Predictive Value of D-Dimer Test for Recurrent Venous Thromboembolism After Anticoagulation Withdrawal in Subjects With a Previous Idiopathic Event and in Carriers of Congenital Thrombophilia

Gualtiero Palareti, MD; Cristina Legnani, MS; Benilde Cosmi, MD; Lelia Valdré, MD; Barbara Lunghi, MS; Francesco Bernardi, MS; Sergio Coccheri, MD

Background—We have shown that normal D-dimer levels obtained after the discontinuation of oral anticoagulant treatment (OAT) has a high negative predictive value for recurrent venous thromboembolism (VTE). The aim of the present study was to assess the predictive value of D-dimer for recurrent VTE in subjects with a previous unprovoked event who are either carriers of inherited thrombophilia or not.

Methods and Results—We prospectively evaluated 599 patients (301 males) with a previous VTE episode. They were repeatedly examined for D-dimer levels after OAT withdrawal and were screened for inherited thrombophilic alterations. Alterations were detected in 130 patients (21.7%), factor V Leiden (70 patients; 2 of whom were homozygotes) and prothrombin mutation (38 patients) were the most prevalent ones. Recurrent events were recorded in 58 subjects (9.7%) during a follow-up of 870.7 patient-years. Altered D-dimer levels at 1 month after OAT withdrawal were associated with a higher rate of subsequent recurrence in all subjects investigated, especially in those with an unprovoked qualifying VTE event (hazard ratio, 2.43; 95% confidence interval, 1.18 to 4.61) and in those with thrombophilia (hazard ratio, 8.34; 95% confidence interval, 2.72 to 17.43). The higher relative risk for recurrence of altered d-dimer was confirmed by multivariate analysis after adjustment for other risk factors. The negative predictive value of D-dimer was 92.9% and 95.8% in subjects with an unprovoked qualifying event or with thrombophilia, respectively.

Conclusions—D-dimer levels measured 1 month after OAT withdrawal have a high negative predictive value for recurrence in subjects with unprovoked VTE who are either carriers or not carriers of congenital thrombophilia.

Key Words: thrombophilia • fibrin fibrinogen degradation product • thromboembolism • thrombosis

After a first episode of acute venous thromboembolism (VTE), the risk of recurrence is relatively high and clinical consequences are important because early (fatal in 5% of patients1) and late (post-thrombotic syndrome) complications are frequent. Although long-term treatment with oral anticoagulants is indicated to reduce this risk, the incidence of treatment-associated complications is not negligible because major bleeding can be expected in ≈2% patient-years.2 The duration of anticoagulant treatment should therefore be tailored to optimize the preventive action of treatment with the minimum risk of complications. It is now well accepted that patients whose first VTE event was associated or triggered by a circumsitual risk factor have a lower risk of recurrence and need shorter anticoagulation periods than patients whose event was unprovoked (idiopathic) or who carry persistent risk factors.3 Although in recent years a number of clinical studies have investigated the effects of different treatment periods in subjects after an idiopathic VTE event, the optimal duration of oral anticoagulant treatment (OAT) in these patients is still uncertain.4–7

It is still a matter of debate whether inherited thrombophilia should be considered a persistent risk factor and whether it requires a longer course of OAT.8,9 A higher incidence of recurrence has been shown in patients with an antithrombin, protein C, or protein S deficiency,10 in patients with multiple thrombophilic defects,11,12 and in patients with a homozygosity for factor V Leiden.13–15 It is still debated whether carriers of heterozygous factor V Leiden or the G20210A prothrombin mutation, the two most common thrombophilic alterations, are at higher risk of recurrence.
Such a conclusion has been drawn in some studies but was not confirmed by other authors.

In a recent prospective study in subjects with a previous first VTE event, we showed that normal D-dimer levels obtained after OAT withdrawal had a very high negative predictive value for VTE recurrence. Conversely, increased D-dimer levels were associated with a significantly higher hazard ratio for recurrence.

The aim of the present prospective, inception-cohort study was to investigate the predictive value for recurrent VTE of D-dimer levels measured 1 month after OAT interruption in patients with a previous unprovoked event who were either carriers or not carriers of congenital thrombophilia.

Methods

Patients
As described elsewhere, consecutive patients with a previous first episode of deep vein thrombosis (DVT) of the lower limbs and/or pulmonary embolism (PE) were prospectively investigated after OAT discontinuation. All patients had attended our clinic for the surveillance of OAT and were treated with warfarin or acenocoumarol at a therapeutic intensity of 2.0 to 3.0 International Normalized Ratio (target, 2.5). The overall quality of laboratory anticoagulation control in patients treated for VTE at our clinic is periodically checked by a specific software program that calculates the percentage of time spent within, below, and above the intended therapeutic range.

For the year 2002, the results were 60.1%, 27.9%, and 12.0% for the time spent within, below, and above the therapeutic range, respectively. The decision to stop anticoagulation was made during a periodic examination after a minimum of 3 months from the index event. Physical examinations were scheduled at 3 months after OAT interruption and every 6 months thereafter. Patients were instructed to wear 40-mm Hg elastic stockings and to contact our department immediately if they experienced symptoms attributable to a new or recurrent VTE episode. Follow-up ended on the 21st month after OAT interruption or earlier in case of death, VTE recurrence, or resumption of OAT for any other indication. The patient and/or family doctor were contacted if a periodic examination was missed.

Index VTE events were classified as unprovoked (in the absence of any clinical risk factors), as secondary to nonremovable risk factors (mainly cancer), and as secondary to circumstantial and removable risk factors, such as surgery, trauma, prolonged immobilization, oral contraception or hormonal replacement therapy, pregnancy, or puerperium. Our institutional review committee approved the study, and all the procedures were in accordance with our institutional guidelines. Informed consent was obtained from all patients.

Timing of Blood Sampling and Thrombophilia

Work-Up
Venous blood samples were taken on 3 different occasions: the day OAT was discontinued (T1), 21 to 37 days afterward (T2), and 3 months (±10 days) afterward (T3). For the sake of brevity, only results obtained in T2 samples or, in their absence, in T1, are considered in the present report.

Thrombophilia screening was performed using blood sampled at T2 (on average, 33 days after OAT discontinuation) and only in a few cases during follow-up. The screening included: prothrombin time, activated partial thromboplastin time, fibrinogen plasma levels, antithrombin, protein C, protein S, and activated protein C resistance. DNA analysis for R506Q factor V mutation was performed in all cases with an activated protein C resistance normalized ratio <0.80. Patients also had a DNA analysis for the G20210A mutation of the prothrombin gene and tests for lupus anticoagulant. All tests included in the thrombophilic screening were performed using standard methods.

D-Dimer Measurement

Plasma D-dimer levels were measured by the VIDAS D-Dimer ELISA method (bioMerieux; normal value ≤500 ng/mL). Only D-dimer results obtained in samples collected at T2 were analyzed in the present report (or in T1 in the few cases in which T2 samples were not available). For technical and organizational reasons, it was not possible to test for D-dimer in 8 patients. The results of D-dimer and other blood coagulation tests performed after OAT withdrawal were not used for management purposes.

Outcome Events

Objectively documented DVT recurrence and/or fatal or nonfatal PE (first event or recurrence) were considered outcomes. Patients with DVT symptoms in the same or contralateral leg as the previous event were given a D-dimer test (for its high negative predictive value) and a compression ultrasound, which was compared with the compression ultrasound examination that was customarily performed in all patients at the time of OAT interruption. In cases of suspected DVT recurrence in the same leg as the index event, unequivocal noncompressibility of a previously compressible venous segment or an increase of at least 4 mm in the residual diameters were the criteria considered diagnostic for DVT recurrence. If the ultrasound result was nondiagnostic (and D-dimer was altered), an echo color Doppler or contrast venography was performed. In patients presenting with symptoms compatible with PE (either first event or recurrence), diagnosis was based on the results of objective algorithms using clinical probability, ventilation-perfusion lung scanning, compression ultrasonography (if indicated), and D-dimer testing. Recurrent events were classified as unprovoked or as secondary to known triggering factors.

All deaths were recorded and coded as attributable to a venous event or any other cause. Events were adjudicated by two investigators (G.P. and B.C.) who were unaware of D-dimer and other blood test results.

Statistical Analysis

Differences between groups were assessed by the chi-square test (Yates’ correction). Cumulative incidence and hazard ratios for recurrent VTE in patients with or without high D-dimer levels were calculated by the method of Kaplan and Meier. The 95% confidence intervals (CI) were calculated with an approximate method, and a two-sided P≤0.05 was considered statistically significant. The Prism statistical software package (Version 3.0, Graphpad Software Incorporated) was used for data processing. The relative risks of VTE recurrence associated with normal or altered D-dimer results were analyzed with the Cox regression analysis after adjustment for age, sex, presence/absence of congenital thrombophilia, and nature of index event by using the SPSS statistical package (11.0 release).

Results

Patients
All patients attending our clinic for OAT surveillance after a first episode of DVT and/or PE and in whom OAT was discontinued between February 1995 and February 2002 were considered eligible for the study. Thrombophilia screening was performed in 620 of 635 subjects. Five patients were excluded when a lupus anticoagulant phenomenon was detected. Eight patients were lost to follow-up 3 months after OAT withdrawal because they moved out of town and their new addresses were not available, and in 8 other patients, D-dimer testing was not possible. The present report analyzes the results obtained in 599 patients (301 males). The characteristics of the patients are shown in Table 1. The presence of one or more thrombophilic alterations was detected in 130 patients (21.7%). A detailed list of alterations is reported in Table 1.
During the 870.7 patient-years of follow-up, 58 VTE recurrences (9.7% of patients) were recorded (35 males). The events included 50 patients with DVT (27 ipsilateral and 23 contralateral), 9 of whom also had PE, and 8 patients with apparently isolated PE (with no diagnosis of associated acute DVT). In 36 cases, the recurrent events seemed to be unprovoked. In 16 cases, a condition of malignancy or chronic disease (chronic heart failure and chronic tuberculosis infection, 1 each) was found; 6 patients had a recurrence due to a transient factor (prolonged bed rest, 4 patients; plaster cast, 2 patients). During follow-up, 7 patients died; death was attributed to cancer in 2 patients, chronic heart disease in 2 patients, and to sudden death in 3 patients. The latter cases were considered outcomes for the study, although an autopsy was not performed.

The rate of recurrence was 11.7% in subjects whose index event was idiopathic; it was lower (4.3%) in those with transient risk factors and much higher (23.7%) in subjects whose event was associated with non-removable risk factors. The recurrences were significantly more frequent in patients with thrombophilia (14.6%) than in those without thrombophilia (8.3%), with a hazard ratio of 1.78 (95% CI, 1.04 to 3.66; \( P=0.036 \)).

### D-Dimer Results and VTE Recurrence

Increased D-dimer levels were recorded in 37.1% of subjects (Table 2). The presence of altered rather than normal D-dimer results was associated with a significantly higher rate of recurrences. This was found in all subjects investigated (16.5% recurrences in patients with altered D-dimer versus 5.8% recurrences in patients with normal D-dimer; \( P<0.0001 \)), and in some subgroups, such as patients whose index event was unprovoked (16.5% versus 7.0%; \( P=0.0226 \)), carriers of thrombophilia (27.1% versus 4.2%; \( P<0.0001 \)), and noncarriers of thrombophilia (12.3% versus 6.2%; \( P=0.038 \); Table 2).

The cumulative probabilities for VTE recurrence (Kaplan-Meier curves) of abnormal versus normal D-dimer results 1 month after OAT interruption are shown in Figures 1 and 2. They are calculated in subjects with an unprovoked index event (hazard ratio, 2.43; 95% CI, 1.18 to 4.61; \( P=0.0153 \)) and in subjects with thrombophilia (hazard ratio, 8.34; 95% CI, 2.72 to 17.43; \( P<0.0001 \)) or without thrombophilia (hazard ratio, 2.04; 95% CI, 1.11 to 4.18; \( P=0.0232 \)).

At the multivariate analysis, after adjustment for other relevant factors (Table 3), the presence of altered D-dimer results remained significantly associated with a higher risk of VTE recurrence in all of the investigated subjects, in those with an unprovoked index event, and in subjects with or without thrombophilia. Altered D-dimer levels were not associated with the risk of recurrence in those subjects whose index event was associated with cancer or was secondary to transient factors.

The sensitivity, specificity, and positive and negative predictive values for VTE recurrence of D-dimer levels are shown in Table 4. D-Dimer results at 3 months (data not shown) were altered in a higher percentage of all investigated subjects (47.6%), and sensitivity and negative predictive value were similar to those of tests obtained at 1 month (71.1% [95% CI, 57.0 to 82.9] and 94.9% [95% CI, 91.7 to 97.1], respectively).

### Discussion

The results of the present study, which are in line with those of the previous one, \(^{20}\) confirmed that the rate of VTE recurrence was significantly lower in subjects whose D-dimer levels were normal when obtained 1 month after anticoagulation was stopped. We hypothesize that this test may be relevant for the management of anticoagulant treatment in VTE patients; we believe that results obtained a few weeks after OAT withdrawal can be clinically relevant as criteria to decide whether or not OAT should be resumed. It might be argued that the management of patients with a previous VTE event would be easier if D-dimer levels were tested before discontinuing oral anticoagulation. In our previous article, \(^{20}\) we noted that most patients had normal D-dimer levels at the time of OAT withdrawal. Altered D-dimer levels were found in only 15.6% of subjects at that time, whereas altered levels increased to 40.3% and 46.2% after 1 and 3 months, respectively. This implies, therefore, that most subjects should be retested at least 1 month after from OAT withdrawal.
An important new finding of the present study is that the predictive value of D-dimer can be expected in patients whose qualifying VTE event was idiopathic but not when the index event was associated with cancer or was triggered by circumstantial risk factors. Furthermore, this study showed that D-dimer has a predictive value in patients independent of the presence or absence of congenital thrombophilic alterations. A significantly higher relative risk for VTE recurrence in subjects with altered D-dimer levels was confirmed in all the investigated subjects.

### TABLE 2. D-Dimer Results and Number of Recurrences

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Altered D-Dimer</th>
<th>Normal D-Dimer</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n=599)</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>D-Dimer</td>
<td>222 (37.1)</td>
<td>377 (62.9)</td>
<td></td>
</tr>
<tr>
<td>Recurrences</td>
<td>36 (16.5)</td>
<td>22 (5.8)</td>
<td></td>
</tr>
<tr>
<td>Unprovoked index event (n=282)</td>
<td></td>
<td></td>
<td>0.0226</td>
</tr>
<tr>
<td>D-Dimer</td>
<td>139 (49.3)</td>
<td>143 (50.7)</td>
<td></td>
</tr>
<tr>
<td>Recurrences</td>
<td>23 (16.5)</td>
<td>10 (7.0)</td>
<td></td>
</tr>
<tr>
<td>Cancer-associated index event (n=59)</td>
<td></td>
<td></td>
<td>0.201</td>
</tr>
<tr>
<td>D-Dimer</td>
<td>23 (39)</td>
<td>36 (61)</td>
<td></td>
</tr>
<tr>
<td>Recurrences</td>
<td>8 (34.8)</td>
<td>6 (16.7)</td>
<td></td>
</tr>
<tr>
<td>Removable risk factor (n=254)</td>
<td></td>
<td></td>
<td>0.166</td>
</tr>
<tr>
<td>D-Dimer</td>
<td>59 (23.2)</td>
<td>195 (76.8)</td>
<td></td>
</tr>
<tr>
<td>Recurrences</td>
<td>5 (20)</td>
<td>6 (3.1)</td>
<td></td>
</tr>
<tr>
<td>Without thrombophilia (n= 469)</td>
<td></td>
<td></td>
<td>0.038</td>
</tr>
<tr>
<td>D-Dimer</td>
<td>163 (34.8)</td>
<td>306 (65.2)</td>
<td></td>
</tr>
<tr>
<td>Recurrences</td>
<td>20 (12.3)</td>
<td>19 (6.2)</td>
<td></td>
</tr>
<tr>
<td>With thrombophilia (n=130)</td>
<td></td>
<td></td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>D-Dimer</td>
<td>59 (45.4)</td>
<td>71 (54.6)</td>
<td></td>
</tr>
<tr>
<td>Recurrences</td>
<td>16 (27.1)</td>
<td>3 (4.2)</td>
<td></td>
</tr>
</tbody>
</table>

Types of thrombophilic alterations (No. of recurrences)

- Heterozygosity for factor V Leiden: 33 (13) vs 35 (2)
- Homozygosity for factor V Leiden: 1 vs 1
- Heterozygosity for prothrombin mutation: 13 (2) vs 25 (1)
- Protein C deficiency: 4 (1) vs 6
- Factor V Leiden + prothrombin mutation: 2 vs 2
- Protein S deficiency: 4 vs 0
- Antithrombin III deficiency: 1 vs 0
- Other combined alterations: 1 vs 2

Values are n (%), except for types of alterations, which is number of patients with alteration (number of recurrences).

**Figure 1.** Cumulative probability of recurrence in subjects with an unprovoked qualifying venous thromboembolic event with normal (<500 ng/mL) or altered (>500 ng/mL) D-dimer results obtained 1 month after OAT interruption.

**Figure 2.** Cumulative probability of VTE recurrence in subjects with congenital thrombophilic alterations according to normal (<500 ng/mL) or altered (>500 ng/mL) D-dimer results obtained 1 month after anticoagulation was stopped.
patients and in various subgroups by a multivariate analysis after adjustment for several factors.

In the present study, we found that the rate of VTE recurrence was higher in carriers of inherited thrombophilia (10.2% versus 5.7% for carriers versus noncarriers; \(P<0.05\); hazard ratio, 1.78). However, the risk was not uniform, even in carriers of the same alteration. In fact, thrombophilic subjects with normal D-dimer levels had a very low risk of recurrence (3 events in 71 subjects); the negative predictive value was as high as 95.8% (range, 88.2% to 99.1%). However, carriers with altered D-dimer levels were at high risk for recurrence (16 events in 59 subjects), with a very high hazard ratio (8.34; 95% CI, 2.72 to 17.43; \(P<0.0001\)). This difference was particularly important in carriers of the frequent but clinically mild alterations, such as heterozygosity for factor V Leiden and prothrombin mutation. VTE recurrences occurred in 39.4% and 5.7% of carriers of the former alteration with abnormal or normal D-dimer levels, respectively, and in 15.4% and 8.0% of carriers of the latter. Our data suggest that in thrombophilic subjects, altered D-dimer may indicate the presence of other prothrombotic factors contributing to a more marked hypercoagulable condition. In line with this interpretation is the surprising finding of no recurrence among subjects with antithrombin deficiency or combined alterations during 21 months of follow-up. Although these subjects were candidates for long-term anticoagulation, OAT was not resumed because of refusal by the patient with ATIII deficiency and because in all these cases, the first VTE event was provoked.

Although the predictive value of the D-dimer test was particularly marked in subjects carrying inherited thrombophilia, it was also significant in those without thrombophilia. In fact, VTE recurrences occurred in 12.3% and 6.2% \((P=0.038)\) of nonthrombophilic subjects with altered or normal D-dimer levels, respectively. Furthermore, it should be considered that 5 of the 19 recurrences occurring in this group were secondary to transient risk factors (prolonged bed rest or trauma and plaster cast). Therefore, we postulate that in these patients, a hypercoagulable condition may occur later than a few weeks after OAT is stopped or episodically and cannot always be detected by D-dimer levels obtained after 1 month.

Caution is recommended in evaluating the results of this study. First, although the most important and well-documented thrombophilic conditions were investigated, other alterations, such as hyperhomocystinemia and increased factor VIII and IX, were not. Moreover, during the entire follow-up period, only 58 thrombotic recurrences (≈9.7% of patients) were recorded; this figure is lower than might be expected, although it is similar to those reported in other studies.²³

An Italian collaborative study showed that no long-term clinical benefit was associated with extending the 3-month course of anticoagulant treatment to 1 year in patients with idiopathic proximal DVT. In fact, prolongation of anticoagulation only delays recurrences until anticoagulation is stopped and does not reduce the risk of recurrence.⁶ It seems, therefore, that an important clinical goal is to evaluate patients according to individual and persistent risk of recurrence and to identify those who may or may not need prolonged anticoagulation. It is well known that patients with cancer have a high risk of recurrence and deserve prolonged anticoagulation, whereas those with secondary events have a low risk and need a short period of anticoagulation. Uncertainty remains about the optimal duration of anticoagulation in patients with an idiopathic event and in those with thrombophilic alterations. A recent clinical trial showed that long-term, low-intensity warfarin therapy, after an early period with full-dose treatment, was effective in reducing to 2.6% the rate of VTE recurrences, in comparison to the 7.2% rate recorded in the placebo group.²⁴

The results of the present article indicate that in patients with a previous idiopathic VTE, normal D-dimer results obtained after anticoagulation is stopped may be useful in identifying those subjects who are at low risk of recurrence and excluding them from prolonged anticoagulation.

In conclusion, the present study showed that normal D-dimer levels measured 1 month after OAT discontinuation had a very high negative predictive value for VTE.

### Table 3. Multivariate Regression Analysis of Relative Risks of Altered D-Dimer Results Obtained in All Subjects and in Subgroups

<table>
<thead>
<tr>
<th>Relative Risk (95% CI)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>2.61 (1.45–4.71)</td>
</tr>
<tr>
<td>Idiopathic index event</td>
<td>2.75 (1.24–6.12)</td>
</tr>
<tr>
<td>Cancer-associated index event</td>
<td>2.96 (0.80–10.88)</td>
</tr>
<tr>
<td>Secondary index event</td>
<td>1.90 (0.50–7.28)</td>
</tr>
<tr>
<td>With thrombophilic alterations</td>
<td>5.88 (1.46–23.72)</td>
</tr>
<tr>
<td>Without thrombophilic alterations</td>
<td>2.19 (1.10–4.35)</td>
</tr>
</tbody>
</table>

*Risks were calculated with adjustment for age, sex, duration of previous oral anticoagulant treatment, and presence/absence of congenital thromboembolic alterations or nature of index event, as appropriate.

### Table 4. Sensitivity, Specificity, and Positive and Negative Predictive Values for Venous Thromboembolism Recurrence Based on D-Dimer Results

<table>
<thead>
<tr>
<th></th>
<th>All Subjects</th>
<th>Subjects With an Unprovoked Event</th>
<th>Subjects With Thrombophilia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity, % (95% CI)</td>
<td>62.1 (48.4–74.5)</td>
<td>69.7 (51.3–84.4)</td>
<td>84.2 (60.4–96.6)</td>
</tr>
<tr>
<td>Specificity, % (95% CI)</td>
<td>65.6 (61.4–69.7)</td>
<td>52.2 (45.7–58.6)</td>
<td>61.3 (51.6–70.4)</td>
</tr>
<tr>
<td>Positive predictive value, % (95% CI)</td>
<td>16.2 (11.6–21.7)</td>
<td>16.2 (10.6–23.3)</td>
<td>27.1 (16.4–40.3)</td>
</tr>
<tr>
<td>Negative predictive value, % (95% CI)</td>
<td>94.2 (91.3–96.3)</td>
<td>92.9 (87.3–96.5)</td>
<td>95.8 (88.2–99.1)</td>
</tr>
</tbody>
</table>
recurrence, especially in subjects with an idiopathic previous event and in carriers of congenital thrombophilic alterations. Increased D-dimer levels were associated with a significantly higher hazard ratio for VTE recurrence. These findings suggest that the D-dimer assay may have a role in tailoring the duration of OAT to optimize prevention of recurrent VTE. Specifically designed clinical studies are needed to assess such a possibility.

References
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