

»POINT-OF-CARE« D-DIMER TESTING**»POINT-OF-CARE« TESTIRANJE D-DIMERA**

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Summary: D-dimer testing is efficient in the exclusion of venous thromboembolism (VTE). D-dimer laboratory assays are predominantly performed in centralised laboratories in intra-hospital settings although most patients with suspected VTE are presented in primary care. On the other hand decreasing turnaround time for laboratory testing may significantly improve efficacy in emergency departments. Therefore an introduction of a rapid, easy to perform point of care (POC) assay for the identification of D-dimer may offer improvement in diagnostics flow of VTE both in primary care and emergency departments while it could also improve our diagnostic possibilities in some other severe clinical conditions (e.g. disseminated intravascular coagulation (DIC) and aortic aneurism (AA)) associated with increased D-dimer. Several POC D-dimer assays have been evaluated and majority of them have met the criteria for rapid and safe exclusion of VTE. In our hands three assays (Stratus, Pathfast and Cardiac) have the laboratory performance profile comparable with our routine D-dimer laboratory assay (Tinaquant).

Keywords: D-dimer, VTE, POC

Introduction

The key event in hemostasis is the formation of fibrin. Through a series of steps in which plasma zymogens of serine proteases are transformed into active enzymes, the coagulation system leads to the formation of the thrombin enzyme that catalyzes the

Kratak sadržaj: Određivanje D-dimera omogućava efikasno isključivanje venskog tromboembolizma (VTE). Laboratorijsko testiranje D-dimera pretežno se izvodi u centralizovanim laboratorijama u okviru bolnica, iako se većina pacijenata kod kojih se sumnja na VTE javlja na pregled u ustanove primarne zdravstvene zaštite. Takođe, skraćenje ukupnog trajanja laboratorijskog testiranja bi znatno poboljšalo efikasnost u urgentnim centrima. Stoga bi uvođenje brzog »Point of Care (POC)« D-dimer testa koji se lako izvodi, dovelo do poboljšanja dijagnostike VTE u primarnoj zdravstvenoj zaštiti kao i u urgentnim centrima, ali takođe i u dijagnostici drugih teških kliničkih stanja (diseminovana intravaskularna koagulacija (DIK), aneurizma aorte) u kojima je D-dimer povišen. Većina dostupnih POC D-dimer testova ispunjava kriterijume za brzo i sigurno isključivanje VTE-a. U našem ispitivanju tri testa (Stratus, Pathfast i Cardiac) pokazala su karakteristike slične D-dimer testu koji je u rutinskoj upotrebi u našoj laboratoriji (Tinaquant).

Ključne reči: D-dimer, VTE, POC

transformation of fibrinogen into fibrin. Fibrin, the final product of coagulation, is the main substrate for the fibrinolytic system, the role of which is to locate fibrin clots at the site of an injury and dissolve them (1).

During fibrin formation, fibrinogen is converted into fibrin by enzymatic (thrombin) cleavage of the fibrinopeptides A and B. This is followed by factor XIIIa induced aggregation of the resulting fibrin monomers producing »cross-linked fibrin«. Plasmin proteolysis of »cross-linked fibrin« generates DD and E fragments as terminal products. Proteolysis of fibrinogen or »non-cross-linked fibrin« produces fibrin(ogen) degradation products (FDP), but does not result in release of D-dimers. Therefore, although D-dimer is generated during fibrinolysis, it is an indicator of *in vitro* fibrin formation, rather than a pure fibrinolysis

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marker (2). It circulates in the blood several days after intravascular thrombus formation (the half-life is approximately 8 hours) (3) and is associated with conditions such as: deep venous thrombosis (DVT), pulmonary embolism (PE), disseminated intravascular coagulation, malignancy, postoperative states, trauma and preeclampsia (4). Measurement of D-dimer has been enabled after the development of monoclonal antibodies which distinguish it from fibrinogen degradation products (5).

The clinical utility of D-dimer measurement

The most important role of D-dimer is in the diagnostic approach to venous thromboembolism (VTE). VTE is a common cause of morbidity and mortality in the Western world with the annual incidence of about 1/1000 (6). Since radiological methods (e.g. venography) considered as the golden standard are not widely available and are both costly and invasive, the use of alternative diagnostic approaches, including D-dimer, has been widely evaluated. The increase of D-dimer does not enable the diagnosis of VTE, since it is not specific and could rise in different clinical conditions (e.g. ageing, trauma, pregnancy, malignancy) (2). However, the negative predictive value of D-dimer is high and a normal D-dimer may be used to rule out VTE (7).

The main diagnostic application for the D-dimer in critical care is the diagnosis and monitoring of disseminated intravascular coagulation (DIC) (8). DIC is a life-threatening syndrome associated with different underlying conditions (e.g. sepsis, malignancy, trauma). D-dimer may be used as a fibrin-related marker of the DIC score, which is a tool to establish a DIC diagnosis (9).

Another application is in the diagnosis or exclusion of aortic dissection. Patients with acute chest pain due to an acute coronary syndrome generally display D-dimer levels within or close to the normal range, whereas D-dimer levels are massively elevated in patients with acute aortic dissection (10, 11). As a general rule, patients with acute chest pain and massively elevated D-dimer levels should not receive anticoagulant and antiplatelet agents before aortic dissection has been excluded (8).

D-dimer laboratory assays

Enzyme-linked immunosorbent assays (ELISA) were initially developed for D-dimer detection for research purposes. They are extremely sensitive (98%) with the negative predictive value of >95% (12). However, ELISA assays are complicated, time consuming and labor intensive and could be performed in most laboratories only during daily working hours. Furthermore, most of them are not

designed for single sample testing, and until recently, were not easily automated for clinical use (13). Several technological advances in assay format and instrumentation made ELISA-based assays more convenient for routine use. Vidas ELISA is the most widely used among those assays. It has excellent sensitivity and is capable of detecting elevated D-dimer antigen associated with a variety of clinical disorders (14).

The automated quantitative turbidimetric assays based on latex agglutination were developed next and their sensitivity level is similar to that of ELISAs (15, 16). However those assays are still performed on large laboratory analysers in central and/or hospital based laboratories.

Near patient D-dimer testing

The importance of D-dimer for the diagnosis/exclusion of VTE results in the fact that the majority of requests for D-dimer testing come from patients expressing complaints which could be associated with VTE. Since this is a potentially life-threatening condition, primary care physicians usually refer all such patients to institutions where specialized diagnostic services for objective testing are available and where VTE could be safely and adequately ruled out. However, numerous studies have revealed that 80–90% of these referred patients do not have VTE (7, 17). Therefore, it would be ideal to safely exclude VTE on the level of primary care in a large proportion of these patients, avoiding referral, and consequently decreasing costs (18).

On the other hand emergency department overcrowding and prolonged patient stay are an increasing problem in most hospitals in the Western world. Some types of rapid testing, such as tests for electrolytes, seem to have little impact on emergency department operations, but others, such as cardiac markers, may reduce time in the emergency department and reduce unnecessary hospital admissions (19). Rapid testing of D-dimer could have a similar impact on emergency department operations.

Therefore, the introduction of near patient testing of D-dimer may improve diagnostic flow, reduce time from blood sampling to diagnosis and decrease the number of unnecessary hospitalisations. Additionally, it can also reduce total costs and have a significant impact on both primary care and emergency departments.

Point of care (POC) principles

The point of care (POC) assays have been defined as diagnostic tests performed outside of a laboratory, including different tests from ordinary urine dipsticks, along self-testing analysers for blood glucose and prothrombin time to more complex desktop

analysers (20). Technological advances in POC testing, primarily decreased size and reduction in user operator variables, have led to their increased utility. However, POC testing does not necessarily imply small analysers. This rather means that the laboratory instruments have been adapted; and this type of testing requires little or no sample preparation (e.g. could use whole blood), small series or single samples could be tested, operation is simple and is possible without knowledge of laboratory practice while turnaround time is rapid. The last point is enabled by virtual elimination of delay between sampling and laboratory analysis, while results become available to the clinicians immediately after finishing the analysing cycle.

The most important request for POC testing is to provide clinicians with measurements that are comparable to those obtained from a clinical laboratory (21). Since clinical laboratories have rigorous quality control programs, the potential main problem of point of care assays is inadequate quality control, since assays are most commonly performed by personnel without laboratory training and knowledge of quality control procedures. Assays commonly use whole blood, while such samples for quality control are not available. Therefore, external quality control is either not available or based on non-standard samples, different from the real sample material. Internal quality control is commonly present but it could not completely replace the external surveillance. Supervision of POC testing by the central laboratory, including split samples analysis, may therefore be helpful to identify potential problems (8).

D-dimer POC assays

A number of POC d-Dimer assays have been introduced recently and they are described by manufacturers as highly sensitive for VTE. To validate some of those assays, we at the Clinical Chemistry Laboratory at Karolinska University Hospital, Stockholm, Sweden, have compared some of the most commonly used POC D-dimer assays with our routine laboratory method Tinaquant D-dimer (Roche Diagnostics, Mannheim, Germany). Tinaquant D-dimer is a fully automated quantitative immunoturbidimetric assay that utilizes (micro)latex particles coated with antihuman D-dimer monoclonal antibody. The analysis is based on turbidimetric end-point measurement at 800 nm and is performed on the Sysmex CA 1500 analyser. This method has been shown to have sensitivity > 95% (up to 100% in some studies) and negative predictive values > 98% for the detection of VTE (15, 22, 23).

We have tested five assays: Pathfast D-dimer (Mitsubishi Chemical, Tokyo, Japan), Cardiac D-dimer (Roche Diagnostics, Mannheim, Germany), Vidas D-dimer (Biomerieux, Marcy l'Etoile, France), Stratus CS

D-dimer (Siemens Diagnostics, Marburg, Germany) and NycoCard D-dimer (Nycomed Pharma, Oslo, Norway) (24).

Pathfast D-dimer is a fully automated one-step sandwich immunoassay method performed on the Pathfast analyser which permits single tests, with ready-to-use reagents including magnetic particles covalently conjugated with a D-dimer monoclonal antibody and alkaline phosphatase-conjugated D-dimer monoclonal antibody. Whole blood used for testing is added into a sample well of a Pathfast cartridge. Obtained results are corrected optionally by input of the individual hematocrit. After the formation of a complex between D-dimer antigen contained in the blood sample and the reagents, bound/free separation is performed by the Magrator procedure, and the chemiluminescent signal is measured after the addition of a chemiluminescent substrate to the immune complex (25).

Using 0.5 µg/mL as the cut-off value, we observed a good correlation between Pathfast and Tinaquant ($r=0.70$), while four samples negative with Tinaquant were positive with Pathfast. This corresponds to previous findings, where Pathfast analytical characteristics were described as satisfactory, with sensitivity in diagnosis of DVT as high as 100% (25, 26).

Cardiac D-dimer is an immunological rapid assay based on two D-dimer specific antibodies, which are biotinylated and gold-labeled, respectively. After the application of 150 µL of heparinized whole blood, the antibodies are resolved by the sample and a sandwich complex with D-dimer is formed. Whereas the erythrocytes are adsorbed, the plasma with the antibody D-dimer complex flows to the detection zone. There, the complex is concentrated on the streptavidin signal line by the affinity between streptavidin and biotin. The accumulation of this complex can be detected and the final result can be read 10 min after the application of the blood sample using a portable Cardiac Reader instrument (Roche Diagnostics, Mannheim, Germany) (27).

In our evaluation four samples were discrepant between Tinaquant and Cardiac (cut-off 0.4 µg/mL) (two Tinaquant negative Cardiac positive and two Tinaquant positive Cardiac negative), while the correlation was excellent, $r=0.93$. Our results are also similar to those previously observed in different studies (27–30). Lower sensitivity (89%) and negative predictive value (87%) in the detection of DVT were, however, observed in one study (28), while others reported excellent negative predictive value and sensitivity up to 100%.

Stratus D-dimer is a chromatography-based sandwich ELISA (Radial Partition Immunoassay) performed with a dedicated device. D-dimer present in the sample is bound by a D-dimer monoclonal antibody on a fibreglass paper. The signal antibody, conjugated to alkaline phosphatase, is then added. A

fluorogenic substrate is hydrolysed by the alkaline phosphatase. When using anticoagulated whole blood, the sample is centrifuged by the analyser (4 min) and an aliquot of plasma is automatically pipetted into the cartridge. The device has to be calibrated every 60 days with dedicated cartridges (31).

One Tinaquant negative sample was detected to be positive on Stratus (cut-off 0.4 µg/mL) in our study, while the correlation with Tinaquant was excellent ($r=0.98$). Sensitivity and negative predictive value for DVT were described to be 95, 96 and 99% in three different studies (31–33), while some false negative results were reported predominantly in distal (below knee) DVT.

Vidas D-dimer is a quantitative fully automated ELISA assay with fluorescence detection performed on the Vidas immunoanalyzer. A solid phase receptacle is coated with an anti D-dimer monoclonal antibody and serves both as solid phase and pipetting device. The conjugate is an alkaline phosphatase labeled anti-D-dimer monoclonal antibody. After pipetting of 200 µL of plasma, all steps are managed by the Vidas analyzer. Results are available after 35 min (15). However, since plasma samples have to be used, this is not convenient for use in near patient testing since the majority of institutions do not have a possibility to centrifuge samples.

Vidas D-dimer assay has been present for a long time and due to its extreme sensitivity is often considered the golden standard (14, 15, 22, 34–36). Our results confirm this, since only one Tinaquant negative sample was detected to be positive on Vidas (cut-off 0.5 µg/mL) and the correlation with Tinaquant was excellent ($r=0.94$).

NycoCard D-dimer is based on the application of plasma on a laminated test card containing a thin, porous membrane to which a mouse monoclonal antibody is bound. A conjugate of the same monoclonal antibody coupled with 4 nm-diameter gold colloids is then added, which binds to the captured D-dimer molecules. The intensity of the red color produced by the gold colloids is read at 540 nm with a NycoCard Reader device, a dedicated reflectometer. The entire test procedure takes less than 4 min, but since plasma samples have to be used, the need for centrifugation dramatically increases the turnaround time and makes testing inappropriate for majority of institutions.

A very good profile of D-dimer was observed in the initial studies and evaluations (sensitivity and negative predictive value >95%) (15, 37). However, recent investigation was less convincing. In one study sensitivity in DVT diagnosis was described to be as low as 63% (38). Our results were also disappointing

and prevent us from recommending this assay for routine use. Five samples (out of 29) were negative with NycoCard, although they were positive with Tinaquant, while CV was 41%.

Conclusions

D-dimer is a clinically useful marker of coagulation activation and *in vivo* fibrin formation and may serve to exclude VTE, but to diagnose DIC and aortic aneurism. D-dimer assays are based on monoclonal antibodies against D-dimer epitopes. The automated quantitative turbidimetric assays based on latex agglutination have the same excellent sensitivity level as ELISA assays. However, permanent requests for improvement operations and decreasing cost both in primary care and emergency departments lead to the need for near patient D-dimer testing. It seems that several POC D-dimer assays have the analytical profile (primary sensitivity and negative predictive value) comparable to those obtained using standard laboratory assays. Our evaluation as well as data observed by others indicate that Pathfast D-dimer, Cardiac D-dimer and Stratus CS D-dimer may safely and adequately rule out VTE in out-patients. Nevertheless, clinicians need to be aware of the different performance characteristics of the available D-dimer assays, to make safe and timely therapeutic decisions.

Key messages to take home:

D-dimer is a unique marker of fibrin degradation.

D-dimer is increased in different clinical conditions and therefore its positive predictive value is low.

Sensitivity and negative predictive value of standard ELISA and automated quantitative turbidimetric assays are excellent and therefore negative D-dimer may be used for ruling out VTE.

POC D-dimer assays have a profile comparable to the standard laboratory methods and can therefore be used for near patient testing, improving turnaround time and decreasing costs.

The problem of quality control of POC D-dimer assays is yet to be completely solved.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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