Aetiology and treatment of venous thromboembolism
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Acquired and Inherited Thrombophilic Factors and the Risk for Residual Venous Thrombosis

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Abstract

Acquired and inherited thrombophilic factors increase the risk for (recurrent) venous thrombotic disease. However, little is known about the pathophysiological mechanisms causing these recurrences, or persistence of thrombosis despite adequate treatment. Because residual thrombosis has been associated with worse prognostic outcome, we have studied the prevalence of residual thrombotic lesions after anticoagulant treatment in patients with deep venous thrombosis of the leg and thrombophilia. Thrombotic parameters as assessed by ultrasonography after a 12-week course of anticoagulants were used.

Both thrombophilia in general and acquired thrombophilia in particular were found to be associated with the extent of residual thrombosis. Of the individual thrombophilic factors, protein C deficiency, prothrombin 20210A mutation, active malignant disease and lupus anticoagulant were associated with increased risk of residual thrombotic mass.

Patients with inherited thrombophilia did not differ from patients without any thrombophilic abnormality concerning residual thrombotic mass (RR:1.3 (0.9-1.8)), while acquired thrombophilic disorders increased the risk for residual thrombotic mass as compared to patients without any defect (RR: 1.7 (1.2-2.2)). However, the mechanisms by which various thrombophilic disorders cause persistence of thrombotic lesions remain unknown, and the usefulness of thrombotic parameters for clinical practice will have to be investigated.
Introduction
Acquired and inherited thrombophilic factors are associated with a tendency to develop venous thromboembolism (VTE). Although it is known that some (acquired) thrombophilic states, for example the antiphospholipid syndrome, malignancy and elevated clotting factor VIII:c, as well as persistence of thrombotic obstruction, increase the risk for recurrence of VTE, little is known about the pathophysiological mechanisms causing these recurrences

Since residual thrombosis may be an important risk factor for recurrent thrombotic disease, it would be interesting to know whether the presence of thrombophilia influences the location and extension of thrombotic lesions and their recovery after treatment with antithrombotic agents. One may hypothesise that thrombotic lesions of thrombophilic patients do not resolve as well as lesions in patients without such abnormalities.

To examine whether thrombophilia is associated with the extent of residual thrombosis after anticoagulant treatment, we used the dataset of a clinical trial on secondary prophylaxis of VTE in patients with acute proximal deep venous thrombosis of the leg (DVT).

Methods
Study Population and Design
Patients with symptomatic DVT from two Dutch teaching hospitals participating in a dose-finding study, described in detail elsewhere, comprised the study population. In brief, this study was a multicenter double blind trial on the dose-effect relationship of subcutaneous longacting pentasaccharide (Idraparinux; Organon, Oss, The Netherlands and Sanofi-Synthelabo, Paris, France) versus an oral vitamin K antagonist (Warfarin) in the 12-week treatment of secondary prophylaxis of VTE. Patients were randomised to either Idraparinux 2.5 mg, 5 mg, 7.5 mg, 10.0 mg or vitamin K antagonist after approximately one week of low-molecular weight heparin (Enoxaparin 2dd 0.1 ml/kg). One of the primary efficacy outcome parameters was the composite of change in thrombotic burden as assessed by compression ultrasonography (CUS) and perfusion lung scanning. Ultrasonography, using real time B-mode with compression only, was performed using a standard 5 to 12 MHz linear array transducer and was performed by the same radiologists in each centre. Veins were scanned in the transverse plane only. In all patients common femoral, superficial femoral and popliteal veins were evaluated, without attempts to identify isolated calf vein thrombosis.
The first CUS (CUS1) was performed at the day at which Idraparinux or warfarin were started, the second ultrasound (CUS2) was performed after 12 weeks of treatment.

Patients with a history of DVT in the ipsilateral leg in which acute DVT had been diagnosed could participate if complete normalisation of thrombosis in that leg had been documented prior to the recurrent symptomatic DVT.

For the present analysis, all patients who had completed the study and in whom screening for thrombophilia had been performed, were included. Since the study results showed a comparable efficacy outcome with respect to thrombotic burden (CUS and perfusion lung scan parameters together) in patients treated with Idraparinux 2.5 mg, 5 mg, 7.5 mg and warfarin, we pooled the data for the present analyses.

Residual Thrombotic Lesions

The main outcome for this analysis was residual thrombus mass after 12 weeks of treatment as measured by compression ultrasonography.

‘Residual thrombus mass’ was used to label the state of no full recovery of thrombus mass after three months of treatment. ‘Normalisation’ was defined as a diameter of less than or equal to 2 mm at each of the three sites. ‘Deterioration’ was defined as an increase in diameter of more than 2 mm or a thrombus growth of more than 25% at any site. Other results were classified as ‘no relevant change’.

The thrombus location was defined by the location of non-compressibility at the level of either at the popliteal, superficial femoral or common femoral vein.

Screening for Thrombophilia

Thrombophilia was defined as having active malignant disease or the antiphospholipid syndrome (acquired thrombophilia) or having abnormal test results for other (hereditary) thrombophilic defects. Antithrombin deficiency, protein S deficiency, protein C deficiency, Factor V Leiden mutation, prothrombin 20210A mutation, mild hyperhomocysteinemia and elevated FVIII:c were considered to be hereditary thrombophilic factors.

Antithrombin antigen concentrations were measured using the Asseraplate Antithrombin Kit (Boehringer, Mannheim, Germany)⁹. Protein C activity was measured by using the Protein C Reagent Kit (Behringwerke, Marburg, Germany)⁹. Concentrations of total and free protein S were measured by ELISA using rabbit antiprotein S polyclonal antibody (DAKO, Glostrub, Denmark) and the 15C4 antiprotein S monoclonal antibody (Serbio, Gennevilliers, France)⁹. Lupus anticoagulant was determined using a panel of coagulation tests, including the
activated partial thromboplastin time (aPTT), the dilute Russell viper venom time (dRVVT) and the kaolin clotting time (KCT), while anticardiolipin antibodies were detected and quantified by ELISA\textsuperscript{11}. Factor V Leiden mutation (FVL) and prothrombin 20210A mutation were determined by standard polymerase chain reaction-based assays as described before \textsuperscript{12,13}. Homocysteine measurements including a loading test were performed. Total (free plus protein bound) homocysteine concentrations were measured by using tri-n-butylphosphine as reducing agent and ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphate as the fluorochromophore, followed by high-pressure liquid chromatography with fluorescence detection\textsuperscript{11}. Clotting factor VIII (FVIII:c) was measured by an one-stage clotting assay, three months after the diagnosis of DVT\textsuperscript{15}.

The following reference values were used: antithrombin: 80 to 120%; total protein S: 65% to 120%; free protein S: 20% to 120%; protein C activity: 65% to 130%; elevated FVIII:c: > 150%; homocysteine: fasting res. after loading levels in women <18.3 microMol/L and <56.2 microMol/L and in men <16.9 microMol/L res. <47.0 microMol/L.

Statistical Analysis
Patients with individual thrombophilic factors or with combinations of thrombophilic factors were compared to individuals without thrombophilia (reference population).

For the comparisons among patients with different thrombophilic factors, Chi-square tests were applied for comparing distribution of dichotomous data, and relative risks (RR) and their corresponding 95% confidence intervals (CI) were calculated. P-values of \textless 0.05 were considered to be statistically significant.

Results
Study Population
A total of 64 patients were eligible for analysis (44% male, mean age 54 years). Table 1 lists the observed thrombophilic defects. One or more defects were found in 53% of patients, while 45% of all patients had an inherited thrombophilic defect. Patients with thrombophilia were comparable with respect to sex (p=0.7) and age (p=0.2) to those without thrombophilia. Of the individual thrombophilic defects, factor V Leiden mutation was the most prevalent (20%). Two patients (3%) had an active form of malignant disease. None of the studied patients developed recurrent VTE during the study or one-month follow-up period.
Table 1: Thrombophilic factors in patients (n, %).

<table>
<thead>
<tr>
<th>Thrombophilic factors</th>
<th>Patients with any thrombophilic factor (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V Leiden mutation*</td>
<td>13 (29)</td>
</tr>
<tr>
<td>Mild hyperhomocysteinemia</td>
<td>12 (19)</td>
</tr>
<tr>
<td>Elevated FVIIIc</td>
<td>11 (17)</td>
</tr>
<tr>
<td>Protein S deficiency</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Prothrombin 20210A mutation*</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Positive anticardiolipin antibody IgG</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Positive anticardiolipin antibody IgM</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Protein C deficiency</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Active malignancy</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Patients with a combination of thrombophilic factors (n, %)</td>
<td>11 (17)</td>
</tr>
<tr>
<td>Patients with inherited thrombophilia*</td>
<td>29 (15)</td>
</tr>
<tr>
<td>Patients with acquired thrombophilia*</td>
<td>5 (8)</td>
</tr>
<tr>
<td>Patients without thrombophilic factors</td>
<td>30 (17)</td>
</tr>
</tbody>
</table>

* all were heterozygous carriers.
+ This group includes patients with protein S and C deficiency, factor V Leiden mutation, prothrombin 20210A mutation, mild hyperhomocysteinemia and elevated FVIIIc.
+ This group includes patients with malignancy, positive lupus anticoagulant and positive anticardiolipin antibodies.

**Thrombotic Parameters**

There were no statistically significant ultrasonographic differences on the first compression ultrasonographic assessment between patients with and without thrombophilia.

Of the three locations, the popliteal vein was most frequently non-compressible on both CUS measurements (Table 2). After 12 weeks of anticoagulant treatment residual thrombotic lesions in the popliteal vein were more often present in patients with thrombophilia as compared to patients without thrombophilia (RR: 2.3 (1.6-3.2)). Furthermore, patients with acquired thrombophilia had more often residual thrombotic mass in the popliteal vein as compared to patients with inherited thrombophilia (RR: 1.7 (1.2-2.3)).

There were no differences in the states ‘deterioration’ or ‘no relevant change’ at the various locations when various groups were compared (thrombophilia vs. no thrombophilia; groups of thrombophilic disorders vs. no thrombophilia; individual thrombophilic defects vs. no thrombophilia). For example, ‘deterioration’ occurred in 3% of all patients with thrombophilia as compared to 4% in patients without thrombophilia (RR: 0.9 (CI: 0.2-4.1)) and ‘no relevant change’ was found in 50 percent of all thrombophilic patients compared to 43% of patients without thrombophilia (RR: 1.2 (0.9-1.6)). Patients with one thrombophilic factor did not differ from patients without thrombophilic defects with respect to residual
thrombotic mass on the second CUS (RR: 1.3 (0.9-1.8)). However, patients with three thrombophilic factors had an increased risk for residual thrombotic mass as compared to patients without thrombophilia (RR: 1.7 (1.2-2.2)).

Patients with inherited thrombophilia did not differ from patients without any thrombophilic abnormality (RR: 1.3 (0.9-1.8)), while acquired thrombophilic disorders increased the risk for residual thrombotic mass as compared to patients without thrombophilia (RR: 1.7 (1.2-2.2)). As compared to inherited thrombophilia, acquired thrombophilia increased this risk as well (RR: 1.3 (1.1-1.6)).

When individual thrombophilic factors were compared to the control group, protein C deficiency, prothrombin 20210A mutation, lupus anticoagulant and active malignancy were risk factors for residual thrombotic mass at the second CUS (all relative risks are 1.7 (1.2-2.2)). Transient risk factors for VTE (e.g. immobilisation, use of oral contraceptives), use of pentasaccharide or warfarin, sex, age, and weight were not associated with important effects on ultrasonographic thrombotic parameters.

**Discussion**

The results of this study show that patients with thrombophilia, in particular acquired conditions, have an increased prevalence of residual thrombotic mass in the central venous tract, as assessed by CUS after a 12-week course of antithrombotic therapy. This was most notable in the popliteal vein. Of the individual thrombophilic factors,
protein C deficiency, prothrombin 20210A mutation, active malignant disease and lupus anticoagulant were associated with increased risk of persistence of thrombotic burden.

Our findings are consistent with the observed increased recurrence risk in patients with malignancy or lupus anticoagulant, and the observation in a recently published study that the risk for recurrence is considerably higher in patients with residual venous thrombosis on ultrasonography as compared to patients with complete normalisation. The prospective study performed by Prandoni et al. found also an association between thrombophilia and residual thrombosis, although screening for thrombophilia was less extensive than in our study, patients with malignancy were not studied, and the primary outcome was recurrent thromboembolic disease.

The mechanisms by which various thrombophilic disorders cause persistence of thrombotic lesions however remain unknown.

Some methodological issues warrant comment. First, although our study had a modest sample size, we were able to detect significant risk increases. Second, we used a subsample of screened patients with DVT participating in a randomised controlled study. The observed prevalence of thrombophilic defects in our study sample was however consistent with known cohorts of consecutive patients with venous thromboembolism.

In conclusion, patients with in particular acquired thrombophilia have an increased prevalence of residual thrombotic mass, which may explain their higher risk for recurrent venous thrombosis.

References
5. The Persist investigators. A novel long-acting synthetic factor Xa inhibitor (SanOrg31006 to replace warfarin for secondary prevention in deep vein thrombosis. A phase II evaluation. Accepted for publication (see Chapter 10 of this thesis).


