Hereditary afibrinogenaemia: from genetics to treatments

Alessandro Casini, Service d'angiologie et hémostase, hôpitaux universitaires de Genève, Genève, Suisse
Françoise Bridey, Pharmacien biologiste, ancien interne des hôpitaux de Paris, France
Philippe de Moerloose, Faculté de médecine, université de Genève, Genève, Suisse

Correspondence : A. Casini
alessandro.casini@hcuge.ch

Afibrinogénémie héréditaire : de la génétique aux traitements

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Abstract

Hereditary afibrinogenaemia is a rare coagulation deficiency characterised by the complete absence of fibrinogen. Most mutations are null mutations affecting the synthesis, intracellular assembly, or secretion of fibrinogen. Bleeding is the main symptom, often during the neonatal period with bleeding from the umbilical cord. The bleeding phenotype is severe, characterised by frequent muscle haematomas, haemarthroses and cerebral bleeds. Paradoxically, patients with afibrinogenaemia also suffer from thrombotic events. Both arterial and venous regions are involved, suggesting a common physiopathology. Other symptoms, such as bone cysts, delayed wound healing and spontaneous spleen ruptures are observed and affect patients' health-related quality of life. Several fibrinogen concentrates are available, with similar pharmacokinetic properties, efficacy and safety profiles. In case of bleeding, fibrinogen supplementation is determined by the severity and source of the bleeding, targeting a fibrinogen level of 1–1.5 g/L. Thrombotic events are challenging to manage as they require fibrinogen prophylaxis together with antithrombotic therapy. Pregnancy and child birth are high-risk clinical situations. A multidisciplinary approach and greater fibrinogen supplementation throughout pregnancy is mandatory.

Résumé

L'afibrinogénémie héréditaire est une maladie rare de la coagulation caractérisée par l'absence complète de fibrinogène. La plupart des anomalies génétiques a l'origine de l'afibrinogénémie sont des mutations nulles empêchant la synthèse, l'assemblage intracellulaire ou la sécrétion du fibrinogène dans la circulation. La symptomatologie hémorragique est au premier plan, souvent dès la naissance, avec des saignements du cordon ombilical. Le phénotype hémorragique est sévère, avec des manifestations fréquentes, en particulier aux niveaux musculaire, articulaire et cérébral. Paradoxalement, les patients avec afibrinogénémie sont aussi è risque d'évènements thrombotiques, parfois dès le jeune âge. Les territoires veineux et artériels sont atteints de manière similaire, suggérant un mécanisme physiopathologique commun. D'autres symptômes tels que des kystes osseux, un retard de cicatrisation ou des ruptures spontanées de la rate sont observés et affectent également la qualité de vie des patients. Plusieurs concentrés en fibrinogène sont disponibles, dont les propriétés pharmacocinétiques, l'efficacité et le profil de sécurité sont semblables. En cas de saignement, la supplémentation en fibrinogène est déterminée par la sévérité et le type de saignement en visant une concentration de fibrinogène cible > 1-1,5 g/L. La prise en charge d'un événement thrombotique nécessite à la fois la mise en place d'une supplémentation en fibrinogène et l'introduction d'un des situations particulièrement à risque. Elles nécessitent une prise en charge multidisciplinaire et une supplémentation en fibrinogène avec une augmentation progressive tout au long de la grossesse.

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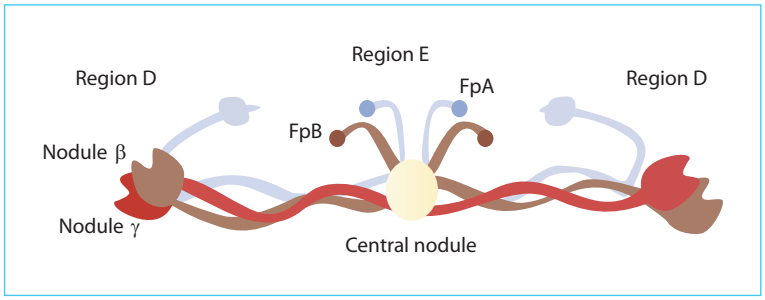
A fibrinogenaemia is characterised by the complete absence of fibrinogen. It is a rare inherited bleeding disorder (defined as fewer than five cases per 10,000 people; Orpha: 335), the varied clinical manifestations of which reflect the importance of fibrinogen in haemostasis [1]. Given the rarity of this condition, the variety of symptoms, and the lack of randomised studies, managing patients with afibrinogenaemia remains a challenge. After reviewing the synthesis and structure of fibrinogen, we discuss various aspects of afibrinogenaemia such as epidemiology, diagnosis, genetics, clinical symptoms and treatment of bleeding and thrombotic complications, as well as the management of pregnancy.

Synthesis and structure of fibrinogen

Fibrinogen is a hexamer formed by two pairs of three chains, the Aα, Bβ and γ chains, encoded by the *FGA, FGB* and *FGG* genes, respectively, located in a 50-kb region of chromosome 4 (q28-q31). The fibrinogen genes are coregulated to maintain a constant basal expression and thus allow a significant and rapid increase in fibrinogen levels during an acute phase reaction. Fibrinogen mRNAs are translated into polypeptides, the signal peptide of which is cleaved in the lumen of the hepatocyte endoplasmic reticulum, where the sequential assembly of the three fibrinogen chains takes place. While properly assembled fibrinogen is secreted as a 340-kDa glycoprotein, incomplete proteins are retained intracellularly and degraded [2]. Circulating fibrinogen is involved in several physiological processes such as immunity and angiogenesis, but it is particularly important as a terminal component of coagulation. Fibrinogen is composed of a central region (E) containing the central globular nodule formed by the NH_2-terminal portions of the Aα, Bβ and γ chains, and two lateral regions (D) formed by the COOH-terminal portions of the Bβ and γ chains (*figure 1*).

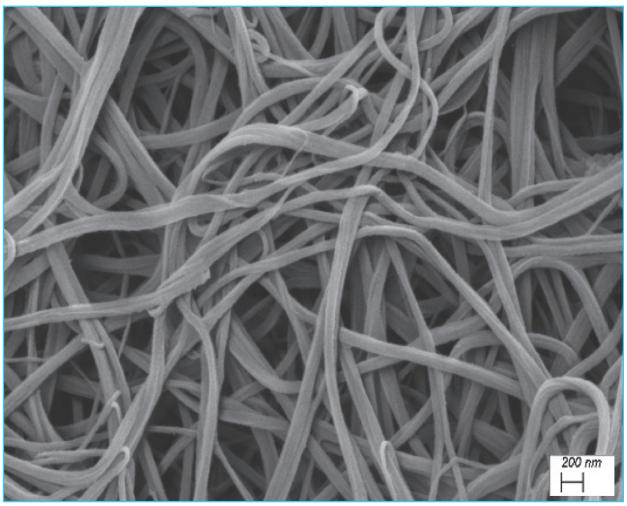
Fibrin polymerisation is initiated after cleavage of fibrinopeptides A and B by thrombin, which exposes binding sites in the E region, complementary to attachment sites in the D regions, allowing the formation of fibrin monomers. The fibrin monomers then interact spontaneously to form oligomers that assemble into protofibrils, the lateral aggregation of which will eventually form fibrin fibres. The elongation and thickening of the fibrin fibres is accompanied by branching leading to the formation of a cross-linked network which, together with platelets, red blood cells and activated factor XIII, provides the structural integrity of the fibrin clot [3]. As an example, *Figure 2* shows the mesh size of fibrin fibres visualised by scanning electron microscopy.

FIGURE 1



Schematic representation of fibrinogen. The Aα chain is shown in blue, the Bβ chain in brown and the γ chain in red. FpA: fibrinopeptide A; FpB: fibrinopeptide B.

Figure 2



Fibrin clot visualised by scanning electron microscopy.

Epidemiology

Although the first description of afibrinogenaemia dates back to 1920, epidemiological data remain relatively poor. National and international registers indicate that quantitative fibrinogen defects account for up to 10% of severe constitutional coagulation factor deficiencies [4]. The prevalence of afibrinogenaemia is higher in countries with high levels of consanguinity. Therefore, while the prevalence is estimated at 0.7 per 1,000,000 inhabitants in France (http://francecoag.org), it could be 10 to 100 times higher in certain countries, and more specifically in certain regions of Pakistan, Lebanon, Iran, Turkey and Egypt. A systematic analysis of the genome of 140,000 individuals in the Genome Aggregation Database (https://gnomad.broadinstitute.org) shows that the prevalence of fibrinogen deficiency of any severity may be much higher than estimated, also in the European population [5].

Diagnosis

Afibrinogenaemia is one of a broad spectrum of inherited fibrinogen defects summarised in *table 1*. The diagnosis of afibrinogenaemia is relatively easy: the complete absence of fibrinogen makes the plasma sample incoagulable on standard coagulation tests such as the prothrombin time test and activated partial thromboplastin time test. The absence of fibrinogen demonstrated by coagulation and immunological methods will confirm the diagnosis (*figure 3*). With the exception of rare cases of severe hypofibrinogenaemia with fibrinogen concentrations below the detection limit, the diagnosis of afibrinogenaemia does not require further investigation [6]. Acquired causes of fibrinogen deficiency, including drug-induced (e.g. asparaginase therapy or thrombolytic therapy), rarely result in total fibrinogen depletion. The differential diagnosis of afibrinogenaemia is thus limited to exceptional situations of acute defibrination syndrome, such as after a snake bite. To our knowledge, no cases of antibody-acquired afibrinogenaemia have been identified to date.

Genetics

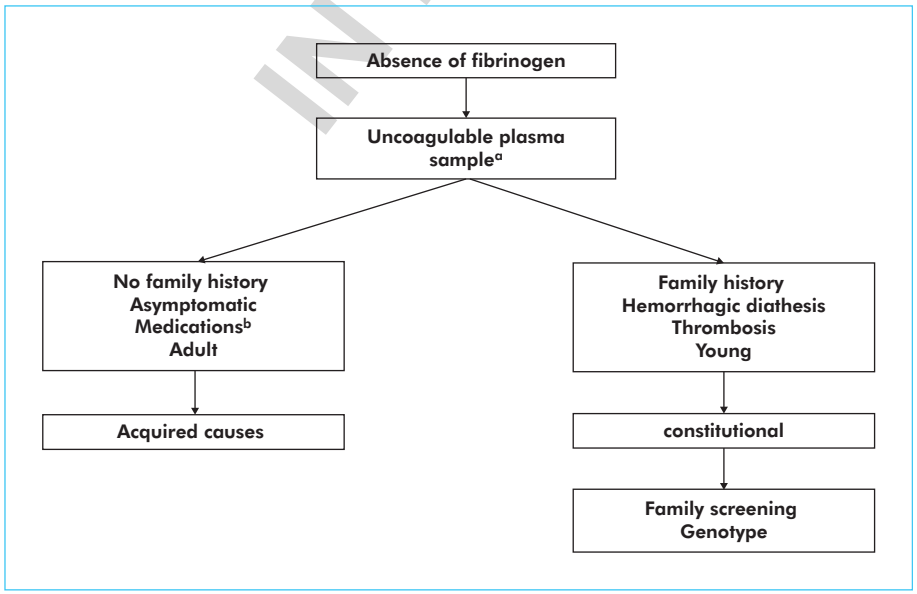
Afibrinogenaemia is an autosomal recessive disease. Since the first mutation was identified in 1999, more than 200 distinct mutations have been identified, in both

Table 1

Classification and biological phenotype of inherited fibrinogen defects.

Type	Afibrinogenaemia	Hypofibrinogenaemia	Dysfibrinogenaemia	Hypodysfibrinogenaemia
Subtypes	Type 1A: haemorrhagic or asymptomatic Type 1B: thrombotic	Type 2A: severe (activity < 0.5 g/L) Type 2B: moderate (0.5-0.9 g/L) Type 2C: mild (1-lower limit) Type 2D: with liver inclusions	Type 3A: haemorrhagic, thrombotic or asymptomatic Type 3B: with mutations associated with high thrombotic risk	Type 4A: severe (antigen < 0.5 g/L) Type 4B: moderate (0.5-0.9 g/L) Type 4C: mild (1-lower limit)
Prothrombin and activated partial thromboplastin time	Incoagulable	Decreased depending on fibrinogen concentration	Decreased depending on fibrinogen concentration, variant and method used	Decreased depending on fibrinogen concentration, variant and method used
Fibrinogen activity and antigen	Undetectable	Proportionally reduced	Discordance between decreased activity and normal antigen depending on variant and method used	Decreased with activity and antigen discordance depending on variant and method used
Genotype	Homozygous or composite heterozygous, null mutation	Heterozygous, null or missense mutation	Heterozygous, missense mutation	Homozygous or heterozygous, null or missense mutation

Figure 3



Diagnostic algorithm in the absence of fibrinogen. [a]Prothrombin level and partial thromboplastin time are incoagulable. [b]Medications that can significantly decrease fibrinogen levels (*e.g.* asparaginase or thrombolytic therapy).

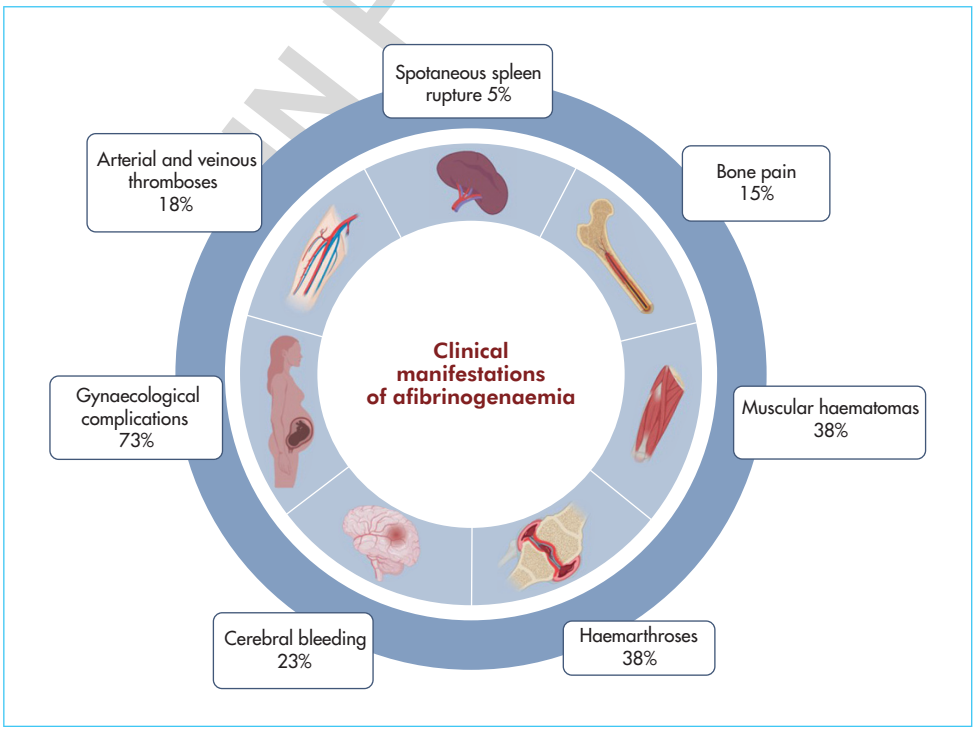
homozygous and composite heterozygous states [7]. A regularly updated list can be found in the online database of the *Groupe Française d'Etude sur l'Hémostase et la Thrombose* (http://site.geht.org/base-fibrinogen/). These are almost exclusively null mutations (large deletions, nonsense mutations, splice-site mutations, frame-shift mutations) that cause fibrinogen deficiency by altering the individual production of one of the fibrinogen chains, the assembly of the hexamer, or the secretion of fibrinogen into the circulation. Interestingly, a few rare missense mutations (<2%) have been identified in the COOH-terminal portions of the Bβ and γ chains, reflecting the importance of these structures in controlling fibrinogen biosynthesis. Two mutations in the *FGA* gene are particularly prevalent. These are a large deletion (11 kb) and the c.510 + 1G > T splice site mutation, which together account for about 20% of the mutations causing afibrinogenaemia [8]. It is important to emphasise that although the penetrance of the biological phenotype (absence of fibrinogen) is complete in afibrinogenaemia, the genotype does not predict the clinical manifestations. Furthermore, this complete lack of fibrin does not allow the functional study of the fibrin clot to predict clinical risk, as is proposed for qualitative fibrinogen abnormalities.

Genetic diagnosis is most useful for confirmation of diagnosis, family screening and prenatal diagnosis. The majority of mutations can be identified by simple polymerase chain reaction with amplification of the exons and intron-exon junctions of the fibrinogen genes or with exome sequencing, although deep intronic mutations or complex rearrangements may require investigation in specialised laboratories [9].

Clinical symptoms

As shown in *figure 4*, the spectrum of clinical manifestations in afibrinogenaemia is very broad. Haemorrhagic symptomatology is at the forefront, often from birth,

FIGURE 4



Clinical manifestations of afibrinogenaemia, adapted from Casini *et al.* [11].

with bleeding when the umbilical cord falls off or during capillary punctures [10,11]. Recently, in an international observational study including 204 patients (119 adults and 85 children) with afibrinogenaemia from different countries, the median value of the International Society of Thrombosis and Haemostasis (ISTH) bleeding score was 14 points (standard: < 3 children, < 4 men, < 6 women) [12]. The frequency of bleeding was significant, with about one-third of patients suffering a bleeding episode each month. The severity of the bleeding phenotype was marked by the number of patients with bleeding requiring medical intervention (82%) as well as by the type of bleeding, namely in muscle (38%), joints (38%) and the brain (23%). The majority of women reported menorrhagia requiring hormonal treatment (74%). It is important to note that in this multicentre study, only 35% of patients were receiving fibrinogen supplementation as prophylaxis [12].

Paradoxically, the risk of thrombosis is also high in patients with afibrinogenaemia. Indeed, in the above-mentioned study, 18% of patients experienced a thrombotic event in the venous and/or arterial territories. Thrombosis in unusual sites was common (*e.g.* aortic, splanchnic or renal thrombosis). Thrombotic episodes were observed in young subjects, with a mean age of 27 years (venous territory) and 36 years (arterial territory). Recurrences occurred in 40% of patients, sometimes repeatedly [12]. The aetiology of this procoagulant state is not fully understood, but an increase in circulating thrombin may play an important role. Indeed, although patients with afibrinogenaemia can still generate thrombin, it cannot be absorbed by fibrinogen and fibrin (which act as antithrombin) and is therefore present in excess. Supporting this hypothesis, the basal increase in prothrombin fragment 1 + 2 and thrombin-antithrombin complex levels measured in patients with afibrinogenaemia is corrected with fibrinogen supplementation [13].

Other less well described and probably under-diagnosed symptoms are worth mentioning. Bone pain due to bone cysts is particularly common in adolescents and young adults (about 15% of patients), probably in connection with growth. These cysts are usually diagnosed by magnetic resonance imaging in the trabeculae of long bones and can be relieved by fibrinogen supplementation [14]. Delayed healing, especially in skin lesions, is also common. This results from a defect in tissue repair related to the lack of fibrinogen and fibrin, which are necessary to support the extracellular matrix and promote cell proliferation and migration [15]. In the absence of fibrinogen, a decrease in the activation of factor XIII, a key element in wound healing, probably also contributes to the failure to heal [16]. Spontaneous spleen rupture is a dreaded complication occurring in about 5% of patients. It is not clear whether the initial cause is a splenic capsule haemorrhage or whether it is a splanchnic thrombosis causing pressure overload of the spleen. Although this is a medical emergency, conservative treatment should be offered whenever possible, given the increased risk of splanchnic thrombosis following splenectomy in patients with afibrinogenaemia [1]. Fibrinogen has many physiological functions other than haemostasis. However, it is currently not clear whether patients with afibrinogenaemia might also be affected by the absence of fibrinogen in immunological, neurological or tumoural terms [17].

Treatment of bleeding and thrombotic complications

Guidelines for managing patients with afibrinogenaemia are based mainly on expert opinion, with some data from non-randomised clinical trials of fibrinogen concentrates [18]. The general management guidelines for patients with severe haemophilia should apply to patients with afibrinogenaemia. Thus, the follow-up of a patient with afibrinogenaemia should be done by a *Centre de Référence de l'Hémophilie* (haemophilia comprehensive care centre). Patients should carry an emergency card with information about their illness, the treatments to be used and

Table 2

Pharmacokinetic parameters of fibrinogen concentrates in patients with afibrinogenaemia (with marketing authorisation in France).

			Manco-Johnson *et al.* 2009 [28]	Djambas-Khayat *et al.* 2019 [20]	Ross *et al.* 2018 [29]
Concentrate	Trade name:				
	- France - other European countries		Riastap Haemocomplettan®	Clottafact FibCLOT	Fibryga Fibryga
	Manufacturer		CSL Behring	LFB	Octapharma
	Raw source		Cryoprecipitate	Supernatant of cryoprecipitate	Cryoprecipitate
	Viral securing		+60 °C, 20 hours	SD/35 nm filtration / + 80 °C, 72 hours	SD/20 nm filtration
	Fibrinogen/reconstitution volume[a]		1 g/50 mL, 2 g/ 100 mL	1.5 g/100 mL	1 g/50 mL
Method	Dose of fibrinogen injected		70 mg/kg	60 mg/kg	70 mg/kg
	(Measuring method)		(Coagulable: PhEu)	(Coagulable: PhEu)	(Activity: Clauss)
	Number of patients		15[b]	14[c]	22[d]
	Average age [min-max]		30 [8–61] years old	7 and 17 years, N = 5 ≥ 18 years, N = 9	26 [12–53] years old
	Sampling time after injection		0.5, 1, 2, 4, 8, 24 hours and 2, 4, 6, 9, 13 days	1, 3, 6, 24 hours and 3, 6, 10, 14 days	0.5, 1, 2, 4, 8 hours and 2, 3, 5, 7, 10, 14 days
	(Measuring method)		(Clauss activity)	(Clauss activity)	(Clauss activity)
Primary PK parameters	Clearance (mL/h/kg)	Median [min-max]	0.55 [0.45–0.86]	0.57 [0.38–0.77]	0.63 [0.40–1.17]
	Volume of distribution (mL/kg)	Median [min–max]	52.7 [36.22–67.67]	53.5 [36.3–60.4]	61.04 [36.89–149.11]
Secondary PK parameters	T_{max} (hours) (end of infusion)	Median [min–max]	-	1.0 [1.0–6.0]	2.0 [0.5–4.1]
	C_{max} (g/L)	Median [min–max]	1.3 [1.00–2.10]	1.34 [1.06–2.19]	1.24 e [0.75–1.96]
	Area under curve 0- (g.h/L)	Median [min–max]	126,8e [81.7-156.4]	105 [78.2–167]	111,14e [59.7-175.5]
	Half-life $t_{1/2}$ (hours)	Median [min–max]	77.1 [55.73–117.26]	67.9 [51.0–99.9]	72.85 [40.03–156.96]
In vivo recovery of injected fibrinogen	Dose dependent (mg/dL per mg/kg)	Median [min–max]	1.7 [1.30–2.73]	2.22 [1.77–3.65]	1.77 [1.08–2.62]
	By plasma volume (%)	Median [min–max]	61.8 [52.45–97.43]	89.0 [69.5–133.0]	64.83 [40.89–88.13]

SD: standard deviation, ND: not available, PhEu: European Pharmacopoeia, PK: pharmacokinetics, SD: solvent/detergent, Tmax: time to maximum concentration.

[a] Value (in grams) determined based on coagulable fibrinogen, according to the European Pharmacopoeia: 008:0024.

[b] PK data reported in 14/15 patients due to a technical problem with the plasma samples.

[c] Some PK parameters for five patients were not calculated due to an insufficient number of quantifiable values.

[d] PK data was reported in 21/22 patients due to an incorrectly administered dose of fibrinogen.

[e] For patients scheduled to receive a fibrinogen dose of 70 mg/kg and who received a different dose, the plasma assay results were standardised for a 70-mg/kg dose and the statistical variables calculated accordingly.

the contact details of the service to be contacted in an emergency. The list of centres in France specialising in the long-term care of these patients is available on the "*maladies hémorragiques constitutionnelles*" website [https://mhemo.fr].

Management of a bleeding event is based on fibrinogen supplementation. Given the high prevalence of brain haemorrhage and the favourable safety profile of fibrinogen concentrates, the administration of fibrinogen as primary or secondary prophylaxis may be preferred to treatment on demand, although there is no evidence to date to justify one option over another. The different fibrinogen concentrates marketed (three are authorised in France to date) have a similar pharmacokinetic profile with a recovery rate *in vivo* of injected fibrinogen in the order of 17–23 g/L per g/kg (1.7–2.3 mg/dL per mg/kg) and a half-life of approximately 70–80 hours in adults and adolescents. The main characteristics of the concentrates are summarised in *table 2*. There are significant inter-individual differences, especially according to age category and weight, which justify a personalised pharmacokinetic study whenever possible [19]. The dosage and duration of fibrinogen supplementation must be individually adapted, depending in particular on the extent and location of the bleeding. Based on the results of clinical studies of fibrinogen concentrates, supplementation is proposed to target a minimum fibrinogen concentration of 1 g/L for minor bleeding and 1.5 g/L for major bleeding [20]. Recently, a group of 21 international experts, the majority of whom were French, made suggestions for management based on a Delphi consensus study [18]. Among the various proposals, the importance of preventive administration of fibrinogen in case of surgery was stressed, targeting a concentration $>$ 1.5 g/L in major surgery and ensuring a basal concentration $>$ 0.5 g/L until healing. The administration of 50–70 mg/kg is usually sufficient in adolescents and adults. If the recovery rate *in vivo* of fibrinogen is known beforehand by carrying out a personalised study, the following formula can be applied for the first injection:

Quantity (g) of fibrinogen = target concentration(g/L) x [(1/rate of recovery (g/L)/(g/kg)) x body weight (kg)]

For example, if a fibrinogen concentration of 1 g/L is targeted in a 50 kg patient with an *in vivo* recovery of injected fibrinogen calculated at 20 g/L per g/kg, the amount to be injected is 2.5 g (50 mg/kg). It is important to note that higher doses are needed in the very young. Indeed, in the subgroup of patients under six years of age, the median recovery rate of injected fibrinogen varies, depending on the study, between 1.83 (range: 1.63–2.37) [21] and 1.32 mg/dL per mg/kg (range: 1.28–1.44) [22], indicating doses of approximately 55 and 76 mg/kg, respectively, to raise fibrinogen levels by 1.0 g/L.

The treatment of thrombotic complications is particularly difficult, as both the risk of thrombotic recurrence and the bleeding risk of anticoagulant treatment must be considered. The introduction or continuation of fibrinogen supplementation is necessary throughout the course of antithrombotic therapy. A short duration of anticoagulation should be preferred. It is difficult to monitor the international normalised ratio (INR) in patients with afibrinogenaemia and low-molecular-weight heparins are the treatment of choice. However, the use of direct oral anticoagulants, described on several occasions, can be considered an effective alternative [23].

Pregnancy

Fibrinogen plays a fundamental role from the beginning of gestation. While fibrinogen is not required for ovulation and fertilisation, a minimal concentration of fibrinogen is necessary to support and maintain embryonic implantation, stabilise uteroplacental binding and promote foeto-maternal vascularisation [24]. Fibrinogen supplementation is necessary throughout pregnancy to avoid

miscarriage and retroplacental haematoma [25]. Fibrinogen requirements increase progressively due to increased fibrinogen clearance in the second and third trimesters of pregnancy [26]. Target concentrations >1–1.5 g/L in the first trimester and >1.5–2 g/L at the time of labour have been proposed on the basis of a few case descriptions [27]. In the postpartum period, particular attention should be paid to the risk of thrombosis, and thromboprophylaxis should be offered.

Conclusion

Afibrinogenaemia is a rare and severe disease. Considerable efforts have been made in recent years to study and describe the genetics and epidemiology of the disease. However, many questions remain, particularly in relation to patient management. We do not know the impact of prophylaxis on patients' quality of life. Similarly, we do not know the targets for fibrinogen concentration in acute bleeding or prophylaxis, for example, during surgery or pregnancy. Global haemostasis tests as well as viscoelastic tests may enable the identification of patients at risk of thrombotic complications and thus individualise fibrinogen supplementation more precisely. Finally, while spectacular progress is being made in gene therapy for haemophilia, no such approach has yet been evaluated for afibrinogenaemia.

Conflicts of interest: the authors have no conflicts of interest to declare.]

References

[1] Casini A, Neerman-Arbez M, de Moerloose P. Heterogeneity of congenital afibrinogenemia, from epidemiology to clinical consequences and management. *Blood Rev* 2020 ; 100793.

[2] Redman CM, Xia H. Fibrinogen biosynthesis. Assembly, intracellular degradation, and association with lipid synthesis and secretion. *Ann N Y Acad Sci* 2001 ; 936 : 480-95.

[3] Weisel JW, Litvinov RI. Mechanisms of fibrin polymerization and clinical implications. *Blood* 2013 ; 121 (10): 1712-9.

[4] Palla R, Peyvandi F, Shapiro AD. Rare bleeding disorders: diagnosis and treatment. *Blood* 2015 ; 125 (13): 2052-61.

[5] Paraboschi EM, Duga S, Asselta R. Fibrinogen as a pleiotropic protein causing human diseases: the mutational burden of aalpha, bbeta, and gamma chains. *Int J Mol Sci* 2017 ; 18 (12): 2711.

[6] Casini A, Undas A, Palla R, Thachil J, de Moerloose P. Subcommittee on factor FXIII, Fibrinogen. Diagnosis and classification of congenital fibrinogen disorders: communication from the SSC of the ISTH. *J Thromb Haemost* 2018 ; 16 (9): 1887-90.

[7] Hanss M, Biot F. A database for human fibrinogen variants. *Ann N Y Acad Sci* 2001 ; 936 : 89-90.

[8] Neerman-Arbez M, Casini A. Clinical consequences and molecular bases of low fibrinogen levels. *Int J Mol Sci* 2018 ; 19 (1): 192.

[9] Casini A, Blondon M, Tintillier V, *et al.* Mutational epidemiology of congenital fibrinogen disorders. *Thromb Haemost* 2018 ; 118 (11): 1867-74.

[10] Peyvandi F, Haertel S, Knaub S, Mannucci PM. Incidence of bleeding symptoms in 100 patients with inherited afibrinogenemia or hypofibrinogenemia; *J Thromb Haemost* 2006 ; 4 (7): 1634-7.

[11] Lak M, Keihani M, Elahi F, Peyvandi F, Mannucci PM. Bleeding and thrombosis in 55 patients with inherited afibrinogenaemia. *Br J Haematol* 1999 ; 107 (1): 204-6.

[12] 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): 3127-36.

[13] Mosesson MW. Update on antithrombin I (fibrin). *Thromb Haemost* 2007 ; 98 (1): 105-8.

[14] van Meegeren ME, de Rooy JW, Schreuder HW, Brons PP. Bone cysts in patients with afibrinogenaemia: a literature review and two new cases. *Haemophilia* 2014 ; 20 (2): 244-8.

[15] Drew AF, Liu H, Davidson JM, Daugherty CC, Degen JL. Wound-healing defects in mice lacking fibrinogen. *Blood* 2001 ; 97 (12): 3691-8.

[16] Bridey F, Negrier C, Duval C, Ariens R, de Moerloose P, Casini A. Impaired factor XIII activation in patients with congenital afibrinogenemia. *Haematologica* 2019 ; 104 (3): e111-3.

[17] Vilar R, Fish RJ, Casini A, Neerman-Arbez M. Fibrin(ogen) in human disease: both friend and foe. *Haematologica* 2020 ; 105 (2): 284-96.

[18] Casini A, de Moerloose P, Congenital Fibrinogen Disorders Group. Management of congenital quantitative fibrinogen disorders: a Delphi consensus. *Haemophilia* 2016 ; 22 (6): 898-905.

[19] Casini A, de Moerloose P. Fibrinogen concentrates in hereditary fibrinogen disorders: past, present and future. *Haemophilia* 2020 ; 26 (1): 25-32.

[20] Djambas Khayat C, El Khorassani M, Lambert T, *et al.* Clinical pharmacology, efficacy and safety study of a triple-secured fibrinogen concentrate in adults and adolescent patients with congenital fibrinogen deficiency. *J Thromb Haemost* 2019 ; 17 (4): 635-44.

[21] Djambas Khayat C, El Khorassani M, Aytac S, *et al.* Pharmacology, efficacy and safety of a triple-secured fibrinogen concentrate in children less than or equal to 12 years with afibrinogenaemia. *Thromb Haemost* 2020 ; 120 (6): 957-67.

[22] Djambas Khayat C, Lohade S, D'Souza F, *et al.* Efficacy and safety of fibrinogen concentrate for on-demand treatment of bleeding and surgical prophylaxis in paediatric patients with congenital fibrinogen deficiency. *Haemophilia* 2021 ; 27 (2): 283-92.

[23] Santoro C, Massaro F, Venosi S, *et al.* Severe thrombotic complications in congenital afibrinogenemia: a pathophysiological and management dilemma. *Semin Thromb Hemost* 2016 ; 42 (5): 577-82.

[24] Iwaki T, Sandoval-Cooper MJ, Paiva M, Kobayashi T, Ploplis VA, Castellino FJ. Fibrinogen stabilizes placental-maternal attachment during embryonic development in the mouse. *Am J Pathol* 2002 ; 160 (3): 1021-34.

[25] Mumford AD, Ackroyd S, Alikhan R, *et al.* Guideline for the diagnosis and management of the rare coagulation disorders: a United Kingdom haemophilia centre doctors' organization guideline on behalf of the British committee for standards in haematology. *Br J Haematol* 2014 ; 167 (3): 304-26.

[26] Saes JL, Laros-van Gorkom BAP, Coppens M, Schols SEM. Pregnancy outcome in afibrinogenemia: are we giving enough fibrinogen concentrate? A case series. *Res Pract Thromb Haemost* 2020 ; 4 (2): 343-6.

[27] Karimi M, Bordbar M, Aali M, Bazrafshan A, Tavoosi H, Gerdabi J. Successful delivery in an patient with afibrinogenemia after three abortions: a case report and review of the literature. *Haemophilia* 2018 ; 24 (2): e63-6.

[28] Manco-Johnson MJ, Dimichele D, Castaman G, *et al.* Pharmacokinetics and safety of fibrinogen concentrate. *J Thromb Haemost* 2009 ; 7 (12): 2064-9.

[29] Ross C, Rangarajan S, Karimi M, *et al.* Pharmacokinetics, clot strength and safety of a new fibrinogen concentrate: randomized comparison with active control in congenital fibrinogen deficiency. *J Thromb Haemost* 2018 ; 16 (2): 253-61.