Prediction of recurrent venous thromboembolism by measuring ProC Global

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Summary

In patients with venous thromboembolism (VTE) a laboratory assay that globally measures the overall thrombophilic tendency is not available. We hypothesized that determination of ProC Global®, a plasma assay which tests the global function of the protein C pathway, could be used to stratify patients according to their risk of recurrent VTE. We prospectively followed 774 patients with first spontaneous VTE for a mean time of 52 months. ProC Global normalized ratio (NR) was measured in plasma by use of a commercially available assay based on activated partial thromboplastin time. Ninety-eight of the 774 patients had recurrent VTE. Patients with ProC Global NR ≥ 0.75 had a relative risk of recurrence of 0.59 (95% CI 0.40–0.87) as compared with those with lower ratio. After four years, cumulative probability of recurrence was 8.6% in patients with ProC Global NR ≥ 0.75 and 17.4% in patients with a lower ratio (p=0.006). Patients with a high ProC Global NR have a low risk of recurrent VTE. ProC Global NR can be used to stratify patients with a first unprovoked VTE according to their risk of recurrence.

Keywords

Venous thrombosis, recurrence, ProC Global

Introduction

Human coagulation consists of a highly regulated system of pro- and anticoagulant forces that insure maintenance of blood fluidity and localize thrombus formation to the site of vascular injury. Any disturbance in the balance of these forces can lead to states of hypocoagulability, clinically manifested as bleeding, or hypercoagulability, clinically manifested as thrombosis. Routine coagulation assays, such as activated partial thromboplastin time (APTT) or prothrombin time, and platelet counting, can detect severe hypocoagulable conditions in most patients, but are less reliable for diagnosing hypercoagulable disorders (1).

Evidence is increasing that global coagulation markers could be useful to identify patients at risk of thrombosis. Patients with first venous thromboembolism (VTE) and low D-dimer after discontinuation of thromboprophylaxis with vitamin K-antagonists have a significantly lower risk of recurrence than those with high D-dimer (2, 3). In a case-control study, an association between shortened APTT and risk of first VTE was found (4). Thrombosis patients without recurrent VTE also have APTT ratios (patient – to normal coagulation times) greater than those with recurrence (5). It has also been shown that by measuring thrombin generation patients at increased risk of first venous thrombosis (6) and at low risk of recurrent VTE can be identified (7, 8).

Other coagulation tests with the potential to more globally detect an imbalance between procoagulant and anticoagulant forces are under investigation. ProC® Global is an APTT-based plasma assay which tests the global function of the protein C pathway after its activation by viper venom from Agkistrodon contortix (9). The assay is thus suitable to diagnose hereditary or acquired defects in the protein C system, including protein C and S deficiency, resistance to activated protein C, and factor V Leiden. In a retrospective study, an abnormal (below 0.75) ProC Global result was associated with a three-fold increased risk of recurrent VTE (10).

We hypothesized that determination of ProC Global could be useful to stratify patients according to their risk of recurrent VTE. We measured ProC Global in a prospective cohort of 774 patients with a first VTE and assessed the relation between this parameter and the risk of recurrence.
Materials and methods

Patients
The study is part of the Austrian Study on Recurrent Venous Thromboembolism (AUREC), an ongoing multicenter cohort study, aimed to identify incidence and risk factors of recurrent VTE (11). Between July 1992 and May 2005, 2,974 patients older than 18 years with objectively confirmed VTE, who had been treated with vitamin K-antagonists for at least three months, were enrolled. Deep vein thrombosis (DVT) had to be established by venography or ultrasonography (in case of proximal vein thrombosis), and pulmonary embolism (PE) by ventilation-perfusion scanning or spiral computed tomography (CT). Patients with both DVT and PE were classified as having PE. A total of 2,200 patients were excluded because of requirement of long-term antithrombotic treatment for reasons other than VTE (451), cancer (459), surgery, trauma, or pregnancy within three months of VTE (460), more than one previous VTE (378), deficiency of a protein C, protein S, antithrombin (72), or the lupus anticoagulant (75). We also excluded 279 patients in whom material for laboratory testing was not available, and 26 patients with factor VIII levels greater than 230 IU/dl who were included in another prospective study. The study was approved by the local ethics committee and written informed consent was obtained from all patients prior to inclusion. Patients entered the study at the time of withdrawal of vitamin K-antagonists and were seen at the investigating center at three months intervals during the first year and every six months thereafter. They were instructed to report in case of symptoms suggestive of recurrent VTE. All women were advised to refrain from female hormone intake.

Outcome measures
The study endpoints were recurrent symptomatic DVT or recurrent PE. DVT was considered to have recurred if the patient had a thrombus in the leg or arm not affected by the previous thromboembolic event; a thrombus in another deep vein in the leg or arm affected by the previous event; or a thrombus in the same venous system affected in the previous event, with proximal extension of the thrombus (if the upper limit of the original thrombus had been visible) or with a constant-filling defect surrounded by contrast medium (if the upper limit of the original thrombus had not been visible). The diagnosis had to be established by venography or ultrasonography. Recurrent PE had to be confirmed by ventilation-perfusion scanning or spiral CT.

The diagnosis was established by an adjudication committee consisting of independent clinicians and radiologists.

Laboratory analysis
Venous blood was collected into 0.1 vol of 0.11 mM trisodium citrate and was centrifuged for 20 minutes at 2,000 g. Plasma was stored at −80°C until time of assay and DNA isolated from leukocytes using standard methods. For measurement of protein C, protein S, antithrombin, the lupus anticoagulant, factor VIII, factor V Leiden and factor II G20210A venous blood was collected three weeks after discontinuation of vitamin K-antagonists after an overnight fast. For measurement of ProC Global blood was collected at a median of 13 months (interquartile range 5 to 25 months) after the acute VTE. Antithrombin, protein C, and protein S were determined according to routine laboratory methods. The presence of the lupus anticoagulant was assessed according to the criteria of the International Society of Thrombosis and Haemostasis (12). Screening for factor V Leiden and for factor II G20210A was carried out as described (3, 11). Factor VIII was measured by one-stage clotting assays as recently described (3, 11).

Determination of ProC® Global
ProC Global was performed by use of a commercially available assay (ProC Global, Dade Behring, Marburg, Germany) on Blood Coagulation System (BCS Dade Behring, Marburg, Germany) analyser. This assay is based on the APTT which is determined before [protein C activation time (PCAT)-0] and after activation of plasma protein C by Protac, a snake venom (PCAT). For PCAT-0, 50 µl of APTT reagent and 50 µl of buffer were added to 50 µl of undiluted plasma. For PCAT, 50 µl of APTT reagent and 50 µl of Protac were added to 50 µl of diluted plasma. After incubation for 3 min. at 37°C samples were measured. Values are given as a normalized ratio (NR) by dividing the PCAT ratio (PCAT/PCAT-0) of the sample plasma by a PCAT ratio of lyophilized standard human plasma and multiplying by a lot-specific sensitivity value defined for each batch by the manufacturer.

Statistical analysis
Time to recurrence (possibly censored) was analyzed according to survival methods (13). The probability of recurrence was estimated according to the method of Kaplan and Meier (14). To test for homogeneity between strata we applied the log-rank test. Values of ProC Global Normalized Ratio (NR) were analyzed in Cox-proportional-hazard models as a continuous variable and as a dichotomized variable to compare the relative risks of recurrence associated with different levels of the respective parameter. Data was adjusted for age and sex. Continuous data were checked for homogeneity using nonparametric tests (Mann-Whitney-U test) and categorical data using contingency-table analyses (Chi²-test). SPSS 12.0.1 was used for statistical analysis. All data are given mean ± SD.

Results
Base-line characteristics of the 774 patients are shown in Table 1. During follow-up, 18 (2%) patients left the study because of cancer, 106 (14%) because they required long-term antithrombotic therapy (including acetylsalicylic acid) for reasons other than VTE, 38 (5%) because of pregnancy, and 13 (2%) because they were lost for follow-up. Sixteen (2%) patients died, two of recurrent VTE. All patients were followed until they left the study or died.

Ninety-eight (13%) of the 774 patients had recurrent VTE (61 DVT, 37 PE) during an average follow-up of 52 months. Patients with recurrence were predominantly male (66% vs. 34%, p<0.001) and had higher clotting factor VIII (172 ± 43 IU/dl vs. 165 ± 45 IU/dl, p=0.3) and factor IX (126 ± 29 IU/dl vs. 116 ± 24 IU/dl, p=0.002) levels than those without recurrence. Carriers of factor V Leiden were more frequent among patients with recurrence as compared with those without recurrence (43% vs.
ProC Global and risk of recurrent VTE

Patients without recurrence had a higher ProC Global NR than those with recurrence (0.78 ± 0.19 vs. 0.74 ± 0.19, p=0.03). In a Cox proportional hazard model, patients with a ProC Global NR ≥ 0.75 had a 40% lower relative risk (RR) than patients with a ProC Global NR < 0.75 (RR 0.58, 95% CI 0.39–0.86, p=0.007). After adjustment for age and sex, RR remained almost unchanged (0.59, 95% CI 0.40–0.87, p=0.009). At four years, cumulative probability of recurrence was 8.6% in patients with

ProC Global NR ≥ 0.75 and was 17.4% in patients with a lower ratio (p=0.006) (Fig. 1). Risk of recurrence was 2.3/100-patient years among patients with ProC Global NR ≥ 0.75 and was 4.0/100-patient years among patients with lower ProC Global NR.

To investigate whether the association between ProC Global and risk of recurrence was linear or whether a threshold level for ProC Global and recurrence risk existed, we calculated RR of recurrence in groups of patients according to quintiles of ProC Global NR values. As shown in Table 2, a linear decrease in RR of recurrence with increasing ProC Global NR values was found.

The sensitivity and specificity of ProC Global NR cut-off level of 0.75 was 52.0% (95% CI 41.7%-62.1%) and 62.7% (95% CI 58.9%-66.3%), respectively. The negative predictive value of high (≥ 0.75) ProC Global NR was as high as 90.0% (95% CI 86.9%-92.5%). The positive predictive value of low (< 0.75) ProC Global NR was 16.8% (95% CI 12.9%-21.6%).

Patients with ProC Global NR < 0.75 were older (45 ± 17 vs. 47 ± 15 years, p=0.07) and had a slightly shorter observation time (51 ± 38 vs. 53 ± 39 months, p=0.6) than those with values equal or greater than 0.75. The proportion of carriers of factor V Leiden was significantly higher among patients with ProC Global NR < 0.75 as compared with those with higher ProC Global NR (75% vs. 1%, p<0.001). No significant difference between patients with high and low ProC Global NR was seen with regard to frequency of prothrombin 20210A (6% vs. 7%, p=0.8).

Table 1: Characteristics of 774 patients with first venous thromboembolism (VTE).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at first VTE (yrs)</td>
<td>46 ± 16</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>312/462</td>
</tr>
<tr>
<td>Site of thrombosis, no. (%)</td>
<td>418 (54%)</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>356 (46%)</td>
</tr>
<tr>
<td>Factor V Leiden – no. (%)</td>
<td>233 (30%)</td>
</tr>
<tr>
<td>Prothrombin G20210A – no. (%)</td>
<td>51 (7%)</td>
</tr>
<tr>
<td>Factor VIII (IU/dl)</td>
<td>166 ± 45</td>
</tr>
<tr>
<td>Factor IX (IU/dl)</td>
<td>118 ± 25</td>
</tr>
<tr>
<td>Duration of oral anticoagulation (months)</td>
<td>8 ± 11</td>
</tr>
<tr>
<td>Observation time (months)</td>
<td>52 ± 38</td>
</tr>
</tbody>
</table>

Figure 1: Kaplan-Meier method estimates of the probability of recurrent venous thromboembolism (VTE) according to ProC Global Normalized Ratio (NR).
Table 2: Relative risk (RR) of recurrence according to different ranges (quintiles) of ProC Global Normalized Ratio (NR).

<table>
<thead>
<tr>
<th>ProC Global NR</th>
<th>No. of patients</th>
<th>No. of recurrences</th>
<th>Unadjusted RR (95% CI)</th>
<th>Adjusted RR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.58</td>
<td>154</td>
<td>26</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>=0.58–0.74</td>
<td>156</td>
<td>26</td>
<td>0.98 (0.57–1.68)</td>
<td>1.03 (0.60–1.78)</td>
</tr>
<tr>
<td>=0.75–0.85</td>
<td>155</td>
<td>15</td>
<td>0.62 (0.33–1.16)</td>
<td>0.66 (0.35–1.25)</td>
</tr>
<tr>
<td>=0.86–0.92</td>
<td>154</td>
<td>16</td>
<td>0.56 (0.30–1.05)</td>
<td>0.53 (0.28–0.99)</td>
</tr>
<tr>
<td>≥ 0.93</td>
<td>155</td>
<td>15</td>
<td>0.54 (0.29–1.02)</td>
<td>0.61 (0.32–1.16)</td>
</tr>
</tbody>
</table>

*adjusted for age and gender.


greater than 0.75 was no longer significant (RR 0.67, 95% CI 0.34–1.33, p = 0.3). We next excluded patients carrying factor V Leiden from our analysis. Five hundred thirty-six patients had wild-type factor V. At four years, cumulative probability of recurrence was 8.0% (95% CI 5.1%–10.9%) in non-carriers of factor V Leiden with ProC Global NR ≥ 0.75 and was 17.5% (95% CI 7.9%–27.1%) in non-carriers with a lower ratio (p = 0.03). RR of recurrence was 0.48 (95% CI 0.25–0.94, p = 0.03) in patients without factor V Leiden and ProC Global NR ≥ 0.75 as compared to patients without the mutation and ProC Global NR ≤ 0.75. The decreased RR remained almost unchanged after adjustment for age and sex (RR 0.53, 95% CI 0.27–1.04, p = 0.07).

Discussion

Assessing the risk of recurrent VTE is essential to determine the optimal duration of anticoagulation. Extended anticoagulation is generally indicated in patients in whom the risk of recurrence outweighs the risk of bleeding during anticoagulation. It has been attempted to determine the risk of recurrence by measuring a panel of laboratory markers of thrombophilia. Several of these markers, however, do not predict increased risk of recurrence (1, 15). Moreover, thrombotic risk is determined to a considerable extent by circumstantial and clinical factors, and the majority of thrombosis patients carries multiple risk factors (16). Thus, screening of laboratory markers of thrombophilia to assess recurrence risk turned out to be futile. At present, risk of recurrence is estimated on individual patient’s basis by assessing single thrombophilic risk factors and clinical conditions, an approach which is time-consuming and cumbersome in daily routine practice. Our study shows that by use of a simple global coagulation assay, i.e. determination of ProC Global, patients with first unprovoked VTE can be stratified into low and high risk groups of recurrence. Patients with ProC Global NR ≥ 0.75 had a RR of recurrence of 0.59 (95% CI 0.40–0.87) as compared with those with lower ratio which translates into a 40% lower RR of recurrent VTE. The probability of recurrence in patients with high ProC Global NR after four years was as low as 8.6%. These patients most likely will not benefit from prolonged anticoagulation, since risk of bleeding might outweigh risk of recurrence. Importantly, the negative predictive value of high ProC Global NR was high (90%), while the positive predictive value of low ProC Global NR was only 16%.

ProC Global assay is designed to detect hereditary or acquired defects in the protein C-system, including deficiencies of protein C and S as well as factor V Leiden (9, 17). We cannot comment on patients with protein C or protein S deficiency since these patients were excluded from our study, because they are candidates for extended anticoagulant therapy already after their first thrombotic event. The assay was well suited to discriminate carriers and non-carriers of factor V Leiden among our patients as only 1% of carriers of factor V Leiden were represented in the group of patients with ProC Global NR ≥ 0.75. In this cohort, factor V Leiden was more prevalent among patients with recurrence than among those without recurrence. Indeed, RR of recurrence in patients with low ProC Global NR lost significance after adjustment for factor V Leiden. However, when carriers of the mutation were excluded, probability of recurrence still significantly differed between patients with high and low ProC Global NR. RR of recurrence was about 50% lower among non-carriers of factor V Leiden and high ProC Global NR compared with non-carriers and a low ProC Global NR. In a European multicenter evaluation, 40% of patients with VTE without a thrombotic risk factor had abnormally low result on ProC Global testing (18). Furthermore, in a study of women with VTE during female hormone intake, the proportion of women with low ProC Global NR was similar between patients with thrombophilia and those without thrombophilia (19). These findings suggest that the assay detects alterations in anticoagulant capacity of the protein C-system other than protein C and S deficiency or factor V Leiden.

Patients with antithrombin deficiency, the lupus anticoagulant or cancer were also excluded from the study for the same reason as were patients with low protein C or S levels. Patients with VTE provoked by surgery or trauma were excluded because they are at known low risk of recurrence (20). The results of the study can therefore not be extrapolated to these patient groups.

Our study generates the hypothesis that determination of ProC Global can be used to stratify patients with a first unprovoked VTE according to their risk of recurrence. It remains to be investigated whether combining ProC Global with other global coagulation assays improves identification of high and low risk patients.

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References