Objective. To determine the incidence of deep venous thrombosis (DVT) recurrence in young people, and its association with some genetic polymorphisms (FV G1691A, FII G20210A, MTHFR C677T, PAI-1 4G/5G).

Methods. A database was established prospectively to follow-up a cohort of unselected patients who had had a first episode of objectively proven DVT under the age of 40 years. All patients had DNA analysis for heritable thrombophilia. We excluded patients with deficiency of antithrombin, protein C or protein S, malignant disease, antiphospholipid syndrome, or a requirement for long-term antithrombotic treatment. The end-point was objective evidence of symptomatic DVT recurrence.

Results. Eighty-seven patients were enrolled in the study. Mean duration of follow-up was 4.07 years. At 2 years, the cumulative recurrence rate was 19.3%. The risk of recurrence was not related to presence or absence of laboratory evidence of genetic polymorphisms: FV G1691A (HR 1.26 [95%CI: 0.64–2.46]; p = 0.51), FII G20210A (HR 0.81 [95%CI: 0.35–1.89]; p = 0.62), MTHFR C677T (HR 1.26 [95%CI: 0.56–2.81]; p = 0.58), PAI-1 4G/5G (0.84 [95%CI: 0.35–2.05]; p = 0.71).

Conclusion. In this study, the risk of recurrent deep venous thrombosis in young people was not related with the presence of FV G1691A, FII G20210A, MTHFR C677T or PAI-1 4G/5G polymorphisms.

Keywords: Venous thrombosis; Thrombophilia; Genetic polymorphism; Recurrence.

Introduction

Patients who have suffered symptomatic deep vein thrombosis (DVT) remain at risk for recurrent venous thromboembolism despite adequate anticoagulation treatment. After a first episode of DVT, patients are usually treated with oral anticoagulation for 6 weeks to 6 months. When treatment is stopped, the frequency of recurrence can be 12–18% after 2 years. Of these patients, approximately 5% die from pulmonary embolism. The risk of recurrence is highest soon after the acute episode and it declines with time. Continued treatment with oral anticoagulation therapy will prevent most episodes of recurrence but there is a substantial risk of major bleeding associated with prolonged treatment. In theory, anticoagulant treatment should be continued until the risk outweighs the benefit. However, the optimum duration of treatment is uncertain because the risk of bleeding associated with anticoagulation and the risk of recurrent DVT after stopping treatment are not easily predicted on an individual basis. Heritable thrombophilic defects, particularly some genetic polymorphisms, are identified in many patients with acute DVT. Data concerning the influence of these on the risk of recurrent DVT are controversial owing to the different study designs and patient cohorts which have been examined. In this prospective cohort study, we aimed to determine the incidence of DVT recurrence in young people, and its association with some genetic polymorphisms: Factor V Leiden (FV G1691A), prothrombin G20210A mutation (FII G20210A), C677T mutation in the 5,10-methylenetetrahydrofolate reductase gene (MTHFR C677T) and 4G/5G sequence polymorphism in the promoter of plasminogen activator inhibitor-1 gene (PAI-1 4G/5G).

Materials and Methods

Inception cohort

All outpatients who were referred to S. João University Hospital, between October 1997 and November 2002,
because of a first episode of objectively confirmed DVT under the age of 40 years, were registered on a clinical outcome audit database (Oporto thrombophilia study—PORtromb project). Of the 99 consecutive young patients with a first episode of proven DVT, 12 were excluded from the study. Reasons for exclusion were malignant disease (n=1), antiphospholipid syndrome (n=4), deficiency of antithrombin, protein C or protein S (n=6), need for long-term treatment with antithrombotic drugs (n=1). No patient died or was lost to follow-up. Thus, there were 87 (88%) patients available for final analysis. Mean age in this cohort was 27 years (range 16–40) and 58 (67%) were women. The distribution of the initial thrombotic events in the cohort was: Proximal leg vein, 71 (82%); calf vein, 10 (11%) and arm vein, 6 (7%).

Patients were questioned regarding the circumstances under which they experienced their first DVT. Surgery, trauma, temporary immobilization, contraceptive oestrogen pills and pregnancy were considered as temporary causes associated with DVT and in all other patients the DVT episode was defined as idiopathic or due to a permanent risk factor. We found 34 patients (39.1%) with idiopathic presentation and 53 secondary to known risk factors (60.9%).

Thrombophilia testing was offered to all patients after informed consent. All 87 patients were genotyped for FV G1691A, FII G20210A and MTHFR C677T. Seventy-one (82%) of these were also genotyped for PAI-1 4G/5G.

Patients were told the test results might help to explain why they had suffered venous thrombosis, but that results might not affect their subsequent treatment. All included patients were Caucasians and came from the same geographical area, the North of Portugal.

The study protocol was approved by the Ethics Committee of our Hospital.

Study design

DVT was objectively confirmed by colour duplex ultrasonography or contrast venography. Pulmonary embolism was diagnosed by ventilation-perfusion lung scanning, CT, or pulmonary angiography.

Patients were treated with therapeutic doses of low-molecular-weight or unfractionated heparin, European class 2 compression stockings, and warfarin for 6 months (target international normalized ratio—INR, 2.5; range, 2.0–3.0).

The patients were followed by frequent out-patient visits (every 6 months), after inclusion. The patients were asked to report immediately if symptoms suggestive of recurrent DVT developed and in case of a suspected episode the participating physicians were instructed to use objective methods for an appropriate diagnosis according to the criteria in the study. In all patients, the radiological evidence for recurrence was reviewed by one investigator who was unaware of the results of the thrombophilia tests.

All patients were told their results and advised regarding acquired risks for DVT and the need for short-term antithrombotic prophylaxis at times of high risk. All women were strongly discouraged from using contraceptive pills regardless of whether they had a history of an association between the use of these hormones and the initial episode of DVT.

Laboratory methods

DNA was extracted using QIAamp (Hilden, Germany) and polymerase chain reaction (PCR) real-time using LightCycler™ (Roche Molecular Biochemicals, Manheim, Germany) was applied. Hybridization probes technique (sequence-specific fluorescent detection with oligonucleotide hybridization probes that are coupled to suitable fluorophores) was used. PCRs were performed in the LightCycler glass capillaries.

For the Factor V Leiden (G1691A), prothrombin G20210A mutation and MTHFR C677T, commercial kits (Roche) were used according to the manufacturer’s instructions.

For the PAI-1 polymorphism two mixes, one for each purpose, were constituted: 2 μl genomic DNA (100 ng), 10.8 μl H2O, 1.6 μl MgCl2 (3 mM), 1 μl of each primer (10 μmol), 0.8 μl of each probe (5 μmol) and 2 μl DNA-Master hybridization probes. The oligonucleotide primers and probes were synthesised by TIB MOLBIOL (Berlin, Germany). The wild-type complementary detection probes 3'-labelled with fluorescein covered the mutation site (sensor) and the adjacent anchor probes were 5'-labelled with the LC-Red640 dye (anchor). Cycling conditions included initial denaturation (95 °C for 600 s), followed by 45 cycles with denaturation at 95 °C for 0 s, hybridization at 59 °C for 5 s and extension at 72 °C for 2 s. Melting curves were generated by slowly heating the sample to 95 °C.
75 °C. The melting points of the two alleles were at 59 and 65 °C. In different runs, the positions and distances of the melting peaks were identical and differed by less than 1 °C for the same allele. For a better discrimination of heterozygotes, the amplified products were run on agarose gel.

**Statistical analysis**

Time-to-event analysis was done with Kaplan–Meier curves and compared by a log-rank test. Univariate and multivariate Cox proportional hazards models were used to assess the association between DVT recurrence and genetic polymorphisms, determining the hazard ratios (HR) and their 95% confidence intervals (95%CI). A p-value < 0.05 was considered statistically significant.

All computations were performed with the use of SPSS® software, version 12.0.

**Results**

Two years of follow-up was completed in 75 patients (86.2%) and mean duration of follow-up was 4.07 years. For the total cohort the cumulative recurrence rate was 19.3% (standard error 0.045) at 2 years and 23.2% (standard error 0.051) at 4 years, by Kaplan–Meier analysis (Fig. 1). Most of the recurrences were in the leg involved in first episode (63%). Table 1 shows the risk of recurrent DVT in relation to presence or absence of laboratory evidence of a heritable defect. When we analysed the impact of the presence or absence of the FV G1619A mutation, there was no evidence of a significant difference (HR 1.26 [95%CI: 0.64–2.46]; p = 0.51). Likewise, when we investigated the FII G20210A mutation, we noted no evidence of a difference between carriers and non-carriers (HR 0.81 [95%CI: 0.35–1.89]; p = 0.62). The same results are obtained for presence or absence of the C677T mutation in the MTHFR gene, even when only the homozygous mutant genotype was considered (HR 1.26 [95%CI: 0.56–2.81]; p = 0.58), or for presence or absence of the PAI-1 4G/5G polymorphism in homozygous 4G state (HR 0.84 [95%CI: 0.35–2.05]; p = 0.71).

The risk of pulmonary embolism was not significantly different between carriers and non-carriers of these genetic polymorphisms.

**Discussion**

The pathogenesis of a first episode of DVT and of its recurrence is multifactorial, depending on the severity and number of hereditary and circumstantial factors.17 The risk of recurrent venous thrombosis is greatly increased among patients who have had more than one thromboembolic episode or with an idiopathic first DVT.1,3,4,6 Debate continues about the use of testing for evidence of thrombophilia; however, as results of more studies are reported, the value and indications for such testing becomes more controversial.19,20

The results of our prospective study show that young patients with DVT who are heterozygous carriers of the prothrombin or factor V gene mutation, or homozygous carriers of the MTHFR C677T or PAI-1 4G/5G polymorphism, are not at higher risk for further venous thrombotic complications than patients without a known thrombophilic condition. We believe than this cohort is representative of young patients presenting with DVT for whom the optimum duration of anticoagulation is uncertain. In fact, only patients with a first episode of symptomatic DVT without conditions confounding the risk for recurrences were prospectively enrolled and treated with

**Table 1. Genetic polymorphisms and risk of recurrence**

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Patients</th>
<th>HR (95%CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FV G1619A†</td>
<td>18/87</td>
<td>1.26 [0.64–2.46]</td>
<td>0.51</td>
</tr>
<tr>
<td>FII G20210A†</td>
<td>8/87</td>
<td>0.81 [0.35–1.89]</td>
<td>0.62</td>
</tr>
<tr>
<td>MTHFR C677T‡</td>
<td>9/87</td>
<td>1.26 [0.56–2.81]</td>
<td>0.58</td>
</tr>
<tr>
<td>PAI-1 4G/5G§</td>
<td>20/71</td>
<td>0.84 [0.35–2.05]</td>
<td>0.71</td>
</tr>
</tbody>
</table>

† One homozygote and 17 heterozygotes.
‡ All heterozygotes.
§ Only homozygous 677TT were included.

*Fig. 1. Estimated freedom from recurrent deep venous thrombosis (dotted line—standard error ≥10%).*
antiocoagulants for a period of 6 months. Interpretation bias was avoided by having the adjudication of recurrent events and genetic testing performed by operators unaware of other patient details. Patients were not selected on the basis of family history of thrombosis, but rather from a cohort of unselected young patients from which those with malignant disease, antiphospholipid syndrome or deficiency of a natural anticoagulant (antithrombin, protein C or protein S) had been excluded. The overall cumulative recurrence rate of 19.3% at 2 years, is similar to other reports. The incidence of thrombophilic defects (the frequency of the factor V Leiden and FII G20210A gene mutations was 20.7 and 9.2%, respectively) also suggests that this cohort is typical of an unselected patient group. Another important argument is that incidence of recurrent DVT is highly dependent on the extension of the initial thrombosis and we have 82% of enrolled patients with proximal-vein thrombosis.

One advantage of this study is the genetically homogenous sample used, living in the same geographical area of Portugal. This might be important due to prevalence variability of these polymorphisms in different populations.

Because of the small size of the sample only one patient carried the FV Leiden mutation in homozygous state and none of the patients was homozygotic for prothrombin gene mutation. These facts precluded an adequate analysis in the context of homozygosity for these two mutations.

Our findings are in keeping with the results of other studies in patients with a first episode of DVT, despite the singularity of a cohort of young patients. In contrast, in other reports, an association was found between the presence of the factor V or prothrombin gene mutations and recurrent DVT. The incidence of recurrent DVT is highly dependent on the extension of the initial thrombosis and we have 82% of enrolled patients with proximal-vein thrombosis.

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References


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