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Bridey, Françoise; Négrier, Claude; Duval, Cedric; Ariëns, Robert; De Moerloose, Philippe; Casini, Alessandro

How to cite

BRIDEY, Françoise et al. Impaired factor XIII activation in patients with congenital afibrinogenemia. In: Haematologica, 2019, vol. 104, n° 3, p. e111–e113. doi: 10.3324/haematol.2018.203901

This publication URL: https://archive-ouverte.unige.ch/unige:125939

Publication DOI: 10.3324/haematol.2018.203901

Impaired factor XIII activation in patients with congenital afibrinogenemia

Afibrinogenemia is a congenital fibrinogen disorder characterized by the complete lack of fibrinogen.[1] In addition to spontaneous severe bleeding, a defective wound healing has been reported in afibrinogenemic patients, although only a few clinical cases have clearly described poor tissue repair.[1] Fibrin(ogen) is thought to play an important role in tissue repair by providing an initial matrix that can stabilize wound fields and support local cell proliferation and migration.[2] Additional molecules such as fibronectin appear to affect the adhesion and migration of cells at sites of fibrin deposition, thereby contributing to wound healing.[3] Activated factor XIII (FXIIIa) crosslinks fibrin by introducing covalent bonds between fibrin γ-γ, γ-α, and α-α chains, making the fibrin clot stiffer, more compact and hence more resistant to fibrinolysis.[4] In addition to its major role in hemostasis, FXIII is critical for wound healing and angiogenesis.[5] As the activation of FXIII is enhanced by polymerizing fibrin, lack of fibrin(ogen) could reduce activity levels of FXIIIa.[6] In this study, plasma FXIII activity and antigen concentrations were measured before and after fibrinogen replacement in afibrinogenemic patients.

After informed consent, fibrinogen and FXIII levels were determined in 5 afibrinogenemic patients over a period of 14 days following a single administration of a fibrinogen concentrate, Clottafact® (LFB, Les Ulis, France) at 0.06 g/kg, infused at 4 mL/min maximum. All tests were performed on frozen plasma samples drawn in citrate tubes (1 volume of citrate and 9 volumes of whole blood) before (T0), and after the end of infusion at T1 hours (h), T3h, T6h, T24h, Day 3, Day 6, Day 10 and Day 14. Fibrinogen activity and antigen were measured by the Clauss method (STAR automate; Stago, Asnieres, France) and by the immunonephelemetric method (BNII; Dade Behring GmbH, Marburg, Germany), respectively. The plasmatic FXIII antigen was measured by an ELISA method (anti FXIII-A subunit) provided by Affinity Biologicals Inc. (Ancaster, Canada). The plasmatic FXIII activity was measured by the Berichrom® chromogenic ammonia release assay (Dade Behring, Marburg, Germany). In both assays, Standard Human Plasma (Dade Behring GmbH, Marburg, Germany) was used as the calibrator for the calculation of FXIII activity and antigen. Results were expressed as percentage of normal (100% or 1 U/mL represents the concentration or the activity of FXIII present in 1 mL of normal plasma). FXIII (i.e. FXIII-A2B2) concentration in fibrinogen concentrate was assayed by an ELISA method using polyclonal purified IgG anti-human FXIII antibodies (Cedarlane Laboratories, Hornby, ON, Canada).

The overall plasmatic FXIII activity and antigen and fibrinogen activity after infusion of 0.06 g/kg of the fibrinogen concentrate are indicated in Figure 1. Individual FXIII activity and antigen levels are indicated in Tables 1 and 2. The mean baseline FXIII antigen was 99%. A maximum increase in FXIII antigen was observed at T24h (mean 109%). Mean levels varied between 96% and 109% up to 14 days. Intra-individual variability ranged between 8% and 11% (mean 10%). Mean FXIII activity

Table 1. Plasma factor XIII antigen levels (%) following the administration of 0.06 g/kg of fibrinogen concentrate.

Patient	T0	T1h	T3h	T6h	T24h (D1)	D3	D6	D10	D14
1	142	104	107	121	131	109	102	117	122
2	86	116	119	110	122	99	109	111	117
3	89	90	82	79	101	93	95	NA	100
4	87	77	83	91	94	84	89	94	103
5	91	103	109	116	95	97	107	101	98
Mean (SD)	99 (24)	98 (15)	100 (17)	103 (18)	109 (17)	96 (9)	100 (8)	106 (10)	108 (11)
Median	89	103	107	110	101	97	102	106	103
Q1-Q3	87-91	90-104	83-109	91-116	95-122	93-99	95-107	98-114	100-117
Range	86-142	77-116	82-119	79-121	94-131	84-109	89-109	94-117	98-122

T: time; h: hours; D: day; NA: not available; SD: Standard Deviation; Q: quartile.

Table 2. Plasma factor XIII activity levels (%) following the administration of 0.06 g/kg fibrinogen concentrate.

Patient	T0	T1h	T3h	T6h	T24h (D1)	D3	D6	D10	D14
1	11	74	66	69	61	41	26	15	19
2	18	89	83	86	60	49	32	17	17
3	17	79	76	69	67	47	30	NA	17
4	13	67	58	55	36	33	28	12	10
5	26	90	87	92	85	73	47	38	39
Mean (SD)	17 (6)	80 (10)	74 (12)	74 (15)	62 (18)	49 (15)	33 (8)	21 (12)	20 (11)
Median	17	79	76	69	61	47	30	16	17
Q1-Q3	13-18	74-89	66-83	69-86	60-67	41-49	28-32	14-28	17-19
Range	11-26	67-90	58-87	55-92	36-85	33-73	26-47	12-38	10-39

T: time; h: hours; D: day; NA: not available; SD: Standard Deviation; Q: quartile.

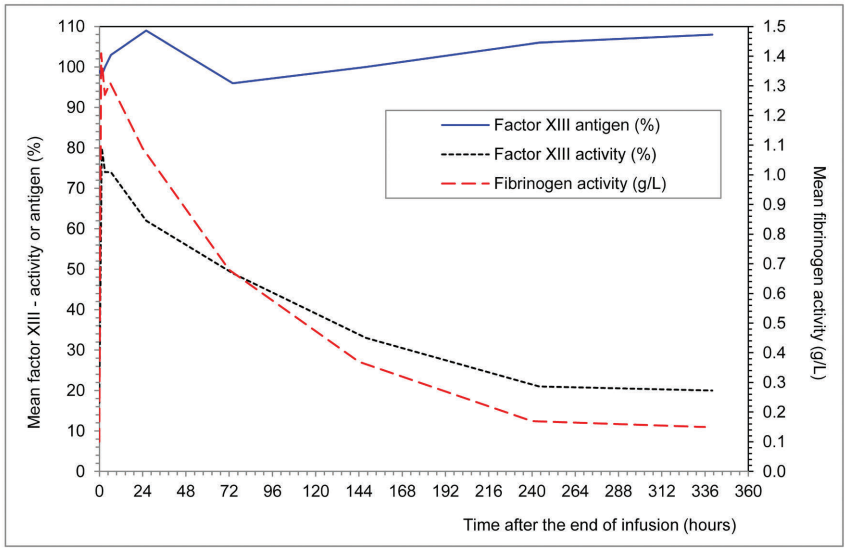

Figure 1. Time course of factor XIII (FXIII) antigen, FXIII activity and fibrinogen activity.

at baseline was markedly low (17%±6%). The FXIII activation appeared rapidly after the administration of fibrinogen. The maximum activity level (80%±10%) was measured 1 h after infusion. FXIII activity levels then decreased until D10 in parallel to the fibrinogen concentration. The mean maximal concentration of fibrinogen and FXIII activities was observed at 1 h post infusion for both proteins following parallel time profiles. Following the administration of the fibrinogen concentrate, a strong positive correlation (r=0.935) between FXIII activity and the presence of fibrinogen was observed (*Online Supplementary Figure S1*). As the Berichrom® assay could underestimate the FXIII activity in afibrinogenemia, we then tested plasma from 8 additional afibrinogenemic patients using a plate-based amine incorporation into casein assay.[7] All patients had decreased FXIII activity with a mean 17.1% (3.7-48.2), indicating that FXIII function was effectively decreased. The kinetics of FXIII cross-linking activity (as measured by biotinylated pentylamine incorporation into casein-coated plates) was faster for the control sample (normal pool) than the patient samples (*Online Supplementary Figure S2*).

FXIII is found in the circulation as heterotetrameric proenzyme FXIII-A_2B_2.[5] FXIII-A_2B_2 is activated by the thrombin-mediated cleavage of an N-terminal 37-amino acid activation peptide from the FXIII-A subunits, which enhances the calcium-dependent dissociation of the inhibitory FXIII-B subunits, leading to the functionally activated FXIII-A_2 (FXIII-A_2*).[8] Our results confirm the essential role of fibrin(ogen) for the full activation of FXIII.[6,9] First, fibrin(ogen) accelerates by 100-fold the thrombin catalyzed cleavage of FXIII-A_2B_2 by enhancing proteolysis of FXIII at Arg37-Gly38 polypeptide bond.[8,10] Second, fibrinogen residues γ390-396 enhance FXIII-A2B2 activation in a FXIII-B dependent mechanism.[11,12] Third, generation of FXIII-A_2* at residues γ390-396 conveniently localizes FXIIIa near the γ-chain crosslinking sites, which are the first fibrin residues to undergo cross-linking.[13] Finally, fibrin(ogen) brings down the Ca^{2+} requirement for the activation of truncated FXIII-A2, acting as a physiologically important Ca^{2+} modulator protein.[14] In our study, the median baseline levels of FXIII activity in patients with afibrinogenemia were markedly low with two different FXIII activity methods. As confirmed by the time-course of the generation of FXIII activity after activation, the FXIII activation was impaired in the absence of fibrinogen. Interestingly, the FXIII antigen was normal, suggesting that association to fibrinogen is not necessary to keep FXIII in the circulation and that, very likely, such an association does not increase the half-life of FXIII in the plasma. FXIII activity was observed immediately after the administration of the fibrinogen concentrate (mean 80%) and pointing out the role of fibrin(ogen) in the activation of FXIII by thrombin. While FXIII antigen remained stable, FXIII activity and fibrinogen levels decreased until day 10, displaying parallel time profiles. Therefore, the dosage of FXIII activity was an indirect way of showing the functional activity of the fibrinogen concentrate. The marked increase in FXIII activity was not linked to the FXIII infusion within the fibrinogen concentrate. Indeed, the median FXIII-A_2B_2 content of the fibrinogen concentrate was 0.0747 mg/mL (3.56 U/mL) and the median FXIII content administered with fibrinogen concentrate was 0.31 mg/kg. The amount of FXIII infused was thus negligible compared to the plasma concentration of FXIII in healthy adults which is between 14 and 28 mg/L.

The observed impaired FXIII activation in afibrinogenemic patients could be clinically relevant. As observed in patients with severe FXIII deficiency,[5] FXIII is essential in wound repair and angiogenesis.[15] In FXIII-deficient mice, the cellular and tissue defects can be corrected by treatment with FXIII.[15] Similarly, in fibrinogen-deficient mice the fibrin(ogen) was crucial for an appropriate cellular migration and organization within wound fields, and in initially establishing wound strength and stability.[2] We can speculate that, in afibrinogenemic patients without fibrinogen replacement, the concomitant impairment of the FXIII activation can contribute to the defective wound healing (e.g. prolonged umbilical cord bleeding) and to the severity of the bleeding phenotype. These observations support the expert's recommendation to maintain target trough fibrinogen level of at least 0.5 g/L until hemostasis is secured.[1] In conclusion, our findings show the essential role of fibrinogen in maintaining FXIII activity in the normal range while it is not necessary to maintain FXIII antigen in circulation.

Françoise Bridey,[1] Claude Négrier,[2] Cedric Duval,[3] Robert Ariëns,[3] Philippe de Moerloose[4] and Alessandro Casini[5]

[1]Clinical Development, Laboratoire Français du Fractionnement et des Biotechnologies (LFB), Les Ulis, France; [2]Hemostasis Unit, Hematology Division, Louis Pradel University Hospital, University

Claude Bernard, Lyon, France; [3]Thrombosis and Tissue Repair Group, Department of Discovery and Translational Science, Leeds Institute of Cardiovascular and Metabolic Medicine, Faculty of Medicine and Health, University of Leeds, UK; [4]Faculty of Medicine, University Hospitals of Geneva, Switzerland and [5]Division of Angiology and Haemostasis, Faculty of Medicine, University Hospitals of Geneva, Switzerland

Correspondence: FRANÇOISE BRIDEY - bridey@lfb.fr
doi:10.3324/haematol.2018.203901

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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