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Review

Heterogeneity of congenital afibrinogenemia, from epidemiology to clinical consequences and management

Alessandro Casini [a,b,*], Marguerite Neerman-Arbez [c], Philippe de Moerloose [b]

[a] Division of Angiology and Hemostasis, University Hospitals of Geneva, Geneva, Switzerland
[b] Faculty of Medicine, University of Geneva, Rue Gabrielle-Perret-Gentil 4, 1205 Geneva, Switzerland
[c] Department of Genetic Medicine and Development, Faculty of Medicine, University of Geneva, 1 Rue Michel Servet, 1211 Geneva, Switzerland

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ABSTRACT

Fibrinogen is a complex protein playing a major role in coagulation. Congenital afibrinogenemia, characterized by the complete absence of fibrinogen, is associated with major hemostatic defects. Even though the clinical course is unpredictable and can be completely different among patients, severe bleeding is the prominent symptom. Patients are also at increased risk of thrombosis and sometimes suffer from spontaneous spleen rupture, bone cysts and defective wound healing. Due to the relative rarity of afibrinogenemia, there are no evidence-based strategies for helping physicians in care of these patients. Fibrinogen supplementation is the keystone to prevent or treat bleeding events. In addition, fibrinogen, a pleiotropic protein with numerous physiological roles in immunity, angiogenesis and tissue repair, is involved in many diseases. Indeed, depletion of fibrinogen in animal models of infections, tumors and neurological diseases has an effect on the clinical course. The consequences for patients with afibrinogenemia still need to be investigated.

1. Introduction

Fibrinogen plays a crucial role in primary and secondary hemostasis as support for platelet aggregation and substrate for fibrin clot formation [1]. In addition, fibrinogen participates in several other essential biological actions such as angiogenesis, tissue repair and the immune response. Cellular and molecular mechanisms underlying the roles of fibrinogen are revealing that it is at the nexus of a complex vascular-immune-inflammatory interplay [2]. Fibrinogen displays diverse functions from direct cell signaling to host defense indicating that it plays a role that goes far beyond coagulation, reflecting its ancestral functions [3,4]. Congenital afibrinogenemia is a hereditary fibrinogen disorder characterised by the complete absence of fibrinogen [5]. Given that fibrinogen is a central player of coagulation, the complete absence of fibrinogen leads to a broad range of symptoms from life-threatening bleeding to thrombosis [6]. However, lack of fibrinogen is likely to be associated with diseases beyond the coagulation system. The purpose of this review is to summarize the current knowledge on afibrinogenemia going through the structure and genetics of fibrinogen and the heterogeneous clinical aspects of this disease.

2. Fibrinogen biosynthesis

Fibrinogen is a hexameric glycoprotein made of two sets of three homologous polypeptide chains: Aα, Bβ and γ [7]. The three fibrinogen chains are encoded by three genes *FGB*, *FGA* and *FGG*, ordered from centromere to telomere on the long arm of human chromosome 4, that are co-regulated to generate mRNA at balanced levels to sustain fibrinogen secretion from the liver [8]. Alternatively spliced mRNAs produce two different isoforms for *FGA*: Aα and AαE, and *FGG* γ and γ' [7]. Epigenetic and post-transcriptional regulation by miRNAs also contribute to the overall rate of fibrinogen synthesis [9,10]. Each gene is separately transcribed and translated to produce a nascent polypeptide including a signal peptide, which is cleaved from each chain during translocation of the single chains into the lumen of the endoplasmic reticulum (ER) [11]. The three component chains are secreted as an assembled hexamer $(A\alpha B\beta\gamma)_2$ primarily from hepatocytes [11]. In addition, a separate small fibrinogen pool with structural differences due to absence of the γ' isoform is also endocytosed in platelet alpha-granules [12]. Fibrinogen chain assembly is a complex machinery proceeding in the lumen of ER in a stepwise manner under the control of chaperones and glycosylation enzymes that efficiently support the

* Corresponding author at: Division of Angiology and Hemostasis, University Hospitals of Geneva, Rue Gabrielle-Perret-Gentil 4, 1205 Geneva, Switzerland.
E-mail addresses: alessandro.casini@hcuge.ch (A. Casini), marguerite.neerman-arbez@unige.ch (M. Neerman-Arbez), philippe.demoerloose@unige.ch (P. de Moerloose).

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correct assembly and folding of the protein [13–15] Misfolded, misassembled and surplus proteins are retained in the ER and, in the large majority of cases, ultimately degraded by lysosomes and proteasomes [16]. Hexamers containing mutant chains which escape this quality control can lead to Fibrinogen Storage Disease [17,18].

As shown in Fig. 1, the fibrinogen structure comprises two terminal D regions containing the γ-nodule and the β nodule formed by the COOH-terminal portions of the Bβ and γ chains, respectively as well as adjacent portions of the coiled coils. One central E region contains the central globular nodule formed by the NH2-terminal portions of all six chains and two adjacent portions of the coiled coil [19,20]. Fibrin polymerisation is initiated by the thrombin-mediated cleavage of fibrinopeptides A (FpA) and B with subsequent exposure of binding sites 'A' and 'B' in the E region complementary to constitutive sites 'a' and 'b' in the D regions [20]. Fibrin monomer molecules produced by the release of FpA interact with each other in a half-staggered manner via the knob-hole interactions resulting in larger fibrin oligomers growing in length to protofibrils [21]. The lateral aggregation of protofibrils supports their packing into fibrin fibers with a 22.5-nm periodic cross-striation [22]. The elongation and the thickening of fibrin fibers are accompanied by branching which leads to formation of a 3-dimensional fibrin clot network finally cross-linked by factor XIIIa which, together with platelets and red blood cells, provides structural integrity to the growing thrombus [23].

3. Epidemiology of congenital afibrinogenemia

The epidemiology of afibrinogenemia is only partially known

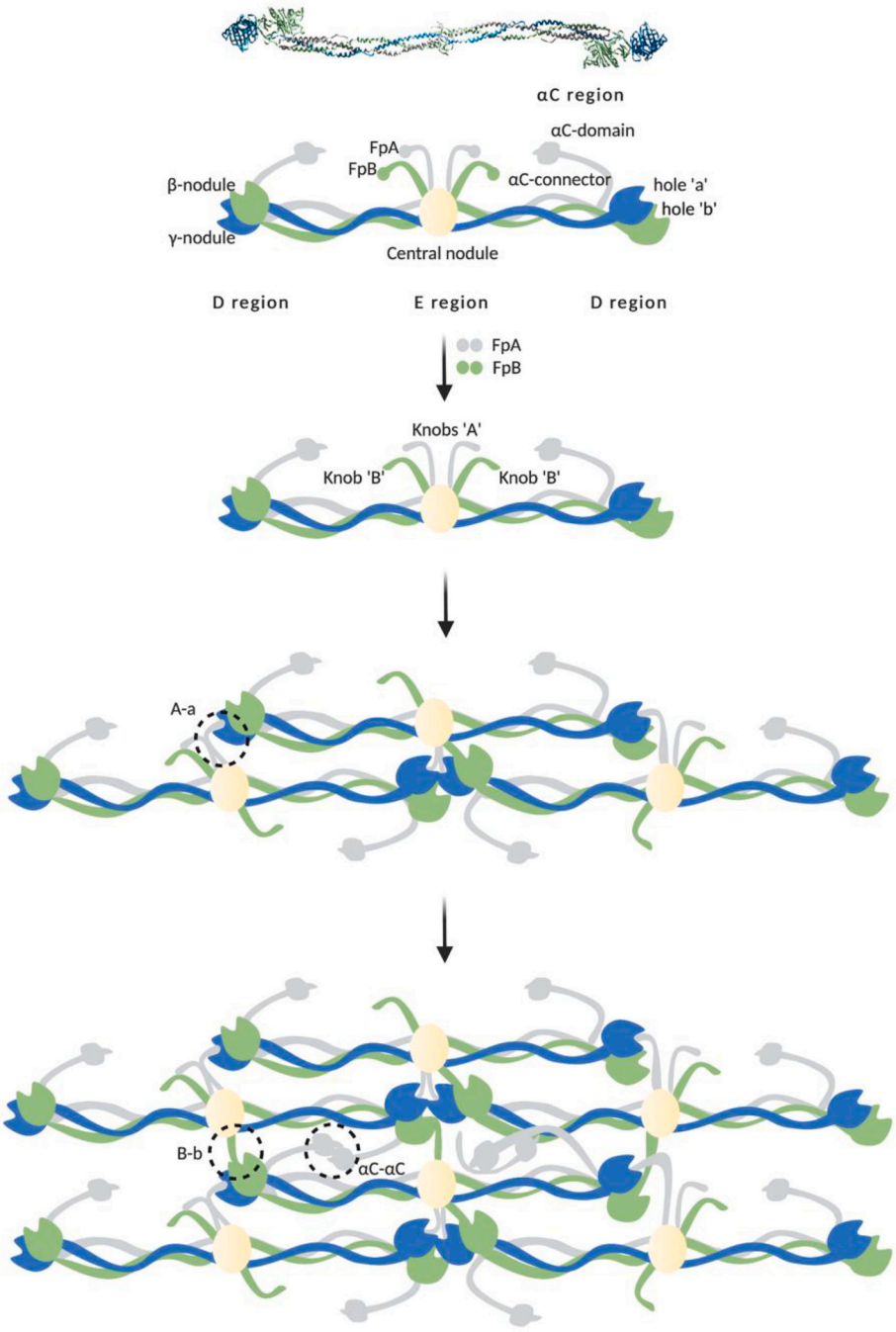

Fig. 1. Fibrinogen structure, fibrin formation and polymerization. The fibrinogen crystal structure is presented in the upper panel with its schematic representation displayed below. Fibrin formation starts with the cleavage of fibrinopeptides A (FpA) by thrombin to expose knobs 'A' which interact with holes 'a' in the C-terminal regions of the γ chains (indicated by dotted circle) to induce fibrin polymerization. Subsequent cleavage of fibrinopeptides B (FpB) by thrombin exposes knobs 'B' which can interact with holes 'b' in the C-terminal regions of β chains (indicated by dotted circle). These are thought to contribute to the lateral aggregation of protofibrils together with 'αC- αC' interactions (indicated by dotted circle). This image was prepared using BioRender.com with the fibrinogen crystal structure originally produced using PDB entry 3GHG [153], the Swiss-PdbViewer 4.1.0 and POV-Ray 3.7 software. (A)α, (B)β and γ chains are colored in grey, green and blue, respectively.

[24,25]. Based on national and international databases, afibrinogenemia could represent up to 10% of rare bleeding disorders [26]. The prevalence is estimated to be around 1 in 10^6 individuals, ranging from 0.7 in 10^6 in France (http://www.francecoag.org, last access 14.04.2020) to almost 50 in 10^6 in Lebanon [27], and probably even more in countries with the highest frequency of consanguinity [28]. The 2018 World Federation of Hemophilia annual global survey recorded 2768 patients with partial or complete fibrinogen deficiency (http://www1.wfh.org/publications/files/pdf-1731.pdf). However, these registries lack prospective and systematic evaluations and probably underestimate the true prevalence [29]. Indeed, a systematic analysis of exome/genome data from about 140,000 individuals listed in the gnomAD Database (https://gnomad.broadinstitute.org/) suggested that the worldwide prevalence for recessively inherited fibrinogen deficiencies could be 10-fold higher than that reported so far with prevalences varying from 1 in 10^6 in East Asians to 24.5 in 10^6 in non-Finnish Europeans [30].

Recently, the Community Counts national surveillance system, including the American Thrombosis & Haemostasis Network, the Centers for Disease Control and Prevention and the Haemophilia Treatment Center Network, has reported that the number of cases of afibrinogenemia was lower by 36% compared to the published global prevalence [29].

4. Diagnostic work-up of congenital afibrinogenemia as compared with other fibrinogen disorders

Diagnosis of afibrinogenemia is relatively easy. Due to the complete absence of fibrinogen, all coagulation assays with fibrin as endpoint are infinitely prolonged [24] and fibrinogen activity and fibrinogen antigen are undetectable [31]. Fibrinogen activity is measured by the Clauss method or from the PT (i.e. PT-derived fibrinogen). Gravimetric assays such as clottable assays which are time consuming and require a certain expertise, have longtime been considered as the gold standard to assess the fibrinogen concentration [32]. A variety of immunological assays are also available to assess the fibrinogen antigen [33]. Differential diagnosis includes other congenital fibrinogen disorders, i.e hypofibrinogenemia, dysfibrinogenemia and hypodysfibrinogenemia [34]. As indicated in Table 1, their biological phenotype is markedly different from afibrinogenemia. Hypofibrinogenemia is defined by proportional decreased levels of functional and antigen fibrinogen levels, while qualitative diseases are characterized by a discrepancy between decreased functional fibrinogen levels and normal (dysfibrinogenemia) or decreased antigenic fibrinogen levels (hypodysfibrinogenemia) [35]. Due to the lower detection limit of functional assays for fibrinogen [33], it can be difficult to distinguish afibrinogenemia from severe hypofibrinogenemia on standard coagulation assays [33]. The distinction is important since compared to patients with afibrinogenemia, most heterozygous patients with hypofibrinogenemia are still able to express fibrinogen from the normal allele, which allows to increase fibrinogen levels in case of trauma, surgery or pregnancy. Acquired deficiencies of fibrinogen are rarely severe enough to cause a complete absence of fibrinogen [36]. Common conditions such as disseminated intravascular coagulation [37], end-stage liver disease [38], intake of drugs (fibrinolytics or asparaginase) [39] or snake bites [40] can cause hypofibrinogenemia by decreased synthesis or increased consumption. To differentiate an acquired fibrinogen deficiency from afibrinogenemia is generally not difficult after study of the personal and familial histories as well as the clinical setting.

Genotyping allows to confirm the diagnosis (Table 1). Afibrinogenemia is an autosomal recessive disorder resulting from homozygosity or combined heterozygosity for causative mutations [41]. Since the report of the first mutation in 1999 [5], more than 200 distinct causative mutations have been identified [42]. Mutations leading to afibrinogenemia are mainly null mutations (i.e large deletions, splice-site mutations, frameshift mutations and nonsense mutations) which affect the individual chain production [14], the normal assembly of the fibrinogen hexamer or the secretion of the fibrinogen molecule [15]. Two recurrent mutations in *FGA* are of particular interest for patients of Caucasian origin [41]. In a cohort of 74 probands, the 11 kb deletion of *FGA* and the donor splice site mutation in *FGA* c.510+1G>T represented 12.2 and 23.6% of the mutated alleles, respectively [43]. The 11 Kb deletion eliminates most of the *FGA* gene giving rise to a complete absence of fibrinogen [44]. Functional analysis of the c.510+1G→T mutation in transfected COS cells demonstrated that it abolishes the normal donor site, leading to aberrant usage of an alternative downstream donor site and a consequent 4-bp frameshift in the majority of transcripts [45]. Haplotype studies have shown that these mutations are recurrent, or ancient mutations, as they are found on multiple discrete haplotypes with very closely linked markers [46]. Other mutational clusters have been identified, for instance the nonsense mutations *FGA* c.635T>G (p.Leu212X) and *FGG* c.400C>T (p.Arg134X) are relatively common in Lebanon [47,48], the nonsense mutation *FGA* c.718C>T (p.Gln240X) has been found in several cases in Egypt [49] while the splice-site *FGA* c.364+1G>A mutation and frameshift *FGG* c.554delA mutation are found in multiple cases in India [50].

Briefly, as indicated in Table 1, patients with hypofibrinogenemia are mostly heterozygous for null mutations or missense mutations in *FGB* or *FGG*, whereas patients with dysfibrinogenemia are mostly heterozygous for missense mutations in *FGA* or *FGG* [51]. A subset of patients with hypofibrinogenemia also have a liver disease known as Fibrinogen Storage Disease, caused by heterozygous mutations in *FGG* which lead to the production of aberrantly folded γ-chains which are not cleared by the ER quality control pathway [52]. Finally, patients with hypodysfibrinogenemia usually have mutations in *FGA* or *FGG* in heterozygous, compound heterozygous or homozygous states [53].

5. Clinical features

One of the most striking characteristics of afibrinogenemia is that the clinical course can be completely different among patients [54] (Fig. 2). Even though bleeding is the main symptom, its severity ranges from life threatening cerebral bleeding to minor occasional bleeding [55]. Furthermore, patients with afibrinogenemia are paradoxically at

Table 1
Coagulation and main genetic characteristics of fibrinogen disorders.

Type of disorder	aPTT	PT	Fibrinogen activity	Fibrinogen antigen	Genotype
Afibrinogenemia	Infinitely prolonged	Infinitely prolonged	Undetectable	Undetectable	Homozygous or compound heterozygous null* mutations
Hypofibrinogenemia	Prolonged depending on fibrinogen levels	Prolonged depending on fibrinogen levels	Decreased	Decreased	Heterozygous null* or missense mutations
Dysfibrinogenemia	Generally prolonged	Generally prolonged	Decreased	Normal	Heterozygous missense mutations
Hypodysfibrinogenemia	Generally prolonged	Generally prolonged	Decreased	Decreased	Homozygous or heterozygous mutations
Acquired fibrinogen deficiency	Prolonged depending on fibrinogen levels	Prolonged depending on fibrinogen levels	Decreased	Decreased	No causative mutation

aPTT: activated partial thromboplastin time; PT: prothrombin time; *: large deletions, splice-site mutations, frameshift mutations and nonsense mutations

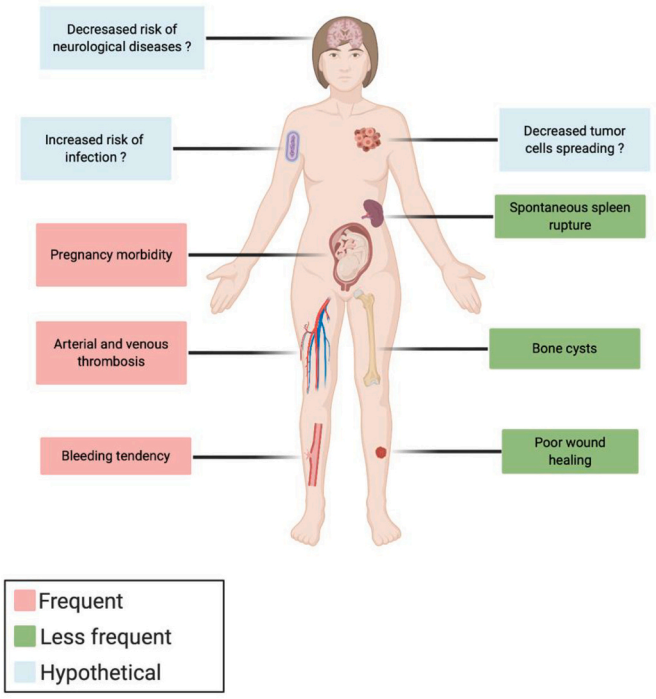

Fig. 2. Frequent, less frequent and hypothetical clinical manifestations of afibrinogenemia. Bleeding and thrombosis are frequent. Pregnancy morbidity includes early fetal loss, placental abruption, vaginal bleeding, postpartum hemorrhage and post-partum thrombosis. Miscellaneous manifestations vary in frequency. Some hypothetical manifestations are not yet supported in clinical studies or described in human subjects but based on observations from animal models. Created with BioRender.com.

increased risk of arterial and venous thromboses [56]. Less frequent symptoms, probably underdiagnosed, can also affect the quality of life of patients with afibrinogenemia [6].

5.1. Bleeding

Bleeding typically starts at birth [57]. In a systematic review on molecular defects in quantitative fibrinogen disorders, among the 52 patients analysed, 18 (34%) reported an umbilical cord bleeding in the neonatal period [13]. The bleeding phenotype in afibrinogenemia is generally severe. For example, in a series of 19 patients from Pakistan [58], the mean bleeding assessment tool score was 20.1 (normal <4 for males and 6 for females) [59]. Table 2 summarises the data of retrospective series reporting the bleeding pattern in afibrinogenemia, which emphasizes that bleeding may occur in almost all tissues. Muscular bleeding and hemarthroses are common [60] and may be severe [61]. Cerebral bleeding is frequent, often recurrent and probably the major cause of morbidity and mortality [62,63]. In a Turkish series of ten patients with afibrinogenemia, six (33.3%) reported a cerebral bleeding, most of the time post-traumatic [64]. More unusual bleeding symptoms have also been described such as hematuria [27], spontaneous hepatic hematoma [65], hemoperitoneum [66] and hemoptysis [67]. The precise incidence of bleeding in patients with afibrinogenemia is not known. In a retrospective questionnaire survey-based study on 100 patients (including 27 patients with hypofibrinogenemia) from ten countries, the mean annual incidence of bleeding episodes was 0.5 for patients on prophylaxis (range 0–2.6) and 0.7 for patients treated on-demand (range 0–16.5). Of the 517 reported bleeding episodes, the most frequent hemorrhages were hemarthrosis (25%) and muscle hematomas (17%) [62]. A prospective post-authorization safety study on a fibrinogen concentrate reported data on 14 patients followed for one year. Among the nine patients treated with prophylaxis, four

Table 2
Bleeding and thrombotic data from retrospective series of patients with afibrinogenemia.

Year, country	Patients, n	Females, n (%)	Umbilical bleeding, n (%)	Muscle bleeding, n (%)	Joint bleeding, n (%)	CNS bleeding, n (%)	Oral cavity*, n (%)	Menorrhagia**, n (%)	Cutaneous, n (%)	Miscellaneous***, n (%)	Thromboses, n (%)
1999, Iran [60]	55	28 (50)	45 (85)	40 (72)	30 (55)	3 (10)	40 (72)	14 (50)	NA	23 (40)	2 (4)
2006, Italy [146]	4	4 (100)	0	2 (50)	0	2 (50)	0	4 (100)	4 (100)	2 (50)	0
2007, Various [147]	6	3 (50)	0	2 (33)	1 (17)	1 (17)	3 (50)	0	1 (17)	0	0
2013, Various [72]	110	65 (59)	66 (60)	51 (46)	33 (30)	22 (20)	32 (29)	36 (55)	51 (46)	39 (36)	24 (21)
2013, India [148]	20	8 (40)	13 (65)	0	1 (5)	5 (25)	7 (35)	3 (38)	17 (85)	6 (30)	0
2015, Various [149]	13****	8 (61)	8 (62)	0	0	1 (8)	8 (62)	1 (8)	8 (62)	6 (46)	0
2016, Switzerland [81]	4	1 (25)	2 (50)	4 (100)	4 (100)	2 (50)	2 (50)	1 (100)	2 (50)	1 (25)	4 (100)
2017, Spain [150]	4	1 (25)	1 (25)	1 (50)	2 (50)	1 (25)	3 (75)	0	1 (25)	3 (75)	2 (50)
2018, United States [136]	4	2 (50)	4 (100)	4 (100)	3 (75)	1 (25)	3 (75)	NA	4 (100)	2 (50)	1 (25)
2019, Sudan [151]	11	NA	5 (45.5)	2 (18.2)	NA	NA	6 (54.6)	2 (18.2)	4 (36.4)	0	NA

* Including gingival bleeding and epistaxis.
** % of women.
*** Including hematuria, postsurgical, retroperitoneal, gastro-intestinal bleeding and hemoptysis.
**** Clinical data available for 10 patients; CNS: central nervous system; NA: not available.

experienced ten hemorrhagic situations while the five patients treated on-demand had 49 hemorrhagic events, including three severe [68]. It is important however to note that some suffer only from minor bleeding and can even be asymptomatic [69]. Long asymptomatic periods are not uncommon, and patients are less likely to have a major disability without replacement therapy than those with severe hemophilia A and B [70].

5.2. Thrombosis

Paradoxically, as shown in Table 2, the clinical course of afibrinogenemia is characterized also by thrombotic events [71]. The exact prevalence of thrombosis is not known, but could be up to 20% [72]. In a series of 25 patients suffering from thrombosis, Korte et al. identified 11 and 16 events in arterial and venous territories, respectively [73]. Acute limb ischemia and toe necrosis are typical arterial thrombotic complications [74–76]. Pulmonary embolisms are also frequently observed [77]. Unusual venous thromboses such as in cerebral [78], splanchnic [79] and renal venous vessels have also been reported [80]. It should be noted that thromboses often occur at a young age and are often recurrent. A systematic review of the literature revealed 48 reports of thromboses with a median of 31 years age at first event and about 15% with recurrences [81].

The underlying pathogenesis of thrombosis in patients with afibrinogenemia remains to be elucidated [73]. Even in the absence of fibrinogen, patients are still able to generate thrombin, both in the initial phase of limited production and in the secondary burst of thrombin generation [82]. In this setting, increases in prothrombin activation fragments and thrombin-antithrombin complexes have been reported [74]. Thrombin, when not trapped within the fibrin clot [83], is available for platelet activation and smooth muscle cell migration and proliferation, especially in the arterial vessel wall [84]. In fibrinogen-deficient mice thrombus formation is maintained, since other adhesive proteins containing an arginine-glycine-aspartic acid sequence such as fibronectin, vitronectin and von Willebrand Factor can serve as ligands to $\alpha_{IIb}\beta_{3.}$ However, the thrombus is unstable and has a tendency to embolize. Using an *in vivo* thrombosis model, Ni et al. analyzed the thrombus growth in wild-type mice and in fibrinogen knockout mice [85]. The number of embolized thrombi was six-fold higher in the knockout mice than that in wild-type mice, with large emboli very often leading to vessel occlusion [85]. Along this line, Remijn et al. have shown that the absence of fibrinogen in human plasma results in large but loosely packed thrombi under flow conditions. Thrombi were essentially formed by many single dendritic platelets in contact with one another through filopodia [86]. A co-existing inherited or acquired plasma hypercoagulability may also contribute to the overall patient's phenotype [87].

5.3. Miscellaneous symptoms

Beside bleeding and thrombosis, patients with afibrinogenemia sometimes suffer from characteristic symptoms such as defective wound healing, spontaneous spleen rupture and bone cysts.

Regarding defective wound healing [88], fibrin(ogen) plays an important role in tissue repair by providing an initial matrix that can stabilize wound fields and support local cell proliferation and migration [89]. It has been shown that granulation tissue in fibrinogen-deficient mice fails to adequately close the wound gap, resulting in persistent open wounds or partially covered sinus tracts [89]. Factor XIII is also essential in wound repair and angiogenesis [90] but its activation is defective in the absence of fibrinogen [91]. Defective wound healing, also a characteristic feature of congenital FXIII deficiency, can lead to skin disorders. Leg ulcers with unusual characteristics such as hemosiderin deposit and localisation have been reported [92]. In such situations, application of fibrin glue could be considered [93].

Spontaneous spleen rupture is one of the most severe, possibly fatal complication of afibrinogenemia. The underlying pathogenesis of spleen rupture in afibrinogenemia remains unclear, but the prevalence seems higher than in patients with other bleeding disorders [94]. In case of minor traumatisms or infections known to cause splenomegaly, microvascular injury can progress to hematoma and capsular rupture in the absence of fibrinogen [95]. In a retrospective series, with a median age of 12 years [96], 8/9 patients were treated by splenectomy, but this approach can increase the potential consecutive risk of splanchnic thrombosis and therefore conservative management should be considered [97].

Skeletal changes in afibrinogenemia have been first reported by Strijthem et al. [98]. They identified juxtatrabecular hemorrhages in metaphysis, leading to formation of intraosseous cysts and remodeling of bone trabeculae Similar findings were successively reported in ten additional patients [99]. Most of these patients develop bone cysts in long bones during childhood, suggesting that this complication could be related to the growing process [100]. The appearance of bone cysts is comparable to intraosseous pseudotumors observed in patients with hemophilia although in afibrinogenemia cysts differ in terms of extent and location. In afibrinogenemia, hemorrhages occur in the immediate vicinity of the cortex or trabecula, locally dissecting away the bone marrow. In this location, the fixation of the venous sinus wall to the surrounding bone and marrow structure might prevent the active contraction of the vessel, which is essential to maintain hemostasis in afibrinogenemia [101]. In addition, the distribution of scattered hemorrhages arranged along the trabeculae according to the lines of stress suggest that they are provoked by mechanical forces. Given that thrombin stimulates production of osteoclastogenic factors by osteoblastic cells and inhibits the early stages of RANKL-induced osteoclast differentiation, a dysregulated thrombin-fibrin axis could lead to cyst formation [102]. Further studies are necessary to better understand the physiopathology of bone cysts in afibrinogenemia. Bone cysts are probably underdiagnosed and should be investigated by magnetic resonance imaging in patients complaining from rheumatic pains in extremities [99].

5.4. Health problems in women

Women are particularly exposed to adverse outcomes during the childbearing period [103,104]. Menorrhagia is the prominent symptom reported by most afibrinogenemic women in reproductive age [60]. Ruptured corpus luteum cyst in luteal phase [105] can lead to massive hemoperitoneum requiring surgical interventions and even oophorectomy [66].

Pregnancy is a very high-risk clinical situation for all women suffering from fibrinogen disorders [106]. Studies with fibrinogen knockout mice have shown that fibrinogen and fibrin are essential components for the maintenance of the foetal-maternal interaction and the attachment of the placenta to the uterine wall by reducing the bleeding at the moment of the spreading of cytotrophoblasts and of maternal vessel remodelling [107,108]. In addition, fibrin stimulates trophoblast proliferation through growth factor binding and integrin upregulation [109] and contributes to placental repair [108]. Human studies have demonstrated that ovulation, fertilization and implantation may occur even in the absence of fibrinogen [110,111] but miscarriages occur early in pregnancy in the absence of fibrinogen. Therefore, all successful pregnancies reported so far have been treated with a fibrinogen supplementation [112]. A systemic literature review has recently analysed 35 pregnancies from 14 women with afibrinogenemia. About half of pregnancies resulted in live births under a fibrinogen supplementation started early in the first trimester [103]. Obstetrical complications included vaginal bleeding [113], rethrochorionic hematoma [114], placenta abruption [111], pre-eclampsia [115] and post-partum thrombosis [115].

6. Management of patients with afibrinogenemia

Patients with afibrinogenemia should be treated in Hemophilia centers with similar principles of care as for patients with hemophilia [116]. For instance, all invasive procedures should be avoided and performed only upon approval of hematologists; nonsteroidal anti-inflammatory drugs are contraindicated; an annual visit should be programmed for all patients [117].

It is now well established that fibrinogen supplementation with plasma-derived fibrinogen concentrates is effective to treat bleeding episodes [118]. In two recent clinical trials, efficacy after a bleeding episode was considered as excellent or good by both the investigators and the independent data monitoring and endpoint adjudication committee [119,120]. As the pharmacokinetics of fibrinogen is variable among the different fibrinogen concentrates and according to the patient's age, weight and individual characteristics, the recovery of fibrinogen activity after infusion of fibrinogen should be individually established [121,122]. Alternative sources for fibrinogen supplementation include fresh frozen plasma and cryoprecipitates [55]. Compared to the latter, fibrinogen concentrates have several advantages: they are safer due to multistep viral inactivation procedures, they provide a precise amount of fibrinogen concentration and they are rapidly available [123]. Management of patients with afibrinogenemia is particularly difficult [124]. There are no data issued from evidence-based medicine studies and only a few guidelines have been published by national Societies (Table 3). Additional recommendations are provided by experts' consensus. In a retrospective study on 100 patients with quantitative fibrinogen disorders, the peak fibrinogen level most often recommended for on-demand treatment of minor bleeding was approximately 1 g/L, and the target level for major episodes such as central nervous system bleeding was 1.5 g/L [62]. Minor episodes, like epistaxis, were usually treated with target peaks of 0.5-0.7 g/L [62]. In a Delphi-like exercise, European experts have proposed statements on five specific clinical settings. Regarding the management of bleeding, the agreement was to aim for a minimal target peak fibrinogen level of $\geq$ 1.5 g/L in case of cerebral bleeding, $\geq$ 1 g/L in case of hemarthrosis and $\geq$ 0.5 g/L in case of muscular bleeding without compartment syndrome [125]. The place of prophylaxis versus on demand therapy in afibrinogenemia is still debated [126,127]. In case of prophylaxis, the trough fibrinogen level to be targeted is unknown [125]. As fibrinogen infusion has been potentially linked to a thrombotic risk, one should aim for the minimal effective fibrinogen level. [128].

Management of women with afibrinogenemia should be conducted by a multidisciplinary team. Taking into account the thrombotic history and the thrombotic risk factors, estrogen-containing or progestin-only regimens should be considered as prophylaxis in patients with menorrhagia [129] and oral contraceptives also appear to be effective for the prevention of follicle rupture-induced hemoperitoneum [130]. The optimal fibrinogen threshold to be targeted during pregnancy is not known (Table 3). Most experts suggest maintaining at least fibrinogen levels >0.5 g/L at the beginning of the pregnancy and to increase fibrinogen dosage throughout the pregnancy up to >1 g/L, maintaining >1.5 g/L at the time of labor and delivery [131,132]. In view of the risk of thrombosis in the post-partum, especially in the case of repeated and prolonged fibrinogen infusion, an accurate thromboprophylaxis should be proposed [87].

Table 3
National Society recommendations on management of bleeding and pregnancy in patients with afibrinogenemia.

	Mild bleeding	Severe bleeding	Pregnancy
UK Haemophilia Centre Doctors' Organization [124]	TXA 15–20 mg/kg or 1 g four times daily alone	FC 50–100 mg/kg, with smaller doses repeated if necessary, at 2–4 days intervals to maintain fibrinogen activity >1 g/L	Prophylaxis throughout pregnancy with FC initially 50–100 mg/kg twice per week, adjusted to maintain trough fibrinogen activity >1 g/l. Peri- and postpartum: FC to ensure a fibrinogen activity >1.5 g/l for at least 3 days
Italian Association of Haemophilia Centres [152]	NA	FC at doses able to increase and maintain circulating factor levels at least up to 1 g/L. The dose can be subsequently reduced, maintaining fibrinogen levels >0.5 g/L, until complete resolution of bleeding	Early prophylaxis aiming at maintaining fibrinogen levels of approximately 1 g/L

TXA: tranexamic acid; FC: fibrinogen concentrate; NA: not available

7. Future directions

Congenital afibrinogenemia is a rare and complex disorder [2]. From the biological point of view, many efforts have been performed to identify the molecular defects and their impact on the biosynthesis of fibrinogen [41]. However, the main challenge derives from our difficulty to identify patients at particular risk of clinical complications, and especially those who are more prone to develop thrombotic complications [133]. Growing evidence suggests that global hemostatic assays could help to better determine the patient's clinical phenotype [134]. Viscoelastic assays are generally used as surrogate markers to evaluate fibrin clots after fibrinogen infusion [135]. Thrombin generation could be of particular interest in afibrinogenemia as it reflects the individual's overall coagulation potential [136]. Sub-samplings studies (i.e, measurement of free thrombin in the fluid phase only) have shown an excess of thrombin in patients with afibrinogenemia [137], that can be normalized with fibrinogen substitution [138]. Variations of thrombin generation among patients may be translated into an individualized treatment prognostic tool for adverse clinical outcomes. They could raise the possibility of tailoring treatment on global hemostatic response rather than normalization of fibrinogen levels.

In addition to its major hemostatic role, fibrinogen has a large range of physiological functions [2]. Indeed, fibrinogen displays diverse functions from direct cell signaling to host defense mechanisms and is at the nexus of a complex vascular-immune-inflammatory interplay [2]. As an example, when skin testing for delayed hypersensitivity is performed in patients with afibrinogenemia there is no induration because of the lack of fibrin deposition [139]. In several cancer models, fibrinogen-deficient mice are protected against tumor spreading [140]. Multiple fibrinogen-cell interactions, including binding with integrin receptor αMβ2, modulates the cancer microenvironment [141]. As a provisional extracellular matrix protein, fibrinogen acts as a physical barrier, a scaffold for immune cell migration, and a spatially defined signal to drive inflammatory cell activation, contributing to overall host defence [142]. Indeed, it has been recently shown *in vivo* and *ex vivo* that fibrin forms a protective film covering the external surface of the clot at the air-wound interface defending the organism against microbial invasion. The lack of fibrin film formation in afibrinogenemia may lead to increased risk of persistent infections from minor dermal lesions [143]. Recent studies have also uncovered the pleiotropic roles for fibrin(ogen) in the activation of central nervous system inflammation, induction of scar formation in the brain, promotion of cognitive decline and inhibition of repair [4]. Fibrinogen infiltration and deposits are critical components in amplifying inflammatory reactivity and causing neuronal damage in the inflamed Alzheimer's disease brain [144]. Fibrin deposition is a prominent feature of multiple sclerosis pathology in both active lesions and chronic plaques [145]. Animal models have provided

evidence that depletion of fibrin(ogen) could be protective in the development and worsening of neurological diseases [4]. Important efforts have recently been made to better assess the epidemiology and the management of afibrinogenemia but clinical features beyond coagulation have not been investigated. Fascinating research questions remain to be explored in this area. Are patients with afibrinogenemia less prone to tumor spreading and metastasis? Are patients with afibrinogenemia less protected against infections? Are patients with afibrinogenemia less at risk to develop neurodegenerative diseases? Only translational research, international collaborations and continuous collection of clinical and biological data will allow to unravel the pleiotropic roles of fibrin(ogen) in such a rare disease.

8. Conclusions

Congenital afibrinogenemia is a rare disease with heterogenous clinical symptoms reflecting the complexity of fibrin(ogen) and its implication in several steps of hemostasis. While bleeding and thrombosis are the most frequent symptoms, miscellaneous complications such as poor would healing, spontaneous spleen rupture and bone cysts are probably underdiagnosed. Management is challenging and requires an accurate collaboration between specialists. Further studies are necessary to determine how one can better predict the clinical course of patients with afibrinogenemia and to investigate the consequences of lack of fibrinogen beyond hemostasis.

Practice points

- Diagnosis of afibrinogenemia is based on the complete absence of fibrinogen which in turn leads to infinitely prolonged activated partial thromboplastin time and prothrombin time.
- The clinical course of patients with afibrinogenemia is usually characterized by a bleeding phenotype but also by thrombosis and more rarely by defective wound healing, spontaneous spleen rupture and painful bone cysts.
- Fibrinogen concentrates should be preferentially chosen over fresh frozen plasma or cryoprecipitates to treat or prevent bleeding episodes.
- Pregnancy is a very high-risk situation requiring a multidisciplinary approach and a fibrinogen supplementation starting early in pregnancy.

Research agenda

- Defining the role of global hemostasis assays and fibrin clot properties to better assess the patient's phenotype and tailor the patient's management.
- Collaborative studies to measure the prevalence of atypical adverse outcomes.
- Determining the optimal fibrinogen thresholds to target in case of acute bleeding, surgery and pregnancy.
- Rationally designed clinical trials to assess the optimal strategy of long-term management (prophylaxis vs on demand therapy).
- The positive or negative effects of the complete absence of fibrinogen on clinical conditions not directly related to coagulation should be further investigated.

Declaration of Competing Interest

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