The place of the determination of D-dimer and its improvement

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D–dimer (DD) assay is a widely used laboratory test in thrombosis-related conditions because it is rapid and easy to perform. This test is highly sensitive to thrombus formation and degradation. However DD levels are increased in many clinical conditions so that its positive predictive value is poor. Improvements of its usefulness have been mainly realised by combining the test with clinical scores and by adapting positive threshold to particular settings of patients. In this article, different methods of DD testing are presented with the aim to review their benefits and pitfalls in various clinical applications.

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Introduction

D-dimer (DD) is a specific product of fibrin degradation. It is formed at the end of the coagulation cascade when cross-linked fibrin is broken by plasmin. A variety of different qualitative and quantitative assays are available for DD testing and are all based on the use of monoclonal antibodies. DD assays are performed by using different techniques: latex particle agglutination either performed manually (semi-quantitative method) or in automated assays using turbidimetry on a coagulometer, and enzyme linked inmmunosorbent assay (ELISA). ELISA and automated latex immunoassays are quantitative assays with a similar sensitivity.[1] DD assays are not standardised so that references ranges and clinical cut-off are not comparable from one assay to the other. Performances of different tests are also widely variable.[1] Most common clinical applications of DD determination are: the diagnosis of venous thromboembolism (VTE) and pulmorary embolism (PE) and the identification of patients at risk of VTE or PE recurrence. It is also a potential tool to determine the optimal duration of anticoagulation treatment and the diagnosis and monitoring of disseminated intravascular coagulation (DIC).

Physiology of D-dimer generation

Figure 1 summarises the step wise process of formation of fibrin and its degradation to generate DD fragments and other products of fibrin degradation.

VTE and PE diagnosis

Bounameaux et al. were the first to suggest a potential usefulness of DD assay to exclude PE.[3] Nowadays it is not recommended to use DD as a standalone test but to request it in combination with a pretest clinical probability (PCP) to exclude VTE or PE.[4] This obviates the need for imaging in a significant number of patients. The most used PCP is the "Well's scoring system".[5] In a recent review over D-Dimer testing, this score was adapted by Tripodi, so that it can stratify patients as having a low, moderate or high probability of DVT or PE.[6] Quantification of DD by Elisa or automated turbidimetric assays has been shown to be highly sensitive in acute DVT or PE (sensitivity>98%).[3] The cut-off value or DD

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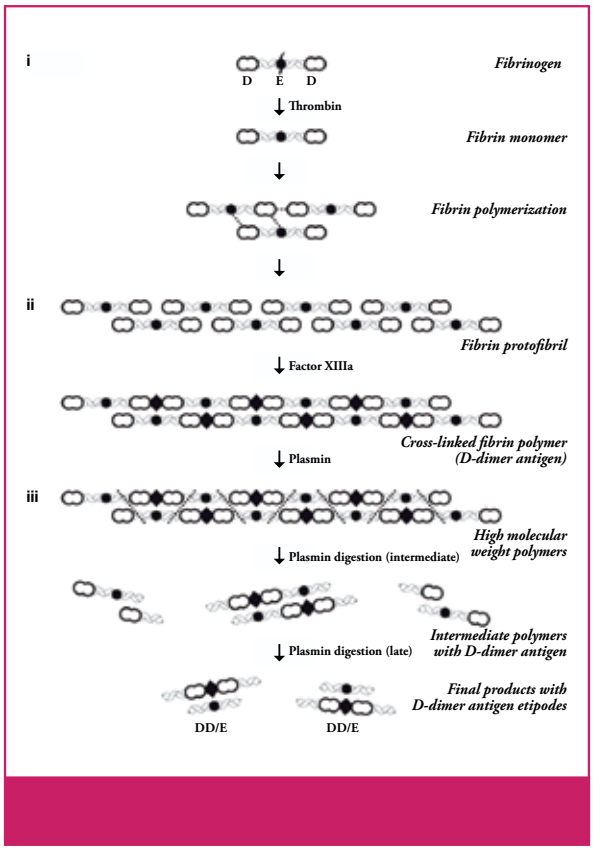

Figure 1. The stepwise process of Fibrin polymerization. The three major steps of D-dimer antigen formation are shown. **(i)** The fibrinogen molecule is cleaved by thrombin to produce fibrin monomers. These monomers associate with fibrinogen or fibrin to form protofibrils. They are held together by noncovalent forces shown as dotted lines between the intermolecular D-domain and D-E domains. **(ii)** Factor XIIIa formed by thrombin on fibrin polymers then covalently attaches D domains and inserts a covalent intermolecular linkage designated by the diamond-shaped figure. **(iii)** Plasmin must degrade fibrin at multiple sites to release fibrin degradation products, which then expose the D-dimer antigen epitope. The initial fragments are high-molecular-weight complexes followed by further degradation to produce the terminal D-dimer-E complex, which contains the dimer antigen. The 3 phases of this process are labeled on the right side of the diagram, and the different molecular forms of fibrinogen and its subsequent transformation by thrombin, factor XIIIa, and plasmin are shown on the left side of the diagram. This is a schematic representation of just one protofibril. Multiple protofibrils are aligned side by side and undergo branching to make a fibrin gel (Image and legend from Adam S et al, with permission).[2]

threshold is established in patients with suspected VTE in clinical studies. It is important to note that this cut-off could vary from one assay to the other. A DD level below this cut-off rules out patients with a low or intermediate clinical probability.[4,7] Whole-blood agglutination assays have a lower sensitivity (85%) and can only rule out DVT or PE in patients with a low clinical probability.[8] Sensitive DD measurement in the presence of a high clinical probability has a lower negative predictive value, and imaging is first recommended in this situation. If imaging is negative, ACCP guidelines recommends additional testing with a highly sensitive DD assay.[7]

The main pitfall of DD testing in VTE diagnosis is its low specificity, limiting its use in different settings as hospitalised patients, inflammation, cancer, infection, pregnancy, trauma, necrosis, ageing, recent surgery. As a consequence, positive DD level above the defined cut-off cannot safely rule in the diagnosis of VTE or PE and may reflect other causes. Only negative values can rule out VTE/PE. To further improve the specificity of the test without decreasing its high negative predictive value, some authors attempted to adjust the DD cut-off based on the pretest probability in PE or to adapt it in particular clinical settings as ageing or cancer.[9,10,11]

Key messages for clinical practice

- **DD testing should always be performed and interpreted in combination with a pretest clinical probability in outpatients suspected of VTE and/or PE.**
- **DD can remain below the cut-off level in patients with delayed diagnosed thrombosis.**
- **DD testing should be interpreted in the particular clinical conditions. For example, aged, cancer and hospitalized patients may have falsely positive DD level, unless the cutoff has been adapted and validated.**
- **DD cut-off is assay dependant and should not be extrapolated from one study to another or to various clinical settings if the assays in use in practice are different.**

Cancer patients are at high risk of VTE and PE. False positive DD levels are common in cancer patients, reducing the specificity and negative predictive value. Low PCP is uncommon in cancer patients with more patients submitted to imaging.[10] On the other hand, Rege et al. suggested that a low positive DD score in patients with DVT is a strong negative predictor for malignancy.[12]

VTE recurrence

Some patients with a first episode of VTE are at increased risk for recurrence. Those patients are eligible to resume their anticoagulation treatment after the initial recommended duration. Identification of such high-risk patients is still not well established, except for cancer or antiphospholipid syndrome. A specific biomarker able to exclude recurrence is potentially highly helpful in the decision to withdraw the treatment or not. High DD level at time of anticoagulation discontinuation seems to be predictive of recurrence as first shown by Palareti et al.[13] In a study performed in 2004, Rathbun found a lower incidence of VTE relapse after 3 months anticoagulation in the group with a negative DD (0.75%).[14]

They concluded that DD level below cutoff was useful to exclude recurrence. According to this study, it seems to be safe to discontinue anticoagulation in patients with a stable or slightly increased vein diameter and negative DD. For patients with positive DD level further evaluation is needed.[15] A review of 7 studies showed that the annual risk of recurrence in patients with unprovoked DVT was 3.5% for patients with DD level below cut-off level and 8.9% in patients above cut-off.[16] In another study, it was shown that normal DD level combined with the absence of any residual thrombosis after treatment discontinuation were associated with a lower risk of recurrent VTE events.[17] A step further was the use of DD level to determine the optimal duration of anticoagulation by Palareti et al.[18] Theses authors measured the DD level 1 month after stopping anticoagulation in patients with unprovoked VTE after 3 months of oral anticoagulation. Anticoagulation was stopped in patients with DD below the cut-off. Patients above the cut-off were randomized either to stop or to resume anticoagulation. After 1.5 years of follow-up, the group who did not resume anticoagulation had a significantly higher level of VTE recurrence.

Key message

- **Patients with DD level above cut-off one month after stopping anticoagulation, are at higher risk of recurrence and benefit for extended prophylaxis.**

DIC

Following the DIC Scientific and Standardization Committee in the International Society of Thrombosis and Haemostasis (ISTH) "DIC is an acquired syndrome characterized by the intravascular activation of coagulation with loss of localization arising from different causes.[19] It can originate from and cause damage to the microvasculature, which if sufficiently severe, can produce organ dysfunction". A variety of clinical conditions can cause DIC. Among them, sepsis is the most frequent one. Complications of pregnancy and malignancy are other common causes. DIC is always secondary and must be diagnosed on both clinical presentation and laboratory findings.[20] A scoring system based on laboratory tests has been proposed by the DIC Scientific and Standardization Committee of the ISTH.[19] This scoring system allows the distinction between overt DIC and non-overt-DIC, and can only be applied in clinical conditions known to be associated with DIC. Among the laboratory tests used in this scoring system, elevated fibrin-related markers mentioned are not specifically DD, but are either direct degradation products like soluble fibrin monomers (FM) either indirect assays such as fibrin degradation product (FDP) or DD. However, DD testing is the most widely used in clinical laboratories

Key messages

- **As other markers of fibrin formation and degradation products, DD should not be used alone but as part of a scoring system integrating both clinical condition and laboratory tests**
- **It is widely used in laboratory practice because it is rapid and easy to perform**
- **Earlier markers of fibrin-degradation product like FM are nevertheless earlier marker of coagulation activation.**

although soluble FM are more early markers of fibrin formation than DD or FDP's and thus more susceptible to be elevated in non-overt DIC. Indeed a recent study explored DD and FM in a DIC score for patients entering intensive care unit for septic shock. DD, FM and the DIC score were associated with a poor 90-day outcome.[21] Only significant gradual increase of the risk of death was observed with FM.

Conclusion

Available DD assays are highly heterogeneous with varying performances in terms of sensitivity and specificity rendering inter-laboratory comparison very difficult. Nevertheless DD assay is a sensitive marker of thrombus formation and is an important diagnostic tool in thrombosis-related clinical conditions. The use of highly sensitive DD assays like Elisa or automated turbidimetric assay has a better performance than semi-quantitative assays. The main improvement has been the combination of clinical scores with DD determinations in the diagnosis of thrombosis-related conditions. Attempts to adapt DD thresholds in particular clinical situations is a step further, with the caution that it cannot be extrapolated from one study to another.

Practically, current RIZIV/INAMI rules allow reimbursement of DD by semi-quantitative or quantitative test only in case of suspected VTE, PE, pregnancy complication or DIC.[22]

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