

D-Dimer testing in suspected venous thromboembolism: an update

H. BOUNAMEAUX, P. DE MOERLOOSE, A. PERRIER and M.-J. MIRON

From the Division of Angiology and Haemostasis, Department of Internal Medicine, University Hospital of Geneva, Geneva, Switzerland

Introduction

Fibrin is the main thrombus component. Its production is followed by activation of the fibrinolytic system, resulting in plasmin generation and subsequent fibrin lysis (Figure 1). Dissolution of crosslinked fibrin leads to formation of specific degradation products, including D-dimer (DD),¹ which can be easily detected and measured in both whole blood and plasma using monoclonal antibodies directed against epitopes located in the DD fragment. In the past decade, DD testing has been established as a useful aid in the diagnosis of venous thromboembolism (VTE).² The present review updates experience on the use of DD testing for diagnosis of deep-vein thrombosis (DVT) of the lower limbs and pulmonary embolism (PE). Briefly, DD was found in the late 1980s and early 1990s to be highly sensitive (but non-specific) to the presence of VTE where clinically suspected in out-patients. In this population, the negative predictive value of a plasma DD concentration below a certain cutoff (usually about 500 µg/l) was more than 95%, thereby allowing one to rule out the disease in a substantial proportion (about one third) of patients. However, the diagnostic performances were strongly dependent upon the assay used, latex tests being definitely less sensitive than the more cumbersome enzyme-linked immunosorbent assays (ELISA), which in turn were not suited for emergency use.² Moreover, this heterogeneity of tests raised uncertainty among clinicians, who called for rigorous evaluation and standardization of the various assays.³ Lastly, the utility of the measurement was dependent on the population to which it was applied, the specificity

being definitely lower in in-patients than in out-patients.

Methods for measuring DD in blood

The advantages and disadvantages of the various methods that measure DD in blood are displayed in Table 1. For clinical use in emergency situations, rapid tests are mandatory. Among them, a whole blood latex test (Simplired, Agen) that gives a

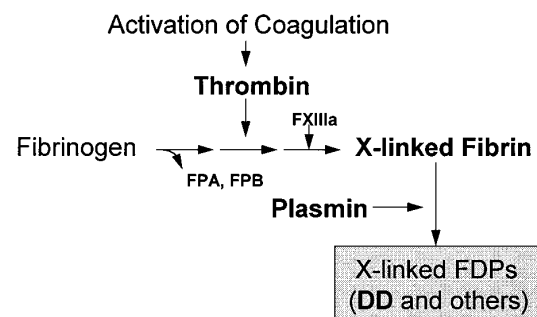


Figure 1. Generation of D-dimer (DD) fragments. Activation of blood coagulation leads to thrombin generation. Thrombin binds to the fibrinogen central domain and liberates fibrinopeptides A and B, resulting in fibrin monomers and polymer formation. The fibrin network is subsequently stabilized ('cross-linked') under the effect of activated coagulation factor XIII. Plasmin induces lysis of cross-linked (X-linked) fibrin, resulting in formation of various X-linked fibrin degradation products (FDPs) including D-dimer (DD) and fragments containing the DD epitope.

Table 1 Comparison of the different assay methods for DD

	Single-test	Rapid	Quantitative	Sensitive	Specific
<i>Latex assays</i>					
Classical latex tests on plasma	+	+	—	—	—
Latex test on whole blood (Simplired®)	+	+	—	+/-	—
<i>ELISA assays</i>					
Classical ELISA tests	—	—	+	+	—
Rapid ELISA test (Vidas DD)	+	+	+	+	—

Table 2 Diagnostic performances of DD measurement in patients suspected of DVT or PE

	Patients (n)	Events (%)	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)
<i>Suspected DVT</i>				
Classical ELISA ²	1337	34.7	97 (95–98)	35 (32–38)
Rapid ELISA (Vidas-DD) ^{6–8}	311	45.7	98 (94–100)	54 (47–62)
Classical latex tests ²	733	36.7	83 (78–87)	68 (63–72)
Whole blood latex test (Simplired) ^{8–11}	612	39.1	82 (77–87)	71 (66–75)
<i>Suspected PE</i>				
Classical ELISA ^{2,12}	1579	34.1	98 (96–99)	43 (40–46)
Rapid ELISA (Vidas-DD) ¹³	195	23.6	100 (92–100)	38 (29–45)
Classical latex tests ²	364	45.9	92 (88–96)	55 (48–62)
Whole blood latex test (Simplired) ^{10,14}	140	25.0	97 (85–100)	63 (54–72)

Sensitivity and specificity are given as weighted means with 95% CIs; *n* represents the number of patients, and Events the percentage of patients with confirmed deep-vein thrombosis (DVT) or pulmonary embolism (PE).

qualitative result in 2 min, and a rapid ELISA assay on plasma (Vidas DD, bioMerieux) that requires 35 min to produce a quantitative result, were submitted to clinical testing over the past few years. Unfortunately, latex tests, including the whole blood Simplired test, have the disadvantages of the visual reading (with potential interobserver disagreement) and a limited sensitivity to the presence of VTE (Table 2). On the other hand, the rapid ELISA test Vidas DD is highly sensitive to the presence of VTE, but needs to be run on a commercial automated device. Other new quick tests, based on the ELISA or latex agglutination principle, should undergo systematic testing in clinical settings before recommending their use in suspected venous thromboembolism.

DD for ruling out deep-vein thrombosis (DVT)

In patients with clinical signs and symptoms, B-mode venous compression ultrasonography or duplex scanning are presently used in clinical practice,⁴ contrast venography still being the diagnostic gold standard. On the other hand, with increasing fear for DVT and its immediate and late consequences (pulmonary embolism and post-thrombotic syndrome), patients

are increasingly referred to diagnostic centres, in spite of a low or very low clinical suspicion. This change in practice resulted in a decrease in the prevalence of DVT among clinically-suspected patients, which has dropped in our centre from 50% to 25% over the past 15 years. Even though non-invasive diagnostic tests are less harmful and much cheaper than venography, they are still relatively expensive and require technical skills. Thus, a highly sensitive and simple test used as initial screening and ruling out DVT in a substantial proportion of subjects, might save time and money, as demonstrated in the setting of PE diagnosis.⁵ The more specific ultrasonographic test would be applied only in patients presenting with a DD concentration above the cutoff.

Table 2 summarizes the diagnostic performances of the various methods to assay DD in suspected DVT. ELISA tests have a superior sensitivity to the presence of DVT compared with latex tests. At present time, the superiority of the Simplired test over other latex tests is far from evident in patients with suspected DVT. Published reports of sensitivity vary widely from paper to paper,^{8–11} ranging from an extremely poor 61%⁸ up to 100%.¹⁰ However, data are still limited, and studies with larger numbers of patients and adequate methodology are urgently needed.

DD for ruling out pulmonary embolism (PE)

Clinical diagnosis of PE is unreliable, and the diagnostic gold standard, pulmonary angiography, is cumbersome, invasive and not without risk. Perfusion/ventilation lung scintigraphy allows ruling out or ruling in PE in less than half of cases, and patients with an abnormal, non-high-probability lung scan pattern should theoretically undergo pulmonary angiography. Recently, strategies were developed that include lower-limbs venous compression ultrasonography, which is highly specific for venous thromboembolism. Such policies reduce substantially the need for pulmonary angiography.^{15,16} On the other hand, because sensitivity of DD to the presence of PE is extremely high (Table 2), a concentration below the cutoff allows ruling out the disease with a negative predictive value of more than 95% when established methods are used.

Table 2 summarizes the diagnostic performances of the various methods to assay DD in suspected PE. Sensitivity of all assays appears to be better for PE than for DVT, which may reflect a larger amount of fibrin formed and degraded in the former. In addition, ELISA assays and the whole-blood latex test seem to exhibit comparable performances (sensitivity to presence of PE of >95%) but confidence intervals for sensitivity of the new tests are still wide, because of the relatively small patient populations studied so far.

Other applications of DD measurement in suspected venous thromboembolism

Screening of DVT in asymptomatic high-risk patients

In a prospective trial of 185 consecutive patients undergoing elective abdominal surgery who were submitted to bilateral ascending venography on the 8th postoperative day, a plasma DD cutoff of 3000 µg/l was able to discriminate between patients with and without postoperative DVT with a sensitivity and a specificity of 89% and 48%, respectively.¹⁷ We, therefore suggested that plasma measurement of DD might be useful in thromboprophylactic studies for initial screening of patients, a level below 3000 µg/l excluding DVT (negative predictive value of 93%) whereas a concentration above the cutoff would require phlebographic confirmation. Similar results have been reported by others in general surgery patients¹⁸ and by our group in patients undergoing total hip arthroplasty.¹⁹ Such an approach would be particularly valuable in thromboprophylac-

tic trials but still needs to be evaluated properly in specially designed, prospective studies.

Diagnosis of recurrent venous thromboembolism

It may be very difficult to distinguish recurrent venous thromboembolism from the sequelae of a previous event. Sié *et al.* showed that DD levels returned to normal values within 3 months after an acute DVT²⁰ in most subjects, thereby implying that a low DD concentration measured in a patient suspected of a recurrent event could be used to rule out recurrence.

Potential use of DD testing for confirming PE

The poor specificity of elevated DD plasma concentration is well known.^{2,21} Indeed, several conditions are associated with fibrin formation and degradation, such as infectious or inflammatory diseases, and cancer. Only 20% or even less of patients who are admitted with these conditions will present with a DD level below the critical cutoff of 500 µg/l, which decreases the usefulness of testing in case of concomitant suspected venous thrombosis.²¹

Nonetheless, in out-patients clinically suspected of PE, the specificity of the classical ELISA was 41.4% (95% CI 37.0%–45.9%) in a large series of 671 patients.¹² This figure was greatly influenced by age, ranging from 72% (30–39 year-old group) to 9% (>80 years of age). As a consequence, DD measurement would allow excluding PE in 2/3 out-patients without the disease under 60 years of age, but only in 1/5 patients older than 60.

The overall specificity of 41.4% of DD for the presence of PE was obtained for a cutoff of 500 µg/l (for the ELISA assay Asserachrom DD from Stago) which was associated with a sensitivity of 99.5% (95% CI 97.2%–100%). Setting the cutoff at 4000 µg/l would result in a dramatic increase of specificity (93.1%) while sensitivity would be lowered to 49.5%.¹² Detailed operating characteristics of DD in suspected PE are displayed in Table 3. These data imply that an out-patient clinically suspected of PE who presents with a DD level above 4000 µg/l has a 5:1 likelihood of having PE, which may be sufficient to initiate anticoagulant treatment, provided the prior clinical probability is sufficiently high.

Standardization of DD assays: necessity or wishful thinking?

Different methods, different results

Several types of assays for DD are available (Table 1) and within each type, numerous commercial kits

Table 3 Characteristics of DD measurement in outpatients suspected of PE

DD cutoff (µg/l)	Sensitivity (%; 95%CI)	Specificity (%; 95%CI)
300	100 (98.1–100)	24.0 (20.2–27.8)
500	99.5 (97.2–100)	41.4 (37.0–45.9)
1000	86.2 (81.4–91.0)	72.4 (68.4–76.4)
2000	75.0 (68.9–81.1)	82.5 (79.1–85.9)
4000	49.5 (42.5–56.5)	93.1 (90.4–95.2)
10000	10.7 (6.4–15.0)	98.3 (96.7–99.3)

Adapted from reference 12.

exist which differ in many aspects, including the capture and the tagging antibody, the required sample dilution, the detection limit, and the incubation time. In a systematic comparison of four ELISA assays in 151 plasma samples from patients clinically suspected of pulmonary embolism, van Beek *et al.* found intra-assay coefficients of variation in the median values of 3.5% to 17%²² while the inter-assay variation coefficients were very similar among the four assays (15–20%). This rather poor reproducibility might account, at least in part, for the only fair correlation (regression coefficients of about 0.60) among the various ELISA assays. Finally, the normal values in a reference healthy population differed considerably between the tests.

On the other hand, because the assays recognize more or less different components in plasma or in blood and because various techniques are used, heterogeneity of the results might have been anticipated. Thus, rather than calling for an unlikely standardization, efforts should focus on determining a critical cutoff for each individual assay, based on the test performances established in clinical studies that were conducted according to the four steps described hereafter.

Four-step evaluation of a diagnostic test

Systematic evaluation of a new diagnostic test in suspected venous thromboembolism²³ includes (i) technical description of the method; (ii) systematic comparison with a diagnostic standard in order to establish the critical cutoff of DD concentration (using Receiver Operating Characteristics curve analysis), and values of sensitivity and specificity of the test to the presence of venous thromboembolism, for the cutoff chosen; (iii) use of the test in so-called management trials, in which anticoagulation is withheld in patients with a DD concentration below the cutoff. A systematic three-month follow-up would allow detection of delayed events and establishment of the true diagnostic performance of the test;

(iv) cost-effectiveness analyses comparing the 'new' strategy with other management policies.

The place of DD testing in an integrated diagnostic approach of venous thromboembolism

No single diagnostic test has a sufficiently high sensitivity and specificity to be used alone in the diagnostic approach of patients suspected of venous thromboembolism, and this is particularly true for DD testing, which is non-specific. Thus, test combinations will be used depending upon their performances but also upon their availability, and the costs. These tests include, besides DD measurement, venous compression ultrasonography,⁴ ventilation/perfusion lung scintigraphy,²⁴ to some extent also echocardiography and spiral CT, and lastly, contrast venography of lower limbs and pulmonary angiography. Test combinations will also be based on the fact that DVT and PE represent two clinical pictures of a common condition, venous thromboembolic disease, and that diagnosing DVT in a patient clinically suspected of PE allows to diagnose PE. In addition, the treatment of DVT and PE is basically the same.

Finally, a diagnostic strategy should be viewed in a Bayesian perspective, every test result being integrated with the clinical probability of having the disease determined prior to any test.^{2,25,26} Figure 2 displays two examples of simple, sequential diagnostic algorithms for suspected DVT or PE which are presently being tested in a large-scale Swiss-Canadian management trial. The sequences were derived from a clinical decision-making model,²⁵ a subsequent management trial¹⁶ and a cost-effectiveness analysis.⁵ In the strategy chosen for suspected PE, DD testing as an initial screening, followed by venous compression ultrasonography, yielded a 10% incremental cost reduction and a 40% reduction of necessary angiograms compared to the reference strategy.⁵ This sequence would in addition permit district hospitals without lung-scan facilities to manage approximately 50% of outpatients with suspected PE without referral.

Conclusions and perspectives

The place of DD measurement in the diagnostic workup of suspected venous thromboembolism is now well established. When using assays assessed in well-conducted clinical trials, the test can safely rule out DVT and PE if the concentration falls below a certain, assay-dependent cutoff. When used as the initial diagnostic step in an out-patient population with a prevalence of the disease of about 25%, this

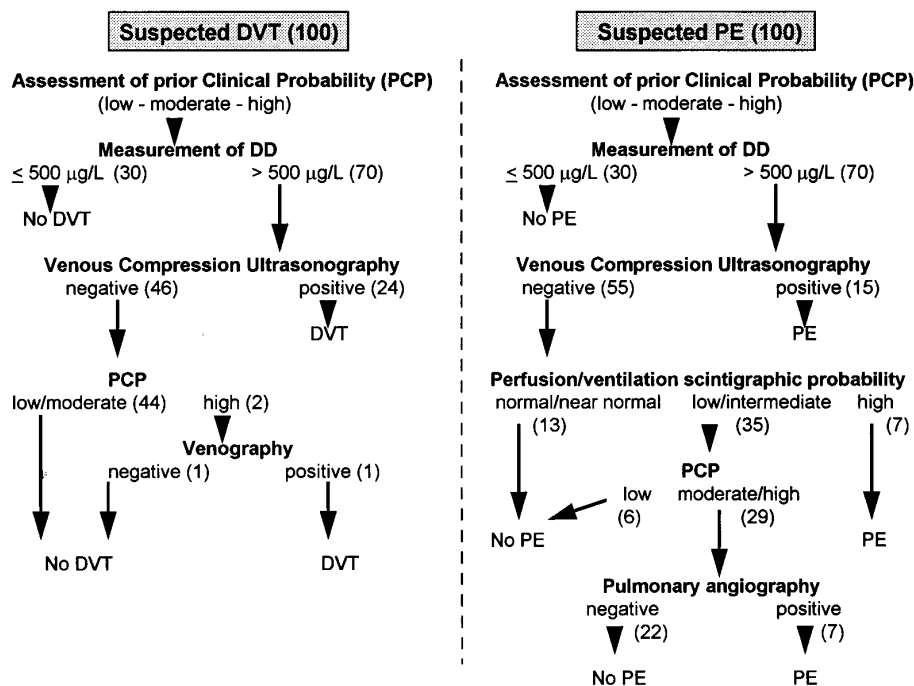


Figure 2. D-Dimer (DD) testing in diagnostic algorithms of suspected DVT or PE. Due to its high sensitivity to venous thromboembolism, DD measurement can be used early in the diagnostic approach of deep-vein thrombosis (DVT) or pulmonary embolism (PE), a value below the critical cutoff allowing to rule out these diseases. If the DD concentration is above the cutoff, more specific tests are to be used along with prior clinical probability (PCP) assessment. The figures between brackets represent the numbers of patients with the corresponding outcome in a hypothetical cohort of 100 outpatients with clinically suspected DVT or PE, given a prevalence of the disease of about 25% in the population studied (estimations from data obtained in our institution, published in part^{11,15}).

simple test will exclude VTE in about 30% of patients at a low cost. A widespread clinical application was made possible thanks the development of rapid, single-test, and quantitative DD assays (and, possibly, more reliable latex tests) that can be used in the Emergency Department.

Further potential uses of the test include diagnosis of VTE in symptomatic in-patients, screening of asymptomatic high-risk patients in thromboprophylactic trials, ruling out suspected recurrent VTE, and ruling in VTE in suspected outpatients when DD concentration is *above* a certain cutoff. These applications look promising but deserve further investigation. Lastly, attempts to standardize the various DD assays should be abandoned, and replaced by systematic studies of the diagnostic performances of each individual assay in various clinical settings.

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