Different cut-off values of quantitative D-dimer methods to predict the risk of venous thromboembolism recurrence: a post-hoc analysis of the PROLONG study

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ABSTRACT

Background
The PROLONG study showed that patients with venous thromboembolism who had qualitatively abnormal results in a D-dimer assay (Clearview Simplify D-dimer) after discontinuation of vitamin K antagonism benefit from resumption of treatment with vitamin K antagonism. The objective of this study was to evaluate the possible advantage of using quantitative D-dimer assays.

Design and Methods
VIDAS D-dimer Exclusion (bioMerieux), Innovance D-DIMER (Dade Behring), HemosIL D-dimer HS (Instrumentation Laboratory) and STA Liatest D-dimer (Diagnostica Stago) assays were performed in plasma aliquots sampled 30±10 days after cessation of vitamin K antagonism in 321 patients enrolled in the PROLONG study.

Results
During the follow-up without vitamin K antagonism, 25 patients had recurrent venous thromboembolism. The cut-off levels of the quantitative assays giving results most comparable with those of the qualitative test were: VIDAS = 800 ng/mL; Innovance = 800 ng/mL; HemosIL HS = 300 ng/mL; STA Liatest = 700 ng/mL. When the effect of the patients' age (≤70 *vs.* >70 years) was analyzed, it was found that only in younger patients was the rate of recurrence of venous thromboembolism significantly higher in patients with abnormal D-dimer levels. However, using the quantitative assays and age-specific cut-off levels it was possible to determine statistically significant hazard ratios also in elderly patients (VIDAS = 600 and 1200 ng/mL, Innovance = 500 and 900 ng/mL, HemosIL HS = 250 and 450 ng/mL, STA Liatest = 700 and 1000 ng/mL, in patients aged ≤70 and >70 years, respectively).

Conclusions
Quantitative D-dimer assays may provide information useful for evaluating the individual risk of recurrent venous thromboembolism. They seem particularly advantageous since they allow the selection of different cut-off levels according to the age or other characteristics of the patients.

Key words: anticoagulation, D-dimer, recurrence, risk factors, venous thromboembolism.

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Introduction

Unprovoked venous thromboembolism (VTE) recurs frequently after discontinuation of treatment with vitamin K antagonists with about 50% of patients suffering from recurrence within 10 years of the incident event. The incidence of VTE recurrence does, however, vary with time and is highest (about 10-15%) over the 6-12 month period after the incident event.[1] Treatment with vitamin K antagonists is effective in preventing recurrent VTE,[2-4] but the duration of anticoagulation does not seem to affect the risk of recurrence once primary therapy for the incident event is stopped.[5-7] Since the risk of vitamin K antagonist-related bleeding does not decrease with time 3 months after the start of therapy[8] (but indeed increases with age), the benefit of prolonged vitamin K antagonism diminishes over time. Indefinite secondary prophylaxis with vitamin K antagonists in all patients with previous unprovoked VTE should, therefore, be carefully assessed and should be limited to those patients with the highest risk of recurrence.

Earlier prospective studies in patients with VTE[9-12] demonstrated that D-dimer levels have a strong predictive value for the occurrence of subsequent episodes. These studies suggested that D-dimer measurement may have a role in gauging the duration of anticoagulation in patients with VTE. The recently published PROLONG study,[13] a prospective, randomized, multicenter study investigating patients after a first episode of symptomatic unprovoked VTE, showed that patients with abnormal D-dimer levels after withdrawal of anticoagulation had a higher rate of VTE recurrence than those with normal D-dimer levels and benefited from resumption of anticoagulation.

Since PROLONG was a multicenter study and D-dimer measurements needed to be performed with the same test in all participant centers, a qualitative D-dimer assay was chosen (Clearview Simplify D-dimer by Inverness Medical Professional Diagnostics). This test can be performed on citrated whole blood and the result (negative or positive) evaluated visually. In principle, however, the accuracy of D-dimer assays may be enhanced by using quantitative tests with appropriate cut-off values.

The present study retrospectively analyzed a subgroup of patients enrolled in the PROLONG study. Its main aims were to assess the performance of four quantitative D-dimer methods to predict the risk of VTE recurrence and to verify whether the adoption of different cut-off values may help to distinguish better between subjects with higher or lower risk of recurrence.

Design and Methods

Patients and study design

As described in detail elsewhere,[13] the PROLONG study was a multicenter prospective study performed in 30 centers belonging to the Italian Federation of Thrombosis Centers (FCSA). In brief, between September 2002 and January 2005, consecutive patients referred for a first episode of symptomatic unprovoked VTE, including proximal deep vein thrombosis (DVT) of the legs and/or pulmonary embolism (PE), and who were treated with a vitamin K antagonist for a minimum of 3 months were eligible. Unprovoked VTE was defined as episodes not associated with pregnancy or puerperium, recent (i.e. within 3 months) fracture or plaster casting of a leg, immobilization with confinement to bed for 3 or more consecutive days, surgery with general anesthesia lasting 30 or more minutes, active cancer, antiphospholipid antibody syndrome, or antithrombin deficiency. At the end of the vitamin K antagonist treatment, candidate subjects had a medical examination to assess their baseline clinical condition. Patients were instructed to stop oral anticoagulation immediately and refrain from taking any other antithrombotic drugs until the next visit, scheduled after 30±10 days. At that visit, D-dimer levels were assessed using the qualitative Clearview Simplify D-dimer assay. Patients with normal Clearview Simplify D-dimer results (n=385) did not resume anticoagulation, whereas those with abnormal results were randomized to either resume (n=103) or not (n=120) anticoagulation with a vitamin K antagonist, with the aim of maintaining the International Normalized Ratio (INR) between 2.0 and 3.0, during the entire follow-up period of 18 months. From the assignment visit, all patients were followed up for a maximum duration of 18 months and were seen at the clinical center at 3- to 6-month intervals. The results were analyzed according to the intention-to-treat principle. The Ethics Committee of all participating clinical centers approved the study. All enrolled patients provided written informed consent.

At the visit scheduled 30±10 days after interruption of vitamin K antagonist treatment, besides performing the Clearview Simplify D-dimer assay, the participating centers were also asked to collect plasma aliquots for further centralized analyses; a total of 386 plasma aliquots were collected and stored in 14 participating centers. The present analysis was centrally performed using plasma samples from 321 patients, 240 with normal Clearview Simplify D-dimer results and 81 with abnormal results; the 65 samples collected from subjects with abnormal Clearview Simplify D-dimer results who had been randomized to resume treatment with vitamin K antagonist were excluded.

This analysis considered the same outcome as in the PROLONG study: objectively documented recurrence of DVT and/or PE.[13]

Blood sampling and D-dimer tests

Blood was collected from the antecubital vein into 0.129 mmol/L trisodium citrate; plasma was prepared by centrifugation for 20 min at 2000 g at room temperature. Platelet-poor plasma was distributed into coded plastic tubes, snap-frozen and stored locally at -70°C. Frozen aliquots were then sent by courier to the coordinating center in dry ice and stored at -70°C. The Clearview Simplify D-dimer assay (Inverness Medical

Professional Diagnostics, Bedford, UK and Luisville, Colorado, USA; kindly provided by Instrumentation Laboratory, Milan, Italy) was performed locally in each center on fresh citrated whole blood.

In the present study, we report the results obtained with four quantitative D-dimer assays which were performed centrally in the laboratory of the co-ordinating center at the end of the follow-up of the PROLONG study. These D-dimer tests were performed (after thawing the frozen aliquots for 5 minutes in a water-bath at 37°C) by technicians unaware of the clinical characteristics of the patients. The following assays were performed: (i) VIDAS D-dimer Exclusion (bioMerieux, Lyon, France; kindly provided by the manufacturer) on a VIDAS apparatus (bioMerieux); the calibration curve stored in the memory for each lot was reset every 14 days using a provided calibrator; (ii) Innovance D-DIMER (Dade Behring, Marburg, Germany; kindly provided by the manufacturer) on a BCS instrument (Dade Behring); calibration was performed using the calibrator included in the kit and was carried out monthly or when a new lot was used; (iii) HemosIL D-dimer HS (Instrumentation Laboratory; kindly provided by the manufacturer) on an ACL TOP instrument (Instrumentation Laboratory); calibration was performed using the D-dimer calibrator included in the kit and was carried out monthly or when a new lot was used; and (iv) STA Liatest D-dimer (Diagnostica Stago, Asnieres-sur-Seine, France; kindly provided by Roche Diagnostics, Monza, Italy) on a STA Compact instrument (Diagnostica Stago); a calibration curve stored in the memory for each lot was used.

The results of VIDAS D-dimer Exclusion, Innovance D-DIMER and STA Liatest D-dimer are expressed in ng/mL of fibrinogen equivalent units, since these assays use crude plasmin digested lysates of cross-linked fibrin clots as calibrators. The calibrator used in the HemosIL D-dimer HS assay, however, consists of purified D-dimer fragments obtained from a plasmin-digested clot and so the results of this assay are expressed in ng/mL of D-dimer units. Since one D-dimer unit corresponds roughly to two fibrinogen equivalent units, the values obtained with the HemosIL D-dimer HS are expected to be about half those of the other assays.

Statistical analysis

Continuous variables are presented as median (range). Hazard ratios (HR) and 95% confidence intervals (CI) were calculated using a Cox proportional-hazard model. The SPSS statistical software package (Version 11.0, Chicago, Ill, USA) was used for data processing.

Results

The demographic and clinical characteristics of the 321 patients whose plasma samples were analyzed in the present study are presented in Table 1. As shown in the table, no differences were found in the characteristics between these patients and those included in the original PROLONG study who did not resume treatment with a vitamin K antagonist (n=505).

The Clearview Simplify D-dimer test gave normal result for 240 (74.8%) patients and abnormal results for 81 (25.2%) patients who were randomized not to resume vitamin K antagonist treatment. During follow-up, 25 patients had recurrent VTE (7.8%; 95%CI: 5.9-11.3) [DVT in 21 (14 contralateral, 2 with PE) and isolated PE in 4 cases; 16 males]. The incidence rate was 5.5 per 100 person-years. There was no significant difference in duration of vitamin K antagonist treatment between patients with [8.0 months (range: 3.0-33.0)] or without [8.5 months (range: 4.9-105)] recurrence. VTE recurrence occurred in 13 (5.4%) of the 240 patients with normal Clearview Simplify D-dimer test results and in 12 (14.8%) of the 81 with abnormal results (HR=2.94; 95%CI: 1.34-6.45). The incidence rate of VTE recurrence was 11.0 and 3.7 per 100 person-years in patients with abnormal and normal Clearview Simplify D-dimer results, respectively.

Table 2 shows the results calculated for each quantitative D-dimer assay using the cut-off value indicated by the manufacturers for diagnostic strategies regarding exclusion of VTE in symptomatic patients. The results, also calculated at different cut-off levels (*see Figure 1*),

Table 1. Baseline characteristics of the 321 enrolled patients.

	Present study	*Original PROLONG study*
Sex [male/female]	173/148	262/243
Age, years [median (range)]	67 (20-84)	65 (19-84)
> 70 years, n. (%)	125 (38.9)	198 (39.2)
Site of the first VTE		
Proximal DVT, n, (%)	194 (60.5)	314 (62.2)
Proximal DVT + PE, n, (%)	64 (19.9)	93 (18.4)
Isolated PE, n, (%)	63 (19.6)	98 (19.4)
Congenital thrombophilic alterations		
Factor V Leiden mutation, n, (%)	30 (9.3)	45 (8.9)
G20210A prothrombin mutation, n, (%)	20 (6.2)	33 (6.5)
Combined alterations or homozygous mut., n. (%)	8 (2.5)	9 (1.8)
Duration of previous VKA treatment		
≤ 6 months, n. (%)	62 (19.3)	81 (16.0)
> 6 months, n. (%)	259 (80.7)	424 (84.0)
Clearview Simplify D-dimer results		
Normal, n. (%)	240 (74.8)	385 (76.2)
Abnormal, n. (%)	81 (25.2)	120 (23.8)
Total duration of follow-up, years	458.9	692.0
Follow-up, days [median (range)]	600 (3-600)	600 (3-600)
VTE recurrences		
n (%)	25 (7.8)	42 (8.3)
% patients/years	5.5	6.1

No differences were found between the characteristics of the patients considered in the present study and those patients (n=505) included in the original PROLONG study who did not resume VKA treatment.
VKA: vitamin K antagonists; VTE: venous thromboembolism; DVT: deep vein thrombosis; PE: pulmonary embolism.

were similar for all the evaluated tests. By increasing the cut-off level fewer patients had abnormal D-dimer results and the percentage of VTE recurrences in these patients increased; however, the absolute number of VTE recurrences in this kind of patient decreased. In patients with normal D-dimer levels the absolute number of VTE recurrences raised in step with increasing cut-off values; however, the percentage of VTE recurrences did not change significantly. Indeed, the negative predictive values at the lowest and highest cut-off levels were, respectively: VIDAS D-dimer Exclusion = 97.4% and 95.1%; Innovance D-DIMER = 95.7% and 93.8%; HemosIL D-dimer HS = 95.8% and 94.4%; and STA Liatest D-dimer = 95.9% and 94.4%. Table 2 also reports the results obtained using as the cut-off level the one giving results most comparable to those obtained with the Clearview Simplify D-dimer assay: VIDAS D-dimer Exclusion = 800 ng/mL; Innovance D-DIMER = 800 ng/mL; HemosIL D-dimer HS = 300 ng/mL; and STA Liatest D-dimer = 700 ng/mL. We also calculated the incidence rate of VTE recurrence per 100 person-years in patients with abnormal and normal D-dimers results using different cut-off levels for each quantitative D-dimer assay (Figure 2). At the cut-off levels providing the results most comparable to those obtained with the Clearview Simplify D-dimer assay,

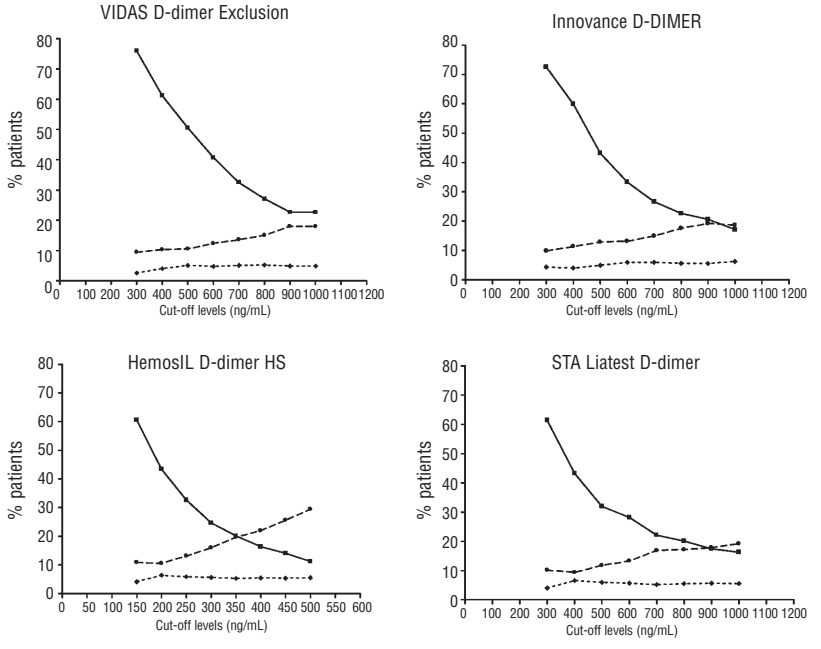

Figure 1. Results of quantitative D-dimer assays calculated using different cut-off levels. The solid lines represent the percentage of patients with D-dimer above the corresponding cut-off level (abnormal D-dimer); the dashed and dotted lines represent the percentages of recurrent venous thromboembolism in patients with abnormal or normal D-dimer levels, respectively.

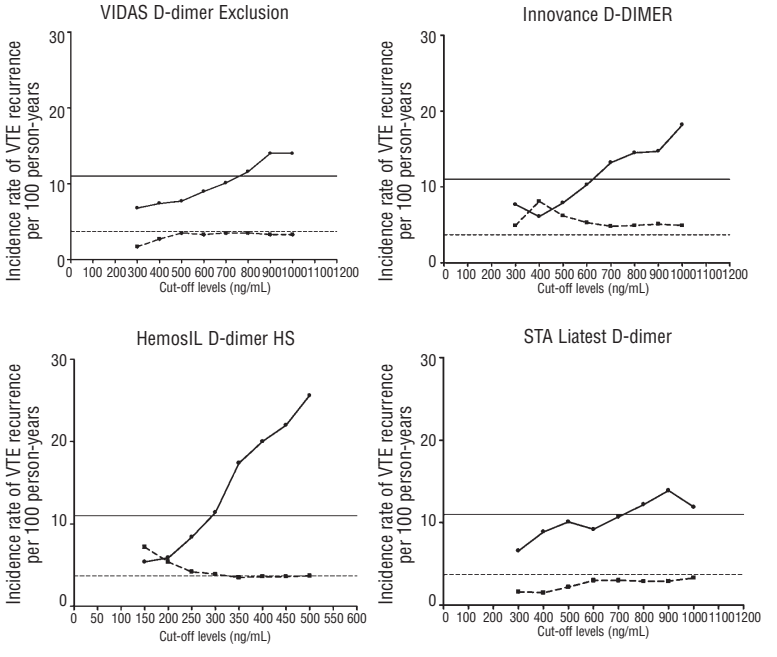

Figure 2. Incidence rate of venous thromboembolism recurrence per 100 person-years calculated using different cut-off levels for quantitative D-dimer assays. The solid lines represent the incidence rates in patients with D-dimer above the corresponding cut-off level (abnormal D-dimer); the dotted lines represent the incidence rates in patients with D-dimer below the corresponding cut-off level (normal D-dimer). The solid and dotted horizontal lines indicate the incidence rates of recurrent venous thromboembolism in patients with, respectively, abnormal or normal results in the Clearview Simplify D-dimer, assay.

the incidence rates in patients with abnormal/normal D-dimer levels were: VIDAS D-dimer Exclusion = 11.6/3.5 per 100 person-years; Innovance D-DIMER = 12.2/2.9 per 100 person-years; HemosIL D-dimer HS = 11.4/3.9 per 100 person-years; and STA Liatest D-dimer = 13.2/4.8 per 100 person-years.

Hazard ratios were also calculated according to the patients' age (≤70 *vs.* >70 years). For quantitative D-dimer assays, results were calculated using a unique cut-off level (that giving results most comparable to those obtained with the Clearview Simplify D-dimer assay, mentioned above and presented in Table 2). As shown in Table 3, the risk of VTE recurrence was significantly higher in patients with abnormal *vs.* normal D-dimer levels according to both the qualitative and all the quantitative D-dimer assays only among the patients ≤70 years old. Furthermore, the percentage of patients with abnormal D-dimer levels in both qualitative and quantitative D-dimer assays was significantly higher (2- to 3-fold) in patients aged over 70 than in those ≤70 years old.

Results were also calculated for the quantitative D-dimer assays using different cut-off levels according to the patients' age (*data not shown*). Table 4 reports the results obtained in patients aged ≤70 and > 70 years. For each assay the results calculated using the lowest cut-off level giving a statistically significant hazard ratio are reported (patients aged ≤70 years: VIDAS D-dimer Exclusion = 600 ng/mL, Innovance D-DIMER = 500 ng/mL, HemosIL D-dimer HS = 250 ng/mL, and STA Liatest D-dimer=700 ng/mL; patients aged >70 years: VIDAS D-dimer Exclusion=1200 ng/mL; Innovance D-DIMER=900 ng/mL; HemosIL D-dimer HS = 450 ng/mL; STA Liatest D-dimer = 1000 ng/mL). As shown in Table 4, to obtain statistically significant hazard ratios higher cut-off levels should be used for elderly patients.

Discussion

D-dimer levels are currently the most widely used laboratory marker for *in vivo* clotting activation, mainly due to their important role in excluding VTE in symptomatic patients.[14-17] Despite the widespread use of D-dimer measurement, clinicians are puzzled by the high variability in numerical results produced by the different methods now available. Indeed, one of the main problems of D-dimer assays is that it is not currently possible to standardize results from different assays,[18-23] making it difficult to extrapolate results from one setting to another. Each assay should, therefore, be validated separately by rigorous clinical and management studies.

Recently, the PROLONG study[13] evaluated the role of D-dimer levels in tailoring the duration of treatment with a vitamin K antagonist in patients with a previous unprovoked VTE episode. The results of that study indicate that patients with altered D-dimer levels, assessed 1 month after withdrawal of vitamin K antagonism, have a significantly higher risk of VTE recurrence and benefit from prolonged treatment with a vitamin K antagonist. As PROLONG was a multicenter study, the same D-dimer test needed to be used in all participating centers. The qualitative Clearview Simplify D-dimer assay was chosen for this purpose; this assay can be performed on citrated whole blood and does not require any instrumentation. The use of qualitative D-dimer tests is not generally recommended; observer-independent tests should be preferred in daily clinical practice in which operating conditions are less stringent than those in clinical studies. On the other hand, the choice of any other quantitative test would not have solved the question of between-assay comparability due to poor standardization of D-dimer methods. Given the poor standardization, if a D-dimer

Table 2. Results obtained with the different D-dimer assays in all patients. The table reports the results calculated using the cut-off levels indicated by the manufacturers to be used in diagnostic strategies for the exclusion of venous thromboembolism in symptomatic patients and the cut-off levels giving the results most comparable to those obtained with the qualitative Clearview Simplify D-dimer assay.

D-dimer assay (n. of tested patients)	*Cut-off*	*N. (%) of patients with abnormal D-dimer*	*N. (%) of VTE recurrences in patients with abnormal D-dimer*	*N. (%) of VTE recurrences in patients with normal D-dimer*	*HR (95%CI)*
Clearview Simplify D-dimer (n=321)	------	81 (25.2)	12 (14.8)	13 (5.4)	2.94 (1.34-6.45)
VIDAS D-dimer Exclusion (n=317)	500 ng/mL[a]	160 (50.5)	17 (10.6)	8 (5.1)	2.08 (0.93-4.69)
	800 ng/mL[b]	86 (27.1)	13 (15.1)	12 (5.2)	3.23 (1.47-7.08)
Innovance D-DIMER (n=252)	500 ng/mL[a]	109 (43.2)	14 (12.8)	7 (4.9)	2.62 (1.10-6.28)
	800 ng/mL[b]	57 (22.6)	10 (17.5)	11 (5.6)	3.41 (1.45-8.03)
HemosIL D-dimer HS (n=304)	230 ng/mL[a]	99 (32.6)	13 (13.1)	12 (5.9)	2.24 (1.06-4.73)
	300 ng/mL[b]	75 (24.7)	12 (16.0)	13 (5.7)	3.10 (1.41-6.80)
STA Liatest D-dimer (n=319)	500 ng/mL[a]	102 (32.0)	12 (11.8)	13 (6.0)	1.96 (0.93-4.15)
	700 ng/mL[b]	71 (22.2)	12 (16.9)	13 (5.2)	3.58 (1.63-7.85)

[a]Results calculated using the cut-off level indicated by the manufacturers for VTE exclusion; [b]results calculated using the cut-off level giving the results most comparable to those obtained with the qualitative Clearview Simplify D-dimer assay. VTE: venous thromboembolism; HR: hazard ratio; CI: confidence interval.

test is to be used in clinical practice to stratify the risk of VTE recurrence, assays other than that used in the PROLONG study need to be independently assessed with a similar management study. Some indications can, however, be drawn from the present study, which evaluated different quantitative D-dimer tests using frozen plasma from patients enrolled in the PROLONG study.

The present analysis shows that quantitative D-dimer assays may be useful in evaluating a patient's individual risk of VTE recurrence following a first unprovoked event. These assays seem particularly advantageous since they allow the selection of different cut-off levels according to a patient's characteristics. We found that when the cut-off levels validated for use in diagnostic strategies for exclusion of VTE in symptomatic patients were used, even when assessing VTE recurrence risk, the percentage of subjects with abnormal D-dimer results was higher than that recorded in the qualitative assay; as a consequence, the rate of VTE recurrence in subjects with abnormal D-dimer levels was lower than that recorded in the PROLONG study. These data suggest that slightly higher cut-off levels than those used in diagnostic strategies used to rule out

Table 3. Results obtained with the different D-dimer assays according to the patients' age. Data were calculated for each assay using the cut-off levels shown in Table 2.

	N. (%) of patients with abnormal D-dimer	*N. (%) of VTE recurrences in patients with abnormal D-dimer*	*N. (%) of VTE recurrences in patients with normal D-dimer*	*HR (95%CI)*
Clearview Simplify D-dimer				
Patients ≤70 years (n=196)	33 (16.8)	5 (15.1)	6 (3.7)	4.52 (1.38-14.8)
Patients >70 years (n=125)	48 (38.4)	7 (14.6)	7 (9.1)	1.69 (0.59-4.81)
VIDAS D-dimer Exclusion Cut-off = 800 ng/mL				
Patients ≤70 years (n=192)	30 (15.0)	4 (13.3)	7 (4.3)	3.50 (1.02-12.0)
Patients > 70 years (n=125)	56 (44.8)	9 (16.1)	5 (7.2)	2.35 (0.79-7.00)
Innovance D-DIMER Cut-off = 800 ng/mL				
Patients ≤70 years (n=115)	15 (13.0)	2 (13.3)	3 (3.0)	4.77 (0.80-28.6)
Patients > 70 years (n=137)	42 (30.7)	8 (19.0)	8 (8.4)	2.49 (0.94-6.65)
HemosIL D-dimer HS Cut-off = 300 ng/mL				
Patients ≤70 years (n=185)	24 (13.0)	4 (16.7)	7 (4.3)	4.35 (1.27-14.9)
Patients > 70 years (n=119)	51 (42.8)	8 (15.7)	6 (8.8)	1.90 (0.66-5.47)
STA Liatest D-dimer Cut-off = 700 ng/mL				
Patients ≤70 years (n=194)	24 (12.4)	4 (16.7)	7 (4.1)	4.62 (1.35-15.8)
Patients > 70 years (n=125)	47 (37.6)	8 (17.0)	6 (7.7)	2.39 (0.83-6.89)

VTE: venous thromboembolism; HR: hazard ratio; CI: confidence interval.

Table 4. Results obtained with the different D-dimer assays in patients aged ≤70 or >70 years. For each assay the results obtained using the lowest cut-off level giving a statistically significant hazard ratio are reported.

D-dimer assay	*Cut-off*	*N. (%) of patients with abnormal D-dimer*	*(%) of VTE recurrences in patients with abnormal D-dimer*	*N. (%) of VTE recurrences in patients with normal D-dimer*	*HR (95%CI)*
			Patients aged ≤ 70 (n=196)		
Clearview Simplify D-dimer	–	33 (16.8)	5 (15.1)	6 (3.7)	4.52 (1.38-14.8)
VIDAS D-dimer Exclusion	600 ng/mL	54 (28.1)	6 (11.1)	5 (3.6)	3.29 (1.00-10.8)
Innovance D-DIMER	500 ng/mL	34 (29.6)	4 (11.8)	1 (1.2)	9.79 (1.09-87.6)
HemosIL D-dimer HS	250 ng/mL	36 (19.4)	5 (13.9)	6 (4.0)	3.74 (1.14-12.3)
STA Liatest D-dimer	700 ng/mL	24 (12.4)	4 (16.7)	7 (4.1)	4.62 (1.35-15.8)
			Patients aged > 70 (n=125)		
Clearview Simplify D-dimer	–	48 (38.4)	7 (14.6)	7 (9.1)	1.69 (0.59-4.81)
VIDAS D-dimer Exclusion	1200 ng/mL	39 (31.2)	8 (20.5)	6 (7.0)	3.23 (1.12-9.33)
Innovance D-DIMER	900 ng/mL	40 (29.2)	8 (20.0)	8 (8.2)	2.73 (1.02-7.27)
HemosIL D-dimer HS	450 ng/mL	29 (24.4)	7 (24.1)	7 (7.8)	3.45 (1.21-9.85)
STA Liatest D-dimer	1000 ng/mL	35 (28.0)	7 (20.0)	7 (7.8)	2.84 (1.00-8.11)

VTE: venous thromboembolism; HR: hazard ratio; CI: confidence interval.

VTE (usually around 500 ng/mL for D-dimer assays whose results are expressed in fibrinogen equivalent units and around 250 ng/mL for those whose results are expressed in D-dimer units)[24] may be better suited for predicting the risk of recurrence.

As shown in Figures 1 and 2, when cut-off levels were increased, the percentage of subjects with altered results decreased and the rate of recurrence increased; in contrast, the rate of recurrence in subjects with normal D-dimer levels remained substantially unchanged. It seems, therefore, that selection of a progressively higher D-dimer cut-off does not translate into a lower negative predictive value. Our interpretation of these data is that an abnormal D-dimer level is a stronger positive predictor for the risk of VTE recurrence than a normal D-dimer level is a negative predictor.

It is well known that D-dimer levels increase with age, particularly in the presence of co-existing functional impairment, because of a combination of factors, including reduced renal clearance, increased fibrinogen levels and the presence of occult disease.[25,26] It has been shown that in healthy subjects D-dimer levels are approximately four times higher in the highest age quartile.[25] As a result, it has been reported that the specificity and hence diagnostic utility of D-dimer measurements for excluding VTE is lower in older patients.[27-29] In one study, it was shown that the specificity of the VIDAS D-dimer assay improved when a higher cut-off level was used in elderly patients without any decrease in sensitivity.[30] The PROLONG study showed that abnormal Clearview Simplify D-dimer results were significantly associated with an increased risk of VTE recurrence; however, analyzing the results according to the age of the patients, it was found that the association did not reach statistical significance in patients aged >70 years. Similar results have been obtained for quantitative assays when a single cut-off level is used for all patients. A theoretical advantage of using quantitative D-dimer tests is that different thresholds discriminating between abnormal and normal can be used according to the patients' characteristics. The present results support the use of different cut-off levels depending on a patient's age. Indeed, using a higher cut-off value, abnormal D-dimer results in the study were associated with a significant increase in the risk of VTE recurrence even in elderly patients.

Some important limitations of the present study need to be pointed out. First and foremost, this was a post-hoc analysis of a prospective study. It is likely that the hazard ratios calculated for the quantitative assays were exaggerated since cut-off levels were determined knowing which subjects had or had not had recurrent VTE. Second, not all the patients enrolled in the PROLONG study could be evaluated in the present analysis because of the unavailability of frozen plasma aliquots and the exclusion of those patients who had been randomized to resume treatment with a vitamin K antagonist. The number of patients examined was, therefore, lower than that in the PROLONG study; in particular the number of recurrent VTE events was low, thus affecting the accuracy of the findings and widening the confidence intervals of estimates. This limitation is even greater when different subgroups of patients were analyzed according to age. Finally, each D-dimer assay should be evaluated in specifically designed management studies to prospectively validate their use in assessing the risk of VTE recurrence.[31] We do, however, believe that the present results can be of some use for further prospective studies.

Appendix

All the centers that participated in this study are affiliated to the Italian Federation of Thrombosis Centers – FCSA. The following is a list of study sites involved and the principal investigators. The number of patients enrolled is given in parentheses:

U.O. Angiologia e Malattie della Coagulazione "Marino Golinelli", Policlinico S.Orsola-Malpighi, Bologna – G. Palareti (n=112, Coordinating Center); I° Div. di Medicina Interna - Centro Emostasi e Trombosi, Arcispedale Santa Maria Nuova, Reggio-Emilia – A. Ghirarduzzi (n=51); A.O. Istituti Ospitalieri, Cremona – S. Testa (n=48); Centro Regionale Malattie Emorragiche e Trombotiche - Div. Ematologia, Ospedale S. Bortolo, Vicenza – A. Tosetto (n=21); Sezione Trasfusionale, Ospedale San Leopoldo Mandic, Merate – N. Erba (n=15); Servizio di Prevenzione e Terapia della Trombosi, Ospedale "Ex Busonera", Padova – V. Pengo (n=15); Centro Sorveglianza Anticoagulati - Malattie della Coagulazione e Angiologia Medica - Div. di Med. Interna, Ospedale "S. Cuore di Gesu'", Gallipoli – L. Ria (n=13); Centro Emostasi, Ospedale Regionale, Parma – C. Pattacini (n=10); Laboratorio Analisi - Ambulatorio per il Controllo della Terapia Anticoagulante Orale, Presidio Ospedaliero di Faenza – E. Bucherini (n=9); Laboratorio Analisi - Divisione di Cardiologia, Ospedale di Bentivoglio – E. Cerè (n=9); Laboratorio di Patologia Clinica, Presidio Ospedaliero S.Maria Incoronata dell'Olmo, Cava dei Tirreni – C. Villani (n=8); Centro Trombosi, A.O. di Careggi Universita' di Firenze – D. Prisco (n=4); U.O. Medicina Interna, Policlinico Universitario Messina – A. Trifiletti (n=4); Centro per lo Studio delle Coagulopatie a Rischio Trombotico, Osp. Galliera, Genova – A. Schenone (n=2).

Authorship and Disclosures

CL analyzed the data and wrote the paper; GP designed and promoted the study; BC was responsible for the enrollment of the patients and follow-up; MC performed the D-dimer assays; ATo and ATr contributed to data interpretation and helped revise the manuscript; all authors checked the final version of the manuscript. CL, GP, BC, ATo and ATr received lecture fees from Instrumentation Laboratory. CL and GP received lecture fees from Roche Diagnostics. There are no other actual or potential conflicts of interest relevant to this article.

References

1. Prandoni P, Noventa F, Ghirarduzzi A, Pengo V, Bernardi E, Pesavento R, et al. The risk of recurrent venous thromboembolism after discontinuing anticoagulation in patients with acute proximal deep vein thrombosis or pulmonary embolism. A prospective cohort study in 1,626 patients. Haematologica 2007;92:199-205.
2. Schulman S, Granqvist S, Holmstrom M, Carlsson A, Lindmarker P, Nicol P, et al. The duration of oral anticoagulant therapy after a second episode of venous thromboembolism. N Engl J Med 1997;336:393-8.
3. Kearon C, Gent M, Hirsh J, Weitz J, Kovacs MJ, Anderson DR, et al. A comparison of three months of anticoagulation with extended anticoagulation for a first episode of idiopathic venous thromboembolism. N Engl J Med 1999;340:901-7.
4. Agnelli G, Prandoni P, Becattini C, Silingardi M, Taliani MR, Miccio M, et al. Extended oral anticoagulant therapy after a first episode of pulmonary embolism. Ann Intern Med 2003;139:19-25.
5. Agnelli G, Prandoni P, Santamaria MG, Bagatella P, Iorio A, Bazzan M, et al. Three months versus one year of oral anticoagulant therapy for idiopathic deep venous thrombosis. N Engl J Med 2001;345:165-9.
6. Pinede L, Ninet J, Duhaut P, Chabaud S, DemolombeRague S, Durieu I, et al. Comparison of 3 and 6 months of oral anticoagulant therapy after a first episode of proximal deep vein thrombosis or pulmonary embolism and comparison of 6 and 12 weeks of therapy after isolated calf deep vein thrombosis. Circulation 2001;103: 2453-60.
7. van Dongen CJJ, Hutten BA, Buller HR, Prins MH. The incidence of recurrent venous thromboembolism after treatment with vitamin K antagonists in relation to time since first event - a meta-analysis. Arch Intern Med 2003;163:1285-93.
8. Palareti G, Leali N, Coccheri S, Poggi M, Manotti C, D'Angelo A, et al. Bleeding complications of oral anticoagulant treatment: an inception-cohort, prospective collaborative study (ISCOAT). Italian Study on Complications of Oral Anticoagulant Therapy. Lancet 1996;348:423-8.
9. Palareti G, Legnani C, Cosmi B, Guazzaloca G, Pancani C, Coccheri S. Risk of venous thromboembolism recurrence: high negative predictive value of D-dimer performed after oral anticoagulation is stopped. Thromb Haemost 2002;87:7-12.
10. Eichinger S, Minar E, Bialonczyk C, Hirschl M, Quehenbergen P, Schneider B, et al. D-dimer levels and risk of recurrent venous thromboembolism. JAMA 2003;290:1071-4.
11. Palareti G, Legnani C, Cosmi B, Valdre L, Lunghi B, Bernardi F, et al. 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. Circulation 2003; 108:313-8.
12. Shrivastava S, Ridker PM, Glynn RJ, Goldhaber SZ, Moll S, Bounameaux H, et al. D-dimer, factor VIII coagulant activity, low-intensity warfarin and the risk of recurrent venous thromboembolism. J Thromb Haemost 2006;4:1208-14.
13. Palareti G, Cosmi B, Legnani C, Tosetto A, Brusi C, Iorio A, et al. D-dimer testing to determine the duration of anticoagulation therapy. N Engl J Med 2006;355:1780-9.
14. Michiels JJ, Freyburger G, van der Graaf F, Janssen M, Oortwijn W, van Beek EJR. Strategies for the safe and effective exclusion and diagnosis of deep vein thrombosis by the sequential use of clinical score, D-dimer testing, and compression ultrasonography. Semin Thromb Hemost 2000; 26:657-67.
15. Heim SW, Schectman JM, Siadaty MS, Philbrick JT. D-dimer testing for deep venous thrombosis: a meta-analysis. Clin Chem 2004;50:1136-47.
16. Stein PD, Hull RD, Patel KC, Olson RE, Ghali WA, Brant R, et al. D-dimer for the exclusion of acute venous thrombosis and pulmonary embolism - a systematic review. Ann Intern Med 2004;140:589-602.
17. Perrier A, Palareti G. D-dimer testing and venous thromboembolism: four view points. J Thromb Haemost 2005;3:382-4.
18. Gaffney PJ. Distinction between fibrinogen and fibrin degradation products in plasma. Clin Chim Acta 1975; 65:109-15.
19. Adema E, Gebert U. Pooled patient samples as reference material for D-dimer. Thromb Res 1995;80:85-8.
20. Nieuwenhuizen W. A reference material for harmonisation of D-dimer assays. Thromb Haemost 1997;77:1031-3.
21. Dempfle CE, Zips S, Ergul H, Heene DL. The Fibrin Assay Comparison Trial (FACT) - Evaluation of 23 quantitative D-dimer assays as basis for the development of D-dimer calibrators. Thromb Haemost 2001;85:671-8.
22. Dempfle CE. D-dimer: standardization versus harmonization. Thromb Haemost 2006;95:399-400.
23. Meijer P, Haverkate F, Kluft C, deMoerloose P, Verbruggen B, Spannagl M. A model for the harmonisation of test results of different quantitative D-dimer methods. Thromb Haemost 2006;95:567-72.
24. Palareti G, Cosmi B, Legnani C. Diagnosis of deep vein thrombosis. Semin Thromb Hemost 2006;32: 659-72.
25. Hager K, Platt D. Fibrin degeneration product concentrations (D-dimers) in the course of ageing. Gerontology 1995;41:159-65.
26. Pieper CF, Rao KMK, Currie MS, Harris TB, Cohen HJ. Age, functional status, and racial differences in plasma D-dimer levels in community-dwelling elderly persons. J Gerontol A Biol Sci Med Sci 2000; 55: M649-M57.
27. Bounameaux H, Demoerloose P, Perrier A, Miron MJ. D-dimer testing in suspected venous thromboembolism: an update. QJM 1997;90: 437-42.
28. Tardy B, Tardyponcet B, Viallon A, Lafond P, Page V, Venet C, et al. Evaluation of D-dimer ELISA test in elderly patients with suspected pulmonary embolism. Thromb Haemost 1998;79:38-41.
29. Righini M, Goehring C, Bounameaux H, Perrier A. Effects of age on the performance of common diagnostic tests for pulmonary embolism. Am J Med 2000;109:357-61.
30. Le Blanche AF, Siguret V, Settegrana C, Bohus S, Le Masne de Chermont E, Andreux JP, et al. Ruling out acute deep vein thrombosis by ELISA plasma D-dimer assay versus ultrasound in inpatients more than 70 years old. Angiology 1999;50:873-82.
31. Baglin T. Value of D-dimer testing to decide duration of anticoagulation after deep vein thrombosis: not yet. J Thromb Haemost 2006;4:2530-2.