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Risk of deep vein thrombosis: interaction between oral contraceptives and high factor VIII levels

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A B S T R A C T

Background and Objectives. High levels of factor VIII are a common risk factor for venous thromboembolism (VTE). The aim of this study was to evaluate the possible interaction between the presence of high factor VIII levels and oral contraceptive (OC) use.

Design and Methods. Factor VIII levels were measured by a chromogenic assay in 174 women who suffered from VTE in reproductive age and in 484 healthy women. Subjects carrying thrombophilic alterations were excluded.

Results. Factor VIII levels were stratified into quartiles. The adjusted odds ratio (OR) of VTE in subjects with factor VIII levels in the upper quartile (>1.95 IU/mL) was 7.45 (95%CI: 3.80-14.6). Among the 174 patients, 85 had experienced VTE during OC use. The 179 healthy women who had used OC for at least 6 months in the two years before presentation but had stopped OC at least 3 months before the blood sampling were considered as OC users. The risk of VTE among OC users with factor VIII levels in the upper quartile was increased about 13-fold (OR: 13.0, 95% CI: 4.92-34.3).

Interpretation and Conclusions. Our results showed that there is an increased risk of VTE due to oral contraceptive use in women with elevated factor VIII and, as has been previously described for factor V Leiden and G20210A prothrombin mutations, the raised level of the coagulation factor and oral contraceptive use seem to have a synergistic effect.

Key words: deep vein thrombosis, factor VIII, oral contraceptives, risk factors.

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Oral contraceptive use is associated with a 2- to 4-fold increase in the risk of VTE.[1] It has been previously reported that some thrombophilic alterations such as factor V Leiden and G20210A prothrombin mutations display a synergistic interaction with oral contraceptive use. Therefore, heterozygous carriers of these mutations who use oral contraceptives have a 20- to 40-fold higher risk of thrombosis than non-users who have a normal genotype.[2-7]

High levels of factor VIII are another common risk factor for VTE. In the Leiden Thrombophilia Study Factor VIII procoagulant activity levels in the upper quartile were associated with a 5-fold increase in the risk of venous thrombosis when compared to the risk in subjects with lower levels.[8] Similar findings have been reported in several independent studies.[9-11] More recently, it was shown that high factor VIII levels increase the risk of venous thrombosis in women using oral contraceptives; however, unlike factor V Leiden and G20210A prothrombin mutations, elevated factor VIII levels seem to have only an additive effect with oral contraceptives.[12]

In this study we evaluated the possible interaction between the presence of high Factor VIII levels and oral contraceptive use. Whereas factor VIII levels were measured by the commonly used one-stage clotting method we used a chromogenic assay in this study.

Design and Methods

Subjects

Among the 596 unrelated consecutive women referred to our Unit for investigation of possible thrombophilic states after expe-

riencing at least one objectively confirmed venous thromboembolic event (by Doppler ultrasonography, venography, ventilation-perfusion lung and/or CT scanning) between January 1998 and January 2003, only those who had experienced a single episode of deep venous thrombosis (DVT) of a lower limb (with/without pulmonary embolism) during reproductive age were considered for the present analysis (n=326). All subjects (n=131) who proved to be carriers of antithrombin, protein C or protein S deficiency, factor V Leiden or G20210A prothrombin mutation, lupus anticoagulant (LAC), with abnormal liver function, overt evidence of autoimmune or neoplastic disease at presentation were excluded. Blood was sampled at least 3 months after the thrombotic episode and 3 weeks after withdrawal of any antithrombotic treatment. None of the patients included in the present analysis was on oral contraceptives or hormonal replacement therapy at the time of the examination.

During the same period 794 apparently healthy women from the general population were given a thrombophilia work-up. They were referred to our Unit by Family Planning Clinics of the city of Bologna or family Doctors, usually for screening before prescription of oral contraception or hormone replacement treatment. As controls we considered those who were in reproductive age, not on oral contraceptives, not pregnant and not within 36 days post-partum at the time of thrombophilic screening (n=527). Women who resulted carriers of antithrombin, protein C or protein S deficiency, factor V Leiden or G20210A prothrombin mutation, or LAC were also excluded (n=39). The controls were from the same geographical area as the patients and had no genetic relationship with them. The study was approved by the local Ethics Committee and informed consent was obtained from all subjects.

Blood sampling, thrombophilia investigations and factor VIII measurements

Blood was collected from the antecubital vein into 0.109 mmol/L trisodium citrate; plasma was prepared by centrifugation for 20 min at 2000 g at room temperature; aliquots of plasma and blood for DNA extraction were snap frozen and stored at -70°C. The following tests were used to identify a thrombophilic condition: prothrombin time; activated partial thromboplastin time; fibrinogen plasma levels, antithrombin, protein C and protein S concentrations; APC resistance, test for diagnosing LAC. DNA analysis for factor V Leiden mutation was performed in all cases with an APC resistance normalized ratio < 0.80; the presence of G20210A mutation of the prothrombin gene was also tested. All tests included in the thrombophilic screening were performed using standard methods, as previously described.[7] Factor VIII was measured by a chromogenic method using a commercial assay (Coamate Factor VIII, Chromogenix by Instrumentation Laboratory, Milan, Italy), as described elsewhere.[13] Calibration curves were created using pooled normal plasma locally calibrated against the World Health Organization standard 91/666 for factor VIII and the results were expressed as IU/mL. The normal plasma pool was prepared locally mixing plasma samples from 60 apparently healthy subjects. The test was performed on an ACL instrument (Instrumentation Laboratory). Since the calibration curve obtained with this method is linear from 0.125 to 1.50 IU/mL but the expected values in the patients were higher than 1.50 IU/mL, test samples were pre-diluted 1:2 with the buffer provided by the manufacturer.

Statistical analysis

Continuous variables are presented as medians and ranges. The Mann-Whitney U-test and the χ^2 test were used for group comparisons. All p values less than 0.05 were considered to indicate statistical significance. Factor VIII levels were stratified into quartiles. Crude odds ratios (OR) and 95 percent confidence intervals (CI) were calculated with Woolf's approximation as estimates of the relative risk for VTE. Multivariate analysis by unconditional logistic regression was used to adjust for age and activated protein C resistance values. Since oral contraceptive use is incompatible with pregnancy or a post-partum state, the proportion of women taking oral contraceptives was calculated after excluding pregnant or post-partum subjects, while the proportion of pregnant women was calculated after excluding oral contraceptive users. For statistical analysis the SPSS software package (SPSS, Chicago, Ill, USA) was used.

Results

In 21 of the 195 patients and in 4 of the 488 controls who met all the inclusion criteria, it was not possible to measure factor VIII levels. The remaining 174 patients and 484 controls were considered for the present analysis. Their detailed characteristics are reported in Table 1.

The median age at the time of blood sampling was significantly higher in patients (37, 19-58 years) than in controls (34, 15-50 years) (p<0.001). No significant difference, however, was found when the age at VTE episode of patients (33 years, 17-50 years) was compared with the age of controls at presentation. A family history for VTE (including DVT, pulmonary embolism and superficial thrombophlebitis) was more frequent in patients (24.7%) than in controls (17.6%), but the difference did not reach statistical significance (p=0.057). Oral contraceptive use was the most frequent circumstantial risk factor for the VTE episode. In fact, among the 174 patients, 85 (56.7%, after exclusion of the 24

Table 1. Characteristics of the 174 women with a previous venous thromboembolic event (VTE) and the 484 healthy women.

	Women with VTE (n=174)	*Healthy women (n=484)*	*P*
Age at presentation, y [median (range)]	37 (19-58)	34 (15-50)	<0.001
Age at first VTE, y [median (range)]	33 (17-50)		n.s.
Time elapsed since last episode, mo [median (range)	11 (4-97)		
Type of episode			
DVT, n (%)	151 (86.8%)		
DVT + PE, n (%)	23 (13.2%)		
Family history of venous thrombosis			
Yes, n (%)	43 (24.7%)	85 (17.6%)	0.057
No, n (%)	131 (75.3%)	399 (82.4%)	
Circumstantial risk factors at first episode			
None, n (%)	33 (19.0%)		
Recent surgery, n (%)	13 (7.5%)		
Trauma/immobilization fracture, n (%)	19 (10.9%)		
Pregnancy/puerperium, n (%)[#]	24 (27.0%)[#]		
Oral contraceptives, n (%)[§]	85 (56.7%)[§]	179 (37.0%)[§]	<0.001

DVT: deep vein thrombosis; PE: pulmonary embolism; #Prevalence was calculated after exclusion of those women who had VTE during oral contraceptive use: §Prevalence was calculated after exclusion of those women who had VTE during pregnancy or puerperium: $Women who had used oral contraceptive for at least 6 months in the two years before presentation but had stopped the treatment at least 3 months before the time of blood sampling.

women who had had thrombosis during pregnancy or puerperium) had experienced VTE while using oral contraceptives. In the control group, the 179 (37.0%) women who had used oral contraceptives for at least 6 months in the two years before presentation but had stopped such a treatment at least 3 months before the time of blood sampling were considered as oral contraceptive users. The risk of VTE conferred by oral contraceptive use was 2.23 (95% CI: 1.54-3.23). Given the high prevalence of the 3rd generation pills (containing gestodene or desogestrel as progestins) both in patients (91.8%) and controls (79.1%), it was impossible to evaluate the association of VTE with different types of oral contraceptive.

The median factor VIII levels were 1.89 (range: 0.45-4.18 IU/mL) for the patients and 1.41 (range: 0.38-4.57 IU/mL) for the controls, and the difference was statistically significant ($p<0.0001$). Table 2 shows the risks (OR) for VTE after stratification of factor VIII levels into quartiles. Women with altered activated APC resistance (≤ 0.90 normalized ratio) in the absence of the factor V Leiden mutation were included in the analysis [67/174 (38.5%) patients and 98/484 (20.2%) controls). Since we previously reported that altered APC resistance in women not carrying the factor V Leiden mutation significantly increased the risk for VTE and that oral contraceptive use in those women further increased the risk,[14] OR were adjusted for APC resistance values in the multivariate analysis. The OR are relative to the reference category (factor VIII levels below 1.20 IU/mL, OR=1). Of the 174 patients, 76 (43.7%) had a factor VIII level above 1.95 IU/mL, compared with 87/484 (18.0%) in the control group ($p<0.0001$). The crude and adjusted OR of VTE for subjects with a factor VIII level above 1.95 IU/mL as compared to those in the reference category (factor VIII levels below 1.20 IU/mL) were 9.17 (95%CI: 4.89-17.2) and 7.45 (95%CI: 3.80-14.6), respectively.

As shown in Figure 1, the association between factor VIII levels and risk of VTE is dose-related. After stratification of patients and controls into deciles according to factor VIII levels, the OR for venous thrombosis steadily increased as the factor VIII levels increased. A total of 38 out of 174 (21.8%) patients had a factor VIII level above the 90th percentile (2.47 IU/mL) whereas only 25/484 (5.2%) of the controls did so. In contrast, 8 patients (4.6%) and 57 controls (11.8%) had a factor VIII level below the 10th percentile (< 0.93 IU/mL). The crude and adjusted OR for VTE for subjects with a factor VIII level above the 90th percentile were 10.8 (95%CI: 4.42-26.5) and 8.23 (95%CI: 3.14-21.5), respectively. Table 3 shows the separate and combined effects of factor VIII and oral

Table 2. Risk of venous thromboembolism (VTE) in women stratified according to quartiles of factor VIII levels.

Factor VIII (IU/mL)	*Percentile*	*Women with VTE (n=174)*	*Healthy women (n=484)*	*Unadjusted OR (95% CI)*	*Adjusted OR # (95% CI)*
< 1.20	Below 25th	14 (8.0%)	147 (30.4%)	1 (Reference)	1 (Reference)
1.20-1.54	26th-50th	35 (20.1%)	135 (27.9%)	2.72 (1.40-5.28)	2.55 (1.30-5.00)
1.55-1.95	51st-75th	49 (28.2%)	115 (23.7%)	4.47 (2.35-8.50)	3.96 (2.03-7.73)
> 1.95	>75th	76 (43.7%)	87 (18.0%)	9.17 (4.89-17.2)	7.45 (3.80-14.6)

#Adjusted for age and activated protein C resistance values.

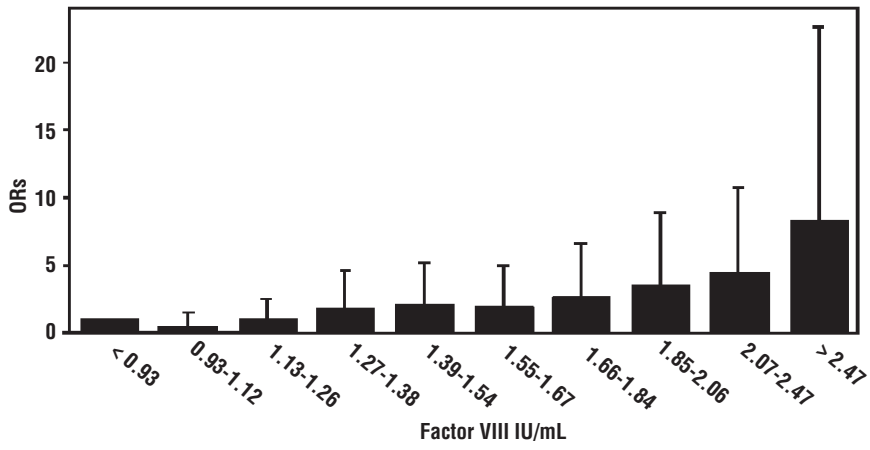

Figure 1. Adjusted (age and activated protein C resistance values) ORs for venous thrombosis according to factor VIII levels (reference category factor VIII < 0.93 IU/ml). The 95% CI are represented by error bars.

contraceptive use. In this analysis, only the two extreme strata of factor VIII levels were considered: > 1.95 IU/mL vs < 1.20 IU/mL. Crude and adjusted OR were calculated using the women with low factor VIII (below 1.20 IU/mL) who did not use oral contraceptives as the reference category (OR=1). As shown in the table, the risk of VTE in women who did not use oral contraceptives was increased 5-fold if their factor VIII level was in the upper quartile. In the women with low factor VIII levels, the risk of VTE was not significantly increased by the use of oral contraception. The risk of VTE in women who used oral contraception and had elevated factor VIII was about 13-fold higher than that in the reference category (crude OR: 15.0, 95%CI: 6.34-35.6; adjusted OR: 13.0, 95%CI: 4.92-34.3).

Table 3. Distribution of cases and controls according to the use of oral contraceptives (OC) and presence of low or high factor VIII levels (< 1.20 IU/mL vs > 1.95 IU/mL).

	Women with VTE	*Healthy women*	*Unadjusted OR (95% CI)*	*Adjusted OR* (95% CI)*
Factor VIII < 1.20 IU/mL No OC	8	88	1 (Reference)	1 (Reference)
Factor VIII < 1.20 IU/mL OC	6	59	1.12 (0.37-3.39)	1.14 (0.37-3.50)
Factor VIII > 1.95 IU/mL No OC	35	57	6.75 (2.92-15.6)	5.22 (2.09-13.1)
Factor VIII > 1.95 IU/mL OC	41	30	15.0 (6.34-35.6)	13.0 (4.92-34.3)

**Adjusted for age and activated protein C resistance values.*

Discussion

Elevated plasma factor VIII levels are an established risk factor for VTE.[8-11] In the Leiden Thrombophilia Study, plasma factor VIII levels equal to or greater than 150 IU/dL were shown to be independently associated with a 4.7-fold increased risk of venous thrombosis, after exclusion of possible confounders.[8]

In line with the studies previously published,[8-11] we confirm that elevated factor VIII levels are a strong risk factor for VTE. In fact, women with a factor VIII level in the upper quartile (> 1.95 UI/mL) had a 7.4-fold higher risk than did subjects with factor VIII levels in the lower quartile (< 1.20 IU/mL). Furthermore, our data show that the association between factor VIII levels and risk of VTE has a dose-dependent relationship and that the risk of venous thrombosis steadily increases as the levels of factor VIII increase.

The specific aim of this study was to evaluate the effect of oral contraceptive use on the risk of VTE in women with elevated factor VIII levels. Since factor VIII may be increased during oral contraceptive treatment,[15] control women who were investigated for thrombophilia during oral contraceptive use were excluded. In the control group we considered as oral contraceptive users only those women who had used the pill for at least 6 months in the two years before presentation but had stopped this treatment at least 3 months before the time of blood sampling to exclude an acute effect of the oral contraceptive. Unlike Bloemenkamp *et al.*,[12] who found that the combination of oral contraceptive use and high factor VIII levels had an additive effect on the risk of VTE, our data show that the joint presence of both risk factors has a synergistic effect. In fact, the observed OR of VTE in women with elevated factor VIII levels using oral contraceptives (OR=13.0) greatly exceeds the sum of the separate effects of the two risk factors and appears to be more than a multiplication of the two effects.

In the previous studies evaluating the relationship between factor VIII levels and VTE,[8-11,16] factor VIII was measured by modifications of the activated partial thromboplastin time (one-stage clotting assay). It has been reported that activation of the coagulation system during blood collection and/or storage conditions and the possible presence of components other than factor VIII, which could contribute to or partially oppose the measured activity, may affect the results of factor VIII measurements.[17-19] Furthermore, the reproducibility of clotting assays has been reported to be poor, especially at high factor VIII concentrations.[20-23] In the present study factor VIII was measured by a chromogenic assay, which can be more specific than the clotting assay. However, in a recent paper on the effects of high factor VIII levels on the risk of VTE recurrence we measured factor VIII by both methods and could not confirm that the chromogenic method gives more reproducible results than the clotting one, as previously reported;[20-23] furthermore, we did not find any clinically significant difference in results obtained with the two different assays.[13]

Some potential limitations of our study should be taken into account. Our controls were selected from healthy women who were referred to our Unit by Family Planning Clinics or general practitioner, usually for screening before starting to use oral contraceptives. Although there is no explicit recommendation, several gynecologists and general practitioners in our region routinely request thrombophilia screening for those women who intend to start the pill or hormone replacement treatment, especially if first-time users. However, screening was not performed in all women before oral contraceptive use but was limited or mainly applied to those women at increased risk of thrombotic complications during hormonal treatment because of factors such as a positive family history of VTE, being overweight, of an older age, being a current smoker or having diabetes. Furthermore, since Italian women are mainly prescribed 3rd generation oral contraceptives our results can not be extrapolated to other populations in whom the use of 2nd generation oral contraceptives seems to be prevalent.

We can conclude that there is further increase in the risk of VTE due to oral contraceptive use in women with elevated factor VIII levels and, as has been previously described for factor V Leiden and G20210A prothrombin mutations, the risk factors seem to have a synergistic effect.

CL: conception, design and interpretation of data, drafting the article; MC, BC, P, OB: collection, analysis and interpretation of data; GP: critical revision of the paper and final approval of the version to be published. The authors reported no potential conflicts of interest.

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