Elevated factor VIII is a risk factor for idiopathic venous thromboembolism in Canada – is it necessary to define a new upper reference range for factor VIII?

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Summary

Previous studies suggest elevated factor VIII is a common, independent risk factor for venous thromboembolism (VTE); however, these studies included secondary and idiopathic VTE. We sought to explore the association between elevated factor VIII and VTE in Canadian patients with idiopathic thrombosis, and confirm the current upper factor VIII reference range was appropriate. We enrolled 300 consecutive patients with idiopathic VTE who were matched to friend controls by age, sex and ethnicity. Factor VIII levels were measured and compared between cases and controls. The optimal cut-off value to designate factor VIII levels as elevated was determined using a variety of methods. The optimal upper cut-off value for factor VIII levels was 270 IU/dl. In the logistic regression analysis, cases were more likely to have elevated factor VIII levels (OR: 8.76), as were females (OR: 1.93) and older subjects (OR: 1.05). Factor VIII cut-offs for a 95% specificity by age were 238 IU/dl for subjects <40 years, 248 IU/dl age 40–55 years, 261 IU/dl age 56–70 years, and 313 IU/dl age >70. Our findings confirm that elevated factor VIII is associated with an increased risk of idiopathic VTE. In our patients and matched controls, the current upper limit of normal (150 IU/dl) for factor VIII is not of clinical use. We propose that the upper limit be increased to 270 IU/dl or individual labs should establish their upper limit if they wish their assays to be discriminatory in patients with VTE. Age specific cut-offs may be clinically relevant.

Keywords

Case-control, factor VIII, risk factors, thrombophilia, venous thromboembolism

Introduction

Several groups have identified elevated levels of factor VIII as an independent risk factor in the development of venous thromboembolism (VTE) (1–3). In these studies, the prevalence of elevated levels of factor VIII is approximately 20% of individuals with VTE, rivaling factor V Leiden as the most common thrombophilia (4). These high levels of factor VIII are independent of a potential acute phase reactant rise in factor VIII anticipated following acute deep vein thrombosis (DVT) (5) and persist independent of other markers of inflammation (6). One study demonstrated a direct relationship between the level of elevated factor VIII and recurrent thrombotic risk (2), while another noted that appreciable relative risk elevation was observed only at extreme values, which in this case was defined as greater than 234 IU/dl (3).

A limitation of previous studies was the inclusion of patients with both idiopathic and secondary causes of acute VTE including immobility, trauma, surgery and pregnancy (1–4, 7). The main objective of our study was to compare levels of factor VIII in an ethnically diverse Canadian population with idiopathic VTE to those of asymptomatic age, sex and ethnicity matched controls. Our second objective was to determine the optimal factor VIII cut-off value for the determination of patients at risk for first or recurrent VTE.

In many reports the upper limit of normal levels of factor VIII has been defined as greater than 150 IU/dl. Interestingly, studies to date have observed 10–30% of their control populations ex-
ceed the established upper limit of normal, which is well beyond the anticipated 2.5% given a random sample of the population. This bears both therapeutic and diagnostic ramifications in the management of idiopathic and recurrent VTE and suggests that the upper limit of normal for defining individuals with an increased risk for the development and recurrence of VTE remains to be determined.

Materials and methods

This investigation was a component of a prospective case-control study that was conducted at the Ottawa Hospital (8). Cases were recruited from our Thrombosis Clinic, which provides care for a community of approximately 700,000 people. Consecutive patients were eligible for inclusion as cases if they had at least one idiopathic DVT or pulmonary embolism (PE) confirmed by objective diagnostic methods (venography, compression ultrasound, spiral CT, VQ scan, pulmonary angiography), had completed three months of follow-up, and did not have a malignant disorder.

Friends of cases were recruited as control subjects. Friend controls have been used successfully in genetic association studies previously performed in patients with VTE, and allow for accurate age and sex matching (1, 5, 9, 10). Controls were matched to cases by sex, ethnicity, and age, the latter using increments of five years. All potential controls were interviewed with a previously validated questionnaire to exclude venous thromboembolic disease and to ensure they were not misclassified cases (11).

It is well known that factor VIII and other acute phase proteins are elevated following an acute systemic illness; therefore, for case subjects, blood samples were drawn at least three months after the diagnosis of DVT/PE. This time frame has been shown to be adequate to preclude the impact of any acute phase response imparted by the thrombotic event (6).

Blood was collected into vacuum tubes that contained 0.129 M trisodium citrate as an anticoagulant. Within 30 minutes of blood draw, samples were centrifuged at 3000 g for 20 minutes at 4°C to obtain platelet poor plasma, aliquoted into plastic tubes, frozen and stored at minus 70°C. Assays were performed in batches after thawing in a 37°C water bath for five minutes. Factor VIII levels were measured using a one-stage assay, the PTT based Diagnostica Stago (Abbott Diagnostics Canada Limited, Mississauga) on the STA compact® automated coagulation factor analyzer. The laboratory is a member of the Ontario Laboratory Proficiency Testing Program, an industry quality assurance program. Controls and calibrators provided by Diagnostica Stago were run alongside research specimens as per the recommendations of the manufacturer.

Plasma from 120 healthy hospital staff volunteers (mean age ~40 years) was used to establish the normal reference range of 50–150 IU/dl which represents the 95% confidence interval around the mean of 100 IU/dl. Samples outside of the reference range were repeated on the same sample. If the result was the same it was considered accurate but if the value was different it was repeated on another day with the same sample, different aliquot. If this result was different a new sample was obtained.

Statistical analyses were performed with SAS® for Windows version 8.2. Fisher’s exact test was used to compare the proportions of subjects above and below the upper cut-off value of 150 IU/dl for factor VIII in the case and control arms and statistical significance was achieved if \( p < 0.05 \) (two-tailed). The mean factor VIII levels in the two groups were compared using the Student’s t-test with statistical significance achieved if \( p < 0.05 \) (two-tailed). Logistic regression was performed with the dependent variable factor VIII and independent variables including age, gender, factor V Leiden, prothrombin gene variant, use of anticoagulants and number of VTE events (in cases). Using the binomial distribution, 95% confidence intervals (CI) were determined for all reported proportions. In order to investigate the odds ratios (ORs) associated with graded levels of factor VIII, we divided our subjects into five categories of factor VIII results. Using the lowest 25% as the comparator group, we calculated the ORs for DVT associated with factor VIII levels in the 25th to 50th, 51st to 75th, 76th to 90th, and 91st to 100th percentiles. Adapting the method recommended by Magder and Fix (12), we determined the optimal choice of a cut-off point using the minimum relative risk (RR) variance, the minimum OR variance, and then determined the point at which the RR and OR variances were approximately equal, the latter of which may be considered the optimal cut-off point. Finally, we plotted a Receiver Operating Characteristic (ROC) curve and determined the sensitivity and specificity of the factor VIII test in our sample at 300 different levels of factor VIII.

Results

Three hundred cases and 300 controls were enrolled. There were 148 females and 152 males in each group with a mean age of 56.21 years (SD=15.33). In both groups, 97% of subjects were Caucasian. One hundred and ninety cases had one VTE and 110 cases had recurrent VTE. There were 115 cases that had PE, with or without DVT. At the time of the study, 233 cases were on warfarin. There were 40 cases and 49 controls who were current smokers.

Factor VIII levels were available for 296 cases and their matched controls. The optimal cut-off value could be considered at any value over 267 IU/dl since the relative risk variance and the odds ratio variance are essentially the same beyond this value. We chose 270 IU/dl as the cut-off since specificity is 95% at this point. At this cut-off the factor VIII levels were significantly greater in cases than controls, with an overall OR of 6.70 (95% CI: 3.75, 11.97). Using the laboratory derived upper limit of 150 IU/dl, 243 (82%) cases and 169 (57%) controls had elevated values, with an OR of 3.45 (95% CI: 2.37, 5.02). The mean factor VIII level for cases was 227 IU/dl (SD= 84) and for controls was 168 IU/dl (SD= 54). In order to determine if there was a graded risk for VTE according to the level of factor VIII, we divided values into five categories (Table). The ROC curve of the factor VIII levels for cases and controls indicated that 95% specificity and 26% sensitivity would be attained at the optimal cut-off value of 270 IU/dl.

The ORs for factor VIII levels above 270 IU/dl were calculated in cases according to whether they had one, two, or more than two VTE and the ORs were 9.22 (95% CI: 3.82, 22.22), 4.77
Table: Odds ratio for venous thromboembolism according to level of factor VIII.

<table>
<thead>
<tr>
<th>Factor VIII (IU/dl)</th>
<th>Percentile</th>
<th>Cases (n)</th>
<th>Controls (n)</th>
<th>Odds Ratio * (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;141</td>
<td>&lt;25th</td>
<td>42</td>
<td>111</td>
<td>1.0</td>
</tr>
<tr>
<td>141–184</td>
<td>25th–50th</td>
<td>59</td>
<td>85</td>
<td>1.8 (1.13–2.98)</td>
</tr>
<tr>
<td>185–229</td>
<td>51st–75th</td>
<td>78</td>
<td>98</td>
<td>3.0 (1.85–4.83)</td>
</tr>
<tr>
<td>240–297</td>
<td>76th–90th</td>
<td>65</td>
<td>74</td>
<td>7.2 (3.98–12.88)</td>
</tr>
<tr>
<td>&gt;297</td>
<td>&gt;90th</td>
<td>52</td>
<td>7</td>
<td>19.6 (8.26–46.64)</td>
</tr>
</tbody>
</table>

*Odds ratios for venous thromboembolism are for each range of factor VIII levels compared to the referent group (factor VIII levels <141 IU/dl).

(95% CI: 1.81, 12.61), and 5.71 (95% CI: 1.43, 22.77), respectively. Using univariate analysis, factor VIII levels varied significantly by gender, age and anticoagulant use. The mean was 205 IU/dl (SD=80) in females and 189 IU/dl (SD=72) in males when cases and controls were combined (p=0.0081). A significant difference remained when only cases were compared (means of 238 IU/dl and 216 IU/dl for females and males, respectively; p=0.025). Of the 66 cases not on oral anticoagulant (OAC) therapy, the mean factor VIII level was 205 (SD=76) and of the 233 cases on OACs, the mean level was 232 (SD=86; p = 0.021).

The result of the logistic regression demonstrated that thrombolytic history (case or control), age, gender, and factor V Leiden were significant. The dependent variable, factor VIII levels, was dichotomized at 270 IU/dl. In this model, cases were more likely to have elevated factor VIII levels (OR: 8.76; 95% CI: 4.76, 16.10), as were females (OR: 1.93; 95% CI: 1.18, 3.17) and older subjects (OR: 1.05; 95% CI: 1.03, 1.06). Subjects who were factor V Leiden positive were more likely to have factor VIII levels below the 270 IU/dl cut-off (OR: 0.40; 95% CI: 0.19, 0.83). Use of anticoagulants and the prothrombin variant did not significantly influence factor VIII levels in this model. Since age was a significant variable we calculated the factor VIII cut-offs that result in a specificity of 95% in four age groups; the values were 238 IU/dl for subjects <40 years (n=96), 248 IU/dl age 40–55 years (n=183), 261 IU/dl age 56–70 years (n=189), and 313 IU/dl age >70 (n=132).

Discussion

Our study demonstrates there is an increased risk for first idiopathic and recurrent idiopathic thrombosis in patients with elevated factor VIII levels. The OR for factor VIII levels above 270 IU/dl was 8.76 (95% CI: 4.76, 16.10). The increased risk with elevated levels of factor VIII has been observed in previous studies, but our study is unique in that we enrolled consecutive patients with idiopathic VTE (3, 6). All previous studies on factor VIII did not specifically exclude patients with transient risk factors so it is possible the risks associated with elevated factor VIII are not accurate for idiopathic thrombosis. Indeed, other recognized thrombophilias like factor V Leiden have not been found to increase the risk for venous thrombus associated with transient risk factors. Furthermore, our data suggest the upper limit of normal for values of factor VIII in a control population without venous thrombosis, with similar demographics to our VTE population, should be higher than the usual standard laboratory derived limit and that an upper limit of normal could be defined as 270 IU/dl. Since we dichotomized factor VIII levels to determine a value that defines increased risk, as would be done in clinical practice, we performed logistic analysis to determine the important independent variables. We demonstrated that age and gender influence factor VIII levels and that patients with factor V Leiden have lower levels of factor VIII.

Given that we and others have identified elevated factor VIII levels to be associated with an increased risk of first and recurrent thrombosis, accurate characterization of patient risk is critical to guide treatment decisions. Establishment of an appropriate reference range is important to avoid false characterization of patients with VTE as at an increased risk for thrombosis. Review of the literature reveals a disproportionate number of patients without VTE with factor VIII levels in excess of the current standard upper limit of normal (>150 IU/dl). The LETS study found that 11% of controls had factor VIII levels above normal (1) and another study reported levels above 175 IU/dl in 10% of controls (2). Schambeck et al. selected 200 IU/dl as the upper limit of normal, the 98th percentile in 266 healthy blood donors, to guarantee a high specificity (13). Brummel et al. reported only mean levels of factor VIII, but these tended to be high in control subjects, 149 ± 37.4 IU/dl (14). In an epidemiological study examining factors contributing to factor VIII levels, the mean factor VIII level in healthy pre-menopausal women was 175.3 ± 54.4 IU/dl (15). The discrepancy in these values may reside in the selection of control subjects between studies. One study enrolled subjects who were referred for suspected VTE but in whom the diagnosis was refuted by objective testing (2) and others used blood donors (13), while our study and the LETS study (1) used unrelated age-matched healthy individuals. It is probable that one argument for the high factor VIII levels in control groups is that the derivation of a laboratory normal reference range does not reflect the patient population of relevance. In our lab, the mean age of the volunteers used to generate the lab normal range was 40, while the average age of thrombosis patients is closer to 60.

Several factors are known or suspected to influence levels of factor VIII. In our study, as in others, significantly higher values were obtained in women and those with advancing age. Longitudinal epidemiological data from a sample of industrial workers demonstrated factor VIII levels were significantly increased in postmenopausal women compared to premenopausal women (15). Since levels of factor VIII did not differ by use of hormones or oral contraceptives, this study suggested levels of factor VIII may be related to age. This conclusion is supported by the epidemiological data from 1774 men that demonstrated a significant increase in factor VIII with age (15). Our exploratory analysis suggests it may be extremely important to define age-specific cut-off values for factor VIII. Although further investigation may be necessary to confirm our findings before this is adopted into clinical practice, we believe the data is compelling enough to institute age specific cut-offs now. If the overall cut-off of 270 IU/dl is employed in patients aged greater than 70, fewer cases of increased risk will be identified.

There have been a few studies that explored the influence of oral anticoagulants on factor VIII levels. One study found a strong positive correlation between factor VIII levels and the du-
ration of warfarin use, which they speculated to be suggestive of a compensatory mechanism (14). In a study of six patients, factor VIII levels decreased after discontinuation of anticoagulants (16) but a more recent study suggested that this decrease was temporary (17). Nevertheless, our univariate analysis suggested warfarin influenced factor VIII levels but our logistic regression analysis did not demonstrate this to be a significant variable. However, the ideal study to determine the effects of oral anticoagulants on factor VIII levels would require determination of factor VIII levels not only on and then off oral anticoagulants, but also after a rechallenge with anticoagulants.

It is well documented that individuals with non-O blood types have higher levels of factor VIII than those with type O (1, 18), but since we included 600 unselected patients and expected blood groups to be balanced between the cases and controls we did not determine blood groups. C-reactive protein (CRP) and von Willebrand factor (VWF) levels were not measured given that factor VIII levels were determined well after the acute thromboembolic event, and other investigators have demonstrated that factor VIII elevations are independent of CRP and VWF levels (5, 6, 19).

Determining patients with elevated levels of factor VIII that contribute to VTE risk may be important for family risk evaluation. Evidence exists for an independent genetic cause for persistently elevated factor VIII levels (2, 20–22), and a quantitative trait locus (QTL) on chromosome 18 has been identified (23). Use of sequence analysis and association studies of the factor VIII gene have not identified the genetic alterations that correlate with elevated levels of factor VIII (24, 25), but heritability has been demonstrated. Therefore determination of elevated factor VIII levels in patients with VTE should lead to consideration of screening first-degree relatives. Whether screening as a primary prevention would be of benefit is at this point unknown.

We have demonstrated that the upper limit of normal for factor VIII in a qualified hematopathology lab is of little clinical value. These values do not identify patients in whom high levels of factor VIII contributed to the thrombotic event, patients at risk of recurrence, and patients who might have an inherited risk for venous thrombosis. Furthermore, our data and that of others, suggest that age and gender should be considered when labs establish the factor VIII levels that identify risk, and that in our lab measuring factor VIII levels by a one-stage assay (the PTT based Diagnostica Stago on the STA compact® automated coagulation factor analyzer), the overall upper limit of normal for identifying that factor VIII contributed to the thrombotic event is 270 IU/dL. This value will identify a reasonable number of patients with few false positives. Cut-offs may be varied to adjust sensitivity and specificity to individual practice preference. For example, in our population a cut-off of 297 IU/dL will improve specificity to 97.6% and increase the odds ratio for VTE to 19.63, but our analysis suggests the optimal cut-off point was 270 IU/dL for the population as a whole.

Our results suggest the current cut-off for factor VIII levels is not appropriate to identify patients at increased risk of venous thromboembolism. Age appears to be an important factor and should be taken into consideration when developing clinically relevant cut-off values. Physicians wishing to use factor VIII levels to identify patients, or potentially families, at risk for venous thrombosis should use a cut-off similar to ours, or establish centre-specific cut-offs in appropriately-aged controls since it appears the optimal cut point is considerably higher than the usual lab established upper limit values. Without a standardized upper limit we risk falsely classifying patients at increased risk. We cannot determine from our study the appropriateness of determining factor VIII levels but our findings and those of others suggest an increased recurrence risk in individuals with elevated factor VIII. Further studies on lab standardization and clinical outcome studies using age-specific cut-offs are desperately needed.

References