EDUCATIONAL COMMENTARY – STRAIGHT TALK ABOUT THE D-DIMER

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Learning Objectives
Upon completion of this exercise, the participant will be able to:

• List and describe the characteristics of the six D-dimer method categories.
• Define “cut point” and describe how it is utilized in the clinical decision-making process for evaluating possible venous thrombotic events.

D-dimer testing is a confusing subject for the following reasons. The D-dimer is an epitope on fragments of fibrin digestion that vary in size and shape. Over the years, the test has reversed roles in the world of hemostasis. Formerly used in bleeding evaluations, it is now applied to clot detection. It is detected and measured by a number of different methods, using different detection strategies, antibodies, and reporting units. Formerly, a positive result was considered significant. Today a result is significant only when it is negative, and even the term “negative” is confusing. In simple agglutination tests, negative means negative. But for the quantitative tests, negative means beneath a chosen cut point, rather than within the reference range determined by normal population studies. The D-dimer’s role in the diagnostic tool kit continues to be debated.

What You are Testing
At the end of the clotting cascade, fibrin monomer is generated when thrombin removes the fibrinopeptides from fibrinogen. The fibrin monomers polymerize to form fibrin strands and these are crosslinked by the action of activated factor XIII. **Figure 1** shows the often-used model of polymerization and crosslinking action. It is factor XIII that generates the D-dimer by linking the D regions of the fibrin molecules. On digestion by plasmin, the bonds formed by factor XIII are not broken, and fragments of the fibrin polymer are released to the circulation. These fragments—oligomers, meaning few identical units—contain the D-dimer antigen that is recognized by the monoclonal D-dimer antibodies. **Figure 2** (see next page) illustrates the digestion points and the resulting fragments containing the D-dimer epitope.
Detecting Bleeding Disorders

In the mid 1980s, the D-dimer test was proposed and adopted as an improvement to existing latex agglutination tests for fibrin degradation products (FDP). Early FDP tests were used to detect complex acquired bleeding disorders including disseminated intravascular coagulation (DIC). These tests relied on antibodies to the D and E regions of the fibrinogen molecule, and positive results were thought to be a significant indicator of DIC. Special, clot-promoting collection tubes were provided to ensure that all fibrinogen was removed from the serum to prevent false-positive findings.

The D-dimer tests were specific for crosslinked fibrin, eliminating the cross-reactivity of fibrinogen, and allowed for testing on the same plasma sample as other coagulation tests. The D-dimer was initially seen as an improved FDP test to be used to define DIC and other acquired bleeding disorders.

But positive findings for D-dimer offer limited information due to the sensitivity of the test and the number of comorbid conditions. D-dimer is reported to be elevated in many normal conditions (e.g., cigarette smoking, elderly, functionally impaired, postoperative, pregnant, and black population). The list of pathologic states resulting in positive findings is also very long and includes other complex bleeding scenarios. One study of hospitalized patients showed that two-thirds of people who were significantly ill were positive for D-dimer in the absence of DIC. Therefore, the test has little usefulness when applied to hospitalized patients. When the result is negative, it can be used to rule out disorders.

Detecting Thromboses

In the late 1980s and early 1990s, the quantitative enzyme-linked immunosorbent assay (ELISA) for D-dimer was applied to detect venous thrombotic events in the emergency department. It was found to have poor specificity for venous thrombosis when positive but was found to be useful as a negative predictor. That is, if the level of D-dimer was low (negative), one could be reasonably sure that the
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patient did not have a clot and might not require expensive and time-consuming imaging techniques to rule out thrombosis. Studies ultimately determined that the test alone could not rule out thrombosis but when used with a pretest clinical evaluation (e.g., Wells score) it could be quite effective.

The Wells evaluation for deep vein thrombosis (DVT) includes questions on: recent history of cancer treatment; recent surgery that required general anesthesia; lower extremity weakness, paralysis, or immobilization; tenderness and swelling of the leg and calf; previously documented DVT; and likelihood of other diagnoses. In practice, the answers to these questions are assigned point values, and the evaluation is totaled. A final score of 2 or higher indicates that DVT is likely, and scans will be performed regardless of the D-dimer result. A score of <2 indicates that DVT is unlikely; it can be ruled out with a negative D-dimer test, but if the D-dimer result is positive, scans will be necessary to rule out DVT. This strategy is predicted to reduce the overall cost of DVT diagnosis. But the effectiveness is predicted from studies using the time-consuming, batch-oriented ELISA test methods. These are of limited practical use in the emergency department where time is of the essence, and other, rapid D-dimer methods were considered by many different investigators.

Different Tests, Methods, Units, and Opinions

In May 2004, Heim and co-workers completed an analysis of 23 published studies of D-dimer use in evaluating lower-extremity DVT. The studies utilized 21 different assay/manufacturer combinations that broke down into six different method categories. Qualitative and quantitative tests were used. The quantitative tests used different reporting units (either D-dimer units [D-DU] or fibrinogen equivalency units [FEU]). While some latex agglutination tests used the same antibody, the quantitative tests used different antibodies. Cut points—the point at which the test is presumed positive for purposes of clot detection—varied from as high as 1,000 µg/L to as low as 40 µg/L.

Heim analyzed methodology and assay performance characteristics including “negative predictive value” and concluded that no one single method was best. He found that three categories of tests—second-generation latex, membrane ELISA, and automated rapid enzyme-linked fluorescent assay (ELFA)—were quantitative, sufficiently rapid, and had high negative-predictive value. Heim and colleagues stressed that test performance in the analyzed clinical studies may have had more to do with patient selection criteria than with actual test characteristics. The evidence continues to suggest that the D-dimer alone cannot be used to rule out venous thrombotic events and must be used with clinical evaluation criteria.

The Future

Many institutions have embraced the strategy of using D-dimer results within defined clinical evaluation protocols in an effort to save time and money. Others question the efficacy of these strategies, predicting that D-dimer testing will ultimately add to costs and delays. Critics suggest that the increased ease of
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testing will result in inappropriate test utilization, increasing the number of false-positive findings. These positive findings will then require an increased number of unnecessary and expensive scanning procedures.

Meanwhile, in August 2006, a United States patent was granted for the use of humanized D-dimer antibody as “imaging payload agents” that would deliver radiopharmaceuticals or other traditional contrast agents to the clot. These payload agents would presumably enhance imaging in the deep veins and pulmonary vasculature. Data from future studies might once again change our perception of the D-dimer test. It seems that only time and more investigation will tell the whole story of the D-dimer’s usefulness in the world of hemostasis.

<table>
<thead>
<tr>
<th>Assay Category</th>
<th>Type of Detection</th>
<th>Output</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>First-Generation latex agglutination</td>
<td>Observer (agglutination)</td>
<td>Qualitative / semi-quantitative</td>
<td>Rapid and easy to perform</td>
<td>Insensitive to low levels Observer-dependent variation Lower NPV than other methods</td>
</tr>
<tr>
<td>Second-Generation latex agglutination</td>
<td>Analyzer (turbidimetric)</td>
<td>Quantitative</td>
<td>Rapid and sensitive No batching Comparable to ELISA microplate in studies for NPV</td>
<td>Many studies report NPV &lt;95%</td>
</tr>
<tr>
<td>Membrane ELISA</td>
<td>Observer (color change)</td>
<td>Qualitative / semi-quantitative</td>
<td>Rapid</td>
<td>Lower NPV reported by some studies</td>
</tr>
<tr>
<td></td>
<td>Analyzer (Reflectometer) (color change)</td>
<td>Quantitative</td>
<td>Rapid No batching Comparable to ELISA microplate in studies for NPV</td>
<td>Many studies report NPV &lt;95%</td>
</tr>
<tr>
<td>Erythrocyte agglutination</td>
<td>Observer (agglutination)</td>
<td>Qualitative / semi-quantitative</td>
<td>Rapid and used as POCT (performed on whole blood)</td>
<td>Observer-dependent variation NPV &lt;95% reported by most studies</td>
</tr>
<tr>
<td>Automated rapid ELFA</td>
<td>Analyzer (enzyme-linked fluorescence)</td>
<td>Quantitative</td>
<td>Rapid No batching Comparable to ELISA microplate in studies for NPV</td>
<td>Some studies report NPV &lt;95%</td>
</tr>
<tr>
<td>Microplate ELISA</td>
<td>Analyzer (color change)</td>
<td>Quantitative</td>
<td>Gold standard for NPV</td>
<td>Slow (2-4 hours) Requires batching Many studies report NPV &lt;95%</td>
</tr>
</tbody>
</table>

NPV indicates negative predictive value; ELISA, enzyme-linked immunosorbent assay; POCT, point-of-care testing; ELFA, enzyme-linked fluorescent assay.
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**Glossary**
Crosslink: a chemical bond between different chains of atoms in a polymer or other complex molecule (In case of fibrin polymer, these bonds are covalent bonds.)

Dimer: a molecule or molecular complex consisting of two identical molecules linked together

Epitope: the part of an antigen molecule to which an antibody attaches itself (It is also called the antigenic determinant.)

Negative Predictive Value: the percent of the time that the patient was truly healthy, when the test was found negative (Remember that in successful studies, the test is only used when the clinical evaluation indicates a low probability of thrombosis.)

Oligomer: a polymer whose molecules consist of relatively few repeating units

Plasmin: this enzyme destroys blood clots by attacking fibrin (It is derived from plasminogen.)

Polymer: a substance that has a molecular structure consisting chiefly or entirely of a large number of similar units bonded together

**Suggested Reading**


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