

## **Laboratory Markers in the Diagnosis of Venous Thromboembolism**

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## **Abstract**

Selected blood tests may be useful in the diagnosis of venous thromboembolism (VTE), or in the identification of a congenital or acquired defect associated with the development of VTE. Several studies have shown the D-dimer assay to have a high negative predictive value but poor specificity when used in the detection of VTE. Yet in the emergency room setting, the D-dimer test may be useful if a detailed risk factor analysis for each patient is included in the diagnosis. The presence of such genetic thrombophilia markers as factor V Leiden, prothrombin 20210A mutation, and antiphospholipid antibodies significantly increases a patient's risk of a thrombotic event. The relative risk of thrombosis in factor V heterozygotes is at least 3 times higher than in the general population, while the increased risk of thrombosis in homozygotes is estimated to be 50- to 80-fold greater than in those without the defect. Thromboembolic events are reported in approximately one-third of antiphospholipid-positive patients. Other markers such as hyperhomocysteinemia and deficiencies of antithrombin, protein C, or protein S, when combined with the previous mutations, significantly increase a patient's risk of a thrombotic event. We feel that it is important to identify these ultra-high-risk patients in order to provide adequate counseling about the risk of thrombosis before elective surgical procedures. Often, lifelong anticoagulation may be needed for these patients, and family members may need testing before taking birth control pills or hormonal replacement.

**Key words:** thrombosis, genetic markers [**if unacceptable, use genetics**], diagnosis, D-dimer, factor V, prothrombin 20210A, patient counseling [**latter four, if acceptable**]

## **Introduction**

The purpose of this brief review is to discuss the value of selected blood tests that may be useful in the diagnosis of venous thromboembolism (VTE), or in the identification of a congenital or acquired defect associated with the development of VTE. It is extremely important in today's cost-conscious world to make sure that each test that is ordered will be clinically useful in the management of the patient. This approach is emphasized in this review.

## **D-dimer Assay**

This assay involves measuring D-dimer, which is a fibrin-specific degradation product that detects cross-linked fibrin resulting from endogenous fibrinolysis and hence deep vein thrombosis (DVT). Several studies have shown that this assay has a high negative predictive value and is a relatively sensitive but nonspecific marker for DVT.<sup>1-3</sup> Brotman et al evaluated the utility and limitations of D-dimer testing for evaluation of VTE in hospitalized patients.<sup>4</sup> Four different methods of D-dimer testing were used in this study. D-dimer testing had little or no utility in distinguishing patients with thrombosis from those without thrombosis in patients who had been hospitalized for more than 3 days, were older than 60 years, or had C-reactive protein levels in the highest quartile. In unselected patients, D-dimer testing had limited clinical utility due to its poor specificity. The use of the D-dimer assay in an emergency room setting, on the other hand, is appealing since often it is not possible to get definitive testing for venous thrombosis. Efforts to find new screening methods are prompted by the high incidence of negative scans in patients referred to the vascular laboratory for venous duplex scan examination. In our vascular laboratory, less than 20% of all patients seen were found to have a DVT.<sup>5</sup>

Recently, a multicenter prospective cohort study examining the safety and effectiveness of a comprehensive strategy integrating a clinical risk assessment score, D-dimer testing, and compression ultrasonography in patients presenting to the emergency department with symptoms suggestive of DVT was published.<sup>6</sup> Through a published commentary on this article Oswald suggested that the diagnostic strategy employed in this study was successful.<sup>7</sup> Of the 882 individuals in whom proximal DVT was excluded during initial evaluation, only 4 patients (0.5%) were subsequently diagnosed with proximal DVT and none developed pulmonary emboli (PE) during 90 days of follow-up.<sup>6</sup> However, the results section of the original article reveals that an additional 5 patients with calf vein thrombosis were missed on the initial evaluation. In addition, Anderson used only compression ultrasonography, which would be unacceptable in many vascular laboratories in the United States. Examination of the entire leg, not just compression ultrasonography, is the standard of care in our institution and in many other hospitals across the country. All of the large prospective studies evaluating D-dimer assays in low-risk patients compared to duplex ultrasonography have been done using scans that only examine the leg down to the trifurcation below the popliteal fossa. This means that all clots below this level in the calf would have been missed in these studies. Future studies which compare total leg duplex ultrasonography to D-dimer assays should be conducted before final conclusions are made regarding the use of D-dimer alone as a screening tool for all leg thromboses.

Although preventing clinical PE is an important goal, equally important is the prevention of postthrombotic syndrome and recurrent thrombosis.<sup>8</sup> In a 30-year study, Heit observed that the incidence of sudden death following a recurrent PE is 20%.<sup>9</sup>

Prevention of these complications can be achieved only by diagnosing and treating all leg thrombi. In a study conducted by Anderson,<sup>6</sup> when the D-dimer assay alone was used to detect proximal DVT, the overall sensitivity was 82.6% with a specificity of 70%. These numbers are not good enough to justify using the D-dimer test alone for VTE diagnosis. In our emergency room we use the D-dimer assay in low-risk patients with leg symptoms when a duplex scan is not immediately available, such as in the middle of the night. If the D-dimer assay is negative, the patient is brought back in the morning for a duplex scan. If the assay is positive, the patient is given a single dose of low molecular weight heparin (LMWH) and asked to return in the morning for a duplex scan. Moderate- and high-risk patients receive a dose of LMWH until the scan is done the next day; D-dimer tests are not performed in these patients.

D-dimer levels have been successfully used by Wells and colleagues to rule out low-risk patients with suspected PE. The negative predictive value using a combination of a low clinical probability assessment score and negative D-dimer assay in outpatients suspected of having a PE was 99.5%, including long-term follow-up.<sup>10</sup> A more recent study by these authors concluded that DVT may be ruled out and ultrasonography safely omitted where there is low clinical probability of DVT and a negative D-dimer test.<sup>11</sup> Leclercq reported no long-term thromboembolic complications in 64 patients using a combination of the D-dimer assay and clinical probability strategies to rule out PE in the outpatient setting.<sup>12</sup> A study conducted by Dunn et al had the largest cohort of patients with suspected PE undergoing D-dimer assay in the emergency department. The high negative predictive value (99.6%) led these authors to conclude that a negative D-dimer Elisa assay can almost always exclude PE in the emergency department setting.<sup>13</sup> Further

studies are necessary in patients undergoing full leg duplex ultrasonography before adopting this method for venous thrombosis diagnosis. Screening methods emphasizing cost-effectiveness are limited in that even one mistake in diagnosis may result in a preventable death.

## **Thrombophilia**

The term ‘thrombophilia’ was introduced in 1965 by Egeberg to describe a tendency to develop venous thrombosis in a Norwegian family that was subsequently shown to have antithrombin deficiency.<sup>14</sup> Middeldorp et al suggested that thrombophilia is usually diagnosed on clinical grounds and among the more important features are thrombosis at a young age, recurrent thrombosis, heparin resistance, purpura fulminans, warfarin-induced skin necrosis, a family history of thrombosis, and thrombosis in an unusual site.

Thrombotic events during pregnancy are frequently seen in those with thrombophilia defects, which emphasizes the importance of taking a careful obstetrical history in all patients as part of their thrombosis risk-assessment profile. Obstetrical complications such as toxemia, stillbirth, placental insufficiency, and the hemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome may occur.<sup>15</sup> Pregnant patients developing VTE frequently harbor thrombophilia defects (Table 1).<sup>16</sup> Patients developing these problems should be screened for thrombophilia as they may suffer an increased incidence of thrombosis later in life following joint replacement or other major surgery compared to patients without these defects.

## **Factor V Leiden**

Activated protein C resistance (APC-R), first described in 1993,<sup>17</sup> is reported to be the most common cause of familial thrombophilia.<sup>18</sup> In the majority of these cases, the APC-R is due to a single mutation in the factor V gene, which is known as factor V Leiden. This defect, which is inherited as an autosomal dominant, renders factor V much more resistant to proteolytic degradation by activated protein C.<sup>19</sup> This phenomenon results in a hypercoagulable state and is seen in 3% to 7% of Caucasian populations, up to 50% in selected thrombophilic families, and in 20% of unselected venous thrombosis patients.<sup>20</sup>

Unpublished data from my own practice reveal that 17.6% of patients with a history of DVT tested positive for the Leiden defect. In my patients with a history of PE the incidence was 27.3%, in those with a family history of thrombosis it was 30.8%, and in those presenting with current deep venous thrombosis 36% had a positive Leiden mutation. The relative risk of thrombosis in heterozygotes is at least three times higher than in the general population, while the increased risk of thrombosis in homozygotes is estimated to be 50- to 80-fold greater than in those without the defect.<sup>21</sup> While only 2% of patients have this extreme form of the defect, serious discussions to inform the patient of the severity of the risk should be had prior to surgery.

### **Prothrombin 20210A Mutation**

The risk for venous thrombosis is also increased in patients who have the recently recognized mutation in the prothrombin gene (P20210A). This mutation results in elevated levels of prothrombin due to increased prothrombin synthesis, and is associated with a 3-fold increase in the risk for venous thrombosis. The prothrombin gene mutation is second in frequency to the Leiden mutation. Approximately 5.5% of patients with

venous thrombosis will harbor this disorder, and about 1.2% of individuals in the general population will test positive for the defect.<sup>22</sup> Others have suggested that this mutation is found in 5% to 15% of patients presenting with venous thrombosis and about 15% of patients being investigated for thrombophilia.<sup>23,24</sup> In my practice, those with a history of DVT had a 17.6% incidence of the defect, those with a history of PE had an 18.2% incidence, and those with current DVT had a 12% incidence of a positive defect.

### **Antiphospholipid Antibody Syndrome**

Antiphospholipid antibodies are a heterogeneous family of immunoglobulins that include, among others, lupus anticoagulants and anticardiolipin antibodies. Thromboembolic events are reported in approximately one-third of antiphospholipid-positive patients. These events include venous thrombosis, stroke, myocardial infarction, gangrene, recurrent pregnancy loss, and thrombocytopenia. Neurologic manifestations include single or recurrent cerebral infarcts, severe vascular headaches, transient ischemic attacks, and visual disturbances such as amaurosis fugax or retinal artery or vein occlusion. Recurrent strokes are more likely in patients with the antiphospholipid antibody syndrome and other risk factors such as cigarette smoking and hyperlipidemia.<sup>25</sup> The relationship between lupus anticoagulants and anticardiolipin antibodies and thrombosis has been thoroughly investigated, and laboratory and clinical criteria for defining the antiphospholipid syndrome have been published.<sup>26</sup> Recurrent fetal loss has a well-established association with the antiphospholipid syndrome.<sup>27</sup> The incidence of the antiphospholipid antibody syndrome in the Caucasian population is 2% to 3%. The risk of recurrent thrombosis in patients with antiphospholipid antibody syndrome is high, ranging from 22% to 69%.<sup>28</sup> When these patients have an additional prothrombotic

genetic risk factor, life-long anticoagulation may be necessary due to the increased thrombotic risk. These patients should be counseled before having elective surgery because of the degree of postoperative thrombotic risk. The most powerful and effective regime for prophylaxis should be used in these patients despite the fact that sometimes it may be associated with a slightly higher bleeding risk.<sup>29</sup>

## **Hyperhomocysteinemia**

The elevation of homocysteine levels in the blood has become a popular topic due to increasing associations between these elevations and the presence of arterial and/or venous thrombotic events. Such events include venous thrombosis, stroke, and myocardial infarctions. Prospective data investigating these relationships show conflicting results. Certain studies have suggested that elevated homocysteine levels roughly double the incidence of venous thrombosis,<sup>30</sup> Overall, however, there appears to be a weak positive association.<sup>31</sup>

Some clinicians feel it is important to test for this defect in those with stroke, myocardial infarction, DVT, and prior to elective operative procedures. When values are elevated, vitamin therapy can be used to lower these levels and, theoretically, provide protection against recurrent arterial or venous thrombotic events postoperatively. We prefer to measure homocysteine levels instead of testing for the gene that plays a role in the homocysteine metabolism known as methylene tetrahydrofolate reductase (MTHFR). Thirty percent of normal individuals have a heterozygous genetic defect without any increase in risk of thrombosis, and if the defect is homozygous, it is thought to contribute to venous thrombosis only if homocysteine levels are increased. The mechanism whereby homocysteine increases the incidence of vascular events including deep vein thrombosis

is thought to be related to endothelial damage, although more work needs to be done in this area.<sup>32</sup>

## **Deficiencies of Natural Coagulation Inhibitors**

Deficiencies of antithrombin, protein C, and its cofactor protein S were the first genetic causes of venous thrombosis to be discovered. These deficiencies are very uncommon, emerging in less than 1% of the population, although they are frequently discovered in those suffering from a thrombotic event.<sup>33,34</sup> A major clinical problem is that these defects can coexist with other genetic abnormalities such as the Leiden defect, resulting in an enormously increased risk for developing a venous thromboembolic event (Table 2).<sup>35</sup> This is another group of patients where special counseling is recommended during serious illness or prior to major operative procedures due to the very high incidence of VTE.

## **Additional Markers of Thrombophilia**

Reports have appeared implicating disorders of the fibrinolytic system in an increased risk for VTE, including thrombin activatable fibrinolysis factor (TAFI). Elevated levels of certain coagulation factors including factors II, VIII, IX, and XI have been associated with an increased risk of thrombosis. It has been postulated that 10% of the population may fit the definition of an elevated level. Little is known about these abnormalities but it is thought that they are a combination of congenital and acquired factors.<sup>36-40</sup>

## **Clinical Significance**

In patients with a history of thrombosis the question is often asked: Why test patients if the clinical course of action will not be different for those having a heterozygous Leiden

or other similar defect? Many feel that if a patient has additional risk factors such as surgery, immobilization, or serious medical illnesses, most physicians would treat these individuals like any other person with a history of thrombosis, by providing additional prophylaxis relative to those in the general population without a positive thrombotic history. Zwicker reminds us that patients with protein C deficiency and a heterozygous Leiden defect have a 73% incidence of a recurrent venous thrombosis, those with a Leiden defect and protein S deficiency have a 72% incidence of recurrent venous thrombosis, and those with an antithrombin deficiency and a Leiden defect have an incidence of recurrent venous thrombosis of 92%.<sup>35</sup> Unfortunately, these other markers of thrombophilia are not often tested for because they are very uncommon in the general population (1% to 7%).<sup>41</sup>

One can see that if there is a combination of defects present, the risks for VTE may be extremely high and probably requires special counseling of these patients and their families. The presence of additional risk factors in patients with a heterozygous Leiden defect such as pregnancy, oral contraceptives, hormonal replacement, surgery, cancer, age, or other serious medical disease markedly increases thrombotic risk.<sup>42</sup> Naturally emergent operative procedures for cancer, serious vascular, or other medical problems will need to be performed, as is the case in the general population. However, in individuals where the incidence of thrombosis is 50- to 80-fold greater than the incidence in the general population, patients and their families should be counseled regarding the thrombotic risks associated with surgery.

Some investigators conclude that patients with a heterozygous defect of both factor V Leiden and prothrombin 20210A should receive life-long anticoagulation to

prevent recurrent thrombosis.<sup>42</sup> A key question for those who do not agree with routine thrombophilia testing is: How else would individuals like the ones mentioned above with a double genetic defect or homozygous defect be identified? Failure to identify these patients prevents the identification of the true incidence of thromboembolism and eliminates the possibility of properly protecting and advising them prior to performance of elective operative procedures. Patients undergoing elective operative procedures, such as total joint replacement, should be offered the most powerful prophylaxis against thrombosis. Several anticoagulant options are available; those which have been shown in randomized clinical trials to result in the fewest venographically detected thrombi should be used.

It is prudent to measure serum homocysteine levels in patients with a history of stroke prior to elective surgical procedures. Patients with elevated values may be treated with vitamins B6, B12, and folic acid in order to reduce these levels and decrease the risk of VTE. Those suffering from arterial thrombosis should be tested for anticardiolipin antibodies and the presence of serum homocysteine. Other genetic markers have not been convincingly shown to be associated with arterial thrombosis.<sup>43</sup> In cases of thrombosis in unusual sites, most investigators would perform complete testing; a variety of thrombophilic defects have been observed in those with mesenteric, portal, cerebral, or retinal vein thrombosis.<sup>43</sup>

Careful and selective thrombophilia testing should be done in all patients where the results would affect their medical management or provide useful data for the health care of family members.



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