Review

Widely Used Types and Clinical Applications of D-Dimer Assay

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ABSTRACT

D-dimers are formed by the breakdown of fibrinogen and fibrin during fibrinolysis. D-dimer analysis is critical for the diagnosis of deep vein thrombosis, pulmonary embolism, and disseminated intravascular coagulation. Modern assays for D-dimer are monoclonal antibody based. The enzyme-linked immunosorbent assay (ELISA) is the reference method for D-dimer analysis in the central clinical laboratory, but is time consuming to perform. Recently, a number of rapid, point-of-care D-dimer assays have been developed for acute care settings that utilize a variety of methodologies. In view of the diversity of D-dimer assays used in central laboratory and point-of-care settings, several caveats must be taken to assure the proper interpretation and clinical application of the results. These include consideration of preanalytical variables and interfering substances, as well as patient drug therapy and underlying disease. D-dimer assays should also be validated in clinical studies, have established cut-off values, and reported according to the reagent manufacturers recommendations.

Keywords: blood coagulation, venous thromboembolism, venous thrombosis, pulmonary embolism, D-dimer testing, fibrinogen degradation products, point-of-care testing, immunoassay, chemiluminescent immunoassay, disseminated intravascular coagulation, coronary artery disease, heart disease, cancer

The hemostatic system acts to coordinate the delicate balance between bleeding and clot formation. Formation of a blood clot, or thrombus, is essential to prevent bleeding in the event of vascular injury; however, inappropriate thrombus formation can cause significant morbidity and mortality. Arterial and venous systems can develop thrombi, which may cause local obstruction with associated ischemic symptoms but may also break off, or embolize, into the circulation and become lodged in distant vessels. This process is called *thromboembolization*. For example, thrombi that originate in the heart can embolize and become lodged in the small vessels of the brain, leading to a stroke or transient ischemic attack. Similarly, a thrombus that originates as a deep venous thrombosis (DVT) in the large veins of the lower extremities may embolize to the lungs, resulting in pulmonary embolism (PE) or infarction.

Thromboembolic disease, which includes DVTs, PEs, and their associated medical complications, affects more than 600,000 people in the United States each year and generates approximately $10 billion in medical expenditures.[1-3] In fact, PE is the third most common cause of death in the United States, after heart attacks and strokes.[4] The disease manifests a higher prevalence in certain populations, including patients with cancer who have cancer-associated thrombosis, as well as persons who have extended periods of immobility, including long-distance travelers and hospitalized patients.

Despite the significant morbidity and mortality associated with this disease, the coagulation assays that are currently available for diagnosis and prognosis of thromboembolic events are not yet ideal, although they have improved substantially in recent years.[5] Of these assays, the D-dimer is the most widely used and will be the focus of our review in this article.

Abbreviations

VTE, venous thromboembolism; DVT, deep venous thrombosis; PE, pulmonary embolism; DDU, D-dimer unit; FEU, fibrinogen-equivalent units; CV, coefficient of variation; FDPs, fibrinogen degradation products; TPA, tissue plasminogen activator; TAFI, thrombin-activatable fibrinolysis inhibitor; DIC, disseminated intravascular coagulation; TFR, time-resolved fluorometry; CPR, clinical prediction rule; CT, computed tomography; ISTH, International Society of Thrombosis and Haemostasis

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The Coagulation System

Under normal conditions, blood maintains its fluidity as it is propelled through the circulatory system; however, in the event of an injury, clotting must occur to reduce loss of blood from the intravascular space. *Hemostasis* refers to the series of complex physical processes that occur between the blood vessels, platelets, coagulation factors, and other elements that promote clotting and thereby prevent blood loss.

Efforts to prevent hemorrhage after vascular injury begin with constriction of the blood vessels to reduce the flow of blood to the affected area. Platelets aggregate at the affected site by binding the prothrombogenic subendothelial collagen fibers that invariably become exposed after vessel injury. Also, platelet clumping is bolstered further by the binding of plasma protein fibrinogen, which results in an initial platelet plug that temporarily prevents blood loss from the intravascular space. Clumped platelets undergo multiple biochemical changes that result in alteration of platelet morphology and release of extracellular chemicals that activate neighboring platelets and plasma coagulation factors.

Incitement of the coagulation cascade ultimately produces fibrin monomers that strengthen the initial platelet plug by spontaneously aggregating into end-to-end and side-to-side fibrils. The fibrin monomers are generated by the enzyme thrombin, which converts the soluble blood protein fibrinogen into fibrin. Thrombin also stimulates factor XIII to covalently crosslink the fibrin monomers into stable, insoluble fibrin polymers. The stabilizing crosslinks occur between the outer D domains of adjacent fibrin monomers and the central E domain of a third fibrin monomer (**Figure 1**). The adjacent, dimeric D domains are referred to as *D-dimers*.

These clotting modalities are counteracted by a number of mitigating mechanisms that prevent excessive or inappropriate clotting.[6] For example, activated coagulation factors are only effective for milliseconds or seconds before they are diluted by normal blood flow and cleared by the liver. Also, endogenous antithrombotic agents, such as antithrombin and proteins C and S, impede platelet activation and inhibit activated coagulation factors. These mechanisms orchestrate a balance between clotting the blood and maintaining its fluidity, whereas dysfunction of these systems may lead to a propensity for inappropriate thrombus formation or bleeding.

D-Dimer

Fibrin thrombi are primarily comprised of fibrin polymers that are normally broken down by the fibrinolytic system almost as soon as they are formed. The fibrinogen degradation products (FDPs) that result from clot disintegration are currently the most widely used indicators of thrombosis. Also, laboratory evaluation of thrombosis relies on the assessment of levels of factors, such as fibrinogen, that are consumed during coagulation.

The major effector of the fibrinolytic system, namely, plasmin, is generated from an inactivated precursor protein called plasminogen by tissue plasminogen activator (TPA). The breakdown of fibrinogen and fibrin by plasmin is counterbalanced by multiple enzymatic modulators such as thrombin-activatable fibrinolysis inhibitor (TAFI) that modifies fibrin to make it more resistant to plasmin breakdown. Also, α-2-antiplasmin and α-2-macroglobulin enzymes inactivate plasmin to prevent its circulation and thus to restrict fibrinolysis to the area of injury.

Plasmin-mediated cleavage of fibrinogen and fibrin produces a heterogeneous assortment of various-sized breakdown products.[7] The smallest degradation products that are resistant to further plasmin activity are termed *fragments X*, *Y*, *D*, and *E*. However, only fragments originating from fibrin polymers that had undergone factor XIII mediated cross-linking will have an intact covalent bond between 2 adjacent D domains (ie, D-dimers) (**Figure 1**). Therefore, the D-dimer fragment, which contains 2 D domains and an E domain, provides the unique target epitope for a fibrin-specific degradation product that is recognized by most reagent antibodies used in the laboratory assessment of thrombosis.

D-Dimer Assays

Clinical measurement of FDPs began in the early 1970s, using staphylococcal clumping, latex fixation and agglutination, hemagglutinin inhibition, immunoelectrophoresis, immunodiffusion, and other techniques.[8,9] In lieu of more sensitive and specific assays, measurement of FDPs initially was used for the diagnosis and management of disseminated intravascular coagulation

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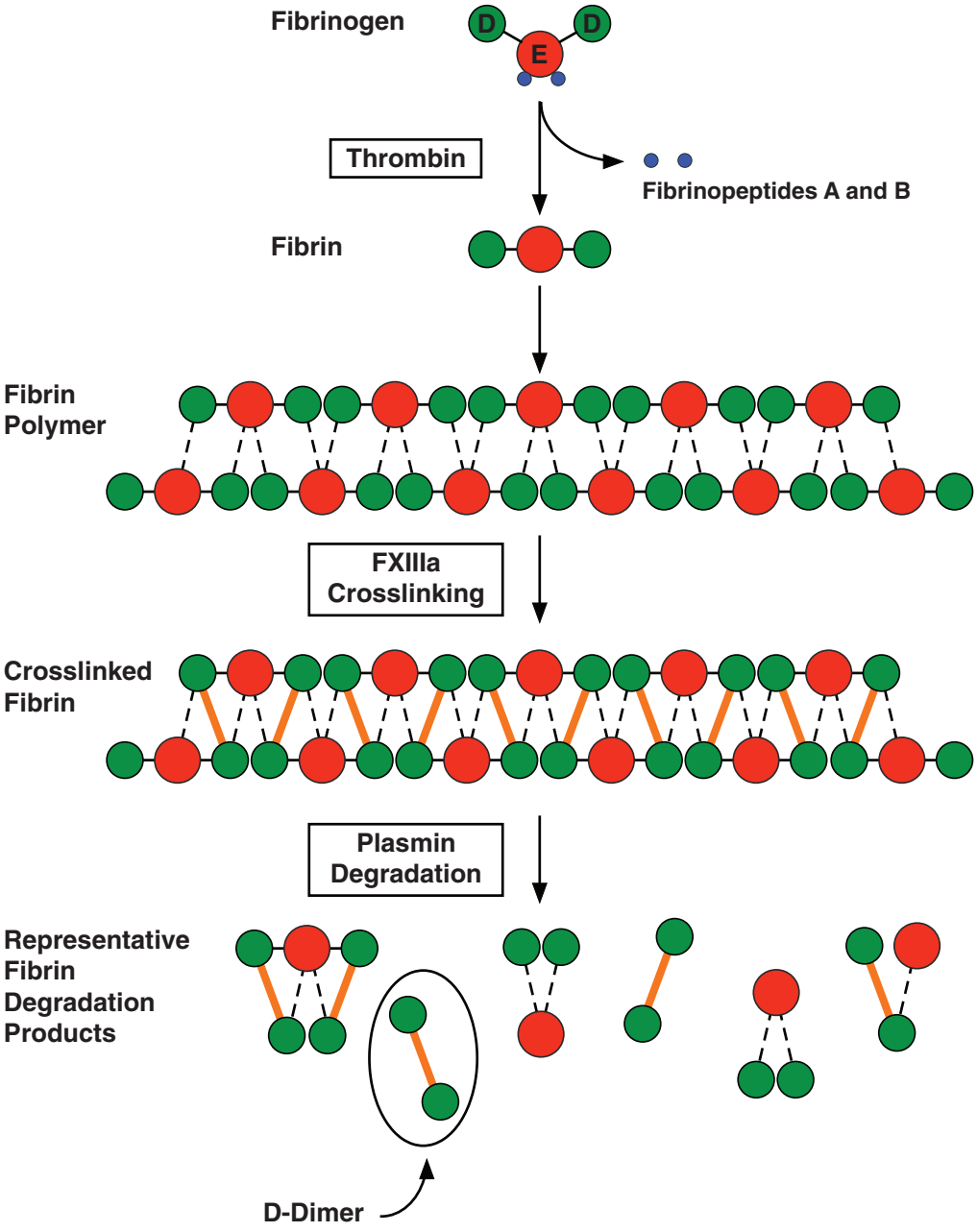

Figure 1

Fibrin formation and degradation. Fibrinogen is transformed to fibrin monomers through the cleavage of 2 small fragments (ie, fibrinopeptides A and B) from the molecule by thrombin. During this process, the negative charge of the E domain of fibrinogen (red circles) is converted to a positive charge, permitting spontaneous polymerization of the fibrin monomers into a polymer stabilized by hydrogen bonds. Thrombin also activates a circulating transglutaminase enzyme, factor XIII, which stabilizes the initial fibrin polymer by catalyzing the formation of crosslinked covalent bonds between adjacent D domains (green circles). Plasmin, a component of the fibrinolytic system, is formed from its circulating inactive precursor, plasminogen, through the activity of a serine protease, tissue plasminogen activator (TPA), released by injured endothelial cells. Plasmin cleaves fibrin into a variety of smaller fragments termed fibrin degradation products (FDPs). The D-dimer, formed from 2 adjacent cross-linked fibrin monomers, is one of the major FDPs. Plasmin also proteolyzes fibrinogen and other plasma proteins. A circulating enzyme, alpha-2-antiplasmin, inactivates plasmin to localize fibrinolysis to the site of injury.

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(DIC) and other thromboembolic diseases. Significant improvement of sensitivity and specificity occurred in the 1980s with the development of monoclonal antibody-based assays that rely on monoclonal immunoglobulins that target specific D-dimer epitopes not found on FDPs or on non–cross-linked fibrin fragments. The early monoclonal assays were primarily qualitative latex slide agglutination assays using latex microparticles coated with monoclonal antibodies specific for D-dimer epitopes. Macroscopically visible agglutination of the particles occurs when they are incubated with plasma containing D-dimers. These unenhanced slide-based latex agglutination assays are rapid and inexpensive, but they lack sufficient sensitivity for detection of D-dimers in critical clinical situations, particularly pulmonary embolism and acute venous thrombosis. Therefore, monoclonal antibody assays have been replaced by other more sensitive and specific methodologies.

Currently, measurement of FDPs largely has been replaced by a variety of more-sensitive commercial D-dimer testing platforms that have been designed for central clinical laboratories and point-of-care applications (**Table 1** and **Table 2**).

The central laboratory quantitative D-dimer assays were initially based on enzyme-linked immunosorbent assay (ELISA) technology but have more recently been adapted to coagulation analyzers and clinical chemistry analyzers, with an endpoint based on immunofluorescence, latex-enhanced immunoturbidimetry, or chemiluminescence. Although these assays are highly sensitive and are economical to perform when analyzing large numbers of specimens, the combination of specimen transportation time and analytical time often results in a prolonged response time of more than 40 minutes. Currently, 8 central laboratory D-dimer assays are approved by the United States Food and Drug Administration (FDA) for the exclusion of PE or DVT with a negative finding in the absence of additional time-consuming and expensive radiographic studies, such as ultrasound, computed tomography (CT), compression ultrasonography, pulmonary angiography, ventilation/perfusing (V/Q) scans, and venography. An additional assay is FDA approved to aid in the diagnosis of thromboembolic disease with the addition of 1 or more radiographic procedures. Several additional central laboratory D-dimer assays are available in Europe and other parts of the world but have not received FDA clearance for application in the United States. Currently, the laboratory testing proficiency program of the College of American Pathologists (CAP) lists 14 different quantitative assays from 7 manufacturers.[10]

The increasing overcrowding of urgent care facilities and the desire for improved patient satisfaction and decreased patient wait time have led to a great interest among physicians for rapid point-of-care (POC) D-dimer assays to screen patients for thromboembolic disease.[11,12] Currently, POC D-dimer assays have received FDA approval to aid in the diagnosis of VTE and PE, and many others are available worldwide. Generally, these assays are homogenous, monoclonal antibody-based sandwich types, with a detection method based on hemagglutination, fluorescence, chemiluminescence, or other technology. A few semiquantitative methods remain in use for POC situations. Whole blood is of the usual specimen type, and the POC D-dimer assays have a short specimen turnaround time of 5 to 20 minutes. Overall, the global D-dimer testing market had an estimated value of 1.5 billion US dollars in 2013 and is expected to reach 1.9 billion US dollars by 2022.[13] In the following paragraphs, we discuss the most widely used central laboratory and POC D-dimer assays in more detail. We also will discuss the clinical applications of the D-dimer assay.

Central Laboratory Assays

Quantitative ELISA assays are considered colloquially to be the reference standard for D-dimer quantitation. This method involves loading plasma specimens into microtiter wells coated with antibodies that have high affinity binding for D-dimers. After incubation, a labeled antibody is then added, and the quantity of bound, labeled substance is measured via colorimetric reaction. The labor-intensive and time-consuming constraints typical of conventional ELISA assays make them impractical for routine clinical laboratory use, which has spurred the development of more rapid, automated, and highly sensitive modified ELISA assays.

Developed in the mid-1990s, the VIDAS D-Dimer (bioMérieux SA) is a rapid assay that uses single-dose, ready-to-use reagents and an antifibrin D-dimer–coated solid-phase receptacle that also serves as a pipetting device for the plasma specimen. The diluent, conjugate, wash solution and other reagents are housed on a strip that is cycled in and out of the receptacle at 37°C to ultimately produce a fluorescent

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Table 1. Selected Central Laboratory D-Dimer Assays

Assay Name	Manufacturer	Methodology	Unit Type	Reported Units	Manufacturer Cut-Off	FDA Approval/Clearance for VTE Evaluation
Advanced D-Dimer	Dade Behring Diagnostics	Quantitative, latex enhanced immunoturbidimetric immunoassay	FEU	mg/L	Instrument dependent	Aid in diagnosis
AQT90 FLEX D-dimer	Radiometer Medical ApS	Quantitative, time-resolved fluorometry	NA	μg/L	500 μg/L	NA
Auto Blue 400 Auto Red 700 D-Dimer	Helena Biosciences	Latex enhanced immunoturbidimetric immunoassay	DDU	ng/mL	200 ng/mL	NA
Diazyme D-Dimer Assay	Diazyme Laboratories	Latex enhanced immunoturbidimetric immunoassay	FEU	μg/mL	<0.5 μg/mL	Aid in diagnosis
HemosIL AcuStar D-Dimer	Instrumentation Laboratory	Emzyme immunoassay, chemiluminescence	FEU	ng/mL	500 ng/mL	Exclusion
HemosIL D-Dimer	Instrumentation Laboratory	Latex enhanced immunoturbidimetric immunoassay	DDU	ng/mL	243 ng/mL	Exclusion
HemosIL D-Dimer HS	Instrumentation Laboratory	Latex enhanced immunoturbidimetric immunoassay	DDU	ng/mL	243 ng/mL	Exclusion
HemosIL D-Dimer HS 500	Instrumentation Laboratory	Quantitative, latex enhanced immunoturbidimetric immunoassay	FEU	ng/mL	500 ng/mL	Exclusion
INNOVANCE D-Dimer	Siemens AG	Quantitative, latex enhanced immunoturbidimetric immunoassay	FEU	ng/mL	500 ng/mL	Exclusion
MDAW D-Dimer	bioMérieux SA	Quantitative, latex enhanced immunoturbidimetric immunoassay	NA	NA	NA	Aid in diagnosis
Nordic Red D-dimer	Nordic Biomarker AB	Quantitative, latex enhanced immunoturbidimetric immunoassay	DDU	ng/mL	200 ng/mL	NA
Nordic Blue D-dimer	Nordic Biomarker AB	Quantitative, latex enhanced immunoturbidimetric immunoassay	DDU	ng/mL	200 ng/mL	NA
STA Liatest D-Dimer	Diagnostica Stago, Inc.	Quantitative, latex enhanced immunoturbidimetric immunoassay	FEU	μg/mL	<0.5 μg/mL	Exclusion
Tina-quant D-Dimer BM	F. Hoffman-La Roche Ltd.	Quantitative, latex-enhanced immunoturbidimetric immunoassay	FEU	μg/mL	<0.5 μg/mL	Exclusion
TriniLIA D-Dimer	Tcoag Ireland Ltd.	Polystyrene microparticle agglutination assay	FEU or DDU	NA	NA	NA
VIDAS D-Dimer	bioMérieux SA	Quantitative, ELISA, sandwich type	FEU	ng/mL	500 ng/mL	Exclusion

FDA, United States Food and Drug Administration; VTE, venous thromboembolism; FEU, fibrinogen-equivalent units; NA, nonapplicable; DDU, data display unit; ELISA, enzyme-linked immunosorbent assay.

product, which is then spectrophotometrically measured at 450 nm. The Vidas D-Dimer assay generates results within 1 hour and has sensitivity of 94% to 100%; specificity of 38%; negative predictive value (NPV) of greater than 99; positive predictive value (PPV) of 60.8% at a cut-off of 500 ng per mL; and reportedly shows no interference from heparin, bilirubin, hemoglobin, FDPs, or plasma turbidity.[14-16]

Latex-enhanced immunoturbidimetric assay is a cost-effective, rapid test that has analytical sensitivity comparable to conventional ELISA. This automated microparticle assay passes a beam of monochromatic light through a suspension of latex microparticles that are coated by monoclonal antibodies specific for D-dimer epitopes. Because the 540-nm wavelength of the light beam is greater than the diameter of the latex microparticles, only a minimal amount of light is absorbed by the latex microparticle solution at baseline. However, when plasma is added to the suspension, any D-dimer present in the specimen causes the latex microparticles to agglutinate, and the congregating aggregates have diameters greater than the wavelength of the light passing through the solution. The resulting increased absorbance of light is measured photometrically and is directly proportional to the amount of D-dimer present in the test specimen. Commercial examples of immunoturbidimetric assays include the STA Liatest D-Dimer (Diagnostica Stago, Inc),[17,18] HemosIL D-Dimer/HemosIL D-Dimer HS/HemosIL D-Dimer HS 500 (Werfen Group IVD),[19] Tina-quant D-Dimer BM (F. Hoffman-La Roche Ltd), Advanced D-Dimer (Dade Behring Diagnostics),[20,21] and

Table 2. Selected Point of Care D-Dimer Assays

Assay Name	Manufacturer	Methodology	Unit Type	Reported Units	Manufacturer Cut-Off	FDA Approval/ Clearance for VTE Evaluation
Triage D-Dimer	Alere	Fluorescence immunoassay	DDU	ng/mL	NA	Aid in diagnosis
AxSym AxSYM D-dimer	Abbott Laboratories, Inc	Quantitative, microparticle enzyme immunoassay (MEIA), chemiluminescence	FEU	ng/mL	500 ng/mL	NA
Roche Cardiac D-Dimer	F. Hoffman-La Roche	Quantitative, latex-enhanced immunoturbidimetric immunoassay	FEU	μg/mL	500 μg/mL	Aid in diagnosis
Clearview Simplify	Sekisui Diagnostics, LLC	Qualitative, solid-phase immunochromatography	NA	Positive or negative	80 ng/mL	CLIA, nonwaived; aid in diagnosis
Dade Dimertest latex assay	Siemens AG	Qualitative or semi-quantitative latex agglutination	NA	Positive or negative	NA	NA
DIMERTEST Latex	Sekisui Diagnostics, LLC	Qualitative or semi-quantitative latex agglutination	NA	Positive or negative	<0.2 mg/L	NA
i-CHROMA D-Dimer	SYCOmed e.K.	Quantitative, fluorescence immunoassay	DDU	ng/mL	300 ng/mL	NA
mLabs D-Dimer	Micropoint Bioscience, Inc.Clara, CA	Quantitative, fluorescence immunoassay	NA	ng/mL	500 ng/mL	NA
NycoCard D-Dimer	Nycomed Pharma	Semiquantitative, immunofiltration	DDU	mg/L	0.5 mg/mL	NA
PATHFAST D-Dimer	Mitsubishi Kagaku Iatron, Inc.	Quantitative, chemiluminescent enzyme immunassay	FEU	μg/mL	0.686 μg/mL	NA
Remel D-Dimer Wellcotest	Thermo Fisher Scientific, Inc.	Semi-quantitative latex agglutination	NA	Positive or negative	NA	NA
SimpliRED D-Dimer	Agen Biomedical Limited	Qualitative, red-blood-cell agglutination	NA	Positive or negative	<120 ng/mL	CLIA, moderate complexity
Stratus CS Acute Care D-Dimer	Siemens AG	Quantitative, fluorescence Immunoassay	FEU	ng/mL	450 ng/mL	Exclusion

FDA, United States Food and Drug Administration; VTE, venous thromboembolism; DDU, data display unit; NA, nonapplicable; FEU, fibrinogen-equivalent units; CLIA, Clinical Laboratory Improvement Amendments

INNOVANCE D-Dimer (Siemens AG).[22,23] The HemosIL AcuStar D-Dimer immunoassay (Werfen Group IVD) is a fully automated chemiluminescence assay that uses a 2-step method for D-dimer quantitation.

Citrated plasma and buffer are incubated with anti-D-Dimer antibody-coated magnetic particles that are washed, magnetically separated, and incubated with isoluminol-labeled anti-D-dimer antibody. Chemiluminescence is measured following a second magnetic separation and washing.

Point-of-Care Assays

The relative insensitivity of latex agglutination assays spurred the development of more-sensitive POC testing, including slide-based D-dimer assays that take advantage of red blood cell (RBC) agglutination principles. A widely used example often observed in acute care settings includes the SimpliRED D-Dimer assay (Agen Biomedical Limited), which incubates a drop of whole blood with a hybrid, bispecific monoclonal antibody that recognizes D-dimer and an RBC membrane antigen. D-dimers present at concentrations higher than the cut-off level will cause visible RBC agglutination that must be visually interpreted by trained personnel. Despite this disadvantage, the test is rapid, inexpensive, and has a reported high sensitivity.

The Nycocard D-Dimer assay (Nycomed Pharma) epitomizes immunofiltration as a POC application for D-dimer quantification. This assay reportedly has sensitivity of 81% to 100% and can be performed in less than 2 minutes. It uses a laminated card that consists of a thin, porous, antibody-coated membrane sandwiched between an underlying absorbent pad and an overlying plastic layer with a central well. Specimen plasma is pipetted into the well and

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enters the membrane, where D-dimer antigens may bind to the antibodies coating the membrane. This step is followed by the addition of colloidal 4-nm-in-diameter gold particles complexed with reagent immunoglobulins that target a different D-dimer epitope. After washing the solution, any remaining gold particles will impart a red color that is directly proportional to the amount of D-dimer in the solution and can be interpreted visually to yield a qualitative result or can be evaluated by a reflectometer or card reader to yield a quantitative result.[24]

Other rapid methodologies that also use D-dimer-specific monoclonal antibodies conjugated to colloidal gold particles include the Clearview Simplify (Sekisui Diagnostics, LLC) and Simplify D-dimer assays (Agen Biomedical Limited). These commercially available qualitative solid-phase immunochromatographic tests rely on a single-use plastic device that houses an acetate cellulose chromatography membrane with immobilized gold-conjugated monoclonal antibody.[25-27]

Several automated fluorescence immunoassays are widely used for D-dimer analysis in POC settings. The Alere (formerly, Biosite) Triage D-Dimer Test (Alere Inc) places several drops of ethylenediaminetetraacetic acid (EDTA)–anticoagulated whole blood or plasma into a single-use test device. In this device, D-dimers form complexes with fluorescent-labeled monoclonal antibodies that are then quantified by a portable fluorometer; the results, expressed in ng per mL (cut-off, 400 ng/mL), are available in approximately 20 minutes.[28] The Stratus CS Acute Care D-Dimer assay (Siemans AG) is a fluorometric immunoassay that uses the Stratus CS STAT Fluorometric Analyzer and incorporates solid-phase radial partition technology into a 2-site sandwich assay. The i-CHROMA D-Dimer Test (SYCOmed e.K.) is a sandwich immunoassay with a nitrocellulose matrix designed for measuring plasma D-dimers within a working range of 50 to 10,000 ng per mL; the emitted fluorescence is measured with the i-CHROMA Reader. D-dimer analyzer AQT90 FLEX (Radiometer Medical ApS,) is another sandwich immunoassay that uses tracer and capture antibodies to form D-dimer sandwich complexes, which are then measured using time-resolved fluorometry (TRF), in which the tracer antibody signal is measured directly from the dried assay solution and calibrated to a D-dimer quantitation within a range of 80 to 100,000 μg per L.[29-31]

Several automated, rapid, quantitative enzyme immunoassays are commercially available for D-dimer analysis. The PATHFAST D-Dimer assay (Mitsubishi Kagaku Iatron, Inc) is a 1-step sandwich immunoassay using D-dimer monoclonal antibody-conjugated magnetic particles, together with alkaline phosphatase–conjugated anti-D-dimer monoclonal antibody.[32,33] The AxSYM D-dimer assay (Abbott Laboratories, Inc) is a similar 2-stage sandwich assay, based on microparticle enzyme immunoassay (MEIA) technology, which produces a fluorescent end product.[34-36]

Clinical Significance of D-Dimers

Thromboembolic disease is caused by dysregulation of complex hemostatic regulatory mechanisms, which leads to the formation of thrombi that may cause local vascular occlusion or may embolize to occlude distal vessels. Because of its severe clinical consequences, many breakdown products of the coagulation system have been studied as potential diagnostic markers for thromboembolic disease. D-dimers represent specific breakdown products of cross-linked fibrin clot formation; to our knowledge, D-dimers are the only clinically beneficial biomarkers for routine use in patients with DVT, PE, and disseminated intravascular coagulation (DIC). Circulating D-dimers are also elevated in patients with coronary artery disease and other cardiovascular diseases, cancer, trauma, pregnancy, infectious and inflammatory diseases, severe renal disease, recent surgical procedure(s), advanced age, and many other conditions, health factors, and diseases. However, because the elevation is less specific than for DVT/PE, laboratory monitoring for D-dimers has limited clinical usefulness in patients with these diseases.[37-39]

The diverse array of POC and central laboratory D-dimer assays has generated confusing, often contradictory streams of interassay comparative studies in patients with thromboembolic disease. In these studies, the sensitivity, specificity, negative and positive predictive values, and other parameters of D-dimer assays have been compared with the results of radiographic studies and with other clinical and laboratory data to attempt to determine the most efficacious and cost-effective means of diagnosis and treatment monitoring of thromboembolic disease. These studies have been particularly relevant in recent years because of the increasing demand for rapid test turnaround time. Some of the more recent comparative studies include those published

by Baker et al in 2010,[40] Perveen et al in 2013,[42] and Sen et al in 2014.[41] Baker and collaborators compared the performance of a POC D-dimer assay, the Biosite Triage (Inverness Medical Innovations, Inc), with that of the Liatest D-dimer (Diagnostica Stago, Inc), performimg those assays on a widely used coagulation analyzer, the STA-R Evolution (Diagnostica Stago Inc).[40] Among 112 patients with suspected DVT, the investigators concluded that the Triage D-dimer assay was comparable to the Liatest in the central laboratory when used in conjunction with the Wells score and compression ultrasonography.

Sen and coworkers reached a similar conclusion in a comparative study of the Alere Triage D-dimer panel (Alere Inc) with the Instrument Laboratory (IL)–tops CTS coagulation analyzer (Werfen Group IVD).[41] Perveen and collaborators compared the VIDAS D-dimer analyzer (bioMérieux SA) with the POC D-dimer analyzer AQT90 FLEX (Radiometer Medical ApS) in 104 patients.[42] All patients with positive imaging were identified correctly by both methods (100% sensitivity); however, the specificity of the AQT90 FLEX (36%) was superior to that of the VIDAS instrument (18%). Hendriksen and collaborators conducted a detailed statistical analysis of the results of 11 published articles comparing 4 user-friendly POC D-dimer assays (Biosite Triage, Biosite Diagnostics [now Alere, Inc]; Roche Cardiac, F. Hoffman-La Roche, Ltd; Axis-Shield Mycocard, Axis-Shield Diagnostics Ltd; and Clearview Simplify, Sekisuki Diagnostics, LLC) to laboratory-based D-dimer tests to determine the long-term cost-effectiveness of the assays in the diagnostic work-up of patients with suspected DVT.[43] They concluded that the overall health outcomes were similar between the POC and central laboratory testing methods but that the POC assays were significantly more cost effective. Lee-Lewandrowski and collaborators specifically evaluated the impact of implementing a POC D-dimer assay (Biosite Triage, Biosite Diagnostics [now Alere Inc) in the emergency department of a large urban academic medical center where the VIDAS D-dimer central laboratory assay (bioMérieux SA) was also used.[44] In this study, the D-dimer turnaround time decreased by 79%, the patient length of stay decreased from 8.46 to 7.14 hours, and length of hospital admissions decreased by nearly 14%. None of the patients with negative D-dimer assay results had developed subsequent VTE by the 3-month follow-up visit.

Interlaboratory variation is a serious problem that further compromises the value of the D-dimer assay. Although small, single-center studies often reveal high sensitivity, specificity, and negative predictive values for commercial D-dimer assays, the results of larger studies have shown that the situation is less ideal. The Coagulation Resource Committee of CAP has taken an active role in the investigation and resolution of this problem. For example, a survey conducted by CAP in 2014, involving 3800 laboratories, discovered that the intermethod coefficient of variance (CV) was unacceptably high, ranging as high as 42% for a single specimen.[45] As discussed in recent reviews by Olson and others, the major reasons for the high interlaboratory and intermethod variability include the following: the heterogeneity of the D-dimer molecular structure; the use of monoclonal antibodies with different antibody specificity required by the commercial reagent manufacturers; the lack of standardized, internationally certified calibrators and quality-control materials; lack of a standardized reporting unit for D-dimers; and the use of inappropriate cut-off values that are too high for the exclusion of thrombosis.[45-47] The lack of standardization of D-dimer reporting units is a serious problem that compromises the use of this important assay. Depending on the testing method, D-dimer results may be reported using 2 different types of units: fibrinogen equivalent unit and D-dimer unit. The fibrinogen equivalent unit (FEU) is based on the mass of fibrinogen (340 kDa) and is approximately 1.75-fold higher than the D-dimer unit (DDU), which is based on the weight of the D-dimer.[47] Also, either type of unit used for D-dimer quantification may be reported with as many as 9 different units of magnitude, including mg/L, mg/dL, ng/dL, ng/mL, μg/L, μg/mL, μg/dL, mg/mL, and ng/L.[48] The problem is compounded by the failure of some laboratories to follow the reporting recommendations of the manufacturers of the reagents they use, leading to the potential for significant errors in interpretation. Also, CAP found that 33% of 1500 laboratories did not comply with the recommendations of the reagent manufacturer for reporting D-dimer results.

The accuracy of the D-dimer assay is further compromised by individual patient heterogeneity in D-dimers determined by patient age, genetic influences, disease factors, hereditary and acquired coagulation deficiencies, the size of the blood clot, and the timing of specimen collection in relation to the thrombotic event.[39,47,49] To address these issues, several systematic studies using meta-analysis have been performed to evaluate the diagnostic efficacy of the D-dimer in large patient populations with suspected

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thromboembolic disease. In an early study of the results of 198 assays reported in 99 different patient groups, the overall sensitivity D-dimer for DVT was 91% and was similar between assays; however, the sensitivity was much lower (55%) and was most dependent on the clinical probability of DVT.[50] In this study, whole blood agglutination assays yielded the highest specificity (68%). A more recent meta-analysis of studies of 4 POC D-dimer assays, involving 23 published studies and nearly 14,000 patients, showed overall sensitivity of 85% to 96% and overall specificity from 48% to 74%, with the quantitative assays outperforming the qualitative tests.[51] A recent meta-analysis of 12,497 older (The paper included patients 51 to >80 years.) patients with suspected VTE revealed the significance of age-adjusted cut-off values. In this study, the specificity of the D-dimer results in specimens from patients of all ages increased with age-adjusted cutoff values but was most dramatic in patients aged 80 years or older, in which specimens the unadjusted cut-off was 14.7% and the age-adjusted cut-off was 35.2%.[52]

In these circumstances, laboratories have many opportunities to advance patient care by assuring the appropriate clinical use, performance, and reporting of the D-dimer assay.[47] According to Olson and collaborators, the Clinical Guidelines Committee of the American College of Physicians,[53] and other authorities, this can be best achieved by the following measures:

- Educating health care professionals in the appropriate use of the D-dimer assay. This assay should be requested only in an appropriate clinical context and should be used with caution in patients with recent trauma or surgical procedure(s), pregnancy, malignant neoplasms, cirrhosis, or severe infection, as well as those who recently received anticoagulation therapy or fibrinolytic drugs.
- Using assays that have been validated in clinical studies and have established cut-off values. Age- and sex-specific cut-off values should be used if possible.
- Being aware of preanalytic variables and interfering substances that can affect the D-dimer assay(s) used in the institution. Common interfering substances include rheumatoid factor, hemoglobin, bilirubin, triglycerides, and some monoclonal proteins. Many drugs can interfere with D-dimer assays but usually not at therapeutic levels.
- Reporting D-dimer results according to the recommendations of the reagent manufacturer.

With these caveats, we discuss, in the following paragraphs, the clinical usefulness of the D-dimer in different diseases.

DVT/PT

Thrombi of the lower extremities usually cause pain, swelling, and discoloration of the affected limb(s). Thrombi originating in the popliteal, femoral, and iliac veins are particularly dangerous due to the high risk of pulmonary embolism.[1] In most patients with possible DVT, a clinical prediction rule (CPR) is used to generate a pretest probability of disease, followed by appropriate laboratory studies if the risk score is high. The Wells CPR is widely used, although several other clinical scores have been developed.[54,55] Conventional laboratory tests for DVT include D-dimer analysis, as well as Doppler ultrasonography, helical CT, contrast venography, and impedance plethysmography. If a PE is suspected, D-dimer analysis, pulmonary angiography, ventilation-perfusion scanning, and helical CT scanning may be performed. However, the use of a D-dimer assay in combination with an evidence-based clinical algorithm in outpatients who have not undergone anticoagulation procedures but are suspected of having DVT or PE can effectively exclude the presence of disease without using more-expensive radiographic studies.

Several studies[56-58] involving large numbers of patients have shown that a low D-dimer level, combined with low CPR scores, has a negative predictive value of greater than 95%. In fact, a meta-analysis of 1660 patients with a normal D-dimer level and “unlikely” Wells CPR showed a negative predictive value of 99.7%.[59] The results of these studies have shown that, of the many D-dimer assays, the quantitative central laboratory assays performed most strongly, whereas the erythrocyte agglutination assays were acceptable for use only in specimens from patients with a low CPR score. In contrast with a negative D-dimer result, a positive D-dimer assay result is nonspecific and can be caused by many diseases. A large retrospective study of 1647 patients, evaluated at a large urban medical center, whose specimens yielded a positive D-dimer result showed infection as the most common cause, followed by VTE, syncope, heart failure, trauma, and cancer. However, the highest D-dimer levels were observed in patients with VTE, particularly among elderly patients.[38]

DIC

DIC is a common disease with high morbidity and mortality; it is characterized by systemic activation of the hemostatic system with intravascular thrombin generation, fibrin formation, and increased fibrinolysis.[60,61] If untreated, it can lead to a depletion of platelets, clotting factors, and inhibitors, resulting in life-threatening bleeding and/or thrombosis. It is most common in hospitalized patients with infectious/inflammatory diseases, malignant neoplasms, trauma, or obstetric diseases. Recent classifications of DIC divide it into nonsymptomatic (pre-DIC), organ failure, bleeding, and massive bleeding subtypes, whereas the International Society of Thrombosis and Haemostasis (ISTH) has harmonized previous diagnostic and treatment guidelines.[62,63] Although the interpretation of D-dimer levels is often compromised by the underlying disease process, the D-dimer is included in the ISTH guidelines of recommended laboratory assays and is most helpful in evaluation of patients with the nonsymptomatic, bleeding, and organ failure subtypes of DIC.[63]

Cardiovascular Disease

Circulating D-dimer levels increase in patients with coronary artery disease, with the highest levels in patients with acute ischemic events, including myocardial infarction and unstable angina. In patients with chest pain, elevated D-dimer is an early marker of coronary ischemia and an independent prognostic factor for myocardial infarction.[64] In a study of 18 biomarkers of coronary heart disease risk in a cohort of more than 27,000 women who have undergone menopause, only the D-dimer level significantly increased the model discrimination, and this factor was independent of other risk factors.[65] More recently, plasma D-dimer levels in patients with coronary artery disease have been reported to independently predict no reflow after primary percutaneous coronary intervention (p-PCI);[66,67] however, that variable was not significantly correlated with long-term prognosis in those patients, nor with reperfusion after thrombolytic therapy.[68-70] Additional studies are needed to develop diagnostic 50 algorithms that incorporate the D-dimer and conventional markers, such as troponin.

Multiple individual studies and several meta-studies have evaluated the role of D-dimer analysis in patients with suspected acute aortic dissection or abdominal aortic aneurysm.[71-77] These studies have shown that D-dimer levels are helpful in the evaluation of these patients, particularly in patients with a low likelihood of disease, but should still be used in conjunction with ultrasound and other aortic imaging modalities.

The results of many studies have revealed elevated levels of D-dimer and other markers of coagulation activation in patients with atrial fibrillation. Also, a high D-dimer level was identified as a risk factor for subsequent thromboembolic and cardiovascular events.[78,79] One study reported that a low D-dimer level was sufficient to exclude the presence of an atrial thrombus and to permit cardioversion to be performed without additional studies.[80]

Other Diseases

Elevated D-dimer levels have been reported in a wide variety of benign and malignant diseases characterized by infection, trauma, ischemia, bleeding, or thrombosis. In many of these diseases, investigators have attempted to use D-dimer levels for diagnosis, prognostication, or treatment. For example, D-dimer levels have been investigated as an adverse prognostic and/or a risk factor for thrombosis in patients with malignant tumors, gastrointestinal bleeding and necrosis, intracerebral hemorrhage, sickle cell disease, migraine headaches, traumatic brain injury, tuberculosis, Cushing disease, asthma, membrane oxygenator failure, and many other diseases.

Of these diseases, cancer has received the greatest interest. Overall, the risk of venous thrombosis in patients with cancer is as high as 7%, presumably due to the prothrombotic effect of malignant neoplasms and treatment-related risk factors such as immobilization, drugs, and surgical intervention.[81,82] However, the common finding of elevated D-dimer levels in patients with cancer in the absence of thrombosis limits the diagnostic usefulness of these findings if used by themselves. However, age-adjusted cut-off values and risk-assessment models that combine the D-dimer with other biomarkers have been demonstrated to improve the diagnostic sensitivity of the D-dimer.[52,83-86] Numerous studies have also shown the adverse prognostic significance of elevated plasma D-dimer levels in patients with cancer of the breast, colon, lung, gastric system, ovaries, prostate, and other organs.[87-96] Nevertheless, the new direct oral factor Xa

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inhibitors show promise in improving the efficacy of treatment for venous thromboembolism in patients with cancer, while avoiding the inconvenience of the subcutaneous injection and/or frequent monitoring required for conventional therapy.[97-99]

Discussion

Thromboembolic disease is a major cause of morbidity and mortality. In response to this serious health concern, many assays have been developed to detect and monitor abnormal blood clotting. Most of these assays use monoclonal antibodies that target specific epitopes on cross-linked D-dimer fragments that are the unique products of cross-linked fibrin degradation. However, serum D-dimer elevation is not specific to thromboembolic disease, and baseline D-dimer elevation can be observed in many other conditions such as DIC, infection, and malignant neoplasms. Evaluation of thromboembolic disease in patients with these complicated conditions often requires incorporation of the D-dimer level into multitest algorithms. Automated multitest instrumentation for assays such as ELISA, latex-enhanced immunoturbidimetry, and chemiluminescence are routinely used by central laboratories, whereas D-dimer assays intended for bedside use in clinics and urgent-care facilities rely on a variety of innovative test methods, such as enzyme immunoassays, immunofiltration, and immunochromatography. Most of these assays have excellent sensitivity and negative predictive values and play an essential role in the evaluation of patients with suspected thromboembolic disease. However, the methodologies that are currently used for D-dimer assessment are plagued with interlaboratory and intermethod variability, as well as a lack of standardized calibrators and reporting units. As a result of these inconsistences, laboratorians and clinicians should be aware of which D-dimer assay their laboratory uses, to assure optimal patient care regarding the diagnosis and management of patients with thromboembolic disease. **LM**

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