D-dimer Testing and Venous Thromboembolism

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Abstract

With its high sensitivity and negative predictive value, D-dimer (DD) testing has gained a role in the diagnostic work-up of suspected venous thromboembolism (VTE) for the exclusion of the disease, potentially reducing the need for imaging tests. The diagnostic yield of DD testing is affected not only by the choice of the appropriate assay for its measurement, but also by patient characteristics. As a consequence, its clinical usefulness for the exclusion of suspected VTE should be carefully evaluated in special clinical settings. There is increasing evidence that DD testing after anticoagulation withdrawal for a first unprovoked VTE episode may be useful to identify patients at higher risk of recurrence, and may help clinicians with the decision of whether to continue or stop anticoagulant treatment. However, further studies are needed to establish the optimal timing of DD testing and the best DD cut-off level that predicts recurrence, and to develop a clinical prediction rule for recurrent VTE.

Keywords

D-dimer, venous thromboembolism, deep vein thrombosis, pulmonary embolism, diagnosis, recurrence

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D-dimer Formation

D-dimer (DD) is the final product of the plasmin-mediated degradation of cross-linked fibrin. Its blood concentration depends on clotting activation with fibrin generation, stabilisation by factor XIIIa and subsequent degradation by the endogenous fibrinolytic system. DD (molecular weight around 180,000Da) consists of two identical subunits derived from two fibrin monomer molecules. Its plasma half-life is approximately eight hours and clearance occurs via the kidney and the reticulo-endothelial system.[1] DD plasma concentration is increased in all physiological and pathological conditions associated with enhanced fibrin formation and subsequent degradation by plasmin (see *Table 1*).

D-dimer Measurement

A large variety of assays is available for DD measurement. All methods are based on the use of monoclonal antibodies that recognise epitopes on DD fragments that are absent on fibrinogen and non-cross-linked fragments of fibrin. The resulting antibody–antigen complexes can be detected by enzyme-linked immunosorbent assay (ELISA) or agglutination techniques.[2] *Table 2* shows a summary of the features of available DD assays: the ELISAs and automated latex turbidimetric tests show the highest sensitivity and no interobserver variability, whereas manual latex and whole-blood assays are less sensitive but have the advantage of a quick and easy bedside execution.

One of the main problems with DD measurement is represented by the difficulty in standardisation of the different available assays.[3] This can be due to several reasons. First is the heterogeneity of the analyte itself: DD is not a single entity but a complex mixture of degradation products of different sizes, with large inter- and intra-individual variability. Second is the type of calibrators used: purified DD fragments, with results expressed as DD concentration, or fibrin-degradation products obtained from controlled plasmin digestion, with results expressed in fibrinogen equivalent units (FEU). Finally, the use of various types of monoclonal antibody with different specificity and affinity means that the same degradation product can be detected to different extents by different assay re-agents. Some mathematical models for the harmonisation of DD test results have been proposed, but no consensus has yet been achieved.[4,5]

D-dimer Testing for Venous Thromboembolism Diagnosis

DD is typically elevated in patients with acute venous thromboembolism (VTE). Indeed, thrombus formation is normally followed by an immediate fibrinolytic response, with the release of fibrin-degradation products into the circulation. It follows that an absence of a rise in DD implies that thrombosis is not occurring. DD is a sensitive but not specific marker for VTE, so a positive DD result has a low capability of establishing the diagnosis of deep vein thrombosis (DVT) or pulmonary embolism (PE). Instead, the value of a DD test is with a negative result, which works to lower the likelihood of the diagnosis. Therefore, with its high sensitivity and negative predictive value, DD testing has gained a role in the diagnostic work-up of VTE for the exclusion of the disease, potentially reducing the need for imaging tests.[6]

Table 1: Conditions Associated with Increased D-dimer Plasma Levels

Non-pathological	Pathological
Age	Trauma
Race (black population)	Pre-eclampsia
Cigarette smoking	Malignancy
Pregnancy and puerperium	Infection
Post-operatively	Chronic inflammatory diseases
	Disseminated intravascular coagulation
	Sickle cell disease
	Arterial or venous thromboembolism
	Acute coronary syndromes
	Stroke
	Peripheral artery disease
	Atrial fibrillation
	Congestive heart failure
	Haemorrhages

Table 2: Features of Available D-dimer Assays

Type of D-dimer Assay	Characteristics
ELISA Assays	
Classic microplate ELISA	High sensitivity, low specificity, observer-independent, not suitable for realtime single testing
Rapid ELISA assays	High sensitivity, low specificity, observer-independent, suitable for realtime single testing
Agglutination Assays	
Semi-quantitative assays	Intermediate sensitivity and specificity, rapid, observer-dependent
Quantitative assays (immunoturbidimetric assays)	High sensitivity, intermediate specificity, rapid, observer-independent
Whole-blood assays	
	High to intermediate sensitivity, intermediate specificity, rapid bedside execution, observer-dependent

ELISA = enzyme-linked immunosorbent assay.

Various integrated strategies for VTE diagnosis have been proposed, in which DD testing has different places in the diagnostic sequence:

- Initial DD testing for the exclusion of VTE with additional imaging tests in patients only with a positive DD result. This strategy was adopted in a large, prospective management study of over 900 consecutive outpatients with suspected DVT or PE referred to the emergency department of the Geneva Hospital.[7]
- DD testing after a first negative imaging test to identify patients who require a new specific evaluation. In this diagnostic strategy for suspected DVT, a positive DD result allows the selection of patients who require a new ultrasonographic evaluation one week after a first negative one to detect extending calf DVT, which may have altered a DD test without being detectable on first ultrasound.[8] A similar use of DD testing has proved to be useful in patients with suspected PE after a first negative lung scan.[9]
- DD testing integrated with pre-test clinical probability (PCP) and subsequent specific tests. Several studies have demonstrated that a negative DD result combined with a low PCP of disease can safely exclude DVT or PE without additional diagnostic testing.[10–14] Recently, a systematic review of 11 management studies using a combination of PCP and DD test results to rule out VTE[15] showed an overall rate of thromboembolic events of 0.45% (95% confidence interval [CI] 0.22–0.83%) among patients in whom anticoagulant treatment was withheld on the basis of a low clinical score and a negative DD result, thus demonstrating the safety of this diagnostic work-up for the exclusion of both DVT and PE. No significant difference in safety was observed with qualitative and quantitative DD tests and with different decision rules. This diagnostic strategy, requiring further specific tests in patients only with positive DD and/or intermediate or high PCP, has also been reported to be the most advantageous in a cost-effectiveness analysis.[16]

The use of DD testing for VTE diagnosis requires the identification of a cut-off level allowing clinicians to exclude the disease. This cut-off value is the point within the measuring range that confers the best sensitivity and specificity to a particular assay, and may not coincide with the upper limit of the reference interval, which is calculated in a healthy population. When choosing a DD assay to be used in the diagnostic work-up of DVT or PE, its sensitivity and cut-off level should be carefully evaluated in order to avoid false-negative results.[17] Specificity is also important because it influences the yield of the test, determining the proportion of false-positive results. The choice of the appropriate method for DD measurement also depends on the place of the test in the diagnostic sequence of VTE. If used as the first step with no additional tests in the event of a negative result, the method of choice should have a sensitivity as close as possible to 100% in order to minimise the proportion of false-negative results. In contrast, if a DD test is used in association with PCP assessment or imaging techniques, a less sensitive test may be accepted.

The performance of several DD tests for either DVT or PE diagnosis was evaluated in a recent meta-analysis by Di Nisio et al.[18] The sensitivities of the enzyme-linked immunofluorescence assay (ELFA) (DVT 96%, PE 97%), microplate ELISA (DVT 94%, PE 95%) and latex quantitative assays (DVT 93%, PE 95%) were superior to those of the whole-blood DD assays (DVT 83%, PE 87%), latex semi-quantitative assays (DVT 85%, PE 88%) and latex qualitative assays (DVT 69%, PE 75%). On the other hand, the latex qualitative and whole-blood DD assays showed the highest specifities (99 and 71% for DVT and 99% and 69% for PE, respectively, compared with approximately 50% for the highly sensitive assays). In a previous meta-analysis by Stein et al.,[19] the sensitivity and negative predictive value of the ELISAs (in particular the quantitative rapid ELISA) were superior to those of other DD tests, including latex quantitative assays.

The usefulness of DD testing in the diagnostic work-up of DVT or PE is affected not only by the choice of the appropriate assay, but also by patient characteristics and clinical context. Extent of VTE, duration of symptoms, anticoagulant treatments, age and co-morbid conditions represent relevant sources of variation in DD tests affecting their sensitivity and specificity. DD levels are related to thrombus extension, being higher in the presence of larger thrombi. This may explain why DD sensitivity has been reported to be lower in distal DVT[20] or subsegmental PE.[21] There is an inverse relationship between DD plasma levels and duration of symptoms. DD concentration tends to decrease when a patient has presented symptoms for several days before testing, reaching 25% of the initial value after one to two weeks.[22] Anticoagulant therapy (with both heparin and vitamin K antagonists) also determines a decrease in DD concentration, which has been estimated at around 25% 24 hours after starting anticoagulation, with a consequent decrease in sensitivity from 95.5% (95% CI 90–99%) to 89.4% (95% CI 84–95%).[23]

Therefore, a DD result below the diagnostic cut-off obtained after starting anticoagulation should be interpreted with caution in order to avoid false-negative results. In several clinical conditions associated with increased DD levels, the specificity of DD testing for VTE diagnosis may be greatly diminished due to a higher number of false-positive results. This is the reason why a reduced diagnostic usefulness of DD testing for VTE exclusion has been reported in surgical and non-surgical inpatients,[24] inflammatory states,[25] pregnancy and post-partum,[26,27] elderly patients,[28] cancer patients[29] and those with previous VTE.[30] Therefore, efforts should be made to avoid inappropriate DD testing, limiting its use to clinical settings in which DD-based management of suspected VTE has been proved to be efficacious and safe.

D-dimer Testing to Predict Venous Thromboembolism Recurrence After Anticoagulation Withdrawal

The optimal duration of anticoagulation after a first episode of unprovoked VTE is still debated.[31] At least six months of anticoagulation have been recommended because of presumed higher rates of recurrence with shorter durations of treatment.[32,33] However, recent randomised trials have indicated that the duration of anticoagulation seems to have little effect on the rate of disease recurrence in patients with unprovoked VTE, and longer treatment only delays recurrence until after anticoagulation is stopped.[34,35] Therefore, the major clinical decision is whether to stop or to continue anticoagulation, because stopping anticoagulant therapy may place some patients at risk of morbidity and mortality due to recurrent VTE, whereas continuing anticoagulation exposes patients to the increased risk of bleeding.

Following the observation that DD plasma levels tend to increase in some patients with previous VTE after oral anticoagulation withdrawal,[36–38] prospective studies initially showed that DD levels have a strong predictive value for the occurrence of recurrent VTE episodes in these patients.[39–41] These studies suggested that a simple and easy-to-implement strategy such as DD measurement after anticoagulation withdrawal could help with the decision of whether to continue or stop anticoagulant treatment. Indeed, a normal DD level may identify patients at low risk of recurrent VTE, in whom anticoagulation therapy may be discontinued, whereas an abnormal DD level may identify patients with a persistent pro-thrombotic tendency who warrant long-term anticoagulation, because they are at relatively high risk of recurrent VTE.

In a prospective, randomised, multicentre trial, the PROLONG study,[42] DD testing was performed one month after stopping anticoagulation in patients with a first unprovoked proximal DVT or PE who had received a vitamin K antagonist for at least three months. Patients with a normal DD level did not resume anticoagulation, whereas those with an abnormal DD level were randomly assigned to either resume or discontinue treatment. The results of this study showed that patients with abnormal DD levels one month after stopping anticoagulation had a higher rate of VTE recurrence at 18-month follow-up than those with normal DD levels, and benefited from resumption of anticoagulation. This finding was confirmed at an extended follow-up of 2.5 years.[43]

Moreover, in a *post hoc* analysis of the PROLONG study,[44] abnormal DD levels one month after anticoagulation withdrawal were found to be predictive of VTE recurrence also in the subgroup of patients with unprovoked PE. Recently, a systematic review by Verhovsek et al.[45] of seven studies that measured DD three to six weeks after stopping anticoagulation showed that in patients who have completed treatment for at least three months for a first episode of unprovoked VTE and after approximately two years of follow-up, the annual rates of recurrent VTE were significantly different between patients with positive and negative DD results (8.9 and 3.5%, respectively). Similarly, a more than two-fold odds of recurrence in patients with unprovoked VTE and elevated DD levels measured one month after anticoagulation withdrawal was found in another recently published meta-analysis.[46]

Despite the increasing evidence of the usefulness of DD testing after anticoagulation withdrawal for discriminating between patients at higher and lower risk of recurrent VTE, some matters still need to be addressed. First, the lack of a worldwide standard for DD testing makes it difficult to compare results obtained with the different available assays. Therefore, each method should be evaluated separately in rigorous management studies to validate their use in clinical practice for assessing the risk of VTE recurrence. Second, the DD cut-off level that best predicts recurrent VTE is not known, especially in patient subgroups in whom baseline DD levels are increased, such as elderly persons.

D-dimer testing is a promising method to identify patients at higher risk of recurrent venous thromboembolism, and could therefore help with the decision of whether to continue or stop anticoagulant treatment.

In the PROLONG study,[42] the qualitative, whole-blood Clearview Simplify DD assay (Inverness Medical Professional Diagnostics) was used. In a *post hoc* analysis of the study,[47] Legnani et al. retrospectively evaluated the performance of four quantitative DD methods to predict the risk of VTE recurrence using plasma samples of a subgroup of patients enrolled in the PROLONG study. This analysis showed that quantitative DD assays may provide useful information for evaluating the individual risk of recurrence, and seem particularly advantageous as they allow selection of different cut-off levels according to age or other characteristics of patients. Further prospective studies are needed to validate these cut-offs.

Another matter to be considered is the timing of DD measurement after oral anticoagulation withdrawal. In the PROLONG study,[42] the DD assay was performed only once, but during follow-up some patients with normal DD levels could have had abnormal results on repeated testing. Therefore, repeated DD assays in patients with an initial normal test may be useful in detecting late hypercoagulability. This matter has been addressed in the PROLONG II prospective cohort study, the results of which are not yet available.

Thus, DD testing is a promising method to identify patients at higher risk of recurrent VTE, and could therefore help with the decision of whether to continue or stop anticoagulant treatment. Other possible predictors of recurrent disease have been evaluated in patients with unprovoked VTE. For patients with DVT, the presence of residual vein obstruction on ultrasonography may predict an increased risk of recurrence,[48] although

some studies did not confirm this observation.[49,50] Male sex is a consistent predictor of recurrent VTE,[51,52] whereas the presence of thrombophilic blood abnormalities does not seem to confer a clinically important increased risk of recurrent VTE.[53–55] Ideally, DD should be part of a clinical prediction rule that incorporates both clinical and laboratory features to better predict recurrent VTE in an individual patient.

Conclusions

In the setting of VTE, DD has gained an important place in the diagnostic work-up of suspected DVT or PE as a simple, non-invasive test that can be useful to rule out the diagnosis, especially in combination with PCP assessment, thus limiting the number of patients requiring further evaluation with imaging techniques. The specificity and clinical utility of DD testing to exclude VTE are reduced in specific patient populations and clinical settings, such as elderly patients, cancer patients, pregnant women and patients with previous VTE, although the test retains its high sensitivity in these situations. Moreover, a DD result below the diagnostic cut-off obtained after starting anticoagulation or in patients having symptoms for several days before testing should be carefully interpreted in order to avoid false-negative results.

There is increasing evidence that DD testing after anticoagulation withdrawal for a first unprovoked VTE episode may also be useful to discriminate between patients at higher and lower risk of recurrence, and may therefore help clinicians with the decision of whether to continue or to stop anticoagulant treatment. However, additional research is needed to establish the optimal interval between stopping anticoagulation and performing DD testing, to identify the optimal DD cut-off level that predicts recurrence and to develop a clinical prediction rule for recurrent VTE. ■

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