EDUCATIONAL COMMENTARY – D-DIMER UPDATE

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Learning Outcomes

On completion of this exercise, the participant should be able to

• discuss the utility of D-dimer testing
• describe current D-dimer assays and reporting units
• analyze how D-dimer results are used in the assessment of clinical situations including pulmonary embolism, deep vein thrombosis, and disseminated intravascular coagulation
• identify how the D-dimer is used in different age groups

Introduction

D-dimer results are used in the evaluation and assessment of disorders that result in the breakdown of fibrin and, in some situations, creation of excessive fibrinogen during fibrinolysis. This includes deep vein thrombosis, pulmonary embolism, and disseminated intravascular coagulation. To provide more specificity for fibrin proteolysis, the monoclonal antibody-based test for the D-dimer was developed in 1980.¹ The D-dimer is a product of clot degradation. It has a plasma half-life of approximately 8 hours and is cleared in the kidneys and reticuloendothelial system.² The D-dimer is a marker of fibrin and indicates that thrombin has been formed and fibrin has been generated from fibrinogen in vivo as a result of activation of the coagulation system.³ Elevated D-dimer levels are found in thrombosis, pulmonary embolism, venous thromboembolism, disseminated intravascular coagulation, myocardial infarction, stroke, liver disease, cancer, and pregnancy.¹

Pathophysiology

In a normal hemostatic system, fibrin clots are formed in response to injury. Thrombin converts fibrinogen into fibrin monomers. This in turn relies on the activation of factor XIII (FXIII) to cross-link monomers into stable insoluble fibrin polymers. Two covalently bound fibrin D domains are cross-linked as a result of the action of FXIII during clot formation.³ During fibrinolysis, fibrin and excessively formed fibrinogen are cleaved by plasmin, resulting in different breakdown products of these polymers. D-dimers form from this breakdown of clots and consist of several fragments, which include X, Y, D and E. The D-dimer fragment contains two D domains, which is the target epitope that is unique for fibrin-specific degradation and is recognized by the monoclonal antibodies used for D-dimer testing.⁴
Clinical Significance of the D-dimer

Increased plasma D-dimer levels are present in many non-thrombotic clinical conditions such as inflammation and pregnancy. Given this, D-dimer is considered a sensitive but nonspecific marker for thrombosis. This results in a high rate of false-positive results, especially in hospitalized patients. Because fibrin is produced in many conditions, D-dimer has a low positive predictive value, which is defined as the probability of a patient actually having the diagnosis screened for if the screening test is positive. Even in situations of thrombosis, D-dimer testing can give false-negative results. This can occur if the thrombosis is present for longer than 7 to 10 days or if the patient was prescribed antithrombotic therapy.4

Owing to the poor specificity of D-dimer testing, the validity of the assay is in its negative predictive value, that is, the ability to rule out the presence of a clot. When the negative predictive value is combined with a validated scoring system, it increases the accuracy of the patient’s pretest probability for ruling out thrombosis. The higher the score, the more likely the patient has a thrombotic condition. See Table 1 for further details. Patients are stratified by their score to determine a low, moderate, or high pretest probability for the presence of a clot. In patients with a low score and a negative D-dimer test, thrombosis can be ruled out, and no further testing is required. However, if the patient has moderate or high pretest probability and a positive D-dimer, additional investigation should be performed, including radiographic or ultrasonographic testing.

Disseminated intravascular coagulation is a consumptive coagulopathy caused by a secondary disorder that leads to the activation of both the coagulation and fibrinolytic systems. There is no single test that can be used to diagnose this coagulopathy; however, laboratory measurements can be helpful. These include platelet counts, fibrinogen and D-dimer levels, and prothrombin time. Patients with disseminated intravascular coagulation will present with elevated D-dimer levels as a result of fibrinolysis.5

A retrospective study reviewed increased D-dimer levels in 759 adults. These patients had D-dimer levels 10 times higher than the cutoff level. In 581 of the 759 cases reviewed, D-dimer levels ranged from 5030 to 239,000 µg/L. The results showed that 32% of patients had a pulmonary embolism and 13% had a deep vein thrombosis; the remainder of the patients had cancer (29%), sepsis (24%), and trauma/surgery (24%). This information suggests that an elevated D-dimer level should be investigated in the context of the patient’s clinical history.6
Table 1. Scoring systems for the D-dimer.

<table>
<thead>
<tr>
<th>Clinical Characteristic</th>
<th>Wells Score&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Geneva Score&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous pulmonary embolism or deep vein thrombosis</td>
<td>+ 1.5</td>
<td>+ 3</td>
</tr>
<tr>
<td>Heart rate &gt;100 bpm</td>
<td>+ 1.5</td>
<td>+ 3 (74-94 bpm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ 5 (&gt;95 bpm)</td>
</tr>
<tr>
<td>Surgery or immobilization within the past 30 days</td>
<td>+ 1.5</td>
<td>2</td>
</tr>
<tr>
<td>Clinical signs of deep vein thrombosis</td>
<td>+ 3</td>
<td>4 Pain/edema</td>
</tr>
<tr>
<td>Alternative diagnosis less likely than pulmonary embolism</td>
<td>+ 3</td>
<td>N/A</td>
</tr>
<tr>
<td>Hemoptysis</td>
<td>+ 1</td>
<td>2</td>
</tr>
<tr>
<td>Cancer treated within the past 6 months</td>
<td>+ 1</td>
<td>2 (1 yr)</td>
</tr>
<tr>
<td>Age older than 65 y</td>
<td>N/A</td>
<td>1</td>
</tr>
<tr>
<td>Unilateral lower limb pain</td>
<td>N/A</td>
<td>3</td>
</tr>
</tbody>
</table>

**Clinical Probability of Pulmonary Embolism**

<table>
<thead>
<tr>
<th></th>
<th>Wells Score&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Geneva Score&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0-1</td>
<td>0-3</td>
</tr>
<tr>
<td>Intermediate</td>
<td>2-6</td>
<td>4-10</td>
</tr>
<tr>
<td>High</td>
<td>≥6</td>
<td>&gt;11</td>
</tr>
</tbody>
</table>


**Diagnostic Testing**

Levels of D-dimers can be detected by several methods: latex-based immunoassays, enzyme linked immunosorbent assays (ELISA), immunoturbidometric automated quantitative assays, and point-of-care (POC) testing.<sup>4</sup>

**Latex-Based Immunoassays**

The first generation of latex agglutination assays used latex-coated antibodies and results were based on visual readings. This was a manual test with minimal sensitivity. If agglutination was observed, this was considered a positive result, and samples were serially diluted to provide a semiquantitative estimate of the D-dimer. These assays were limited in detection of minimally increased D-dimer levels. The second generation of immunoassays used a photometric analyzer to provide a quantitative assay with a more sensitive lower limit of detection.<sup>7</sup>
Enzyme-Linked Immunosorbent Assays (ELISA)
A quantitative methodology for the detection of the D-dimer is based on ELISA. This is considered the gold standard of D-dimer testing and has a high sensitivity. In this methodology, plasma is placed onto microtiter wells coated with antibodies with a high-affinity binding for D-dimers. A labelled antibody is then added to the incubated plasma-antibody mixture, and the bound antibody is measured using colorimetry.5

Immunoturbidometric Assays
To provide more widely adapted testing on coagulation analyzers, most assays are based on an end point of immunofluorescence, immunoturbidometry, or chemiluminescence. The most common methodology is immunoturbidometry, in which a beam of light is passed through coated latex microparticles specific for D-dimer epitopes. When plasma is added, agglutination occurs, and absorbance is measured. The absorbance is directly proportional to the amount of D-dimer in the specimen.5

Point of Care
Rapid point-of-care assays for D-dimer are used to screen patients for venous thromboembolism. Several different methodologies are used. With hemagglutination, a drop of whole blood is mixed with hybrid, bispecific monoclonal antibodies that are specific for D-dimer and red blood cells. If D-dimer is present above the cut-off value, visible agglutination will occur. A newer rapid test using immunochromatography has higher sensitivity according to one study.8 Additional POC methodologies include single-use devices that use fluorescent-labelled monoclonal antibodies and sandwich immunoassays.

Reporting D-dimers
Two different units have been used in the reporting of D-dimer levels: fibrinogen equivalent units (FEU) and D-dimer units (DDU).¹ A DDU is based on the amount of D-dimer fragment present. Fibrinogen equivalent units represent the concentration of fibrin degradation products in relation to the mass of fibrinogen from which they were derived. As a result, the mass of a D-dimer fragment in a FEU is twice the mass in a DDU (2 DDU = 1 FEU).⁹ For example, D-dimer assays that use FEUs may have a cutoff of 0-0.500 mg/L FEU (0-500 µg/L FEU), while an equivalent cutoff for assays that use DDU would be 0-0.250 mg/L DDU (0-250 µg/L DDU).

The cutoff value for a D-dimer test is the value at which the test can rule out the presence of a clot. Any level below the cutoff value means the patient does not have a blood clot; anything above the cutoff value
may mean the patient has a clot, but it must be confirmed with additional radiographic testing. Based on the type of study the manufacturer conducts to determine the cutoff value, the D-dimer can be used as an aid in diagnosis or to exclude a thrombotic condition. The clinical study conducted by the manufacturer may utilize one of the scoring systems and score patients for the likelihood of a venous thromboembolism. Patients can then be classified as having a low, moderate, or high pretest probability. The result of the D-dimer assay is compared with the result of an ultrasound and the absence or presence of a clot. A correlation of negative D-dimer results with negative radiography can support a claim to the US Food and Drug Administration that the test can be used to exclude the presence of a clot. This exclusionary claim allows clinicians to use the negative D-dimer results to exclude the presence of a deep vein thrombosis or pulmonary embolism.9

There are several challenges involved in reporting D-dimers. Laboratories must provide a reference range for the D-dimer. D-dimers are detectable in low levels in healthy individuals due to the process in which small amounts of fibrinogen are converted to fibrin physiologically.9 The normal range of the D-dimer test may exceed the value of the cutoff, which can add to the confusion of interpreting the D-dimer result. Other problems with this assay are that reporting units are not standardized and many laboratories fail to clearly identify what units they are using. D-dimer levels can be reported in ng/mL, µg/mL, mg/L, and µg/L, with or without FEU or DDU. One of the largest problems is there is no universal calibrator to standardize D-dimer concentrations across assay types.10

Age-Adjusted D-dimers
D-dimer levels increase with age. A study using healthy individuals revealed that in the upper 95th percentile, D-dimer levels were 2.5 times greater in people older than 70 years than in those younger than 50 years.11 As a result of this, several studies have looked at having an age-adjusted D-dimer cutoff when ruling out thrombosis in patients older than 50 years. This age-adjusted D-dimer cutoff is calculated by using the patient’s age and multiplying by ten when using FEUs and multiplying the age by five when using DDUs. For example, if the patient is 70 years old, the upper limit of normal for that age will be 700 µg/L FEU; if the units are DDU, the upper limit will be 350 µg/L DDU.

Conversely, the concentrations of the D-dimer are lower in young healthy subjects. A study demonstrated that using a fixed threshold of 0.250 mg/L FEU resulted in improved sensitivity for both proximal and isolated distal deep vein thrombosis in patients under age 60 with low to moderate pretest probability. This decreased the rate of false-negative results.12

Using age-adjusted D-dimer cutoffs can increase the specificity of D-dimer testing for each age group, reduce the number of false-positive tests, and prevent unnecessary testing.
Conclusion

The D-dimer test is a sensitive but nonspecific assay for detecting thrombotic conditions. The validity of the test lies in its negative predictive value when used with a scoring system to determine its pretest probability. High D-dimer levels indicate occult disease and warrant additional investigation. With the introduction of testing using monoclonal antibodies, the test has become more sensitive. Laboratory instrumentation is now able to perform rapid testing by immunoturbidometric methods. Although there are many issues with this test, including different reporting units and not having a universal calibrator to standardize testing, the implementation of age-specific reference ranges may help prevent false-negative results and well as additional unnecessary and expensive testing.

References


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