

Laboratory Diagnosis of Dysfibrinogenemia

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● **Dysfibrinogenemia is a coagulation disorder caused by a variety of structural abnormalities in the fibrinogen molecule that result in abnormal fibrinogen function. It can be inherited or acquired. The inherited form is associated with increased risk of bleeding, thrombosis, or both in the same patient or family. Traditionally, dysfibrinogenemia is diagnosed by abnormal tests of fibrin clot formation; the thrombin time and reptilase time are the screening tests, and the fibrinogen clotting activity–antigen ratio is the confirmatory test. The inherited form is diagnosed by demonstrating similar laboratory test abnormalities in family members, and if necessary by analysis of the fibrinogen protein or fibrinogen genes in the patient. The acquired form is diagnosed by demonstrating abnormal liver function tests and by ruling out dysfibrinogenemia in family members. This article reviews the laboratory testing of dysfibrinogenemia and presents an algorithm for sequential test selection that can be used for diagnosis.**

(*Arch Pathol Lab Med.* 2002;126:499–505)

Fibrinogen and its proteolytic cleavage product, fibrin, are critical plasma proteins with multiple functions in blood clotting. These functions include fibrin clot formation, factor XIIIa–mediated fibrin crosslinking, nonsubstrate thrombin binding, platelet aggregation, and fibrinolysis.^{1–3} A variety of structural abnormalities can occur in fibrinogen and can interfere with 1 or more of its hemostatic roles. Structurally abnormal fibrinogens are collectively termed *dysfibrinogenemias*. This article reviews the clinical features, pathophysiology, and laboratory testing of inherited and acquired dysfibrinogenemia.

INHERITED DYSFIBRINOGENEMIA

Nomenclature

Inherited dysfibrinogenemia is caused by mutations in the coding region of the fibrinogen A α , B β , or γ gene. Dysfibrinogenemias are named after the city where the patient lives, or after the city of the hospital where the patient was evaluated. Roman numerals are added after

the city name if there is more than 1 dysfibrinogen from the same city. The term *hypodysfibrinogenemia* is used when the inherited dysfibrinogen is associated with low fibrinogen antigen in plasma. Approximately 245 fibrinogen gene abnormalities with unique name designations have been reported to date. An online database of fibrinogen mutations that is periodically updated is accessible at http://www.geht.org/pages/database_ang.html.

Clinical Features

The prevalence of inherited dysfibrinogenemia among the general population is unknown; however, the prevalence among patients with a history of venous thrombosis is 0.8%.⁴ The pattern of inheritance is almost always autosomal dominant.⁵ Most patients have no history of bleeding or thrombosis (55%), while others develop bleeding (25%) or thrombosis (20%).⁶ Interestingly, 27% of patients who have had a thrombotic event also have a history of bleeding.⁴ Asymptomatic dysfibrinogenemia is usually brought to clinical attention because of an abnormal laboratory test (ie, thrombin time).

Bleeding manifestations include epistaxis, easy bruising, menorrhagia, hematomas, hemarthrosis, postoperative bleeding, postpartum bleeding, antepartum bleeding, and delayed wound healing.⁵ Most bleeding episodes are mild to moderate in severity. Thrombotic manifestations include lower extremity venous thrombosis, thrombophlebitis, pulmonary embolism, arterial thrombosis, and a combination of arterial and venous thrombosis.⁴

Pathophysiology

The pathophysiologic mechanism of bleeding in most cases appears to be defective fibrin clot formation. This defect is caused by impaired thrombin-mediated release of fibrinopeptide A and/or fibrinopeptide B from fibrinogen, as well as impaired fibrin monomer polymerization.⁵

The pathophysiologic basis of thrombosis (ie, thrombophilia) in dysfibrinogenemia has been reviewed.^{4,6–9} Two general mechanisms have been proposed. The first is impaired anticoagulant function. This impairment is caused by mutations that lead to defective thrombin binding to low-affinity nonsubstrate binding sites on fibrin. This defect presumably results in the release of thrombin into the circulation, leading to increased fibrin clot formation and a tendency toward pathologic thrombosis.

The second mechanism is impaired profibrinolytic function. This impairment is caused by mutations that lead to (1) defective binding of profibrinolytic proteins (tissue plasminogen activator, plasminogen) to fibrin or (2) resistance of fibrin to the digestive action of plasmin. These defects cause impaired fibrinolysis and a tendency toward

Accepted for publication November 28, 2001.

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Fibrinogen

Inherited Lesions Associated with Thrombophilia

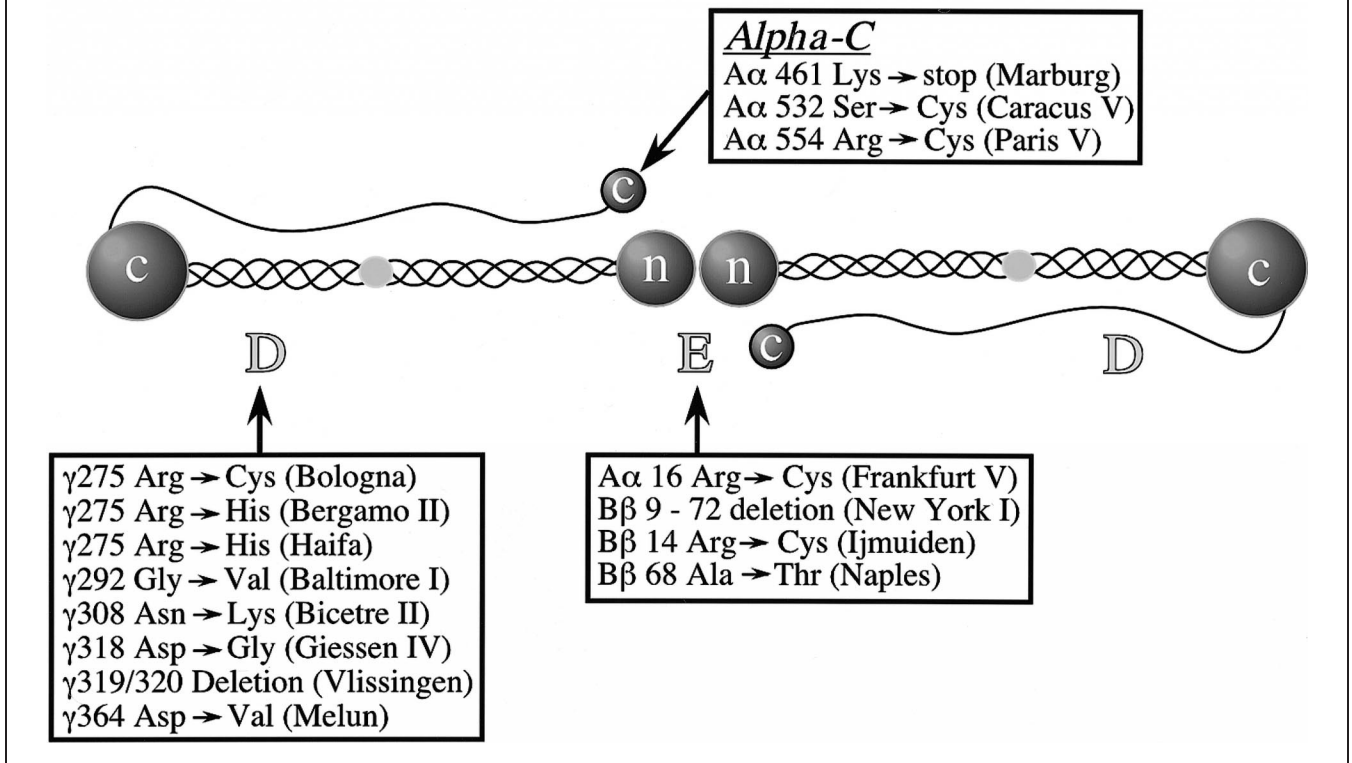


Figure 1. Diagram of the fibrinogen molecule and location of mutations associated with thrombophilia. Fibrinogen is composed of 2 half molecules, each containing 1 Aα, 1 Bβ, and 1 γ chain. Each half molecule associates at the amino terminus (n) to form the central E domain. Each half molecule also has a terminal D domain that contains the carboxy terminus of 1 Bβ and 1 γ chain (c), and a terminal alpha-C domain that contains the carboxy terminus of 1 Aα chain (c). Mutations that are most likely associated with thrombosis (data from Haverkate and Samama⁴) are shown for each domain. The name designation for each mutation is shown in parentheses.

pathologic thrombosis. The specific mutations that are most likely associated with thrombosis are shown in Figure 1.

ACQUIRED DYSFIBRINOGENEMIA

Clinical Features

Acquired dysfibrinogenemia is usually caused by disease of the liver or biliary tract. The specific disease associations include cirrhosis,¹⁰ chronic active liver disease,¹¹ acute liver failure,¹¹ acetaminophen overdose,¹² choledochal cyst of the bile duct,¹³ and miscellaneous causes of obstructive jaundice.¹¹ The prevalence of dysfibrinogenemia is higher in patients with liver disease (76% to 86%) than in those with obstructive jaundice (8%).¹¹ One case report associated acquired dysfibrinogenemia with mithramycin administration.¹⁴

Acquired dysfibrinogenemia is also a paraneoplastic marker of certain malignancies, such as hepatoma and renal cell carcinoma.¹⁵⁻¹⁸ The dysfibrinogen is usually present at the time of diagnosis and disappears when the tu-

mor is in remission. The dysfibrinogen can also reappear after tumor relapse.

Pathophysiology

The mechanism of acquired dysfibrinogenemia involves increased sialylation of carbohydrate side chains of the fibrinogen molecule.^{16,19,20} The additional sialic acid residues are localized to the Bβ and γ chains.²⁰ This chemical change increases the net negative charge of fibrinogen, which promotes charge repulsion between fibrin monomers and decreases the rate of fibrin polymerization.²¹

In vitro data suggest that the mechanism of cancer-associated dysfibrinogenemia involves the synthesis and secretion of abnormal fibrinogen by tumor cells. Several tumor cell lines are capable of fibrinogen synthesis, including HepG2 human hepatoma,²² ME-180 human uterine cervix carcinoma,²³ and MCF-7 human breast carcinoma.²⁴ In the case of MCF-7 cells, the fibrinogen Bβ chain is missing an amino terminal peptide fragment, and the final molecule is defective in assembly.

It is unknown if acquired dysfibrinogenemia is an independent risk factor for bleeding or thrombosis. Only rarely has bleeding been reported.¹⁴ This is a difficult issue to resolve, in part because patients with hepatobiliary disease develop a variety of hemostatic abnormalities that could contribute to bleeding or thrombotic risk.²⁵

Laboratory Diagnosis

A laboratory testing algorithm for the diagnosis of dysfibrinogenemia is shown in Figure 2. It is based in part on criteria established by the International Society of Thrombosis and Haemostasis (Subcommittee on Fibrinogen; Scientific and Standardization Committee).⁴ Testing begins with sensitive but nonspecific screening tests and then proceeds to more specific confirmatory tests. This algorithm can be used in patients with bleeding or thrombosis not explained by more common acquired or inherited entities.

SCREENING TESTS

Thrombin Time

The primary screening test is the thrombin time. The thrombin time measures the rate of fibrin clot formation after the addition of a standard concentration of thrombin to citrated plasma.²⁶ Thrombin mediates fibrin clot formation by cleaving 2 molecules each of fibrinopeptide A and fibrinopeptide B from fibrinogen, producing fibrin monomer. Fibrin monomer then polymerizes in a linear and lateral fashion to form the fibrin clot. Dysfibrinogens prolong the thrombin time by inhibiting fibrinopeptide A and/or B release, or by inhibiting fibrin monomer polymerization.⁴ Only 1 dysfibrinogen has been reported to shorten the thrombin time.⁶

The sensitivity of the thrombin time in dysfibrinogenemia is not well characterized. The specificity of the thrombin time is poor (Table). Some of the conditions listed in the Table, such as low or high plasma fibrinogen concentration, are common in hospitalized patients and need to be considered when interpreting abnormal results. If heparin contamination is a possibility, then protamine or heparinase can be added to plasma to neutralize heparin's effect on the thrombin time.

Reptilase Time

An alternative screening test is the reptilase time.⁴ This test measures the rate of fibrin clot formation after the addition of reptilase to citrated plasma. Reptilase is a snake venom enzyme obtained from *Bothrops jararaca* or *Bothrops atrox*. Its mechanism of action differs from thrombin in that only fibrinopeptide A, and not fibrinopeptide B, is cleaved from fibrinogen.^{27,28}

Dysfibrinogens prolong the reptilase time by inhibiting either fibrinopeptide A release or fibrin monomer polymerization. One advantage of this test is that it is unaffected by heparin. The diagnostic sensitivity of the reptilase time is unknown. Some authorities recommend that both the thrombin time and reptilase time be used for screening of dysfibrinogenemia.²⁹

CONFIRMATORY TEST

Fibrinogen Activity–Antigen Ratio

If either the thrombin time or the reptilase time is positive for dysfibrinogen, then the confirmatory test is the fibrinogen activity–antigen ratio.^{4,30} A fibrinogen activity–

antigen ratio that is below the reference range is considered positive for the diagnosis. The sensitivity and specificity values of this test are not well characterized. Its use is based primarily on tradition.

The fibrinogen activity is based on the measurement of fibrin polymerization function and is most commonly determined by the Clauss method. The Clauss method measures the rate of clot formation after adding a high concentration of thrombin to citrated plasma.³¹ The fibrinogen activity of the patient sample is derived from a standard curve relating the clotting time to plasma standards of known fibrinogen activity.

An alternative approach for measuring fibrinogen activity is the prothrombin time–based method.³² The fibrinogen activity of the patient sample is determined from a standard curve relating the maximum turbidity of the clot (derived from the prothrombin time polymerization curve) to plasma standards of known fibrinogen activity. This test has not been validated for the diagnosis of dysfibrinogenemia, so it cannot be recommended at this time.

The fibrinogen antigen concentration can be determined by immunologic (enzyme-linked immunosorbent assay, radial immunodiffusion),^{30,33} precipitation (heat, sulphite),^{33–35} and thrombin clotting methods.^{33,34} Some of these approaches have sources of error that need to be taken into consideration. For example, fibrin degradation products can cause falsely elevated fibrinogen antigen values when using sulphite precipitation,³⁵ thrombin clotting,³⁵ and some immunologic methods.³³ Falsely decreased fibrinogen antigen values can occur with the heat precipitation method in the presence of fibrin degradation products,³³ cryoglobulins,³⁴ and high plasma viscosity.³⁴

There are some preanalytic and analytic issues to be aware of regarding the fibrinogen activity–antigen ratio. First, the activity and antigen assays should be performed on the same sample because fibrinogen levels can fluctuate from day to day.^{36,37} Second, the activity–antigen ratio should be interpreted against a method-specific reference range because fibrinogen antigen and activity levels are method dependent.³⁸ These variables can be controlled if a single laboratory performs the activity and antigen assays on the same sample and then reports the ratio result along with a method-specific reference range.

Thrombin Time 1:1 Mixing Study

Some cases of acquired dysfibrinogenemia have a normal fibrinogen activity–antigen ratio, despite having a prolonged thrombin time.^{14,39} To document that the cause of the abnormal thrombin time is due to a fibrinogen defect, one can perform a modification of the thrombin time 1:1 mixing study.¹⁴ The modified test is performed in 2 parts. In part 1, a thrombin time is determined on a 1:1 mix of patient plasma and pooled normal plasma. In part 2, a thrombin time is determined on a 1:1 mix of defibrinated patient plasma and pooled normal plasma. The patient plasma is defibrinated by heating at 56°C for 10 minutes. The control for part 2 is a 1:1 mix of buffered saline and pooled normal plasma.

In acquired dysfibrinogenemia, the thrombin time 1:1 mix is prolonged in part 1, and normal (corrected) in part 2. The basis for a prolonged thrombin time in part 1 is that the dysfibrinogen inhibits fibrin clot assembly of normal fibrinogen. The basis for a corrected thrombin time in part 2 is that the inhibitory dysfibrinogen has been re-

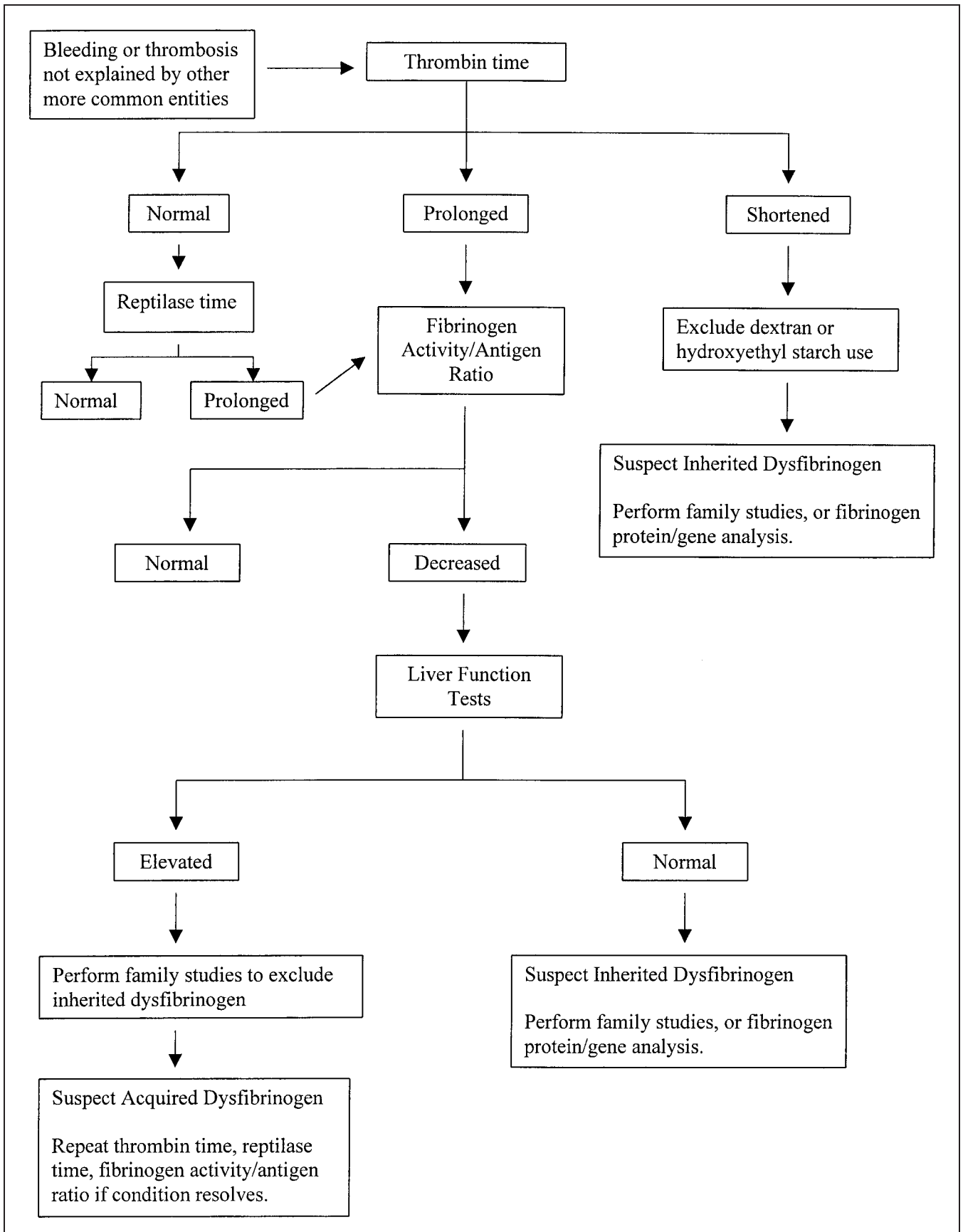


Figure 2. Laboratory testing algorithm for diagnosis of dysfibrinogenemia.

Effect of Various Disorders and Substances on Thrombin Time

Disorder/Substance	Thrombin Time	Mechanism of Effect
Amyloidosis, AL type ⁴²	Prolonged	Inhibition of fibrin clot formation; identity of inhibitor unknown
Drugs		
Argatroban ⁴³	Prolonged	Inhibition of thrombin
Bovine thrombin, topical ⁴⁴⁻⁴⁶	Prolonged	Development of alloantibodies against bovine thrombin; may crossreact with human thrombin
Dextran ^{47,48}	Shortened	Increased fibrin monomer polymerization
Heparin ⁴⁹	Prolonged	Inhibition of thrombin
Hirudin, hirugen, hirulog ^{50,51}	Prolonged	Inhibition of thrombin
Hydroxyethyl starch ⁵²	Shortened	Unknown
Tissue plasminogen activator ⁵³	Prolonged	Inhibition of fibrin monomer polymerization due to production of fibrin degradation products
Urokinase ⁵³	Prolonged	Inhibition of fibrin monomer polymerization due to production of fibrin degradation products
Dysfibrinogenemia		
Inherited⁶	Prolonged	Inhibition of fibrinopeptide A release; inhibition of fibrinopeptide B release; inhibition of fibrin monomer polymerization
	Shortened	Unknown
Acquired ²¹	Prolonged	Inhibition of fibrin monomer polymerization
Fibrin degradation products ⁵⁴⁻⁵⁶	Prolonged	Inhibition of fibrin monomer polymerization
Fibrinogen		
High plasma levels ⁵⁷	Prolonged	Unknown
Low plasma levels ⁵⁸	Prolonged	Decreased fibrinogen substrate
Monoclonal immunoglobulins ⁵⁹⁻⁶¹	Prolonged	Inhibition of thrombin; inhibition of fibrin monomer polymerization
Radiocontrast agents⁶²	Prolonged	Inhibition of fibrin monomer generation and polymerization
Diatrizoate, ioxaglate, ioxithalamate, iopamidol, metrizamide		

moved by heat precipitation. The sensitivity, specificity, and predictive values of this test are unknown.

Fibrinogen Electrophoresis

One-dimensional and 2-dimensional gel electrophoresis are useful in confirming an inherited dysfibrinogen if the mutation alters the apparent molecular weight or isoelectric point of the A α , B β , or γ chain.^{40,41} One-dimensional electrophoresis separates polypeptides based on apparent molecular weight. Two-dimensional electrophoresis separates polypeptides based on apparent molecular weight in the first dimension and isoelectric point in the second dimension. These analyses can be performed either on purified fibrinogen or on plasma if the electrophoresis is followed by immunoblotting with fibrinogen-specific antibodies.

An example in which electrophoresis has been used is fibrinogen Osaka V (γ 375: Gly \rightarrow Arg), which causes a defect in high-affinity calcium binding. In the presence of calcium, fibrinogen Osaka V has a slower migrating γ chain compared to the normal γ chain on 1-dimensional electrophoresis. This difference in protein migration rate allows detection of both the heterozygous and homozygous states.

Distinguishing the Acquired and Inherited Forms

Acquired dysfibrinogenemia is typically diagnosed by demonstrating (1) abnormal laboratory tests of hepatocellular or cholestatic function (ie, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, γ -glutamyltransferase, direct bilirubin) and (2) normal throm-

bin time and/or reptilase time in family members. The diagnosis can be further substantiated by repeat testing after the condition resolves to show that fibrinogen function returns to normal.¹⁷ The possibility of an inherited defect should be considered if fibrinogen dysfunction persists after resolution of the hepatobiliary disease.

If liver function tests are normal, then the dysfibrinogenemia is most likely inherited. The inherited nature of the disease can be confirmed by demonstrating a similar abnormality in a family member, detecting a protein abnormality by fibrinogen electrophoresis, or by identifying a mutation within 1 of the 3 fibrinogen genes by molecular genetic analysis.⁴ Fibrinogen electrophoresis and molecular genetic analysis of fibrinogen genes are beyond the capability of most clinical laboratories, so this testing is generally referred to laboratories that have a special interest in dysfibrinogenemia.

COMMENT

Dysfibrinogenemia is a rare but important cause of inherited bleeding and thrombosis. Bleeding is due to fibrinogen mutations that inhibit fibrinopeptide release or fibrin monomer polymerization. Thrombosis can be due to mutations that inhibit either the anticoagulant or profibrinolytic functions of fibrin. In many cases, the pathogenesis of thrombosis is unknown.

Laboratory testing is critical for accurate diagnosis. The thrombin time and reptilase time are used for screening suspected cases. An important caveat is that these tests are susceptible to a high rate of false positivity due to their nonspecificity. The fibrinogen activity-antigen ratio is use-

ful for confirmation in most cases. Liver function testing and family studies can confirm an acquired etiology. More complex testing by specialized laboratories, such as fibrinogen protein electrophoresis and fibrinogen gene analysis, can provide a more definitive diagnosis of an inherited etiology.

This article was prepared as part of a College of American Pathologists Coagulation Resource Committee project focusing on the laboratory evaluation of coagulation disorders.

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