Usefulness of repeated D-dimer testing after stopping anticoagulation for a first episode of unprovoked venous thromboembolism: the PROLONG II prospective study

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For a complete list of PROLONG II participants, see the Supplemental Appendix.
ABSTRACT
The PROLONG randomized trial showed that a normal D-dimer (D-d) at 1 month after anticoagulation suspension for unprovoked venous thromboembolism (VTE) was associated with a low risk of late recurrences (4.4 percent patient-years). However it is unknown whether D-d changes subsequently. The aim of this prospective multi-center study was to assess D-d time course and its relation with late recurrences in patients with normal D-d at 1 month after anticoagulation suspension for a first episode of unprovoked VTE. D-d was measured with a qualitative method (Clearview Simplify D-dimer, Inverness Medical Professional Diagnostics, Bedford, UK). Patients with a normal D-d at 1 month after stopping anticoagulation repeated D-d testing every two months for a year. D-d was normal in 68% (243/355) patients at 1 month after anticoagulation suspension. Patients in whom D-d became abnormal at the 3rd month and remained abnormal afterwards had a higher risk of recurrence (7/31; 27 percent patient-years; 95% CI:12-48) than patients in whom D-d remained normal at the 3rd month and afterwards (4/149; 2.9 percent patient-years; 95% CI:1-7) (adjusted hazard ratio: 7.9; 95% CI:2.1-30; p=0.002). Repeated D-d testing after anticoagulation suspension for a first episode of unprovoked VTE could help tailor the duration of treatment. This trial is registered at http://clinicaltrials.gov as NCT00266045.
INTRODUCTION

The optimal duration of anticoagulation after a first episode of unprovoked venous thromboembolism (VTE) is still unknown. Recently, the American College of Chest Physicians recommended that patients with unprovoked VTE should receive at least 3 months of anticoagulant therapy and then subjects should be evaluated for the risk-benefit ratio of long-term therapy. Long term treatment is recommended in those in whom risk factors for bleeding are absent and for whom good anticoagulant monitoring is achievable. In this recommendation, a relatively high value is attached to prevention of recurrent VTE and a lower value to the burden of long-term anticoagulant therapy. However, the decision about treatment is ultimately left to the physicians’ subjective evaluation of the risk-benefit ratio in each patient and to patients’ preferences about treatment prolongation. The risk of recurrence is greatest in the first 6 to 12 months after the initial episode while it gradually decreases thereafter. As a result the benefits of extending anticoagulation may be offset over time by the risk of clinically important bleeding associated with vitamin K antagonists (VKAs). The identification of patient characteristics predictive of recurrence would be a valuable aid in the decision about stopping or prolonging VKAs after a first episode of unprovoked VTE.

The multi-center randomized study PROLONG showed that D-dimer (D-d) could play a role in establishing the duration of anticoagulation after a first episode of idiopathic VTE. Patients were treated with VKAs for a minimum of 3 months and D-d was tested at one month after anticoagulation withdrawal. If D-d was normal patients did not resume treatment, whereas those with elevated D-d were randomized to either stop or resume VKAs. Patients with an abnormal D-d had a statistically significant higher risk for recurrence when compared with those with normal D-d. Continued anticoagulation in patients with elevated D-d significantly reduced the risk of recurrent VTE over an extended follow-up of 2.55 years. In subjects with normal D-d the rate of VTE recurrence was 4.4 percent patient-years (pt-y), which is higher than the expected annual rate of major bleeding (2 percent pt-y) with VKAs. As a result, the optimal duration of treatment is an unresolved issue in these patients. In the PROLONG study only a single D-d measurement was performed after stopping anticoagulation and it is unknown whether and how often D-d becomes abnormal afterwards and whether a late hypercoagulability is associated with the risk of recurrences.

To address these questions, we conducted the observational multi-centre prospective PROLONG II study. In this study D-d was tested serially over a year in
METHODS

Study patients

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 deep vein thrombosis (DVT) and/or pulmonary embolism (PE). DVT was to be detected by compression ultrasonography or venography, and PE to be diagnosed on the basis of objective algorithms using clinical probability, ventilation-perfusion or helical computed tomography lung scan, compression ultrasonography (if indicated), and D-dimer testing. Patients were eligible for the study if they had completed at least six months of VKA therapy [either warfarin (Coumadin, Bristol Myers Squibb) or acenocoumarol (Sintrom®, Novartis Pharma)] with a target INR of 2.5 (range 2.0 to 3.0).

Unprovoked VTE was defined as an episode not associated with pregnancy or the puerperium, recent (i.e. within three months) fracture or plaster casting of a leg, immobilization with confinement to bed for ≥3 consecutive days, surgery with general anesthesia lasting ≥30 minutes, active malignancy, antiphospholipid antibody syndrome, or antithrombin deficiency. 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 in accordance with the Declaration of Helsinki.

Study procedures

At the end of the intended anticoagulation period (T0), subjects were assessed for eligibility and baseline clinical conditions. The presence of known co-morbidities such as coronary heart disease, peripheral arterial disease, cerebrovascular disease, chronic intestinal inflammatory diseases, chronic pulmonary obstructive disease, autoimmune disease, diabetes, arterial hypertension, obesity and dyslipidemias was collected on a standardized form. Patients underwent compression ultrasonography (CUS) of the proximal deep veins in both legs to measure the diameter of any residual venous
obstruction (RVO) in the common femoral, superficial femoral and popliteal veins. Patients then underwent blood sampling for INR. If the INR was in the therapeutic range (INR:1.8-3.2), D-d was also measured using the Clearview Simplify D-dimer assay (Inverness Medical Professional Diagnostics, Bedford, UK and Louisville, Colorado, USA; kindly provided by the Instrumentation Laboratory company, Milan, Italy). This is a qualitative, fast, whole blood method, previously shown to perform well in VTE diagnosis with good reproducibility. The test itself is a standard immunochromatographic test in which the D-dimer specific murine monoclonal antibody DD3B6/223 is conjugated to colloidal gold particles. It is performed by mixing 35 μl of capillary or venous blood with two drops of test reagent. A positive test result occurs when D-dimer levels exceed 80 ng/ml (as measured by an ELISA method - DIMERTEST® GOLD EIA) and is indicated by a visible pink-purple coloured line that forms at the test zone. Test results are valid if a pink-purple line is also present at the control zone. The test can be read within 10 minutes.

Patients with abnormal D-d continued VKAs and they were scheduled for a reassessment of D-d after 6 months. Patients with a normal D-d were instructed to stop oral anticoagulation on the same day and refrain from taking any other antithrombotic drugs until the next visit, scheduled after 30±10 days (T30).

At the 30-day visit, venous blood was sampled for D-d assay and thrombophilia tests. Patients with abnormal D-d results resumed anticoagulation with VKAs (INR 2.0 to 3.0).

Patients with a normal D-d test did not resume anticoagulation and underwent bi-monthly D-d testing (±10 days) over a year at the 3rd (T90), 5th (T150), 7th (T210), 9th (T270), 11th (T330) and 13th month (T390).

Testing for lupus anticoagulant phenomenon and/or antiphospholipid antibodies and antithrombin, protein C and protein S activities, factor V Leiden and the G20210A mutation in the prothrombin gene was performed at the enrolling centers, as reported before. Subjects found to have antiphospholipid syndrome or antithrombin deficiency were excluded from further analysis and resumed anticoagulation.

**Follow-up and study outcomes**

All patients with a normal D-d were followed for a maximum duration of 13 months from the assignment visit and were seen at the clinical center every two months over a year when D-d testing was repeated for a maximum of six times.
D-d testing was avoided and rescheduled after 30 days in case of recent trauma, surgery or other conditions that could have influenced the results. Patients were instructed to contact the clinical center immediately if symptoms developed suggestive of VTE or in case of bleeding.

All subjects who continued VKAs for an altered D-d during anticoagulation or who resumed VKAs for an altered D-d at one month after stopping anticoagulation were scheduled for a re-assessment of D-d after at least 3-6 months.

The main study outcome was confirmed recurrent VTE in subjects who stopped anticoagulation for a normal D-d at one month after VKA withdrawal. The secondary outcome was the composite of major bleeding and recurrent VTE in those who continued or resumed VKAs for abnormal D-d.

In cases of suspected DVT recurrence, the results of CUS were compared with those of the last available previous examination. A recurrent DVT was diagnosed if a previously fully compressible segment (contralateral or ipsilateral) was no longer compressible or if an increase of at least 4 mm in the diameter of the residual thrombus during compression was detected. When thrombus diameter changed between 1.1 and 3.9 mm, or in cases of high/moderate clinical probability and normal proximal compression ultrasonography, the examination was repeated 5 to 7 days later.

In patients with suspected PE, diagnosis of recurrence was based on objective algorithms using clinical probability, ventilation-perfusion lung scanning or helical computed tomography, compression ultrasonography and/or D-d if indicated.

Major bleeding events were defined as bleeds that were retroperitoneal or intracranial, or associated with a decrease in the haemoglobin of ≥2.0 g/dL, or required transfusion of ≥2 units of blood, or if surgery or invasive procedures were necessary to stop bleeding.

All suspected outcome events and all deaths were evaluated by a central adjudication committee whose members were unaware of the name of the subject, the enrolling center, the results of D-d, thrombophilia assays, INRs and VKA treatment. All clinical investigation results were also reviewed by the committee members without any indication of the name of the subject, the assigned group, or the city/institution where the testing had been performed.
Statistical analysis

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

Patients with a normal D-d at one month (T30) after stopping anticoagulation were evaluated according to the D-d result at each subsequent time point (T90,T150,T210, T270,T330,T390) and were divided into four groups: 1- patients in whom D-d was normal, being also normal at the previous time point; 2- patients in whom D-d became newly abnormal, being normal at the previous time point; 3- patients in whom D-d was abnormal, being also abnormal at the previous time point; 4- patients in whom D-d became normal, being abnormal at the previous time point. At each time point the rate of recurrence was calculated over the next two months.

Patients with a normal D-d at one month after stopping anticoagulation were also divided into four subgroups according to D-d subsequent time course: 1- patients in whom D-d remained normal at T90 and afterwards (with D-d becoming abnormal only once); 2- patients in whom D-d became abnormal at T90 and remained abnormal persistently or at least twice; 3- patients in whom D-d became abnormal after T90 and remained abnormal persistently or at least twice.

Kaplan-Meier survival curves were plotted to estimate the cumulative incidence of symptomatic recurrent VTE. Hazard ratios and their 95 percent confidence intervals were calculated using the Cox’s proportional hazards model. Initially an unadjusted hazard ratio was calculated with age, sex, duration of anticoagulation, DVT vs. DVT and/or PE as the index event, presence/absence of co-morbidities, presence/absence of thrombophilia, presence/absence of RVO. Then a multivariate model was constructed, introducing only those variables with a p value <0.2 at the univariate analysis.

The data were analyzed using the Prism statistical software package (Version 3.0, GraphPad Software Incorporated, San Diego, CA) and the SPSS statistical package (Version 11.0, SPSS Inc., Chicago, IL).

RESULTS

Patients

A total of 355 patients were enrolled in 15 clinical centers between March 1st 2005 and February 2nd 2008.
The flow chart of the study from T0 to T30 is shown in figure 1. During anticoagulation, D-d was abnormal in 19 subjects (5.3%; 95% CI: 3-8%) in whom VKAs were continued, whereas D-d was normal in the remaining 336 patients and they stopped VKAs. At T30, D-d was abnormal in 85/328 (26%; 95% CI: 21-31%) and these patients resumed VKAs.

The baseline characteristics of the patients are reported in Table 1. Patients (n=104) who prolonged or resumed VKA for an abnormal D-d either at T0 or at T30 were significantly older, had more co-morbidities and had a longer follow-up than patients who stopped VKAs for a normal D-d at both T0 and T30 (n=243).

Follow-up and Recurrent Venous Thromboembolism

Recurrences before T30 were observed in 2 patients who stopped VKA for a normal D-d at T0 (0.59%; 95% CI:0-2%). Among the 243 patients with a normal D-d at T30 who maintained VKA suspension, 8 early recurrences (3.3%; 95% CI: 1-6%) were observed before D-d was determined at T90.

Total follow-up after T30 in patients with normal D-d was 212 patient-years, mean follow-up was 10.6 months (range 1-13 months) and 79% of patients (192/243) had a follow-up of at least 7 months.

Figure 2 shows the rate of conversion from D-d normal to abnormal from T90 to T390. After T90, the rate of newly abnormal D-d remained stable (approximately 10-15%) up to T270. Afterwards the rate of newly abnormal D-d decreased to 8-10%.

Table 2 illustrates study follow-up from T90 to T390. The rate of recurrence was 22.6% (7/31; 95% CI: 10-41%) in those subjects in whom D-d was abnormal at T90 and 4.6% (9/195; 95% CI: 2-9%) in patients in whom D-d was normal at T90 (p=0.0031). Recurrence rates were significantly higher in males than in females (15/136: 11% -95% CI: 6-18% vs. 1/107:1%; 95% CI 0-5% P=0.001), while a trend for higher recurrences was observed in subjects with than in those without co-morbidities (10/89: 11.3%; 95% CI: 6-20% vs. 6/136: 4.4% -95% CI: 2-9%; p=0.06).

Table 2 also shows the events observed in the two months between each measurement according to D-d results at each time-point. At T90 newly abnormal D-d was associated with a significantly higher rate of recurrence when compared to normal D-d. At T210 and at T270, the rate of recurrence was significantly higher in those subjects in whom D-d was abnormal also previously when compared with persistently normal D-d.
Table 3 shows the recurrence rate according to D-d time course from T90. All recurrent events were idiopathic except one event secondary to trauma in a subject with a normal D-d at T90. The rate of recurrence was significantly higher in subjects in whom D-d became abnormal at T90 and remained abnormal afterwards than in subjects in whom D-d was normal at T90 and remained normal afterwards (p=0.001). However the rate of recurrence was also significantly higher in subjects in whom D-d became abnormal after T90 than in subjects in whom D-d was normal at T90 and remained normal afterwards (p=0.035).

Table 4 shows the hazard ratio for the main outcomes associated with the different subgroups according to D-d time course, while the survival curves associated with the different subgroups are indicated in figure 2. Covariates such as age, duration of anticoagulation, DVT vs. DVT and/or PE as the index event, presence/absence of thrombophilia, presence/absence of RVO had a p > 0.20 at the univariate analysis and therefore they were not introduced in the multivariate analysis. Only sex (p=0.025) and presence/absence of co-morbidities (p=0.025) had a significant effect and were introduced in the multivariate model.

Subjects with an abnormal D-d at T90 and in the subsequent time-points after VKA withdrawal had a significantly higher hazard ratio for recurrence than subjects with a normal D-d at T90 and in the subsequent time points and the difference remained significant after correction. After adjustment, no difference was observed between subjects in whom D-d became abnormal after T90 and patients with a normal D-d at T90.

No significant difference was observed in the conversion of D-dimer from normal to abnormal between subjects with PE (isolated or with DVT) (25/81-31%) and DVT without PE (51/145-35%). The risk of recurrence was also similar in subjects with PE (4/81-5%) and those without PE (12/145-8%).

Among the 104 patients in whom VKAs were continued for an abnormal D-d at T0 or at T30, 2 episodes of pulmonary embolism occurred in 2 patients who discontinued VKAs (one for invasive manoeuvres). D-d was re-tested after an additional VKA course of at least 3-6 months in 42 patients of whom 7 had an altered D-d during treatment and therefore continued VKAs. The remaining 35 patients stopped VKAs for a normal D-d during anticoagulation but 30 (85.7%) resumed VKAS because D-d became abnormal at 1 month after stopping anticoagulation. Among the 5 patients who stopped VKAs, 2 recurrent DVTs were observed (one after a month and one after 10 months).
Major bleeding

Major hemorrhages were recorded in 5 of the 104 patients who either continued or resumed VKAs for an abnormal D-d at T0 or at T30 (3 gastro-intestinal hemorrhages, 1 retroperitoneal haematoma, 1 fatal intracranial hemorrhage; 4.8%, 95% CI: 2-11%; 3.3% patient-years, 95% CI: 1-7%). In two patients, VKAs were discontinued for severe chronic anemia. One patient died due to sepsis.

DISCUSSION

The previously reported PROLONG randomized study showed that patients with a first episode of unprovoked VTE who have an abnormal D-d at one month after VKA suspension had a higher rate of recurrent VTE (15 percent, 10.9 percent patient-years), when compared with D-d positive patients who resumed anticoagulation (combined rate of recurrent VTE and major bleeding of 2.9 percent; 2.0 percent patient-years) over a follow-up of 18 months. In the patients with a normal D-d who stopped VKAs, the observed rate of recurrence of 6.2 percent (4.4 percent patient-years) is clinically relevant, although it was not statistically different from that of patients with abnormal D-d who resumed anticoagulation. The role of D-d in predicting late recurrences was also confirmed by a systematic review of randomized and prospective studies in 1888 patients who had completed at least 3 months of anticoagulation for a first episode of unprovoked VTE and after approximately 2 years of follow-up. In the different studies a single D-d test was conducted between 3 weeks and 2 months after stopping VKAs. A negative D-d result was associated with a 3.5 % (95% CI: 2.7-4.3%) annual risk for recurrent disease, whereas a positive D-d result was associated with an 8.9 % (95% CI: 5.8-11.9%) annual risk for recurrence.

The rate of recurrence in D-d negative patients is similar to the rate of recurrence observed after a first VTE episode triggered by a transient risk factor which is usually treated with a short course of anticoagulation. However, the recurrence rate in D-d negative patients might still be considered high enough to warrant prolonging anticoagulation. As a result, the risk/benefit relationship of prolonging anticoagulation in patients with a normal D-d is uncertain, but it is also unknown how often and when an initially single normal D-d may become abnormal over time. To this purpose, the PROLONG II study evaluated the usefulness of repeated D-d testing to assess the frequency and time frame of late hypercoagulability and its association with recurrences in
subjects with a normal D-d at one month after the suspension of VKAs for a first episode of VTE.

The results of the study indicate that an initially normal D-d at one month after VKA suspension becomes abnormal at the 3rd month in about 14% of cases and becomes abnormal at each subsequent time point in about 10-15% of subjects up to T270 and then it decreases to 8-10%. This late hypercoagulability is associated with a significantly increased rate of recurrence between T90 and T150 when compared with subjects with normal D-d, while at the later time points the recurrence rate is significantly higher only for those patients in whom D-d was abnormal also in the previous time point. In those patients in whom D-d remains normal when compared to the previous time point the rate of recurrence is negligible. These results indicate that the rate of recurrence in subjects with a normal D-d during and at one month after VKA withdrawal is highest in the first three-five subsequent months and may warrant repeated testing in this time frame.

When the entire D-d time course over a year is considered, a normal D-d at one month after VKA withdrawal remains normal in the majority of patients (66%) and in these patients the rate of VTE recurrence after T90 is low (2.7 percent-2.9 events percent patient years). In the remaining patients, an initially normal D-d becomes abnormal at the 3rd month and remains persistently abnormal in 14% of subjects and it is associated with a significant higher rate of recurrence when compared with that of patients with normal D-d at T90 in whom D-d remains normal. In 20% of subjects D-d becomes abnormal after the 3rd month and remains persistently abnormal afterwards. In these patients the adjusted hazard ratio for recurrence is not statistically different from that of patients with persistently normal D-d at T90.

PE was present in 34% of patients and they might be more likely to convert from a normal to an abnormal D-dimer and also at higher risk of recurrence. No significant difference was observed either in the conversion of D-dimer from normal to abnormal or recurrence rate between subjects with PE (isolated or with DVT) and those with DVT without PE. However, the sample of subjects with PE was too small to draw definitive conclusions.

In those patients with an abnormal D-d either at T0 or at one month after VKA withdrawal who continued or resumed VKAs and in whom D-d was retested after 3-6 months, D-d was abnormal in the majority of cases and VKAs were either continued or resumed for long term. This indicates that an abnormal D-d at T0 or T30 remains abnormal over time in the majority of patients.
The results of this study should be evaluated with some caution. First, the trial was prospective, and bias could not be completely minimized, in spite of a blinded central adjudication committee. However, the baseline characteristics of patients who stopped anticoagulation were similar to those of patients who resumed anticoagulation except for age and co-morbidities. The rates of events in treated and untreated patients are similar to those reported in the literature, albeit low, as expected, with large confidence intervals.

Second, some very early recurrences were observed in the first month after stopping VKAs and it is unknown whether testing D-d during anticoagulation could also help tailor the duration of treatment. However only about 5-10% of subjects have an abnormal D-d during anticoagulation in the therapeutic range and very large studies would be needed to address this question.

Third, a qualitative method for D-d testing was employed to allow a uniform approach across the participating centers for classifying D-d levels as either normal or abnormal. Although a formal inter-observer variability assessment was not performed and some variability in the interpretation of the test can be expected, the test itself was previously shown to have good reproducibility. However, in a post-hoc analysis we have calculated specific cut-offs of different quantitative D-d methods on frozen plasma samples from the PROLONG study. The prospective management DULCIS (D-dimer and Ultrasound in Combination Italian Study) study is currently ongoing to validate these cut-offs.

Fourth, D-d time course was difficult to describe, as it did not have always a clear pattern. Some patients who had a persistently normal D-d had also a single abnormal test indicating only transient hypercoagulability, while others with an abnormal test had a normal D-d once or twice. Fifth, although a normal D-d at T90 which remains normal over a year after VKA withdrawal identifies a subgroup of patients with unprovoked VTE with a low risk of recurrence (2.7%, 2.9 percent patient-years), the lowest acceptable rate of recurrence which may not warrant prolonging treatment after unprovoked VTE is not yet defined. This low rate of recurrence could be similar to the expected rate of major bleeding (approximately 2 percent patient-years) during anticoagulation. In the present study, the rate of major bleeding in patients who continued or resumed long-term VKAs was very similar (4.8%, 3.3 events percent patient-years) to the rate of recurrence in subjects with a persistently normal D-d over a year but higher than expected. This could be due to the higher risk of bleeding associated with the older age of patients with an abnormal D-d who resumed VKAs and also to their longer exposure time (mean follow-up
18 months vs. 10 months in patients with a normal D-d who stopped VKAs) as the majority of these patients underwent long-term anticoagulation.

In conclusion, the results of this study indicate that repeated D-d testing especially in the first three months after VKA withdrawal for a first episode of unprovoked VTE could identify a subgroup of patients with a low risk of recurrence, which may not warrant prolonged anticoagulation. To address this issue we are currently conducting the multi-centre prospective management study DULCIS (http://clinicaltrials.gov: NCT00954395) in which D-d is tested in the first three months after VKA withdrawal together with RVO. Subjects with repeated normal D-d over 3 months after VKA withdrawal and without significant RVO do not resume VKAs while subjects in whom D-d becomes abnormal over the first 3 months after stopping anticoagulation resume VKAs. The results of the study should be available by the end of 2011.
Acknowledgements

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Authorship Contributions
B. C., G. P., V. P., A. T., A. T., A. G., S. T., D. P., D. P., F. M. designed the study and wrote the protocol, analyzed and interpreted the data, and contributed to writing the manuscript, B. C. performed the research and wrote the manuscript, C. L. collected and analysed the data and performed the statistical analysis.

Disclosure of Conflicts of Interest
Drs. Cosmi, Legnani, Palareti, Pengo, Testa, and Tripodi report having been paid lecture fees by the Instrumentation Laboratory company, Milan, Italy.
REFERENCES


Table 1 Base-Line Characteristics of the Enrolled Patients

|                          | Patients N=355 | D-d normal at T0 or at T30 N=243 | D-d abnormal at T0 or at T30 N=104 | P  
|--------------------------|----------------|----------------------------------|------------------------------------|------
| Female sex n (%)         |                |                                  |                                    |      
|                          | 162 (45.6)     | 107 (44)                         | 53 (51)                            | 0.28 |
| Age                      |                |                                  |                                    |      
| Mean±95%CI yr ≥65 yr n (%) | 62±1.2         | 59±2                             | 69±2                               | <0.0001 |
| Type of venous thromboembolism n (%) |                |                                  |                                    |      
| Proximal DVT (no PE)     | 236 (66.5)     | 155 (63.7)                       | 73 (70.2)                          | 0.22 |
| DVT + symptomatic PE     | 43 (12.1)      | 30 (12.3)                        | 13 (12.5)                          | 0.97 |
| Isolated PE              | 76 (21.4)      | 58 (23.8)                        | 18 (17.3)                          | 0.22 |
| Co-morbidities           |                |                                  |                                    | 0.014 |
| Residual venous thrombosis n (%) |                |                                  |                                    |      
| (n .CUS available, %)    | 124 (35)       | 84 (40.2)                        | 42 (44.2)                          | 0.59 |
| Congenital thrombophilic alteration/s n (%) |          |                                  |                                    |      
| Examined n (%)           | 273 (77)       | 206 (84.8)                       | 67 (64.4)                          | 0.42 |
| All                      | 132 (48.3)     | 97 (47.1)                        | 36 (53.7)                          |      |
| Prot C,S,AT deficiencies, LAC | 5 (1.8)       | 2 (1.0)                          | 3 (4.4)                            | 0.18 |
| Hyperhomocysteinemia     | 60 (22)        | 43 (20.1)                        | 18 (26.8)                          | 0.39 |
| FV Leiden                | 33 (12)        | 25 (12.3)                        | 8 (11.9)                           |      |
| Prothrombin mutation     | 20 (7.3)       | 16 (7.7)                         | 4 (6.0)                            | 0.82 |
| Combined alterations and/or homozygous | 14 (5.1)  | 11(5.3)                          | 3 (4.5)                            | 0.78 |
| Duration of previous anticoagulant treatment n (%) |          |                                  |                                    |      
| < 6 months - n (%)       | 77 (21.6)      | 54 (22.2)                        | 21 (20.2)                          | 0.78 |
| 7-12 months - n (%)      | 164 (46.2)     | 110 (45.2)                       | 49 (47.1)                          | 0.84 |
| > 12 months - n (%)      | 114 (32.1)     | 79 (32.5)                        | 34 (32.7)                          | 0.97 |
| Total duration of follow-up patient-years | 365 | 212 | 153 | <0.0001 |
| Follow-up Mean±95%CI mo. |                | 10.6±0.2                         | 18±16                              |      |

D-d denotes D-dimer; VKA = vitamin K antagonist treatment; FV Leiden = factor V Leiden mutation; DVT = deep vein thrombosis; PE = pulmonary embolism; AT = antithrombin; LAC = lupus anticoagulant

P value refers to chi-squared or Fisher’s exact test or t-test, as appropriate, between normal D-d and abnormal D-d
TABLE 2
D-d time course from T90 to T390 and recurrences observed in the two months between each D-d measurement

<table>
<thead>
<tr>
<th>Time points</th>
<th>N pt.</th>
<th>D-d normal at previous T</th>
<th>D-d newly abnormal at previous T</th>
<th>D-d abnormal but altered at previous T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (%)</td>
<td>Abnormal (%)</td>
<td>N. pt</td>
<td>rVTE (%)</td>
</tr>
<tr>
<td>T90</td>
<td>228 (3)</td>
<td>--</td>
<td>194</td>
<td>4 (2.1)</td>
</tr>
<tr>
<td>T150</td>
<td>209 (1)</td>
<td>174</td>
<td>34 (16.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>T210</td>
<td>193</td>
<td>144</td>
<td>49 (25.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>T270</td>
<td>178 (1)</td>
<td>130</td>
<td>47 (26.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>T330</td>
<td>163 (4)</td>
<td>114</td>
<td>45 (28.3)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>T390</td>
<td>144 (9)</td>
<td>100</td>
<td>35 (26)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

* p = 0.05 vs. D-d normal also previously; ** p = 0.11 vs D-d normal also previously
*** p = 0.07 vs. D-d normal also previously; & p = 0.18 vs. D-d normal also previously
§ p = 0.015 vs. D-d normal also previously; # p = 0.0069 vs D-d normal also previously

Table 3  Recurrent VTE according to D-dimer time course from the 3rd month

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Normal D-d at T90*</th>
<th>Abnormal D-d at T90*</th>
<th>Abnormal D-d after T90*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%) (95% CI)</td>
<td>149 (66) (59-72%)</td>
<td>31 (14) (10-19%)</td>
<td>46 (20.3) (15-26%)</td>
</tr>
<tr>
<td>n (%) (95% CI)</td>
<td>4 (2.7) (1-7)</td>
<td>7 (22.6) (10-41)*</td>
<td>5 (10.9) (4-24)**</td>
</tr>
<tr>
<td>n/100 person-Yr (95% CI)</td>
<td>2.9 (1-7) 138</td>
<td>27 (12-48) 25.6</td>
<td>11.1 (4-24) 45</td>
</tr>
<tr>
<td>Type of recurrent VTE</td>
<td>DVT</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>DVT + PE</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Isolated PE</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

* p = 0.001 vs normal D-d; ** p = 0.035 vs normal D-d

Table 4  Hazard Ratios for Main Outcomes

<table>
<thead>
<tr>
<th>Hazard Ratios (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
</tr>
<tr>
<td>D-d normal at T90</td>
</tr>
<tr>
<td>D-d abnormal at T90</td>
</tr>
<tr>
<td>D-d abnormal after T90</td>
</tr>
</tbody>
</table>

* adjusted for sex (p=0.025 male vs female), and presence vs absence of co-morbidities (p=0.025)
LEGENDS TO FIGURES

Figure 1
The flow chart of the study from T0 to T30

Figure 2
Frequency of conversion from D-dimer normal to abnormal from the 3rd month (T90) to the 13th month (T390)

Figure 3
Cumulative incidence and hazard ratios (HR) of main outcomes according to D-d time course from the 3rd month (T90) to the 13th month (T390):
Group 1- patients in whom D-d was normal at T90 and afterwards (with D-d becoming abnormal only once)
Group 2- patients in whom D-d became abnormal at T90 and afterwards remained altered persistently or at least twice
Group 3- patients in whom D-d became abnormal after T90 and afterwards remained abnormal persistently or at least twice
FIGURE 1

PATIENTS N. = 355

D-dimer (T0)

Abnormal
N= 19 (5.3%; 95% CI:3-8%)

Normal
N= 336
VKAs stopped
For 30±10 dd

VKAs continued

Events occurred before T30:
1 PE , 1 sudden death (rVTE:0.59%)
1 SVT, 5 drop-outs
(2 resumed VKA, 3 withdrew consent)

D-dimer (T 30 d)

Abnormal
N=85 (26%;95% CI:21-31%)

Normal
N= 243
VKAs not resumed

VKAs resumed for 3 - 6 mo

Events occurred before T 90:
6 DVTs (rVTE:3.3%)
2 PE
5 withdrew consent
1 resumed VKA for SVT
1 resumed VKA for LAC

VKA = vitamin K antagonist treatment;  DVT = deep vein thrombosis; PE = pulmonary embolism; SVT = superficial vein thrombosis; LAC = lupus anticoagulant
FIGURE 3

Cumulative incidence of outcomes %

Follow-up (days)

Numbers at risk

<table>
<thead>
<tr>
<th>Group</th>
<th>90</th>
<th>150</th>
<th>210</th>
<th>270</th>
<th>330</th>
<th>390</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>149</td>
<td>147</td>
<td>139</td>
<td>127</td>
<td>116</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>64</td>
</tr>
<tr>
<td>Group 2</td>
<td>31</td>
<td>29</td>
<td>26</td>
<td>24</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Group 3</td>
<td>46</td>
<td>46</td>
<td>45</td>
<td>43</td>
<td>40</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24</td>
</tr>
</tbody>
</table>

HR = 9.38
p = 0.0004

HR = 3.78
p = 0.047
Usefulness of repeated D-dimer testing after stopping anticoagulation for a first episode of unprovoked venous thromboembolism: the PROLONG II prospective study

Benilde Cosmi, Cristina Legnani, Alberto Tosetto, Vittorio Pengo, Angelo Ghirarduzzi, Sophie Testa, Domenico Prisco, Daniela Poli, Armando Tripodi, Francesco Marongiu and Gualtiero Palareti