 DOI: 10.2478/acm-2022-0013

DIAGNOSIS AND CLASSIFICATION OF HEREDITARY FIBRINOGEN DISORDERS

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Abstract

Hereditary fibrinogen disorders (HFDs) are rare bleeding disorders with a wide spectrum of biological and clinical features. While most patients with HFDs are at risk to suffer from mild to severe, sometimes life-threatening bleeding, thrombotic events are also common. Therefore, an appropriate diagnosis is needed to offer the optimal treatment. Diagnosis of HFDs can be challenging and plenty of pitfalls. The sensitivity and specificity of hemostasis routine test are depending on the reagents, the methods, and the fibrinogen variants. To distinguish subtypes of HFDs additional tests are often required. Historically based on the assessment of fibrinogen levels, a recent classification also considers the clinical phenotype and the genotype. In this short review, diagnosis strategies and HFDs classification are reviewed.

INTRODUCTION

Hereditary fibrinogen disorders (HFDs) encompass a relatively large group of fibrinogen deficiency[1]. HFDs are classically subdivided into types 1 (afibrinogenemia and hypofibrinogenemia) and types 2 (dysfibrinogenemia and hypodysfibrinogenemia)[2]. Clinical features vary according to the fibrinogen level and the subtype of HFDs. Afibrinogenemia, characterised by the complete absence of fibrinogen, is typically associated with a severe bleeding phenotype and a paradoxical thrombotic tendency[3,4]. Hypofibrinogenemia, defined by decreased levels of fibrinogen activity and antigen, is associated with a bleeding risk dependent on the fibrinogen concentration[5,6]. In dysfibrinogenemia, normal levels of dysfunctional fibrinogen lead to highly heterogenous clinical features, from mild to life-threatening bleeding to recurrent thrombotic events, even though most patients are asymptomatic at time of diagnosis[7]. Hypodysfibrinogenemia, in which both activity and antigen fibrinogen levels are decreased, is often marked by a strong tendency to bleeding and thrombosis[4,8]. A recent classification identified several sub-types of HFDs considering fibrinogen activity and antigen levels, genotype, and clinical phenotype. Therefore, an accurate diagnosis is essential for the correct identification of patients and to optimize their management.

In this short review we will discuss the stepwise procedure leading to diagnosis, we will highlight some pitfalls, and we will give some details on classification of HFDs.

Diagnosis of hereditary fibrinogen disorders

A fibrinogen disorder is usually suspected in patient addressed for a bleeding tendency with decreased fibrinogen level. However, incidental finding is frequent, especially in patients with mild fibrinogen deficiencies. Before starting investigations of HFD, it is important to rule out an acquired fibrinogen disorder. Liver disease, drugs and cancer, the most common causes, can affect the synthesis, the secretion, or the proteolysis of fibrinogen resulting in decreased levels of fibrinogen activity[9,10]. The familial history is also crucial, often helping to distinguish between a hereditary and acquired fibrinogen disorder.

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Table 1 Biological features of hereditary fibrinogen disorders and International Society Thrombosis and Hemostasis (ISTH) classification

	Fibrinogen activity	Fibrinogen antigen	Genotype	ISTH classification
Afibrinogenemia	No detectable	No detectable	Homozygous or compound heterozygous for a null* mutation	1A. Bleeding phenotype or asymptomatic individuals 1B. Thrombotic phenotype
Hypofibrinogenemia	Decreased	Decreased	Heterozygous for a null or missense mutation	2A. Fibrinogen activity <0.5 g/L (severe) 2B. Fibrinogen activity 0.5 - 0.9 g/L (moderate) 2C. Fibrinogen activity 1- and lower limit of normal value (mild) 2D. Histologically proven accumulation of fibrin in hepatocytes (Fibrinogen storage disease**)
Dysfibrinogenemia	Decreased***	Normal***	Heterozygous for a missense mutation	3A. Bleeding phenotype or with thrombotic phenotype not fulfilling criteria for dysfibrinogenemia 3B or asymptomatic individuals 3B. Carriers of a thrombotic fibrinogen mutation**** or suffering from thrombotic events with a first-degree familial thrombotic history (relatives with the same genotype) without any other thrombophilia
Hypodysfibrinogenemia	Decreased	Decreased	Homozygous or compound heterozygosity for a null and/or a missense mutation	4A. Fibrinogen antigen <0.5 g/L (severe) 4B. Fibrinogen antigen 0.5 - 0.9 g/L (moderate) 4C. Fibrinogen activity 1- and lower limit of normal value (mild)

*: large deletions, splice-site mutations, frameshift mutations and nonsense mutations; **: Fibrinogen Brescia, Fibrinogen AI du Pont, Fibrinogen Pisa, Fibrinogen Ankara, Fibrinogen Angers, Fibrinogen Beograd, Fibrinogen Trabzon; ***: dependent on the reagents, the methods, and the fibrinogen variant; ****: Fibrinogen Dusart, Fibrinogen Caracas V, Fibrinogen Ijmuiden, Fibrinogen New York I, Fibrinogen Nijmegen, Fibrinogen Naples at homozygous state, Fibrinogen Melun.

The first step in diagnosis of HFDs relies on measurement of fibrinogen activity by the Clauss method[11]. As second step, if Clauss assay is decreased, fibrinogen antigen has to be measured. As indicated in table 1, if both activity and antigen are undetectable, afibrinogenemia can be diagnosed. If fibrinogen activity and antigen are proportionally decreased, a hypofibrinogenemia should be considered. Discordance between decreased fibrinogen activity and normal antigen is highly predictive of dysfibrinogenemia. A ratio fibrinogen activity / antigen with a cut-off of 0.7 has historically be proposed to distinguish between quantitative and qualitative fibrinogen disorders, but not fully validated[12]. Recently, a cut-off of 0.55 has also been tested[13]. If fibrinogen activity and antigen are disproportionally decreased, a hypodysfibrinogenemia should be considered.

Thrombin time and reptilase time are not mandatory for the diagnosis of HFDs, even though they provide useful information on the degree of defect in fibrin polymerisation[14]. Derived fibrinogen from the prothrombin time (PT-der) is widely used to assess the fibrinogen concentration[15]. The PT-der is an indirect estimation of fibrinogen concentration. In dysfibrinogenemia, PT-der overestimated the fibrinogen activity but has a very good correlation with antigen value[16]. A ratio PT-der / Clauss with a cut-off of 1.43 seems to provide an excellent specificity and sensitivity for the diagnosis of dysfibrinogenemia[17]. In addition, recent data promotes novel approaches for diagnosis of HFDs. Clot wave form from Clauss could have a similar performance to detect dysfibrinogenemia[13].

It should be noted that in dysfibrinogenemia several variables such as clot detection methods, reagents, and type of fibrinogen variant, influence the measurement of routine hemostasis assays[18]. For instance, Fibrinogen Longmont is better detected by photo-optical endpoint[19,20]. In case of very low values of fibrinogen activity, due the limit of quantification of analysers, it could be difficult to distinguish between afibrinogenemia and hypofibrinogenemia or between hypofibrinogenemia and hypodysfibrinogenemia. In these cases, genotype is necessary to confirm the diagnosis[21]. In the setting of research, analyses of fibrin clots, may complete the fibrinogen work-up in order to better determine the patient's clinical phenotype[22].

Molecular analysis

HFDs result from monoallelic or biallelic mutations in *FGA*, *FGB* and *FGG* genes in chromosome 4[23]. Besides the confirmation of the diagnosis, genotype may help for familial screening, prenatal testing, and prediction of the clinical phenotype. The development of next generation sequencing allows complete analysis of *FGA*, *FGB* and *FGG* exons enhancing the identification of a causative mutation[24]. However, in developing country, Sanger sequencing is still the best option[25]. Usually, afibrinogenemia results by homozygosity for a null mutation[26]. Thus, patients with hypofibrinogenemia are heterozygous carriers of afibrinogenemic allele. These mutations affect either the synthesis or the assembly or the secretion of fibrinogen into circulation. Some mutations are more common, such as the large deletion 11Kb of *FGA* and the splice site mutation *FGA* c.510+1G>T[27,28]. Most of dysfibrinogenemia are due to missenses mutation located in exon 2 of *FGA* or exon 8 of *FGG*[29]. Overall, two hotspot mutations (i.e, *FGA* c.103C>A or c.104G>A and *FGG* c.901C>T or c.902G>A) represent more than 80% of causative mutations in dysfibrinogenemia. These mutations yield to a delay in thrombin-mediated fibrinopeptide cleavage or defective fibrin polymerisation[30,31]. In hypodysfibrinogenemia, a single mutation can affect both the secretion and the function of the fibrinogen molecule. In alternative, a compound mutation can confer a concomitant "hypofibrinogenemia" and "dysfibrinogenemia" trait[4].

A few fibrinogen variants are strongly correlated with a clinical phenotype[32]. Patients with thrombotic-related fibrinogen variants are a very strong risk of thrombosis[33]. Thrombosis

can occur in all vascular territories, including in young patients[34-39]. These mutations affect the fibrin clot structure leading to a procoagulant state[40]. Other mutations, located in exons 8 and 9 of *FGG* cause accumulation of fibrinogen aggregates in hepatocytes resulting in chronic liver inflammation and eventually cirrhosis[41]. In dysfibrinogenemia, mutations modifying specific aspects of the fibrinogen molecule, can decrease the clottability and therefore increase the bleeding tendency. For instance, mutations in the NH2-terminal portion slow the conversion of fibrinogen into monomeric fibrin; mutations adding new N-linked glycosylation sites introduce negatively charged carbohydrate side chains affecting the alignment of fibrin monomers during polymerization; mutations generating unpaired cysteine form extra disulfide bonds and produce highly branched and fragile fibrin networks; truncation mutations in the fibrinogen αC regions impair the lateral fibril aggregation and factor XIII crosslinking[30].

Classification of hereditary fibrinogen disorders

As indicated in Table 1, the International Society Thrombosis and Hemostasis (ISTH) classification introduces several subtypes of HFDs according not only on the fibrinogen levels but also on the clinical pattern and the genotype. Although severe bleeding is the prominent symptom in afibrinogenemia, some patients experience recurrent thrombosis[42]. In a recent large series of patients (n=204), 18% reported a thrombotic event[43]. Management of thrombosis in afibrinogenemia is particularly difficult as physician has to deal with both the thrombotic and the bleeding risk[6]. Patients with afibrinogenemia and a thrombotic phenotype are classified as type 2A.

The bleeding risk in hypofibrinogenemia is strongly correlated to the fibrinogen concentration. In a registry from the Netherlands, the correlation between baseline fibrinogen activity and ISTH bleeding assessment tool score was considered as moderate (r= -0.683)[44]. It is generally well accepted that patients with fibrinogen activity levels >0.8 g/L do not suffer from spontaneous bleeding[45]. Therefore, patients with hypofibrinogenemia are classified as severe (2A), moderate (2B), or mild (2C) according to the fibrinogen activity. A fourth subtype (2D) is hypofibrinogenemia associated with fibrinogen storage disease. This subtype is classically suspected in familial history of mild hypofibrinogenemia and cryptogenic liver disease. The physiopathology is not fully understood. Mutations in fibrinogen γ chain could provoke conformational changes resulting in abnormal exposure of hydrophobic patches that become available for interactions with APOB-lipoproteinemia causing their intracellular retention and impairment of fibrinogen secretion[46].

Most of patients with dysfibrinogenemia will be asymptomatic at the time of diagnosis[47-49] but the natural course of dysfibrinogenemia is nevertheless characterized by a risk of bleeding and thrombosis. In a series of 101 patients with dysfibrinogenemia with a median follow-up of 8.8 years, the cumulative incidence of major bleeding and thrombotic events was 2.5 and 18.7 per 1000 patient-years, respectively, with estimated cumulative incidences at age 50 years of 19.2% and 30.1%[7]. These patients are classified as subtype 3A. As previously mentioned, the thrombotic risk is remarkably more important in patients with thrombotic-related fibrinogen variants (subtype 3B). In such patients, thrombosis usually occur in young patients, including in unusual vascular sites.[50]

In hypodysfibrinogenemia the bleeding risk is proportional to the fibrinogen level, but it is also exacerbated by the dysfunctional fibrinogen. Indeed, hypodysfibrinogenemia is characterized by reduced fibrinogen function but also a less marked reduction of plasma fibrinogen antigen[8]. Therefore, patients with hypodysfibrinogenemia are classified as severe (2A), moderate (2B), or mild (2C) according to the fibrinogen antigen.

CONCLUSIONS

Diagnosis of HFDs is a stepwise procedure. Development and/or integration of alternative techniques to assess the fibrinogen concentration could help in the next future to minimise diagnosis incertitude. A better knowledge of the molecular basis and their correlations with the clinical phenotype will allow to a more subtle classification of HFDs.

REFERENCES

1. Casini A, de Moerloose P, Neerman-Arbez M. Clinical Features and Management of Congenital Fibrinogen Deficiencies. *Semin Thromb Hemost* 2016;42(4):366-374.
2. de Moerloose P, Casini A, Neerman-Arbez M. Congenital fibrinogen disorders: an update. *Semin Thromb Hemost* 2013;39(6):585-595.
3. Casini A, von Mackensen S, Santoro C, et al. Clinical phenotype, fibrinogen supplementation, and health-related quality of life in patients with afibrinogenemia. *Blood* 2021;137(22):3127-3136.
4. Peck RC, Fitzgibbon L, Reilly-Stitt C, Doherty C, Phillips E, Mumford AD. Pseudohomozygous dysfibrinogenemia. *Res Pract Thromb Haemost* 2021;5(6):e12568.
5. Brunclikova M, Simurda T, Zolkova J, et al. Heterogeneity of Genotype-Phenotype in Congenital Hypofibrinogenemia-A Review of Case Reports Associated with Bleeding and Thrombosis. *J Clin Med* 2022;11(4).
6. Simurda T, Asselta R, Zolkova J, et al. Congenital Afibrinogenemia and Hypofibrinogenemia: Laboratory and Genetic Testing in Rare Bleeding Disorders with Life-Threatening Clinical Manifestations and Challenging Management. *Diagnostics (Basel)* 2021;11(11).
7. Casini A, Blondon M, Lebreton A, et al. Natural history of patients with congenital dysfibrinogenemia. *Blood* 2015;125(3):553-561.
8. Casini A, Brungs T, Lavenu-Bombled C, Vilar R, Neerman-Arbez M, de Moerloose P. Genetics, diagnosis and clinical features of congenital hypodysfibrinogenemia: a systematic literature review and report of a novel mutation. *J Thromb Haemost* 2017;15(5):876-888.
9. Arai S, Kamijo T, Takezawa Y, et al. Acquired dysfibrinogenemia: monoclonal lambda-type IgA binding to fibrinogen caused lower functional plasma fibrinogen level and abnormal clot formation. *Int J Hematol* 2020;112(1):96-104.
10. Besser MW, MacDonald SG. Acquired hypofibrinogenemia: current perspectives. *J Blood Med* 2016;7:217-225.
11. Undas A. How to Assess Fibrinogen Levels and Fibrin Clot Properties in Clinical Practice? *Semin Thromb Hemost* 2016;42(4):381-388.
12. Krammer B, Anders O, Nagel HR, Burstein C, Steiner M. Screening of dysfibrinogenaemia using the fibrinogen function versus antigen concentration ratio. *Thromb Res* 1994;76(6):577-579.
13. Suzuki A, Suzuki N, Kanematsu T, et al. Development and validation of a novel qualitative test for plasma fibrinogen utilizing clot waveform analysis. *Sci Rep* 2022;12(1):434.
14. Undas A. Determination of Fibrinogen and Thrombin Time (TT). *Methods Mol Biol* 2017;1646:105-110.
15. Mackie IJ, Kitchen S, Machin SJ, Lowe GD, Haemostasis, Thrombosis Task Force of the British Committee for Standards in H. Guidelines on fibrinogen assays. *Br J Haematol* 2003;121(3):396-404.
16. Skornova I, Simurda T, Stasko J, et al. Use of Fibrinogen Determination Methods in Differential Diagnosis of Hypofibrinogenemia and Dysfibrinogenemia. *Clin Lab* 2021;67(4).
17. Xiang L, Luo M, Yan J, et al. Combined use of Clauss and prothrombin time-derived methods for determining fibrinogen concentrations: Screening for congenital dysfibrinogenemia. *J Clin Lab Anal* 2018;32(4):e22322.
18. Shapiro SE, Phillips E, Manning RA, et al. Clinical phenotype, laboratory features and genotype of 35 patients with heritable dysfibrinogenaemia. *Br J Haematol* 2013;160(2):220-227.
19. Jennings I, Kitchen S, Menegatti M, et al. Potential misdiagnosis of dysfibrinogenaemia: Data from multicentre studies amongst UK NEQAS and PRO-RBDD project laboratories. *Int J Lab Hematol* 2017;39(6):653-662.

20. Leung B, Beggs J, Mason J. Fibrinogen Longmont: A Clinically Heterogeneous Dysfibrinogenemia with Discrepant Fibrinogen Results Influenced by Clot Detection Method and Reagent. *TH Open* 2022;6(1):e18-e20.
21. Casini A, Undas A, Palla R, et al. Diagnosis and classification of congenital fibrinogen disorders: communication from the SSC of the ISTH. *J Thromb Haemost* 2018;16(9):1887-1890.
22. Casini A. From Routine to Research Laboratory: Strategies for the Diagnosis of Congenital Fibrinogen Disorders. *Hamostaseologie* 2020.
23. Richard M, Celeny D, Neerman-Arbez M. Mutations Accounting for Congenital Fibrinogen Disorders: An Update. *Semin Thromb Hemost* 2022.
24. Cao Z, Dong Y, Zeng J, et al. Whole-exome sequencing identified novel mutations in FGA and FGG genes in the patients with decreased fibrinogen. *Thromb Res* 2019;177:79-82.
25. Casini A, Blondon M, Tintillier V, et al. Mutational Epidemiology of Congenital Fibrinogen Disorders. *Thromb Haemost* 2018;118(11):1867-1874.
26. Neerman-Arbez M, de Moerloose P, Casini A. Laboratory and Genetic Investigation of Mutations Accounting for Congenital Fibrinogen Disorders. *Semin Thromb Hemost* 2016;42(4):356-365.
27. Attanasio C, de Moerloose P, Antonarakis SE, Morris MA, Neerman-Arbez M. Activation of multiple cryptic donor splice sites by the common congenital afibrinogenemia mutation, FGA IVS4 + 1 G-->T. *Blood* 2001;97(6):1879-1881.
28. Neerman-Arbez M, Antonarakis SE, Honsberger A, Morris MA. The 11 kb FGA deletion responsible for congenital afibrinogenaemia is mediated by a short direct repeat in the fibrinogen gene cluster. *Eur J Hum Genet* 1999;7(8):897-902.
29. Casini A, de Moerloose P. How I treat dysfibrinogenemia. *Blood* 2021;138(21):2021-2030.
30. Li Y, Ding B, Wang X, Ding Q. Congenital (hypo-)dysfibrinogenemia and bleeding: A systematic literature review. *Thromb Res* 2022;217:36-47.
31. Casini A, Neerman-Arbez M, Ariens RA, de Moerloose P. Dysfibrinogenemia: from molecular anomalies to clinical manifestations and management. *J Thromb Haemost* 2015;13(6):909-919.
32. Casini A, de Moerloose P. Can the phenotype of inherited fibrinogen disorders be predicted? *Haemophilia* 2016;22(5):667-675.
33. Haverkate F, Samama M. Familial dysfibrinogenemia and thrombophilia. Report on a study of the SSC Subcommittee on Fibrinogen. *Thromb Haemost* 1995;73(1):151-161.
34. Engesser L, Koopman J, de Munk G, et al. Fibrinogen Nijmegen: congenital dysfibrinogenemia associated with impaired t-PA mediated plasminogen activation and decreased binding of t-PA. *Thromb Haemost* 1988;60(1):113-120.
35. Koopman J, Haverkate F, Lord ST, Grimbergen J, Mannucci PM. Molecular basis of fibrinogen Naples associated with defective thrombin binding and thrombophilia. Homozygous substitution of B beta 68 Ala----Thr. *J Clin Invest* 1992;90(1):238-244.
36. Marchi R, Lundberg U, Grimbergen J, et al. Fibrinogen Caracas V, an abnormal fibrinogen with an Aalpha 532 Ser-->Cys substitution associated with thrombosis. *Thromb Haemost* 2000;84(2):263-270.
37. Collet JP, Soria J, Mirshahi M, et al. Dusart syndrome: a new concept of the relationship between fibrin clot architecture and fibrin clot degradability: hypofibrinolysis related to an abnormal clot structure. *Blood* 1993;82(8):2462-2469.
38. Tarumi T, Martincic D, Thomas A, et al. Familial thrombophilia associated with fibrinogen paris V: Dusart syndrome. *Blood* 2000;96(3):1191-1193.
39. Wada Y, Lord ST. A correlation between thrombotic disease and a specific fibrinogen abnormality (A alpha 554 Arg-->Cys) in two unrelated kindred, Dusart and Chapel Hill III. *Blood* 1994; 84(11):3709-3714.
40. Koopman J, Haverkate F, Grimbergen J, et al. Molecular basis for fibrinogen Dusart (A alpha 554 Arg-->Cys) and its association with abnormal fibrin polymerization and thrombophilia. *J Clin Invest* 1993;91(4):1637-1643.
41. Asselta R, Paraboschi EM, Duga S. Hereditary Hypofibrinogenemia with Hepatic Storage. *Int J Mol Sci* 2020;21(21).

42. Casini A, Neerman-Arbez M, de Moerloose P. Heterogeneity of congenital afibrinogenemia, from epidemiology to clinical consequences and management. *Blood Rev* 2021;48:100793.
43. Casini A, von Mackensen S, Santoro C, et al. Clinical phenotype, fibrinogen supplementation, and health-related quality of life in patients with afibrinogenemia. *Blood* 2021;137(22):3127-3136.
44. Saes JL, Verhagen MJA, Meijer K, et al. Bleeding severity in patients with rare bleeding disorders: real-life data from the RBiN study. *Blood Adv* 2020;4(20):5025-5034.
45. Peyvandi F, Palla R, Menegatti M, et al. Coagulation factor activity and clinical bleeding severity in rare bleeding disorders: results from the European Network of Rare Bleeding Disorders. *J Thromb Haemost* 2012;10(4):615-621.
46. Callea F, Giovannoni I, Sari S, et al. Fibrinogen Gamma Chain Mutations Provoke Fibrinogen and Apolipoprotein B Plasma Deficiency and Liver Storage. *Int J Mol Sci* 2017;18(12).
47. Castaman G, Giacomelli SH, Biasoli C, Contino L, Radossi P. Risk of bleeding and thrombosis in inherited qualitative fibrinogen disorders. *Eur J Haematol* 2019;103(4):379-384.
48. Wypasek E, Klukowska A, Zdziarska J, et al. Genetic and clinical characterization of congenital fibrinogen disorders in Polish patients: Identification of three novel fibrinogen gamma chain mutations. *Thromb Res* 2019;182:133-140.
49. Zhou J, Ding Q, Chen Y, et al. Clinical features and molecular basis of 102 Chinese patients with congenital dysfibrinogenemia. *Blood Cells Mol Dis* 2015;55(4):308-315.
50. Shen YM, Trang V, Sarode R, Brennan S. Fibrinogen Dusart presenting as recurrent thromboses in the hepatic portal system. *Blood Coagul Fibrinolysis* 2014;25(4):392-394.

Received: November, 1, 2022
Accepted: November, 14, 2022