Laboratory testing for fibrinogen abnormalities

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Fibrinogen is essential for the formation of a fibrin clot. Acquired and congenital disorders of fibrinogen may result in decreased concentration or altered function of fibrinogen, often leading to an increased risk of bleeding. Routine coagulation testing and specialized laboratory investigations can guide diagnosis in patients suspected of having a fibrinogen abnormality. This article summarizes the types of laboratory assays that are used to assess fibrinogen disorders, and key abnormalities found in different types of fibrinogen disorders. Am. J. Hematol. 83:928–931, 2008. © 2008 Wiley-Liss, Inc.

Introduction

Fibrinogen is a 340 kDa soluble glycoprotein, synthesized in the liver, that is a major constituent of normal plasma [1,2]. Fibrinogen circulates as a dimer of its three polypeptide chains (alpha, beta, gamma) at plasma concentrations of 150–400 mg/dl (1.5–4.0 g/l), with a half life of ~4 days [1,2]. Thrombin cleavage removes fibrinopeptides A and B (FPA and FPB) from fibrinogen, which triggers fibrin polymerization. In vitro, the cleavage of fibrinogen by reptilase (a snake venom) removes FPA but not FPB, and this also triggers fibrin polymerization [1,2].

The function and quantity of plasma fibrinogen can be altered by both inherited and acquired disorders [1,3–13]. Increased levels of fibrinogen can be seen in pregnancy [3,4], acute or early liver disease [5], and many acute illnesses [6–8]. Acquired fibrinogen deficiencies can be due to consumptive states (e.g. placental abruption), fibrinolytic therapy [9], hemodilution and severe liver disease [10]. The magnitude of congenital fibrinogen deficiencies range from severe (absent fibrinogen or afibrinogenemia) to moderate and mild (hypofibrinogenemia) [11,12]. In some individuals, the inherited defect in fibrinogen leads to production of a dysfunctional molecule (dysfibrinogenemia), that can be associated with bleeding or thrombosis, or both problems [13]. Artifactually low levels of fibrinogen are seen with samples that contain fibrin clots due to improper collection. Neonatal fibrinogen, and the fibrinogen synthesized in some individuals with liver disease, has an altered content of carbohydrate [14,15] that can mimic dysfibrinogenemia in certain laboratory tests. Elevated fibrinogen due to an acute phase reaction can be associated with prolonged reptilase times and less commonly prolonged thrombin times, possibly due to increased sialic acid and/or phosphorous content [16].

The prevalence of fibrinogen disorders is uncertain, and some fibrinogen deficiencies and dysfunction are not detected by standard coagulation screening tests [such as the prothrombin time (PT) and activated partial thromboplastin time (APTT), Table I], which are often normal provided some fibrinogen is available for clot formation. In this report, we review the methods and findings for commonly used laboratory tests for fibrinogen disorders, and some coagulation abnormalities that could potentially be confused with a fibrinogen abnormality.

Coagulation Tests Used to Assess Fibrinogen

Table I summarizes the principles of coagulation tests that can be abnormal with deficient or dysfunctional fibrinogen. The PT, which is often reported as the International Normalized Ratio (INR), can detect deficiencies of factors VII, X, V, and prothrombin, and mainly severe fibrinogen deficiencies, in a single step [17]. Most PT reagents contain a heparin neutralizer which helps distinguish fibrinogen abnormalities from heparin. Like the PT, the APTT detects mainly severe fibrinogen deficiencies or dysfunction (see Table II). The APTT can also detect deficiencies of the contact factors prekallikrein, high molecular weight kininogen and factor XII, and of factors XI, IX, VIII, X, V, and prothrombin [1,19].

The thrombin clotting time (or thrombin time) evaluates the conversion of fibrinogen to fibrin, and its polymerization, after the addition of an exogenous source of thrombin [1]. This assay, although not standardized, tends to be more sensitive to fibrinogen deficiency and dysfunction than the PT and APTT (Table II). Although thrombin cleaves fibrinogen to liberate FPA and FPB, the snake venom reptilase cleaves only FPA from fibrinogen [1]. Like the thrombin clotting time, the reptilase time is sensitive to fibrinogen deficiency and dysfunction, but unlike the thrombin time, it is not prolonged by heparin or by FPB cleavage defects (see Table II). It is often helpful to distinguish fibrinogen deficiency from dysfunction by performing the thrombin clotting or reptilase times using a diluted sample of pooled or control platelet poor plasma adjusted to the same final fibrinogen concentration as the patient's sample.

There are numerous laboratory methods available for measurement of fibrinogen (Tables I, II). One of the commonly used methods is the assay of functional fibrinogen by the Clauss method, which involves adding a standard and often high concentration of thrombin to platelet poor plasma [20]. The fibrinogen concentration of unknown samples is determined from a reference curve. The reference curve is prepared by testing dilutions of standardized reference platelet poor plasma. This method is easily adapted

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TABLE I. Methods of Laboratory Testing for Fibrinogen Abnormalities

	Principle	Utility	Limitations	Ref.
Prothrombin time (PT)/ International normalized ratio (INR)	Test of extrinsic and common pathways of coagulation. Reagent contains phospholipid, calcium ion, and tissue factor that trigger fibrin clot formation when added to citrated plasma. Time to clot formation is timed.	Prolonged by afibrinogenemia (no clot) and severe hypo- or dysfibrinogenemia.	Poor sensitivity to mild fibrinogen deficiency or dysfunction.	[17]
Activated partial thromboplastin time (APTT)	Test of the intrinsic and common pathways of coagulation. Reagent contains a contact activator and phospholipid. The reagent is incubated with citrated plasma, followed by addition of calcium. Time to clot formation is timed.	Prolonged by afibrinogenemia (no clot) and severe hypo- or dysfibrinogenemia.	Poor sensitivity to mild fibrinogen deficiency or dysfunction.	[1,19]
Thrombin clotting time or thrombin time (TCT or TT)	Test of functional fibrinogen and fibrin polymerization. Reagent contains thrombin, with or without calcium. The reagent is added to citrated plasma. Time to clot formation is timed. The test can be modified to include a heparin neutralizer (e.g. protamine).	Prolonged by FDPs, afibrinogenemia, hypofibrinogenemia, dysfibrinogenemia and thrombin inhibitors. More sensitive than the PT or APTT for quantitative and qualitative defects in fibrinogen.	Poor specificity as the test can be prolonged by: • Presence of heparin • Presence of direct thrombin inhibitor • High concentration of FDPs that interfere with fibrin polymerization • Paraproteins that inhibit fibrin polymerization Test is not standardized. The amount of thrombin in the test varies considerably, resulting in reference ranges that vary considerably between laboratories.	[18]
Reptilase time	Reagent is a snake venom that directly activates fibrinogen by cleaving fibrinopeptide A.	The test is not affected by heparin. The test is prolonged by FDPs, afibrinogenemia, hypofibrinogenemia, and dysfibrinogenemias that are not caused by fibrinopeptide B cleavage abnormalities.	Does not detect all forms of dysfibrinogenemia. Can be prolonged by: • High concentration of FDPs that interfere with fibrin polymerization • Paraproteins that inhibit fibrin polymerization	[18]
Clottable fibrinogen (e.g. Clauss)	Reagent contains a high concentration of thrombin that triggers clot formation when added to citrated plasma. The time to clot formation is recorded and is read off of a reference curve for tests performed with known concentrations of fibrinogen.	Functional assay.	Lipemia and /or elevated bilirubin may interfere with the assay when it is performed on instruments with a photo-optical endpoint analyzer.	[20]
Fibrinogen antigen	Various immunoassays including Laurell rocket assays with polyclonal anti-fibrinogen antibody and ELISA.	Quantitative assay that does not assess function.	Results need to be correlated with findings of other fibrinogen assays.	[22]
Ratio of fibrinogen antigen to clottable fibrinogen	Uses results of functional and immunoassays.	May help to distinguish dysfibrinogenemia (high ratio) from hypofibrinogenemia (ratio close to 1).		

FDP, fibrin degradation products.

TABLE II. Results of Investigations for Fibrinogen Abnormalities in Patients Assessed for Different Clinical Problems

Clinical Problem	Afibrinogenemia	Hypofibrinogenemia	Dysfibrinogenemia	Dysfibrinogenemia with a probable FPB abnormality	Paraprotein inhibitor of fibrin polymerization	Post thrombolytic therapy
	Trauma-related intracranial hemorrhage at age 40. Subsequent trauma- and surgery-related bleeding	Recurrent pregnancy loss from placental abruptions	Menorrhagia; Post-partum hemorrhage requiring transfusion	Menorrhagia; Post-partum hemorrhage	Circulating plasma cells and 70.5 g/L IgG lambda paraprotein	Treatment given for acute myocardial infarction
PT (Normal: 11–14 sec)	No clot detected	14.2	13.0	13.0	21.4	–
INR (Normal: 0.8–1.2)	No clot detected	1.2	1.1	1.1	1.8	2.2
APTT (Normal: 22–35 sec)	No clot detected	32	33	28	24	42
TCT or TT (Normal: 20–30 sec)	No clot detected	46	122	49	72 (1:1 mix 43 sec)	No clot detected
Reptilase time (Normal: 15–27 sec)	>60	33	40	22	–	–
Clottable fibrinogen (Normal: 160–420 mg/dl)	<20	60	120	180	200	20
Fibrinogen antigen (Normal: 160–420 mg/dl)	<10	60	380	350	400	–
Ratio of fibrinogen antigen to clottable fibrinogen	–	1.0	3.2	1.9	2.0	–

Please note the reference ranges for these assays may differ between laboratories.
–, not available.

to automated coagulation analyzers. Many photo-optical coagulation analyzers can determine fibrinogen concentrations using a mathematical derivation of the sample prothrombin time in seconds [21]. However, derived fibrinogens are less accurate and may over-estimate the quantity of fibrinogen relative to the Clauss method [21]. Methods are also available for quantifying fibrinogen antigen, often using immunological electrophoresis (e.g. Laurell rocket) or enzyme-linked immunosorbent assays (ELISA) [22].

Table II summarizes the clinical and laboratory findings for a variety of disorders affecting fibrinogen or its function. In general, screening for a mild fibrinogen deficiency or dysfibrinogenemia often requires a Clauss fibrinogen and a thrombin clotting time, with or without a reptilase time, to determine if there is a potential discrepancy between the amount of fibrinogen and its function. Immunoassays are often performed to evaluate abnormalities detected by these screening tests.

The clinical features of fibrinogen disorders can vary considerably, as illustrated by the brief case vignettes in Table II. Presenting problems range from minimal bleeding symptoms, to significant health problems, including menorrhagia, pregnancy losses, post partum hemorrhage, and bleeding problems typical of severe hemorrhagic disorders, including intracranial hemorrhage and joint bleeds [13,23,24]. Some patients with dysfibrinogenemia suffer from thrombosis, with or without bleeding [23]. Thrombotic complications have been reported in individuals with dysfibrinogenemias associated with impaired release of FPA and/or FPB, or defects in fibrin polymerization, although the relationship between these abnormalities and thrombophilia is unclear [25]. The thrombotic tendency in fibrinogen Caracas V is attributed to an abnormally tight fibrin network that impedes fibrinolysis [26]. Inheritance of a dysfibrinogenemia and another hereditary thrombophilia (e.g. fibrinogen Cedar Rapids with factor V Leiden) is thought to increase thrombotic risks [27].

As the screening tests for fibrinogen disorders are fairly inexpensive, the tests are often included in a laboratory assessment for bleeding problems. Because many inherited dysfibrinogenemia and some hypofibrinogenemias are autosomal dominant, [13,23] it can be helpful to screen for additional affected family members when an index case is identified.

Summary

The possibility of an inherited or acquired fibrinogen disorder should be considered when selecting laboratory tests to evaluate patients with bleeding problems. Although severe disorders of fibrinogen, such as afibrinogenemia, prolong the PT and APTT, these assays are insensitive to milder fibrinogen abnormalities. To exclude milder fibrinogen disorders, it is helpful to perform an assay of clottable fibrinogen, in addition to a thrombin time and/or reptilase time, which can be more prolonged than expected when there are qualitative abnormalities typical of a dysfibrinogenemia. When these assays detect abnormalities suggestive of a fibrinogen disorder, it can be helpful to compare the levels of clottable fibrinogen to fibrinogen antigen to determine if there is a quantitative or qualitative fibrinogen abnormality.

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