The influence of factor V Leiden and G20210A prothrombin mutation on the presence of residual vein obstruction after idiopathic deep-vein thrombosis of the lower limbs

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Summary
It was our aim to assess whether factor V Leiden (FVL) and G20210A prothrombin (FII) mutation are associated with the presence of residual vein obstruction (RVO) after a standard course of anticoagulation for a first episode of idiopathic proximal deep-vein thrombosis (DVT) of the lower limbs, with or without symptomatic pulmonary embolism (PE). Patients were enrolled in two prospective multicentre studies: PROLONG and PROLONG II. RVO was detected by compression ultrasonography according to the method of Prandoni on the day of anticoagulation withdrawal. Patients were also screened for FVL and FII mutation. The presence of FVL and/or FII mutation was determined in 872/963 (90.5%) patients, in 753 of whom RVO was assessed. FVL was significantly less frequent among subjects with isolated PE (7/176:4%) than among patients with either DVT and PE (15/133:11.3%; p=0.0018) or isolated DVT (89/563:15.8%; p<0.0001), confirming the FVL paradox. The rate of FII mutation was similar among patients with isolated PE (11/176:6.2%) and patients with either DVT and PE (12/133:9%) or isolated DVT (52/563:9.2%). FVL and FII mutation were not significantly associated with RVO at the multivariate analysis in all patients, although data suggest that FVL and FII mutation may have a differential effect on RVO in the subgroups of patients with DVT and DVT plus PE patients. Male sex and isolated DVT were significantly associated with RVO in all patients. In conclusion, male sex and isolated DVT are associated with RVO, while FVL and FII mutations are not significantly associated with RVO in this study.

Keywords
Deep-vein thrombosis, pulmonary embolism, thrombophilia, venous thrombosis, residual vein obstruction

Introduction
Residual vein obstruction (RVO) develops in approximately 40% of patients after a standard 3-6 month course of anticoagulation for a first episode of deep-vein thrombosis (DVT) of the lower limbs (1, 2). The incomplete resolution of thrombi and their transformation into RVO may produce permanent damage of deep vein valves and post-thrombotic syndrome might ensue (3-5). RVO has also been shown to be associated with the risk of recurrence by some authors (6, 7), although such an association has not been confirmed by others (8-10).

Factors influencing RVO development may help identify those patients at a higher risk of post-thrombotic syndrome and, possibly, recurrent events. Such factors, however, are not completely elucidated. Thrombus extension, treatment delay after the start of symptoms, anticoagulation quality and duration may be all associated with the incomplete resolution and fibrous organisation of thrombi. Data on the effect of thrombophilia on RVO development are scarce, but they suggest that thrombophilia may be associated with RVO (11, 12). Bank et al. (11) showed that protein C deficiency, G20210A prothrombin (FII) mutation, active malignant disease and lupus anticoagulant were associated with an increased risk of residual thrombotic mass. Subsequently, Spiezia et al. (12) showed that thrombophilia influenced vein recanalisation and thus the development of persistent RVO in 472 patients with...
prospective multicentre studies: PROLONG (18) and PROLONG II (19).

Methods

Study patients

The PROLONG (18) was a multicenter randomised study in which patients aged 18 to 85 years, with a first episode of objectively documented symptomatic idiopathic venous thromboembolism (VTE), either proximal lower extremity DVT and/or PE, were eligible. The PROLONG II study was a prospective cohort study in patients aged 18 to 82 years with a first episode of objectively documented symptomatic idiopathic VTE, either proximal lower extremity DVT and/or PE. Patients were enrolled after receiving at least three (PROLONG) or six (PROLONG II) months of vitamin K antagonists (VKA) therapy [either warfarin (Coumadin, Bristol Myers Squibb) or acenocoumarol (Sintrom®, Novartis Pharma)] with a target international normalised ratio (INR) of 2.5 (range 2.0 to 3.0).

Idiopathic or unprovoked VTE was defined as an episode not associated with a triggering factor or active malignancy, as reported previously, including those events occurring during oral contraceptive (OC) use.

Exclusion criteria were serious liver disease, renal insufficiency (plasma creatinine >2 mg/dl), active cancer or undergoing chemotherapy and/or radiotherapy, other indications for anticoagulation or contraindications for such treatment, limited life expectancy, or geographical inaccessibility. The Ethics Committees of all participating clinical centers approved the study. All enrolled patients provided written informed consent.

Study procedures

Briefly, on the day of VKA discontinuation, compression ultrasoundography (CUS) of the lower limbs was performed to detect any proximal DVT, regardless of the the type of event, DVT localisation and type of thrombophilia.

The association between RVO and thrombophilia may be due to the influence on thrombus structure by common defects, such as factor V Leiden mutation (FVL), the most prevalent genetic factor known to increase the risk of venous thrombosis. FVL has repeatedly been shown to be a stronger risk factor for a first episode of DVT than for pulmonary embolism (PE) and this differential effect is known as the “Factor V Leiden paradox” (13-17). The mechanism of this effect is unknown although it has been hypothesised that FVL may make the thrombus less prone to breakage and therefore to embolisation. Thrombophilic defects could therefore play a role in slowing thrombi resolution and favouring their fibrotic organisation into persistent RVO.

The aim of this study was to assess whether common thrombophilic defects, such as FVL and FII mutation, are associated with RVO presence after a standard course of anticoagulation for a first episode of idiopathic proximal DVT of the lower limbs, with or without symptomatic PE, in consecutive subjects enrolled in two prospective multicentre studies: PROLONG (18) and PROLONG II (19).

Statistical analysis

Baseline differences between groups were assessed by the Chi-square test (Yates’ correction) or McNemar’s test for categorical variables and t-test or Mann-Whitney U test for continuous variables, as appropriate.

Odds ratios (OR) and their 95% confidence intervals (95% CI) were calculated using univariate logistic regression to establish factors associated with RVO development.

A stepwise multivariate model was then constructed including age, sex, duration of anticoagulation before enrollment, type of index event -DVT with or without PE, presence/absence of FVL and/or FII mutation, D-dimer (negative or positive), OC (use or no use). Only the possible interaction between VTE site and thrombophilia was considered; no other subgroup analyses were conducted. To minimise concern about multiple testing, we based our statistical evaluation of the possible interaction between VTE site and thrombophilia (either FVL or FII mutation) on the risk of developing RVO with use of multivariate logistic regression model incorporating terms for the main effect of VTE site, the main effect of FVL and/or FII, and the interaction terms between VTE site and thrombophilia (either FVL or FII mutation) on the risk of developing RVO.

The data were analysed using the Prism statistical software package (Version 3.0, GraphPad Software Incorporated, San Diego, CA, USA) and the SPSS statistical package (Version 13.0, SPSS Inc., Chicago, IL, USA).
Results

Characteristics of patients

Figure 1 shows the flow-chart of the analysis and Table 1 shows the characteristics of the 963 enrolled patients. The majority of patients (617: 64%) had an isolated symptomatic DVT, while 20% (194) had isolated PE and only 16% (152) had both symptomatic DVT and PE. There were significantly less men (37%) than women among patients with isolated PE than among patients with either isolated DVT (57% p<0.001) or DVT and PE (56% p<0.001), even after excluding women with PE during OC (42% men). FVL was significantly less frequent among subjects with isolated PE (4%) than among patients with either isolated DVT (15.8%; p<0.0001) or DVT and PE (11.3%; p=0.0018), thus confirming the “FV Leiden paradox”. Patients with DVT and PE had a significantly lower rate of RVO at the end of treatment than patients with isolated DVT (31% vs 43%; p <0.001).

Features associated with RVO presence in DVT patients

Table 2 shows that males and isolated DVT were more frequent among subjects with RVO when compared with subjects without RVO, although significance was lost after correcting for multiple testing. The frequency of FVL was similar in patients with and without RVO. The FII mutation was less frequent in subjects with isolated PE (4%) than among patients with either isolated DVT (15.8%; p<0.0001) or DVT and PE (11.3%; p=0.0018), thus confirming the “FV Leiden paradox”. Patients with DVT and PE had a significantly lower rate of RVO at the end of treatment than patients with isolated DVT (31% vs 43%; p <0.001).
combination) and also in subjects with DVT and PE and one site with RVO (data not shown).

Table 3 shows the results of the univariate and multivariate stepwise logistic regression analysis of variables associated with RVO in all patients. The effect of FVL was not significant either at the univariate or multivariate analysis, while the FII mutation was associated with a significantly lower probability of developing RVO at the univariate but not at the stepwise multivariate analysis. VTE site (isolated DVT) and male sex were significantly associated with RVO presence both at the univariate and multivariate analysis. A sensitivity analysis was conducted after excluding OC users OR: 1.5; 95% CI: 1.1–2.1; p=0.021. ** after excluding OC users OR: 0.6; 95% CI: 0.3–1.1; p=0.098. *** after excluding OC users OR: 1.7; 95% CI: 1.1–2.7; p=0.023.

Subgroup analyses according to VTE site were conducted as the results in Table 2 suggested that thrombophilia effects on RVO were in the opposite direction in isolated DVT and DVT plus PE patients. Table 4 shows the univariate and multivariate logistic regression analysis of the association of FVL or FII mutation with RVO in the two subgroups of patients with isolated DVT and DVT plus PE. Although the interactions between VTE site and thrombophilia were not statistically significant, the effect of FVL on RVO was of borderline significance with large confidence intervals in subjects with DVT and PE (OR: 3.6, 95% CI: 1.1–12.5; p=0.045), but not significant in patients with isolated DVT (OR: 0.9, 95% CI: 0.6–1.6; p=0.9) at the multivariate analysis. The effect of FII mutation was associated with a lower probability of developing RVO in patients with isolated DVT with borderline significance (OR: 0.5; 95% CI: 0.2–0.9; p=0.048) but not in patients with DVT and PE (OR: 1.1; 95% CI: 0.2–4.6; p=0.1) at the multivariate analysis.

Discussion

The composition of thrombi in lower limb DVT, their tendency to embolise and dissolve completely or not may carry long-term consequences. RVO, alone or in combination to valvular reflux, can lead to chronic venous hypertension and thus to the post-thrombotic syndrome (3-5). Common thrombophilic defects may influence the structure of thrombi and their evolution into persistent RVO (11, 12). FVL has been shown to be associated with a lower frequency of isolated PE than DVT, suggesting that in FVL patients thrombi are more firm and less likely to embolise.

Our post-hoc analysis confirms the FVL paradox but shows that when all patients with DVT and DVT plus PE were considered, FVL had no significant effect on RVO, while the FII mutation was associated with a lower probability of developing RVO which, however, became non significant at the multivariate analysis and...
Residual vein obstruction (RVO) occurs in approximately 40% of patients with deep vein thrombosis (DVT) and pulmonary embolism (PE). Our post-hoc analysis shows that factor V Leiden (FVL) and factor II (FII) mutation were not significantly associated with RVO at the multivariate analysis. This indicates that FVL could influence RVO development in patients with DVT and PE, but this effect estimate is imprecise for the large CIs due to the small sample size of DVT plus PE patients. The effect of FII mutation was associated with a significant lower probability of developing RVO in patients with isolated DVT but not in patients with DVT and PE, probably due to the small sample size of DVT plus PE patients. This effect may be related to the higher risk of developing isolated PE observed in FII mutation carriers, unlike those of FVL, as shown by Martinelli et al. (16), which may imply that FII mutation may favour thrombus dissolution and embolisation.

Our data also show that other factors, such as site of VTE and sex, have a stronger effect on RVO development than thrombophilia. Patients with isolated DVT were more prone to develop RVO when compared to subject with DVT and symptomatic PE. This suggests that in patients with DVT and PE, thrombi can embolise, and thus dissolve more easily, than in subjects with isolated DVT. Males also developed RVO more frequently than females, although the underlying mechanism of the effect of sex on RVO is unknown. However a higher risk of recurrent VTE has been shown in males than in females (23, 24) and the association of RVO with male sex might be a marker of hypercoagulability.

Differences in populations and study methods may explain the different results of our post-hoc analysis when compared with previous studies on the association of thrombophilia and RVO (11, 12). Both previous studies were single-centre studies, while our study is a post-hoc analysis of two multicentre, prospective studies. Banks et al. (11) and also Spiezia et al. (12) enrolled patients with both provoked and unprovoked DVT and protein C, protein S and antithrombin deficiencies and anti-phospholipid antibodies were measured. In our studies patients with unprovoked VTE were enrolled and only FVL and FII mutation were determined. The study by Banks et al. (11) only included 64 patients. The study by Spiezia et al. (12) was retrospective and RVO was determined serially until vein recanalisation was detected.

Some limitations of our study should be considered. The clinical utility of RVO is uncertain, for both recurrence risk and PTS. RVO has been shown to be a marker of recurrence risk by some authors (6, 7), but its predictive role has not been confirmed by other authors (8-10). In a previously published post-hoc analysis of the PROLONG study we did not find any association between RVO and recurrent VTE (9).

Factors which may influence RVO, such as delay in diagnosis and start of treatment, quality of anticoagulation, thrombus extension, were not recorded. As a result, the differences observed may be affected by these unstudied factors. Treatment duration may also influence RVO development and in both studies RVO was as-

| Table 4: Influence of FVL and FII mutation on RVO in the subgroups of patients with isolated DVT and DVT plus PE *.

<table>
<thead>
<tr>
<th></th>
<th>Isolated DVT</th>
<th>DVT plus PE</th>
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<tbody>
<tr>
<td>FVL (present vs. absent)</td>
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<tr>
<td>Unadjusted OR (95% CI)</td>
<td>0.98 (0.6–1.5)</td>
<td>2.8 (0.9–8.6)</td>
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<tr>
<td>Adjusted OR (95% CI)**</td>
<td>0.9 (0.6–1.6)</td>
<td>3.6 (1.1–12.5)</td>
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<td>Interaction between VTE site and FVLS§</td>
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<tr>
<td>Unadjusted p-value</td>
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<tr>
<td>Adjusted p-value**</td>
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<tr>
<td>FII mutation (present vs. absent)</td>
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<td></td>
</tr>
<tr>
<td>Unadjusted OR (95% CI)</td>
<td>0.5 (0.25–0.92)</td>
<td>0.77 (0.2–3.2)</td>
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<tr>
<td>Adjusted OR (95% CI)**</td>
<td>0.5 (0.25–0.99)</td>
<td>1.1 (0.2–4.6)</td>
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<tr>
<td>Interaction between VTE site and FII mutation §</td>
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<tr>
<td>Unadjusted p-value</td>
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<tr>
<td>Adjusted p-value**</td>
<td>0.511</td>
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</table>

* Odds ratios represent the risk of RVO development and were obtained with logistic regression. ** values were adjusted for sex, age (<65 vs. > 65 years), VKA duration (< 6 vs. > 6 months), OC use (no vs. yes), D-dimer (negative vs. positive). § p-values are for the VTE site and thrombophilia interaction term entered in the logistic regression model.

What is known about this topic?
- Residual vein obstruction (RVO) occurs in approximately 40% of patients after a standard 3-6 month course of anticoagulation for a first episode of deep-vein thrombosis (DVT) of the lower limbs.
- RVO can lead to chronic venous hypertension and thus to the post-thrombotic syndrome and it has also been shown to be a marker of higher risk of recurrence by some, but not all, authors.
- Factors influencing the presence of RVO after DVT of the lower limbs are not completely elucidated, and their determination may help identify those patients at a higher risk of developing the post-thrombotic syndrome and possibly recurrent events.
- Data on the role of thrombophilia in RVO development are scarce, indicating that thrombophilia may be associated with RVO.

What does this paper add?
- Our post-hoc analysis shows that factor V Leiden (FVL) and factor II (FII) mutation were not significantly associated with RVO at the multivariate analysis in all patients.
- Data were suggestive for FVL being associated with RVO in patients with DVT plus pulmonary embolism (PE), while FII mutation was associated with a lower frequency of RVO in isolated DVT patients.
- Male sex and isolated DVT were significantly associated with RVO in all patients.
sessed at different time points but always after at least 3-6 months of anticoagulation. Subjects with RVO had a longer mean anticoagulation duration than subjects without RVO, although the difference in anticoagulation duration between the two groups was not significant. This indicates that the duration of treatment beyond 3-6 months does not influence RVO development. Moreover, no standardised and universally accepted method of RVO measurement is available. As a result, the reproducibility of this measurement could be a relevant issue across studies due to the lack of widely accepted criteria for the definition of vein recanalisation. As an effort to standardise RVO measurement across different centres, a video of a practical session showing the measurement of RVO was sent to all the participating centres. However, a formal intra- and inter-observer variation assessment was not conducted in either studies, due to the high number of centres, which in itself, however, may better reflect a wide variety of practicing clinicians.

Valvular reflux was not determined nor PTS development. As a result, the association between RVO and either valvular reflux or

Study sites and investigators of the PROLONG study (all the participating centers are affiliated in the Italian Federation of Anticoagulation Clinics –FCSA):


Study sites and investigators of the PROLONG II study (all the participating centers are affiliated in the Italian Federation of Anticoagulation Clinics –FCSA)

- A.O. Istituti Ospitalieri, Cremona – S. Testa, A. Alatri, P. Oretti;
- Centro Trombosi, A.O. di Careggi Universita’ di Firenze – D. Prisco, D. Poli;
- Centro Trasfusionale e Ambulatorio Emostasi e Trombosi, Ente Ospedaliero di Vimercate Usl 28, Vimercate – L. La Rosa;
- Centro Emostasi e Trombosi, Arcispedale Santa Maria Nuova, Reggio-Emilia – A. Ghirarduzzi;
- Centro Emostasi e Trombosi, Ospedale degli Infermi, Rimini – E. Tiraferri;
- Laboratorio Analisi – Ambulatorio per il Controllo della Terapia Anticoagulante Orale, Presidio Ospedaliero di Faenza – E. Bucherini;
- Centro Emofilia - Servizio Emostasi e Trombosi Azienda Ospedaliera Pugliese-Ciaccio; Catanzaro; R. Santoro;
- Centro Regionale Malattie Emorragiche e Trombotiche – Div. Ematologia, Ospedale S. Bortolo, Vicenza – A. Tosetto;
- Centro Pazienti Anticoagulati, Azienda Spedali Civili di Brescia, Brescia Del Bono Roberto;
- Centro Per Il Controllo Della Terapia Anticoagulante e per lo Studio della Trombosi; Ospedale Civile S. Andrea-Asl N.5 La Spezia, R. Benedetti;
- Centro Emostasi e Trombosi, Ospedale dell’Annunziata, Cosenza – V. Rossi;
- Laboratorio Emostasi E Trombosi Centro Trombosi , Istituto Clinico Humanitas-Inrcs Rozzano, L.Rota;
PTS cannot be determined in this analysis. The effect of thrombophilia on recurrent VTE was not addressed by this study.

PE was looked for in subjects with DVT only in case of symptoms, while asymptomatic PE was not searched systematically. Moreover, only patients with idiopathic VTE were enrolled and factors influencing RVO may be different in subjects with DVT secondary to a triggering factor or to cancer. Patients were enrolled in many centers over several years, and different regional distribution of common thrombophilic defects may also be taken into account in the differential effect of thrombophilia on RVO.

Our data indicate common thrombophilic defects do not significantly influence RVO development, although our data suggest that the effect of common thrombophilic defects on RVO development may differ according to VTE site and also may have an opposite effect in isolated DVT and DVT plus PE patients. However, the estimate of thrombophilia effects is imprecise due to the small sample size of patients with DVT plus PE, and these effects on RVO are lost after excluding OC-related VTE. Larger studies are required to confirm these findings.

VTE site (isolated DVT) and male sex are significantly associated with RVO development, and their influence is stronger than that of thrombophilia in patients with idiopathic DVT of the lower limbs with or without PE. These factors may help identify those subjects with idiopathic DVT more prone to develop RVO, the post-thrombotic syndrome and, possibly, recurrent events. In our study the results are suggestive after consideration of multiple testing and a larger replication study will bring proof.

Conflicts of interest
None declared.

References