The antigen fibrin d-dimer (DD) is the primary enzymatic degradation product of cross-linked fibrin by plasmin. Systemic values of DD are an index of fibrin turnover in the circulation and a single measurement may be adequate to assess the fibrinolytic status. Systemic DD values are raised in a variety of clinical conditions and inclusion of DD testing may provide cost effective diagnostic strategies. In addition to the diagnostic use of DD, it may also be of potential prognostic use in many conditions. Currently, despite the implementation of clinical guidelines, inappropriate DD testing is a significant problem. It is, consequently, valuable for emergency physicians to be knowledgeable about the pathophysiological basis and limitations of DD testing to ensure its appropriate clinical use.

The place of DD measurement in the diagnostic investigation of suspected venous thromboembolism (VTE) is now well established. This review updates experience on DD testing for VTE and discusses potential areas for the extension of indications for DD testing in emergency medicine.

PATHOPHYSIOLOGY OF FIBRIN DD

Plasmin is the fibrinolytic enzyme derived from its inactive precursor, plasminogen, by the action of thrombin and plasminogen activators. The main plasminogen activators are tissue plasminogen activator (tPA) and pro-urokinase, which is activated into urokinase by, among others, the contact system of coagulation. Plasmin is neutralised by α2 antiplasmin thereby restricting its fibrinogenolytic activity and localising the fibrinolysis on the fibrin clot.

Fibrin is the main component of a thrombus. It is formed by the activation of the coagulation system. Its production is followed by activation of the fibrinolytic system, resulting in plasmin generation and subsequent fibrin lysis (Fig 1). Under normal physiological conditions there is a balance of the two opposing processes. Dissolution of crosslinked fibrin (XL-Fg) leads to formation of specific degradation products, including DD, which can be measured in both whole blood and plasma using monoclonal antibodies directed against epitopes located in the DD fragment. The activity of DD is considered to reflect the overall activity of clot formation and lysis. Because DD is not artificially generated in vitro during blood collection, its measurement more consistently reflects in vivo haemostatic activity than do other assays for coagulation or fibrinolytic activities that may be activated in vitro. Its absence excludes the presence of intravascular thrombus.

**Abbreviations:** DD, d-dimer; VTE, venous thromboembolism; tPA, tissue plasminogen activator; XL-Fg, crosslinked fibrin; FDP, fibrin degradation product; EUSA, enzyme linked immunosorbent assay; HMW, high molecular weight; LMW, low molecular weight; FACT, Fibrin Assay Comparison Trial; CT, computed tomography; PE, pulmonary embolism; DVT, deep vein thrombosis; PTP, pretest probability; CHD, coronary heart disease; ACS, acute coronary syndrome; MI, myocardial infarction

**Figure 1** A d-dimer as a reactive marker of the haemostatic balance. Activation of the coagulation system leads to the formation of thrombin. This enzyme cleaves the amino-terminal fibrinopeptides A and B from fibrinogen. The resulting fibrin monomer molecule is then capable to polymerise into an insoluble fibrin network. The fibrin polymers are further stabilised by covalent crosslinks introduced by the action of the enzyme factor XIIIa. Plasmin, the active focal enzyme of the fibrinolytic system, is responsible for the lysis of the crosslinked fibrin clot. This results in the formation of soluble crosslinked FDPs containing a myriad of DD epitopes. (Adapted with permission from Houdijk WPM. Clinical applications of D-dimer. Dev Thromb Hemost 1999;3:1-15.)
During fibrin formation, fibrinogen is converted into fibrin by enzymatic (thrombin) cleavage of the fibrinopeptides A and B (fig 1). This is followed by aggregation of the resulting fibrin monomers. Linkage of C terminal appendages of the γ chains by factor XIIIa results in dimerisation of D-domains of adjacent fibrin monomer units. Plasmin proteolysis of this “crosslinked” fibrin generates fragments DD and E as terminal plasmin digests of fibrinogen. The dimeric D-domain therefore may serve as indicator of in vivo fibrin formation. Monoclonal antibodies generated by immunisation with fragment DD react with XL-Fg in general, including fibrin oligomers of variable size. DD testing has limited specificity because many conditions are associated with fibrin formation (box 1). Local fibrin formation and lysis are part of the inflammatory response, and fibrin degradation products (FDPs), including DD, are terminal products. In contrast, proteolysis of fibrinogen or non-crosslinked fibrin (non-XL-Fg) results in formation of monomeric fragment D. The dimeric D-domain therefore may serve as indicator of in vivo fibrin formation. Monoclonal antibodies generated by immunisation with fragment DD react with XL-Fg in general, including fibrin oligomers of variable size. DD testing has limited specificity because many conditions are associated with fibrin formation (box 1). Local fibrin formation and lysis are part of the inflammatory response, and fibrin degradation products (FDPs), including DD, are terminal products. In contrast, proteolysis of fibrinogen or non-crosslinked fibrin (non-XL-Fg) results in formation of monomeric fragment D. The dimeric D-domain therefore may serve as indicator of in vivo fibrin formation. Monoclonal antibodies generated by immunisation with fragment DD react with XL-Fg in general, including fibrin oligomers of variable size. DD testing has limited specificity because many conditions are associated with fibrin formation (box 1). Local fibrin formation and lysis are part of the inflammatory response, and fibrin degradation products (FDPs), including DD, are terminal products. In contrast, proteolysis of fibrinogen or non-crosslinked fibrin (non-XL-Fg) results in formation of monomeric fragment D. The dimeric D-domain therefore may serve as indicator of in vivo fibrin formation. Monoclonal antibodies generated by immunisation with fragment DD react with XL-Fg in general, including fibrin oligomers of variable size. DD testing has limited specificity because many conditions are associated with fibrin formation (box 1). Local fibrin formation and lysis are part of the inflammatory response, and fibrin degradation products (FDPs), including DD, are terminal products. In contrast, proteolysis of fibrinogen or non-crosslinked fibrin (non-XL-Fg) results in formation of monomeric fragment D. The dimeric D-domain therefore may serve as indicator of in vivo fibrin formation. Monoclonal antibodies generated by immunisation with fragment DD react with XL-Fg in general, including fibrin oligomers of variable size. DD testing has limited specificity because many conditions are associated with fibrin formation (box 1). Local fibrin formation and lysis are part of the inflammatory response, and fibrin degradation products (FDPs), including DD, are terminal products. In contrast, proteolysis of fibrinogen or non-crosslinked fibrin (non-XL-Fg) results in formation of monomeric fragment D. The dimeric D-domain therefore may serve as indicator of in vivo fibrin formation. Monoclonal antibodies generated by immunisation with fragment DD react with XL-Fg in general, including fibrin oligomers of variable size.
a composition of fibrin derivatives similar to that observed in the target clinical plasma samples, immersed in a plasma matrix.

The lack of a DD reference standard can be overcome by conversion of DD values from different assays to a common scale by using a conversion factor related to the median values obtained with a sufficiently large set of clinical plasma samples. This issue has recently been investigated by the Fibrin Assay Comparison Trial (FACT) study. Briefly, the FACT study was performed to generate basic data for development of calibrators and standard preparations of DD. It is an attempt to propose a method of preparation of a universal set of DD calibrators that could potentially be used as reference materials. The difference observed in reactivity between different commercial kits occurred only with artificially created plasma samples and those of patients treated with streptokinase. True patient samples (DIC or deep venous thrombosis), as compared with standardised in vitro fibrin preparations, demonstrate a high degree of concordance by all the methods tested. This indicates that even if different DD assays respond to different epitopes, these epitopes are either present in parallel on the fibrin compounds detected, or fibrin compounds carrying the respective epitopes occur simultaneously in most clinical plasma samples. Differences concerning analyte reactivity will, therefore, become more evident only in samples containing an unusual composition of fibrin derivatives. Nevertheless, no definitive common scale for conversion of DD values from different assays has been formulated by the FACT study; so far. Meanwhile, this study suffers from two important limitations. Firstly, although a number of assays tested used the same antibodies, no attempt was made to classify the data according to the antibodies used. This automatically introduced bias to the results. Secondly, the study was based on a limited number of samples and the materials used for this testing were artificially modified plasma/serum samples created using a very specific procedure with degradation products not existing in vivo. Changing this procedure may significantly alter the results.

**DD assay cut off points**

Because different DD assays recognise more or less different components in plasma or in blood and because various techniques are used, heterogeneity of results is somewhat inevitable. Thus, rather than attempts at standardisation, some investigators suggest efforts should focus on determining a critical cut off for each individual assay, based on the test performances established in clinical studies. Cut off points are critical when using DD assays because they determine the sensitivity and specificity. If the DD cut off is set too low, then the test is too sensitive and not specific, so almost everyone ends up being positive and the test loses meaning. Laboratories can accept meaningful, published cut off points, but should not extrapolate a cut off point from the manufacturer of another assay. Furthermore, to ensure good applicability, when choosing a DD assay it should be verified that the assay has been studied in a patient population similar to that in which it would be used.

**CLINICAL APPLICATIONS OF DD TESTING**

**Pulmonary embolism (PE)**

Despite the existence over the past decade of a decision rule for the evaluation of PE, its diagnostic evaluation, more than any other acute clinical condition, continues to pose a dilemma for emergency physicians. A perfusion-ventilation (V/Q) lung scan followed by pulmonary angiography in conjunction with a low probability lung scan is generally considered to be the standard diagnostic strategy. Although a negative qualitative DD assay does not have the required sensitivity to rule out PE in the ED setting, DD when measured with a quantitative assay, is nearly always increased in acute PE (sensitivity 85%–99%), and a low value virtually rules it out. Furthermore, despite a low specificity (45%–68%), diagnostic strategies incorporating DD testing are cost effective.

Various strategies incorporating DD testing for emergency evaluation of PE have been proposed. These include using a rapid plasma ELISA DD as an initial screening test, using whole blood agglutination DD in combination with spiral computed tomography (CT) and using whole blood agglutination DD in combination with pretest clinical probability (pretest probability; PTP). Nevertheless, there is no universal consensus on the role of DD testing in the emergency evaluation of PE.

A negative ELISA DD result as a single initial screening test, while simultaneously ignoring the PTP, safely eliminates a third of patients with suspected PE from further more expensive and invasive diagnostic tests. None the less, caution should be exercised with this approach, particularly in patients with a high PTP until further confirmatory data are available. A more conservative approach would be to combine the results of the DD assay with PTP and other non-invasive diagnostic tests (duplex ultrasonography of lower limb veins and V/Q scanning). Diagnosis of lower limb deep venous thrombosis (DVT) as an alternative to proving the presence of PE, among patients with suspected PE and inconclusive lung scans, is now a valid concept. A combination of DD measurement and lower limb venous ultrasonography can safely eliminate one third of patients with suspected PE.

Although V/Q scanning is still widely used in many institutions as the definitive test for detecting PE, it is a poor test, providing a definitive clinical answer in only 27% of cases. In a prospective study, patients with suspected PE were randomised either to spiral CT or to V/Q scan as the initial screening test. Although there was no difference in the detection rate of emboli between the two groups, a confident diagnosis was possible with more patients in the CT group than in the group that underwent V/Q scanning. Furthermore, greater out of hours availability makes spiral CT a better primary screening test, than V/Q scanning, for ED patients with suspected PE. Meanwhile, incorporating DD testing (whole blood agglutination assay) is cheaper and further increases clinical efficiency, by reducing the number of spiral CT examinations in patients with suspected PE by 50%.

DD testing (whole blood agglutination assay) in combination with a low PTP obviates the need for further investigation in 47% of ED patients with suspected PE. More importantly, outcome studies have demonstrated that it is safe to withhold further investigation and treatment in patients with a negative DD test, intermediate or low clinical suspicion for PE and a non-diagnostic (intermediate or low probability) V/Q scan.

**Proximal acute lower limb DVT**

Duplex scanning, the present method of choice for the diagnosis of DVT, is comparatively time consuming and expensive. In addition, the majority of imaging studies for patients with suspected DVT, up to 98% in one study, are negative. A highly sensitive and simple test used for initial screening and ruling out DVT in a substantial proportion of subjects, might be quicker and cost effective. The more specific ultrasonographic test would then be applied only in patients presenting with a DD concentration above the appropriate cut off point. Although the precise role of DD tests in assessing suspected cases of DVT has yet to be established, it potentially meets these criteria.

Qualitative whole blood agglutination assays cannot be relied upon to exclude thrombosis in the initial assessment of suspected DVT. In one study, the diagnosis of DVT would have been missed in 20% of ED patients if only a whole blood agglutination DD assay was used. In contrast, quantitative
assays can lead to a significant (30%–50%) reduction in requests for imaging studies. Meanwhile, a clinical scoring system can be used for risk stratification in DVT patients. This can be used in conjunction with ultrasound scanning and DD measurement in the work up of suspected DVT. If the clinical risk of DVT is low, the absence of thrombosis is supported by a negative quantitative whole blood DD test.

To date, the assays with the highest sensitivity in patients with suspected DVT are ELISA methods designed for batch analysis. These have a sensitivity of 97%–100%, and, more recently, an immunochromatographic assay (Simplify, Agen Biochemical) and, more recently, an immunochromatographic assay (Simplify, Agen Biochemical) These both give results within a few minutes and can be used at the bedside. However, the diagnostic performance of the SimpliRED test in suspected DVT is highly variable with a sensitivity, specificity and negative predictive value of 61%–100%, 78%–94%, and 52%–100%, respectively. Although SimpliRED is less sensitive than ELISA assays, it seems adequate to safely rule out DVT in ED patients with a low PTP. Moreover, it is more specific than the ELISA assays, obviating further investigation in 40% of patients with suspected DVT. Currently, there are limited published data on diagnostic performance of the Simplify DD assay besides that obtainable from the manufacturer. However, its interpretation is designed to be less susceptible to inter-observer variation than the SimpliRED assay. With visual interpretation of results very similar to that of a commercially available pregnancy test. In addition, it detects DD in both whole blood and plasma. In comparison with these assays based on visual interpretation of results, the more sensitive quantitative turbidimetric DD immunoassay used in conjunction with PTP is more reliable. The turbidimetric immunoassay takes about 20 minutes longer to perform than a whole blood assay but is not dependent on subjective interpretation. Furthermore, the turbidimetric immunoassay is more sensitive (94.1% compared with 66%) than the whole blood test and is 100% sensitive for proximal acute lower limb DVT.

Calf vein acute DVT
Although the clinical significance of calf vein thrombosis is controversial, many patients present to the ED specifically to clear the differential diagnosis of calf pain and rule out a DVT. Meanwhile, the risk of pulmonary embolism from isolated calf vein thrombosis seems negligible; however, a recurrence rate of 29% and propagation rate into more proximal veins of up to 28% have been reported. It is logical to hypothesise that patients with extending calf DVT have thrombi that are more active biochemically, more likely to produce increased plasma DD values, and therefore, more likely to show an abnormal DD assay result.

In calf DVT, the qualitative whole blood agglutination DD assay has a moderate sensitivity (70%), moderate specificity (77%), a false negative rate of 30%, and NPV of only 85.7%. Meanwhile, a negative quantitative ELISA DD test with a negative compression sonogram of the popliteal and common femoral veins predicts the absence of a major thromboembolic event in the next three months in 97% of patients referred with suspicion of DVT. However, because earlier studies have shown a substantial rate of progression of untreated calf DVT to PE, it is, therefore, important to confirm every reasonable suspicion of thrombotic calf pain quickly and objectively with the aid of a quantitative DD assay.

Recurrent VTE
It may be very difficult to distinguish recurrent VTE from the sequelae of a previous event. DD estimation is potentially useful for this purpose because DD levels return to normal values within three months after an acute lower limb DVT. Thus, in a patient suspected of a recurrent VTE event, a low DD value in conjunction with clinical assessment and appropriate investigation could be used to rule out recurrence.

Coronary ischaemia
DD is positively associated with coronary heart disease (CHD) incidence or recurrence. This association is largely independent of classic risk factors such as smoking and hypercholesterolaemia. CHD risk is about 70% greater in those in the top third of DD values compared with those in the bottom third.

Meanwhile, there remains a need for additional markers in the early stages of coronary thrombosis, a hallmark of acute ischaemic syndromes, because a persistent 2% to 4% of patients discharged from the ED may have a myocardial infarction (MI) within 24–48 hours with current diagnostic tools. Bayes-Genis et al have recently demonstrated that plasma DD values are significantly higher in patients with acute ischaemic events (MI and unstable angina) than in non-ischaemic patients. Moreover, plasma DD values for MI are significantly higher than for unstable angina. DD concentration >500 µg/l has an independent diagnostic value for MI and increases the diagnostic sensitivity of the electrocardiogram and history from 73% to 92%.

Progression of coronary thrombosis may be an important determinant of prognosis in patients with acute coronary syndromes (ACS), judging from the decrease in recurrent ischaemia, infarction and death associated with antithrombotic and antifibrinolytic therapy. Identification of patients at risk of progression of coronary thrombosis has only been possible by coronary angiography, with no clinically applicable laboratory assays to characterise the balance between thrombosis and fibrinolysis. Angiography is an insensitive method of detection of coronary thrombosis when compared with direct thrombus visualisation by angioscopy, an equally invasive procedure. DD assays may permit rapid bedside risk stratification of patients with ACS who are at higher risk of thrombotic complications.

Plasma DD value is a potentially useful marker of CHD risk, an addition to clinical diagnostic models for MI detection in ED patients with acute cardiogenic chest pain and may identify ACS patients who may benefit from more aggressive antithrombotic therapy. Currently the use of DD testing in ACS is limited by the inherent lack of specificity of most assays for the detection of coronary thrombosis. This limitation may at least be partially obviated using diagnostic strategies incorporating other more established markers of myocardial damage such as cardiac specific troponins.

DD AS AN ED PROGNOSTIC MARKER
As clinical decision units and more prolonged periods of treatment become more common in the ED, there is need for rapid bedside adjunctive diagnostic aids in risk stratification of the ED patient population. In addition to ACS, DD is potentially useful as a prognostic marker in conditions such as acute bowel ischaemia, acute upper gastrointestinal haemorrhage, intracranial haemorrhage, cerebral infarction, atrial fibrillation, and bacteraemia.

Increased plasma DD is associated with adverse clinical outcome in acute upper gastrointestinal haemorrhage and intracranial haemorrhage. DD values may be an indicator for emergency laparotomy in acute bowel ischaemia and endoscopy in upper gastrointestinal haemorrhage. In cerebral infarction, DD values may indicate the aetiology. Cardioembolic (diagnosed in the presence of a
detectable cardiac embolic source without large vessel disease) and atherothrombotic (diagnosed in the presence of large vessel disease without a cardiac embolic source) strokes are associated with significantly increased DD values. In contrast, in lacunar stroke (small subcortical infarcts without a cardiac source or large vessel disease) DD values are not significantly changed.

Clinical applicability of current data regarding the prognostic role of DD suffers from several limitations including studies that are retrospective, have small sample sizes, and use qualitative rather than quantitative DD assays. In addition, some studies use the DVT cut off value for diagnosis. The DVT cut off value may not be scientifically valid in the diagnosis of other clinical conditions. Larger prospective studies, using quantitative DD assays with appropriate cut off points, in the ED setting are required before DD values can be routinely used for risk stratification of patients in the ED setting.

CONCLUSION

Measurement of systemic DD, an index of ongoing thrombus formation and lysis, can aid clinical diagnosis in venous thromboembolic conditions and acute coronary syndromes. In addition, it may potentially permit risk stratification in the ED of patients with calf DD, acute upper gastrointestinal haemorrhage, intracranial haemorrhage, and bacteremia, thereby permitting a targeted approach to further investigation and treatment.

DD has limited specificity in hospitalised patients where comorbidity is common. In ED patients, there is mounting evidence that in the absence of an increase in DD, specifically in patients with low PT2, venous thromboembolism is rare, particularly if other non-invasive tests are negative or equivocal.

Although ELISA DD assay is the gold standard, it is too slow and cumbersome for use in emergency medicine. Meanwhile, the diagnostic performance of the less cumbersome and comparatively quick immunoturbidimetric assays compare favourably with ELISA. Turbidimetric immunoassays are therefore currently the best DD assay for use in emergency medicine. Attempts to standardise the various DD assays is ongoing with no definitive answers yet. In the meantime, DD assays should be used in conjunction with clinical assessment. Cut off points and the patient population used in preparing the assay should also be taken into consideration upon interpretation of assay results.

Before definitive incorporation of DD into diagnostic clinical models in emergency medicine, more studies using quantitative DD assays are required to further refine current knowledge, which is largely based on qualitative assays.

ACKNOWLEDGEMENTS

The authors thank Geraldine Healy. Haematology Laboratory, Beaumont Hospital, for reading the manuscript and providing some useful references.

Contributors

Abel Wakai and Aidan Gleeson initiated the review. Abel Wakai, Aidan Gleeson and Desmond Winter produced the final manuscript. Abel Wakai acts as the guarantor for the paper.

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D-dimer in emergency medicine


