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Minimal factor XIII activity level to prevent major spontaneous bleeds

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Minimum factor XIII level to prevent major bleeds

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Essentials:

- A strong association between bleeding severity and FXIII activity level (FXIII:C) was shown.
- The range 5-30 IU/dL of FXIII:C was associated with a high variability of bleeding severity.
- The PROspective study confirmed the association between FXIII:C activity and bleeding severity.

A FXIII:C of 15 IU/dl is a proposed target to start prophylaxis for prevention of major bleeding.

ABSTRACT

Background: Congenital factor XIII (FXIII) deficiency is a rare bleeding disorder associated with significant bleeding manifestations. The European Network of Rare Bleeding Disorders (EN-RBD) study, performed from 2007 to 2010, showed a strong association between bleeding severity and FXIII activity in plasma of patients with FXIII deficiency. Among these patients variable levels of FXIII activity, from undetectable to 30%, were associated with a wide range of bleeding severity. Objectives and patients: The present cross-sectional study, in the frame of the PRO-RBDD project, a prospective cohort study, analyzed data of 64 patients with FXIII deficiency and different types of clinical and laboratory severity. Results: The results of this analysis confirmed that FXIII coagulant activity in plasma is well associated

with clinical severity of patients. In addition, 15 IU/dl of FXIII activity was identified to be the level under which the probability of spontaneous major bleeding sharply increases (from 50% for levels of 15 IU/dL to more than 90% for levels of 5 IU/dL or lower). Conclusion: the PRO-RBDD study suggests a FXIII coagulant activity level of 15 IU/dL as a target to start prophylaxis in order to prevent major bleedings, such as central nervous system or gastrointestinal tract hemorrhages.

KEYWORDS: Factor XIII, Factor XIII deficiency, Haemorrhage, Phenotype, Prophylaxis

INTRODUCTION

Coagulation factor XIII (FXIII) is a plasma protransglutaminase heterotetramer composed of two FXIII-A and two FXIII-B subunits, which following activation by thrombin and Ca^{2+} acts in the last step of the coagulation cascade [1]. The major function of FXIII is to stabilize blood clots by cross-linking fibrin chains and incorporating antifibrinolytic proteins during hemostasis [2]. This makes the blood clot highly resistant to premature degradation by fibrinolytic proteins [3]. FXIII is capable of cross-linking a wide range of proteins and is involved in a number of processes beyond coagulation, including wound healing and tissue repair [4], fibroblast migration and proliferation, phagocytosis, and angiogenesis of the endothelial barrier [5-8]. FXIII is also necessary for the maintenance of pregnancy [9-11].

Congenital FXIII deficiency is transmitted as an autosomal recessive trait, and is caused by defects in the *F13A1* (OMIM #613225) and the *F13B* genes (OMIM #613235). Since its first description in 1960 more than 500 cases of FXIII deficiency

have been identified worldwide, and most of them concern the deficiency of the FXIII A subunit [6,12]. The prevalence of FXIII-A deficiency has been estimated at one case in 2–3 million, however this data may change in different region of the world [13,14]. The clinical symptoms of FXIII deficiency include delayed wound healing, recurrent spontaneous miscarriage, bleeding of soft and subcutaneous tissue and life-threatening spontaneous central nervous system bleeding, which is the primary cause of death in affected patients [14-19]. In cases of severe FXIII deficiency, early manifestation as umbilical cord bleeding occurs frequently during the neonatal period [14].

Despite the severity of FXIII deficiency, the clinical and phenotypic characteristics remain poorly characterized. In 2012, the European Network of Rare Bleeding Disorders (EN-RBD) analyzed data of 489 patients with rare bleeding disorders (RBDs) and reported the results on the association between clotting plasma levels and bleeding severity for each coagulation factor deficiency [20]. This same report showed a strong association between FXIII activity levels and clinical bleeding severity, with a heterogeneous bleeding tendency, in patients with FXIII coagulant activity level (FXIII:C) ranging from undetectable to 30 IU/dL. Patients with FXIII:C ≥30 IU/dL remained almost asymptomatic [20]. Surprisingly, patients with FXIII activity levels between 5 to 30 IU/dL, in addition to spontaneous minor (grade II) or post-traumatic (grade I) bleeding, showed also spontaneous major bleeding (grade III) such as umbilical cord bleeding [20]. Therefore, a definition of minimum FXIII coagulant activity level to prevent spontaneous major bleeding is desirable to better guide therapeutic decision.

Retrospective analyses, clinical trials and, more recently, prospective analyses have shown that prophylactic treatment with FXIII concentrate can produce sufficient FXIII activity levels for normal hemostasis [21-24].

With this as background, a cross-sectional study of FXIII-deficient patients was performed in the frame of the Prospective Rare Bleeding Disorders Database (PRO-RBDD), with the goal of confirming the data obtained by the EN-RBD in a wider and different group of patients, and establishing the minimum FXIII activity level to prevent major spontaneous bleedings. This was an attempt to support clinicians in their decision on which patient should start a prophylactic replacement therapy.

This study was conducted in cooperation with the EUropean HAemophilia NETwork with the support of the European Commission Health Programme through the Executive Agency for Health and Consumers (EAHC) [25].

METHODS

Data collection

The PRO-RBDD project was designed to collect data on demographics, laboratory phenotypes, genotypes, clinical manifestations, surgery, treatment types and the long-term safety and efficacy of treatment in patients affected with RBDs worldwide. A dedicated group consisting of specialists in the field and clinical epidemiologists have worked with informational technology team to implement the previous EN-RBD interactive web-based database to include new variables and to allow for data collection at sequential checkpoints. Ethical approval, in accordance with national

and international ethical standards, was obtained from the ethical review board of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. All enrolled patients or their parents provided written informed consent.

Data collection began in February 2013 and was designed to include two different sets of data: 1) a retrospective data set to be collected at baseline (demographic data and data on the history of patient from birth to the time of enrolment); 2) a prospective data set to be collected at six follow-ups (at least one every six months, over three years). For the purposes of the current study, the analysis focused on the retrospective data set of patients with FXIII deficiency (baseline data).

The previous classification proposed by the EN-RBD was used to classify baseline data to have a standard definition of laboratory phenotype and bleeding severity [20]. The laboratory phenotype severity was classified into severe (undetectable FXIII:C in plasma), moderate (FXIII:C from lower detection limit to 29 IU/dL) and mild (FXIII:C 30 IU/dL to normal levels) FXIII deficiency. The bleeding severity was classified into four categories: asymptomatic (no documented bleeding episodes), grade I bleeding (bleeding after trauma or drug-induced), grade II bleeding (spontaneous minor bleeding – mucocutaneous), and grade III bleeding (spontaneous major bleeding – umbilical, gastrointestinal and central nervous system bleeding, hemarthrosis and hematoma). To better understand the association between clinical severity of the patients and laboratory phenotype, in addition to bleeding severity, data on (1) age at first bleeding, (2) reason for diagnosis or age at diagnosis and (3) type of treatment were also retrieved and analyzed.

Data were collected from 17 centers of 12 countries, including the Czech Republic, Germany (2), Greece, France, Italy, The Netherlands, Pakistan, Serbia, Switzerland, Turkey (2), USA (2) and the United Kingdom (3). According to World Health

Organization Global Health Observatory data [26], all countries were classified as upper middle or high income countries except one, defined as a lower middle income country.

Laboratory analysis

All the 64 patients included in this study were tested locally for FXIII:C. However, partners had the possibility to send centrally plasma for confirmation of diagnosis, free of charge (central laboratory: Division of Clinical Laboratory Science, University of Debrecen, Hungary). This service was offered with the aim of using an alternative laboratory method with a limit of detection lower than that obtained by local methods. This service was not utilized by all centers probably due to problems in finding dry ice or in shipping samples.

For 29 of the 64 patients included in the study (of whom 22 had a severe laboratory phenotype), the baseline FXIII:C values of the local laboratory were confirmed by the central laboratory. After a high correlation between the two measurements was demonstrated, the central lab values were used for our analyses. For all the other patients (n=35, of whom 9 with a moderate and 17 with a mild laboratory phenotype) the FXIII:C values of the local laboratory were used.

FXIII:C was measured using ammonia release assay, as previously described [27]. The concentration levels of FXIII-A2B2 (plasma) [28], FXIII-A (plasma and platelets) [29] and FXIII-B (plasma) [30] subunits were also measured using ELISA assays. Plasma samples were collected at least 21 days after the last treatment (if any).

Statistical analysis

Data are presented as medians and interquartile ranges (IQR) for continuous variables and as counts and percentages for categorical variables. The Pearson's test was used to check the correlation between FXIII:C values of samples tested both locally and at the central laboratory. Chi-square test was used to test differences between categories of laboratory phenotype severity and bleeding severity (from 0 = asymptomatic to 3 = grade III bleeding). Goodman and Kruskal's gamma was used to determine the intensity and direction of associations between ordinal variables. A regression model taking family dependence into account was also performed (three patients had two relatives included in the database, and three patients, one). Linear regression analysis was used to determine the association between FXIII:C (dependent variable) and the age at first bleeding or at diagnosis (continuous variable), with adjustment for sex and country in which the patient was diagnosed. Differences between the median age at first bleeding or at diagnosis according to the three categories of laboratory phenotype severity of FXIII deficiency were assessed using the Kruskal-Wallis test. To find out the level of FXIII:C that better discriminates patients with or without grade III bleeding, a multivariable logistic regression model was built in which grade III bleeding was the outcome and FXIII:C (kept continuous) was the predictor, adjusting for age and sex. Values of FXIII:C below the lowest level of detection in the FXIII:C assay were represented as half of the lowest detection limit (i.e. in case of samples analyzed only locally, where the lowest detection limit was 5 IU/dL a value of 2.5 IU/dL was used; in the samples analyzed at the central laboratory where the lowest detection limit was 1 IU/dL a value of 0.5 IU/dL was used). In the logistic model, restricted cubic splines with three knots were used to search for possible non-linear relationships between FXIII:C and

the outcome, expressed as log odds. Following the relationship between log odds and probability (probability = exp (log odds) / [1 + exp (log odds)], a probability curve was obtained, which represents the predicted probability of grade III bleeding according to different levels of FXIII:C. Receiver operating characteristic (ROC) curve was used to assess what FXIII:C level better discriminated patients with or without grade III bleeding. Sensitivity and specificity for different FXIII:C levels were calculated. The area under the ROC curve (AUC) with 95% CI was used as estimate of the predictive capability of the logistic model. An internal validation of the predictive model was performed with 1000 bootstrap replicates in order to correct for optimism. All p-values are two-sided and correspond to a significance level of <0.05. All analyses were performed using the statistical software R, release 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

For the 29 samples tested both locally and at the central laboratory, a very high correlation between the two FXIII:C values was observed ($\rho=0.97$, $p<0.001$). Baseline characteristics of the 64 patients with FXIII deficiency are shown in Table 1. Thirty-five patients (55%) had grade III bleeding, which mostly occurred in those patients with severe FXIII deficiency, eight (12%) had grade II bleeding, ten (16%) had grade I bleeding. Five patients (8%, all with mild FXIII deficiency) bled only during pregnancy, and 6 patients (9%, all with mild deficiency), remained asymptomatic at the time of their inclusion into the study (median age of 43 years). Seven out of 64 patients had been previously registered in the EN-RBD database. The results showed below, that refer to the entire PRO-RBDD population, did not differ when the analyses were performed excluding these seven patients.

Association between clinical severity and laboratory phenotype severity

Bleeding severity

Grade III bleeding episodes were observed in 90% of patients with undetectable FXIII:C in plasma (Table 1). No patient with FXIII:C <30 IU/dL remained asymptomatic at the time of inclusion in the study. Grade III bleeding episodes were surprisingly observed also in 46% of patients with FXIII:C between the lower detection limit and 29 IU/dL. No symptoms were observed in 43% of patients with mild FXIII deficiency. An association between laboratory phenotype and bleeding severity was confirmed by Chi-Square test and Goodman and Kruskal's gamma (χ^2 = 46, p <0.0001; G = −0.86, 95% CI: −0.97 to −0.74).

Age at first bleeding

The age at first bleeding was recorded in 54 patients. After adjustment for sex and center of diagnosis, linear regression analysis showed a positive association between the age at first bleeding and FXIII:C (β = 0.81 [95% CI: 0.41 to 1.19] for every 1-year increase). The linear regression model taking family dependence into account led to only trivial changes in the 95% CIs. Similarly, the age at first bleeding varied according to laboratory phenotype severity (Fig. 1A, p <0.0001), and most of the patients with undetectable FXIII:C presented bleeding symptoms at neonatal age.

Reason for diagnosis or age at diagnosis

The age at diagnosis was recorded in all patients. Almost all patients with FXIII:C <30 IU/dL were diagnosed due to bleeding episodes, while patients with FXIII:C ≥30IU/dL were diagnosed following either a bleeding episode or screening test (as a

relative of a patient). After adjustment for sex and center of diagnosis, linear regression analysis showed a positive association between the age at diagnosis and FXIII:C (β = 0.57 [95% CI: 0.27 to 0.88] for every 1-year increase). The linear regression model taking into account the family dependence led to only trivial changes in the 95% CIs. Age at diagnosis differed according to laboratory phenotype severity (Fig. 1B, p <0.0001). In patients with undetectable FXIII:C the median age (IQR) at first bleeding was 0 years [0 to 1], lower than that at diagnosis, (5 years [2.5 to 9]).

Type of treatment

Data on type of treatment was retrieved for all 64 FXIII-deficient patients; 19 out of 31 (61%) patients with undetectable FXIII:C and seven out of 13 (54%) patients with FXIII:C between lower limit of detection and 29 IU/dL received prophylactic treatment. Prophylaxis was only provided to 57% of the patients with grade III bleeding episodes (20 out of 35). The 15 patients with grade III bleeding not receiving prophylaxis were from Pakistan (9), Turkey (3), Germany (2) and Italy (1).

Minimal FXIII coagulant activity to prevent grade III bleeding

A linear increase in the log-odds of grade III bleeding with decreasing levels of FXIII activity was observed (Fig. 2A; p = 0.52 for the non-linear component). The probability curve (Fig. 2B) shows that the predicted probability of grade III bleeding steeply increases for FXIII coagulant activity levels <20 IU/dL, with a probability of about 50% for levels of 15 IU/dL and >90% for levels of 5 IU/dL or lower. Those patients suffering from grade III bleeding all had FXIII activity levels ≤16 IU/dL (Fig. 3). The six patients with detectable FXIII:C deficiency and grade III bleeding (Table

1) had FXIII activity levels between 5 and 16 IU/dL. In order to widening the description of this particular group of patients, additional information about genotyping, FXIII:C tests and any possible treatment, if any, were also extrapolated from the PRO-RBDD. Table 2 reports details of this group of patients.

In the ROC analysis, that allowed us to find the FXIII:C level that better discriminates patients with from those without grade III bleeding, the FXIII level of 15 IU/dL corresponded to a sensitivity of 97% (95% CI: 83 to 99%) and a specificity of 76% (95% CI: 58 to 90%). The AUC of the ROC curve sorting from the full logistic model was 0.96 (95% CI: 0.93 to 1.00) (Fig. 4). After correction for optimism (1000 bootstrap replicates), the AUC became 0.94. In a full logistic model where FXIII:C was dichotomized at the cut-off point of 15 IU/dL, the AUC of the ROC curve was very similar (0.95 [95% CI: 0.91 to 0.99]).

DISCUSSION

FXIII deficiency is a rare disorder characterized by episodes of severe bleeding. Because of the low prevalence, studies have historically described individual case reports or small series of patients, limiting what could be learned from the reported findings. In our previous report on the EN-RBD study, we described a new method to classify bleeding severity and laboratory phenotype severity for the first time in a European group of patients [20].This classification made important steps towards standardization of the diagnosis and treatment of FXIII deficiency, but has not been validated in a real-life representative patient population. In this cross-sectional study, we have confirmed the usefulness of the EN-RBD FXIII deficiency classification system by analyzing the baseline data of 64 FXIII-deficient patients from the ongoing PRO-RBDD study. We found that FXIII plasmatic activity correlates well with two

important clinical parameters: age at first bleeding and age at diagnosis, considered two parameters of clinical severity of patients. Almost all patients with undetectable FXIII:C up to 29 IU/dL were diagnosed due to bleeding episodes, while patients with FXIII:C ≥30 IU/dL were diagnosed following an isolated non-major bleeding episode or as family member of an affected patient.

Grade III bleeding was observed in 55% of all patients, similar to the value of 48.5% described in our previous study [20]. We consider this as a validation study of the previous EN-RBD data collection which assessed that patients with FXIII deficiency require a FXIII:C ≥ 30 IU/dL to remain asymptomatic [20]. However, the wide range of FXIII activity (from the lower detection limit to 29 IU/dL) associated with a highly variable bleeding tendency (from grade I to III), led us to identify a FXIII activity level to prevent spontaneous major bleeding. This decision was made aiming to support clinicians in their decision on which patients could benefit from prophylaxis and what FXIII:C level could be considered safe. Our results showed that the probability of grade III bleeding (as umbilical, gastrointestinal and central nervous system bleeding, hemarthrosis and hematoma) steeply increases when FXIII coagulant activity levels fall below 20 IU/dL. In particular, the FXIII cut-off level of 15 IU/dL corresponded to a sensitivity of 97%, thus identifying almost all patients with grade III bleeding (the only one that was not identified had FXIII coagulant activity level of 16 IU/dL). We recognize that the limited number of patients does not allow for a definite clear cut-off for prophylaxis. However, since in this study the highest FXIII level associated with grade III bleeding was 16 IU/dL, and the probability of grade III bleeding increased from 50% to more than 90% for levels of 15 IU/dL or lower, a value of 15 IU/dL seems a reasonable level to be suggested for the initiation of prophylaxis. Interestingly, this result looks similar to the previous data obtained by

Kerlin et al evaluating pharmacokinetics of recombinant FXIII, which showed that the trough geometric mean FXIII activity level was 0.16 IU/mL (i.e.,16 IU/dl) [31]. Whether or not this FXIII cut-off should be maintained as trough level during prophylaxis cannot be answered by the present study, in which all the analyses were based on laboratory and clinical data collected at baseline. The trough level might be lower than 15 IU/dL, since during prophylaxis the period of exposure to very low levels of FXIII is shorter than that in the absence of prophylaxis, and limited to the period immediately before the next dose of FXIII concentrate. This shorter exposure period might be insufficient to trigger spontaneous major haemorrhages. Anyway, the answer to this question will only be possible in a prospective study on FXIII deficient patients receiving prophylaxis, where a correlation between trough FXIII levels and bleeding events (if any) can be made.

Recent prospective studies have suggested that prophylactic treatment of FXIII deficiency with FXIII concentrate is safe and effective in patients at high risk of life-threatening bleeding [22,24]. Additionally, FXIII has a long half-life, therefore it is administered only once every four to six weeks [13]. Despite this, only 61% of patients with severe FXIII deficiency in the PRO-RBDD database were on prophylactic FXIII treatment at enrolment, probably due to the lack of product in some countries.

To our knowledge, no prospective studies with the purpose to collect data on any type of hemorrhagic event (spontaneous, post-trauma, post-surgery or other) and any type of possible treatment for FXIII deficiency is currently ongoing, except the Post Approval Safety Surveillance Mentor™ 6 by NovoNordisk. Analyses of the follow-up data recorded into the PRO-RBDD to determine whether prophylactic treatment could successfully reduce severe clinical bleeding episodes in this FXIII-

deficient patient population are ongoing. It will be also useful to confirm the results of the current analysis (exclusively based on data at enrolment) because less subjected to the possible errors due to investigation of medical history, by its nature less accurate. The main limitation of our study is that clinical records predominantly included patients presenting with bleeding symptoms. With this selection bias, individuals from the general population, with moderate-to-severe FXIII deficiency and few (or no) bleeding symptoms, were less likely to have been included in this study. This could have led to ascertainment bias with an overestimation of the risk of bleeding according to FXIII activity levels. Only studies recruiting index cases and family members that are followed over time will yield more precise risk estimates. However, this limitation is unlikely to affect management strategies, as in a daily clinical practice physicians deal with patients referred because of bleeding symptoms (thus a selected group of individuals with FXIII deficiency), and within this group they should discriminate patients who may benefit from prophylaxis with FXIII concentrates to prevent grade III bleeding. Another limitation is that samples were centralized for a methodologically uniform FXIII measurement in only half of the patients. However, since the main purpose of this re-analysis was to improve the lower limit of FXIII detection, most of the samples re-tested were in the severe laboratory group (i.e., undetectable FXIII), and a very high correlation between the two values of the local and central laboratories was found, the possibility of having misclassified FXIII deficient patients is low. Hence, we are confident that a similar high correlation would also apply to the other samples tested only at the local laboratory.

In conclusion, we have presented an analysis of baseline data from an ongoing study of an international FXIII-deficient patient population. These data allowed us to validate the EN-RBD classification and have helped to indicate a FXIII activity cut-off of 15 IU/dL to prevent spontaneous major bleedings. The continuation of the study including a centralized FXIII testing extended to all patients and strict criteria regarding FXIII washout might confirm this minimal level as a target value to start prophylaxis in patients affected by congenital FXIII deficiency.

AUTHORSHIP CONTRIBUTIONS

M. Menegatti and R. Palla designed the queries, performed quality control on data entered, analyzed the results and wrote the manuscript; M. Boscarino extracted data from the database and performed statistical analysis; P. Bucciarelli performed statistical analysis and contributed in writing the manuscript; S. Halimeh, B. Lachmann, C. Bidlingmaier, H. Platokouki, H. Pergantou, S. M. Siboni, R. E. G. Schutgens, M. Borhany, F. Naveena, D. Mikovic, M. Saracevic, P. de Moerloose, A. Casini, N. Ozdemir, A. Yilmaz, A. Mumford, A. Harvey, J. Payne, A. D. Shapiro, A. Williamson, J. Chapin, F. Hsu enrolled the patients, collected data and revised the manuscript; L. Muszbek and E. Katona performed laboratory analyses and revised laboratory results; M. Makris as project leader of EUHANET project contributed to the revision of the the manuscript; F. Peyvandi designed the study and the case report form, critically revised the results and the manuscript. All authors reviewed the manuscript and approved the final version.

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DISCLOSURE OF CONFLICTS OF INTEREST

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REFERENCES

1. Muszbek L, Bereczky Z, Bagoly Z, Komáromi I, Katona É. Factor XIII: a coagulation factor with multiple plasmatic and cellular functions. *Phys Rev* 2011; **91**: 931-72.
2. Lord ST. Molecular mechanisms affecting fibrin structure and stability. *Arterioscler Thromb Vasc Biol* 2011; **31**: 494-9.
3. Fraser SR, Booth NA, Mutch NJ. The antifibrinolytic function of factor XIII is exclusively expressed through alpha(2)-antiplasmin cross-linking. *Blood* 2011; **117**: 6371-4.
4. Inbal A, Lubetsky A, Krapp T, Castel D, Shaish A, Dickneitte G, Modis L, Muszbek L, Inbal A. Impaired wound healing in factor XIII deficient mice. *Thromb Haemost* 2005; **94**: 432-7.
5. Muszbek L, Bagoly Z, Bereczky Z, Katona E. The involvement of blood coagulation factor XIII in fibrinolysis and thrombosis. *Cardiovasc Hematol Agents Med Chem* 2008; **6**: 190–205.
6. Duckert F, Jung E, Shmerling DH. A hitherto undescribed congenital haemorrhagic diathesis probably due to fibrin stabilizing factor deficiency. *Thromb Diath Haemorrh* 1960; **5**: 179–186.
7. Seitz R, Duckert F, Lopaciuk S, Muszbek L, Rodeghiero F, Seligsohn U; Study Group. ETRO Working Party on Factor XIII questionnaire on congenital factor XIII deficiency in Europe: status and perspectives. *Semin Thromb Hemost* 1996; **22**: 415–8.
8. Dardik R, Loscalzo J, Inbal A. Factor XIII (FXIII) and angiogenesis. *J Thromb Haemost* 2006; **4**: 19–25.

9. Inbal A, Muszbek L. Coagulation factor deficiencies and pregnancy loss. *Semin Thromb Hemost* 2003; **29**: 171–4.

10. Muszbek L, Bagoly Z. Fibrin formation disorders and pregnancy loss. *Thromb Res* 2007; **119**: S69–S70.

11. Koseki-Kuno S, Yamakawa M, Dickneite G, Ichinose A. Factor XIII A subunit-deficient mice developed severe uterine bleeding events and subsequent spontaneous miscarriages. *Blood* 2003; **102**: 4410–2.

12. Biswas A, Ivaskevicius V, Seitz R, Thomas A, Oldenburg J. An update of the mutation profile of Factor 13 A and B genes. *Blood Rev* 2011; **25**: 193-204.

13. Muszbek L, Bagoly Z, Cairo A, Peyvandi F. Novel aspects of factor XIII deficiency. *Curr Opin Hematol* 2011; **18**: 366-72.

14. Dorgalaleh A, Naderi M, Hosseini MS, Alizadeh S, Hosseini S, Tabibian S, Eshghi P. Factor XIII deficiency in Iran: a comprehensive review of the literature. *Semin Thromb Hemost* 2015; **41**: 323-9.

15. Anwar R, Minford A, Gallivan L, Trinh CH, Markham AF. Delayed umbilical bleeding—a presenting feature for factor XIII deficiency: clinical features, genetics, and management. *Pediatrics* 2002; **109**: E32.

16. Lak M, Peyvandi F, Ali Sharifian A, Karimi K, Mannucci PM. Pattern of symptoms in 93 Iranian patients with severe factor XIII deficiency. *J Thromb Haemost*2003; **1**: 1852-3.

17. Ichinose A, Asahina T, Kobayashi T. Congenital blood coagulation factor XIII deficiency and perinatal management. *Curr Drug Targets* 2005; **6**: 541–9.

18. Perez DL, Diamond EL, Castro CM, Diaz A, Buonanno F, Nogueira RG, Sheth K.. Factor XIII deficiency related recurrent spontaneous intracerebral

hemorrhage: a case and literature review. *Clin Neurol Neurosurg* 2011; **113**: 142–5.

19. Shetty S, Shelar T, Mirgal D, Nawadkar V, Pinto P, Shabhag S, Mukaddam A, Kulkarni B, Ghosh K. Rare coagulation factor deficiencies: a countrywide screening data from India. *Haemophilia* 2014; **20**: 575-81.

20. Peyvandi F, Palla R, Menegatti M, Siboni SM, Halimeh S, Faeser B, Pergantou H, Platokouki H, Giangrande P, Peerlinck K, Celkan T, Ozdemir N, Bidlingmaier C, Ingerslev J, Giansily-Blaizot M, Schved JF, Gilmore R, Gadisseur A, Benedik-Dolničar M, Kitanovski L, Mikovic D, Musallam KM, Rosendaal FR; European Network of Rare Bleeding Disorders Group. 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**: 615-21.

21. Lusher J, Pipe SW, Alexander S, Nugent D. Prophylactic therapy with Fibrogammin P is associated with a decreased incidence of bleeding episodes: a retrospective study. *Haemophilia* 2010; **16**: 316-21.

22. Ashley C, Chang E, Davis J, Mangione A, Frame V, Nugent DJ. Efficacy and safety of prophylactic treatment with plasma-derived factor XIII concentrate (human) in patients with congenital factor XIII deficiency. *Haemophilia* 2015; **21**: 102-8.

23. Inbal A, Oldenburg J, Carcao M, Rosholm A, Tehranchi R, Nugent D. Recombinant factor XIII: a safe and novel treatment for congenital factor XIII deficiency. *Blood* 2012; **119**: 5111-7.

24. Dreyfus M, Barrois D, Borg JY, Claeyssens S, Torchet MF, Arnuti B, Pautard B; Groupe d'Etudes Francophone du FXIII. Successful long-term replacement therapy with FXIII concentrate (Fibrogammin(®) P) for severe congenital factor XIII deficiency: a prospective multicentre study. *J Thromb Haemost* 2011; **9**: 1264-6.

25. Makris M, Calizzani G, Fischer K, Gatt A, Gilman E, Hollingsworth R, Lambert T, Lassila R, Mannucci PM, Peyvandi F, Windyga J. The European Haemophilia Network (EUHANET). *Blood Transfus* 2014; **12**: s515-8.

26. World Health Organization. Annex1: Regional and income groupings. *Global Health Observatory (GHO) data*. 2015;http://www.who.int/gho/publications/world_health_statistics/2015/en/.

27. Katona E, Penzes K, Molnar E, Muszbek L. Measurement of factor XIII activity in plasma. *Clin Chem Lab Med* 2012; **50**: 1191-202.

28. Katona E, Haramura G, Karpati L, Fachet J, Muszbek L. A simple, quick one-step ELISA assay for the determination of complex plasma factor XIII (A2B2). *Thromb Haemost* 2000; **83**: 268-73.

29. Katona EE, Ajzner E, Toth K, Karpati L, Muszbek L. Enzyme-linked immunosorbent assay for the determination of blood coagulation factor XIII A-subunit in plasma and in cell lysates. *J Immunol Methods* 2001; **258**: 127-135.

30. Ajzner E, Schlammadinger A, Kerényi A, Bereczky Z, Katona E, Haramura G, Boda Z, Muszbek L. Severe bleeding complications caused by an autoantibody against the B subunit of plasma factor XIII: a novel form of acquired factor XIII deficiency. *Blood* 2009; **113**: 723-5.

31. Kerlin B, Brand B, Inbal A, Halimeh S, Nugent D, Lundblad M, Tehranchi R. Pharmacokinetics of recombinant factor XIII at steady state in patients with

congenital factor XIII A-subunit deficiency. *J Thromb Haemost* 2014; **12**: 2038-43.

TABLE

Table 1. Baseline characteristics of the 64 patients with FXIII deficiency.

Sex (Male/Female), N		31 / 33	
Median age (IQR), yrs		27 (16–40)	
Patients aged ≤12 years, N (%)		8 (13)	
	Severe (undetectable)	**Moderate** (lower detection limit to 29 IU/dL)	**Mild** (≥30 IU/dL)
Laboratory severity[*,§,] N (%)	31 (49)	13 (21)	19 (30)
Bleeding severity[*,¶]			
Asymptomatic, N (%)	0 (-)	0 (-)	6 (43)
Grade I, N (%)	2 (7)	5 (39)	3 (21)
Grade II, N (%)	1 (3)	2 (15)	5 (36)
Grade III, N (%)	28 (90)	6 (46)	0 (-)

* Based on classification according to EN-RBD (Peyvandi et al).

§ Information not available for one patient with grade III bleeding.

¶ Five patients with mild deficiency were not classified because they had exclusively bleeding during pregnancy.

Table 2. Characteristics of the 6 patients with FXIII:C levels between 5 and 16 IU/dL and grade III bleeding.

Patient	Age at diagnosis	Laboratory severity (method - date of measurement)	Bleeding symptoms	Country	Mutation - genotype	Prophylaxis
1	23 months	14 IU/dl (Photometric – local, at diagnosis)	CNS Haematoma Mucocutaneous Umbilical cord	Pakistan	Ser414Leu homozygous	NO
2	24 months	13 IU/dl (Photometric – local, after diagnosis) 16 IU/dl (Ammonia release - central, after diagnosis)	Mucocutaneous During pregnancy Spontaneous abortion Umbilical cord	Pakistan	IVS11+1 G>A- homozygous	NO
3	3 months	6 IU/dl (method NA - local, at diagnosis)	CNS GI Haemarthrosis Haematoma Umbilical cord	Germany	Leu250SerfsX18 homozygous	NO
4	14 years	8 mg/L (ELISA A2B2 - local, at diagnosis) 5 IU/dl (Amine incorporation – local , 21 days from the last prophylactic infusion)	CNS Menorrhagia Mucocutaneous	USA	Arg 78Cys + Arg79Gly compound heterozygous	YES Started one year after the diagnosis (45 U/Kg once/month)
5	1 month	5 IU/dl (Photometric - at diagnosis)	Mucocutaneous Umbilical cord	UK	Phe9fs homozygous	YES Started two months after the diagnosis (500 U once/3 weeks)
6	1 year	7 IU/dl (Amine incorporation - 30 days from the last prophylactic infusion 29/01/2014)	CNS Haemarthrosis Haematoma Mucocutaneous	USA	Arg704Trp + Arg172Stop compound heterozygous	YES Started 13 years after the diagnosis (37 U/Kg once/month)

FIGURE LEGENDS

Figure 1. Distribution of age at first bleeding (panel A) and age at diagnosis (panel B) according to laboratory severity of FXIII CD. The box plots represent the interquartile ranges, the solid horizontal line inside each box plot is the median value and the vertical bars delimit the min and max values of the distribution. The black circles identify single observation.

Figure 2. A: Restricted cubic spline curve showing the age- and sex-adjusted relationship between FXIII activity levels and risk of grade III bleeding, expressed as log odds. The FXIII activity levels are truncated at the value of 30 IU/dL, since patients with values above this point had no grade III bleeding.
B: Probability curve showing the predicted probability of grade III bleeding according to FXIII activity levels. This curve is obtained following the relationship between log odds and probability (probability = exp(log odds) / [1 + exp(log odds)]. The grey areas around the curves represent 95% CI.

Figure 3. Distribution of FXIII activity levels according to presence or absence of grade III bleeding. The box plots represent the interquartile ranges, the solid horizontal line inside each box plot is the median value and the vertical bars delimit the min and max values of the distribution. The black circles identify single observation.

Figure 4. ROC curve showing the predictive ability of the full logistic model (containing FXIII:C levels, age and sex) for the discrimination of patients with or without grade III bleeding. The dashed diagonal line represents the situation of predictive uncertainty (AUC = 0.50).

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