboembolism,[7] it could be a potential confounding factor. However, adjustment for fibrinogen plasma concentration did not alter the association. Moreover, previous studies have shown that an increased factor VIII coagulant level

observed after a venous thromboembolic event was a persistent phenomenon and was independent of the acute phase reaction,[5,7] and therefore, it could be hypothesized that the elevated factor VIII coagulant level represents a prothrombotic tendency and not a secondary reactive phenomenon. These statements support the hypothesis that it is the intrinsic factor VIII coagulant level which is related to venous thromboembolism. The result of the secondary analysis excluding those patients with a known cause of high factor VIII levels, such as surgery, pregnancy and malignancy also strengthens this hypothesis.

To date, the molecular basis of elevated factor VIII coagulant levels is poorly known (genetic, acquired, or a combination of both). ABO blood group is a strong genetic determinant of factor VIII levels, mediated via von Willebrand factor levels. However, factor VIII levels had an effect on risk even when adjusted for blood group[1] suggesting additional determinants of factor VIII levels. In addition, factor VIII coagulant level shows a familial clustering[14] but no molecular basis of elevated levels within the factor VIII gene has been found.[15]

In conclusion, our study adds further evidence in support of an association between high levels of factor VIII coagulant activity and venous thromboembolism, suggesting that the same relation may exist in patients over 70 years.

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Contributions

EO, KL and DM designed the study, EO and KL analyzed the data and drafted the manuscript, MTB and JFA performed or supervised the biological measurements, PVD, LB, JFA, MTB, PYS and DM interpreted the data and revised the manuscript critically. All coauthors approved the final version of the article to be published.

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Disclosures

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Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Vicente Vicente, Deputy Editor. The final decision to accept this paper for publication was taken jointly by Professor Vicente and the Editors. Manuscript received September 19, 2002; accepted February 26, 2003.

In the following paragraphs, the Deputy Editor summarizes the peer-review process and its outcomes.

What is already known on this topic

High levels of coagulant factor VIII have been associated with an increased risk of venous thrombombolism.

What this study adds

This investigation shows that high levels of factor VIII coagulant, are also a risk factor for venous thromboembolism in elderly patients (above 70 years old).