Check for updates

Received: 18 March 2024 | Revised: 14 May 2024 | Accepted: 20 May 2024

DOI: 10.1111/hae.15059

Haemophilia WFH WILEY

ORIGINAL ARTICLE

Rare bleeding disorders

Limited value of testing for factor XIII and α2-antiplasmin deficiency in patients with a bleeding disorder of unknown cause

Sander Ariëns[1] | Albert Huisman[2] | Idske C. L. Kremer Hovinga[1] | Rolf T. Urbanus[1] | Karin P. M. van Galen[1] | Lize F. D. van Vulpen[1] | Kathelijn Fischer[1] | Roger E. G. Schutgens[1]

[1]Center for Benign Haematology, Thrombosis and Haemostasis, van Creveldkliniek, Utrecht, the Netherlands

[2]Central Diagnostic Laboratory, University Medical Center Utrecht and University Utrecht, Utrecht, the Netherlands

Correspondence
Roger E.G. Schutgens, Center for Benign Haematology, Thrombosis and Haemostatis, Van Creveldkliniek, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, the Netherlands.
Email: R.Schutgens@umcutrecht.nl

Testing for FXIII and α2-antiplasmin in BDUC

Abstract

Introduction: In patients with an increased bleeding tendency, extensive diagnostic blood testing is often performed. When results of tier 1 assays of primary haemostasis are normal, protocols recommend additional testing to rule out rare disorders including coagulation factor XIII (FXIII) and α2-antiplasmin (α2AP) deficiency.

Aim: To evaluate the added diagnostic value of FXIII and α2AP levels in patients with a bleeding disorder of unknown cause (BDUC).

Methods: A retrospective monocentre cohort study between August 2011 and August 2023 was conducted. In all patients with bleeding tendencies and normal diagnostic tests for von Willebrand disease and platelet function, FXIII and α2AP were measured.

Results: We included 158 consecutive patients; mean ISTH-BAT scores were 8.2 (SD ± 3.7) in children, 6.2 (SD ± 2.1) in men and 10.6 (SD ± 3.3) in women. Median age was 37 (range 5–79) years, 88.6% of patients were female. Patients displayed median FXIII activity of 111% (IQR = 97–131) and median α2AP activity of 112% (IQR = 103–119).

Three (1.9%) patients had FXIII levels < 50%, respectively 43%, 45% and 46%. Corresponding ISTH-BAT scores were 7, 12 and 14. No α2AP levels < 60% was observed. No significant association was found between FXIII levels and ISTH-BAT scores.

Conclusion: In our cohort of BDUC patients, no clinical relevant FXIII deficiencies were detected; absolute values were well above the 30% cutoff considered adequate for normal haemostasis. No α2AP deficiencies were detected. These data suggest that in BDUC patients, measuring FXIII or AP activity is of limited value.

KEYWORDS

alpha-2-antiplasmin, bleeding disorder of unknown cause (BDUC), factor XIII deficiency, haemorrhagic disorders, primary haemostasis

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2024 The Author(s). *Haemophilia* published by John Wiley & Sons Ltd.

1 | INTRODUCTION

In more than half of the patients referred for assessment of a possible bleeding tendency, no definitive cause will be found. They will receive the more general diagnosis "bleeding disorder of unknown cause" (BDUC).[1] However, a clear definition of BDUC is lacking as is consensus about the diagnostic assays that need to be performed to rule out specific bleeding disorders. As a result, patients are potentially withheld from receiving the most optimal treatment.

Several surveys and scoring systems have been developed to assess bleeding phenotypes objectively, so called bleeding assessment tools (BAT). A broadly used BAT, endorsed by the International Society on Thrombosis and Haemostasis (ISTH), is the ISTH-BAT. Scores of ≥4 for men and ≥6 for women are considered abnormal and indicate an increased bleeding tendency.[2] Whilst no consensus about the definition of BDUC has been reached as of today, an abnormal ISTH-BAT score is often an absolute criterion.[1]

As per standard for patients with a bleeding tendency, extensive laboratory testing is often performed. To rule out known bleeding disorders such as Von Willebrand disease, haemophilia A and B, other coagulation factor deficiencies and platelet disorders, routine tier 1 diagnostic assays are performed. These include a full blood count and blood film, Von Willebrand antigen (VWF:Ag), Von Willebrand ristocetin activity (VWF:RCo), factor VIII, IX, XI, activated partial thromboplastin time (aPTT), prothrombin time (PT) and platelet function.

The British Committee for Standards in Haematology states in its guidelines for rare coagulation disorders, that testing of individual coagulation factors (FII, FV, FVII, FVIII, FIX, FXI and FXIII) is recommended given the severe nature of symptoms that may occur if these coagulation factors are deficient.[3] When results of tier 1 assays of primary haemostasis are normal, local protocols also recommend additional testing to rule out rare disorders including coagulation factor XIII (FXIII) and α2-antiplasmin (α2AP) deficiency, see Figure 1. As both FXIII and α2AP are linked to fibrin-dependent clot stability, these 2 assays are often performed together.

FXIII deficiency is a very rare bleeding disorder with an estimated prevalence of 1 in 2 million people worldwide.[4] FXIII plays a key role in achieving haemostasis by catalyzing the cross-linking of fibrin and thereby stabilizing the blood clot. Although important, research suggests FXIII not only affects proteins within the plasma but also proteins within platelets and vascular matrix for example.[5] Symptoms of FXIII deficiency may include skin bruising, mucosal bleeds and intramuscular bleeding. Levels above 30% are considered adequate for normal haemostasis.[6]

In severe homozygous FXIII deficiency umbilical stump bleeding is a commonly reported symptom in the neonatal period, occurring in up to 80% of cases. Female patients can be diagnosed at a later stage in life after multiple unexplained miscarriages.[4] Spontaneous intracranial bleeding (haemorrhage) is the main cause of disability or death in patients with this disease and incidence rates have been reported to be around 30%.[4,5,7,8]

α2AP acts as the main inhibiting factor of the fibrinolytic pathway. It does so by inhibiting plasmin, an enzyme that degrades fibrin and thereby promotes the breakdown of blood clots.[9] AP deficiencies are extremely rare and very few cases have been described in the literature. Symptoms vary between homozygous and heterozygous patients, with heterozygous patients having no or mild symptoms like prolonged bleeding after dental extraction.[10,11] Homozygous patients often experience more severe bleeding symptoms similar to patients with haemophilia,[12] such as spontaneous, as well as trauma related bleeds into joints and muscles.

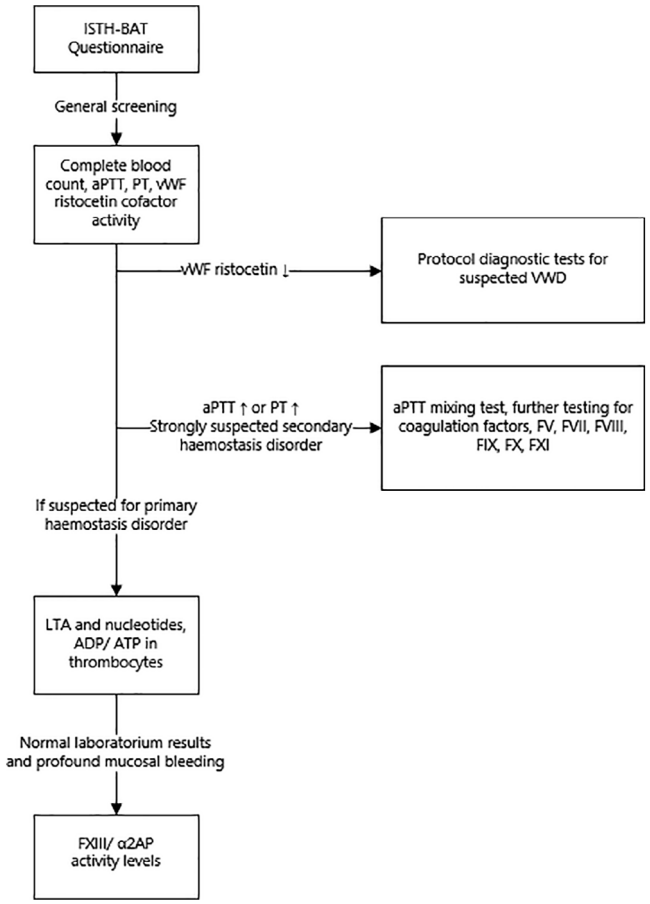

FIGURE 1 Local protocol showing the diagnostic process for a suspected bleeding disorder. aPTT, activated partial thromboplastin time; LTA, light transmission aggregometry; PT, prothrombin time; VWD, Von Willebrand Disease; vWF, von Willebrand-factor.

Prior research suggests BDUC is being diagnosed with increasing frequency.[2] Therefore, making BDUC an ever more present challenge in modern healthcare. With little research data available, it is difficult to state whether testing for these conditions should be standardized in patients with a BDUC. This study aims to evaluate the diagnostic value of FXIII and α2AP activity in patients with a BDUC.

2 | MATERIALS AND METHODS

2.1 | Objective

The primary objective was to evaluate the prevalence of FXIII deficiency and α2AP deficiency in patients with a BDUC.

13652516, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/hae.15059 by Cochrane Netherlands, Wiley Online Library on [17/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

2.2 | Study population

This retrospective study was conducted at the Van Creveldkliniek, Center for Benign Haematology, Thrombosis and Haemostasis, at the University Medical Center (UMC) Utrecht in the Netherlands.

All patients with a BDUC who visited our haemophilia treatment centre between August 2011 and August 2023 were included in the study. Patients with a previous diagnosis of FXIII or α2AP deficiency (before laboratory measurement) were excluded. We only included patients with an ISTH-BAT score and then checked whether tier 1 diagnostic assays for complete blood count, von Willebrand factor activity, aPTT, PT and platelet function were performed and were normal. Patients of all ages were included, both male and female participants. Excluded were potential study subjects who explicitly opposed against the use of their medical data for scientific research purposes, subjects with no available data on ISTH-BAT scores and subjects with abnormal tier 1 assay results.

The ISTH-BAT questionnaire was administered by experienced haematologists. Scores of ≥4 for men and ≥6 for women were considered abnormal and clinically relevant FXIII deficiency was defined as FXIII activity <30%.

α2AP activity <60% were classed as a mild deficiency and activity <10% were classed as a severe deficiency. The study design was approved by the local ethics committee and informed consent was not deemed necessary as it would take a disproportionate effort to contact all patients.

The data of all participants were collected as part of standard care and have been provided to the investigator by the data manager in a coded format.

2.3 | Laboratory measurements

All laboratory assays were performed at the ISO15189 accredited Central Diagnostic Laboratory of the UMC Utrecht. Antiplasmin activity was measured on an ACL Top 750 LAS coagulation analyzer using Hemosil plasmin inhibitor reagent (Werfen Diagnostics, Bedford, MA, USA) and on a STA-Rack-R analyzer using Stachrom antiplasmin reagent (Diagnostica Stago, Asnieres-sur-Seine, France). Factor XIII was measured using Berichrom factor XIII reagent (Siemens Healthcare, Marburg, Germany) on a Selectra analyzer (Elitech, Puteaux, France). Haemoglobin concentration and platelet count were measured using a Cell-Dyn Sapphire hematology analyzer and an Alinity Hq hematology analyzer (Abbott Diagnostics, Santa Clara, CA, USA). PT and aPTT were measured using Hemosil Readiplastin (PT) reagent and Hemosil aPTT SP reagent respectively on an ACL Top 750 LAS coagulation analyzer (Werfen Diagnostics, Bedford, MA, USA) and STA neoplastine CI PT reagent and STA aPTT reagent respectively on a STA-Rack-R analyzer (Diagnostica Stago, Asnieres-sur-Seine, France). Von Willebrand ristocetin cofactor activity was measured using Hemosil von Willebrand factor ristocetin cofactor activity reagent (VWF:GPIbR) on an ACUstar analyzer (Werfen Diagnostics, Bedford, MA, USA). Platelet aggregation was measured on a PAP8 aggregometer (Biodata, Horsham, PA, USA) or a TA8V aggregometer (Diagnostica Stago, Asnieres-sur-Seine, France) using ADP (Sigma Aldrich, St Louis, MO, USA), Arachidonic acid and ristocetin (Diagnostica Stago, Asnieres-sur-Seine, France), epinephrine and U46619 (Hart biologicals, Hartlepool, UK), collagen (Horm, Takeda, Linz, Austria) and TRAP-6 (Bachem, Bubendorf, Switzerland) according to the ISTH recommendations.[13] ADP and ATP content of platelets were measured using the ATPLite assay according to the recommendation of the manufacturer (Perkin Elmer, Shelton, CT, USA). All analyzers were maintained according the recommendations of the manufacturer and results were guaranteed by a rigorous quality control program using internal and external quality control schemes.

2.4 | Statistical analysis

Descriptive data are presented as means with standard deviations or medians with interquartile ranges for continuous data, depending on the distribution of the data. Missing values of diagnostic measurements were imputed with the mean value of data from the remaining cohort. All data were checked for normality and the available data were distributed normally. To assess the association of FXIII activity with ISTH-BAT scores linear regression was used.

Regression rates were adjusted for age because higher age is associated with increased variability in bleeding scores and higher maximum scores due to more exposure to procedures over time.[14] p-Values < .05 were considered as statistically significant. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 29.0.

3 | RESULTS AND DISCUSSION

A total of 158 patients with a BDUC and elevated ISTH-BAT scores were included. Table 1 shows the baseline characteristics of the included patients. The median age at inclusion was 37 years (range 5 to 79) and 88.6% of patients were female. Mean ISTH-BAT scores were 8.2 (SD ± 3.7) in children, 6.2 (SD ± 2.1) in men and 10.6 (SD ± 3.3) in women. The distribution of the different items of the ISTH-BAT is given in Figure 2.

Patients displayed median FXIII activity of 111% (IQR = 97–131) and median α2AP activity of 112% (IQR = 103–119). Although there were no patients with FXIII activity below the clinically relevant cut-off of 30%, three (1.9%) patients had FXIII activity < 50%, that is, 43%, 45% and 46%. Their ISTH-BAT scores were 7, 12 and 14 respectively. No association was found between FXIII activity and ISTH-BAT scores, (Beta Coefficient = −0.006, 95% CI −0.031–0.018; $p = .615$).

No patients with α2AP activity < 60% were observed. The observed prevalence of both FXIII and α2AP deficiency in this study is 0%. These data suggest that in patients with an unexplained bleeding tendency, routinely measuring FXIII and α2AP activity levels is not indicated.

13652516, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/hae.15059 by Cochrane Netherlands, Wiley Online Library on [17/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 1 Patient characteristics.

	Adult men $N = 13$	Adult women $N = 134$	Children $N = 11$	Total $N = 158$
Median age (range)	44 (19–70)	38 (18–79)	13 (5–18)	37 (5–79)
Mean ISTH-BAT score	6.2 (SD ± 2.1)	10.6 (SD ± 3.3)	8.2 (SD ± 3.7)	10 (SD ± 3,5)

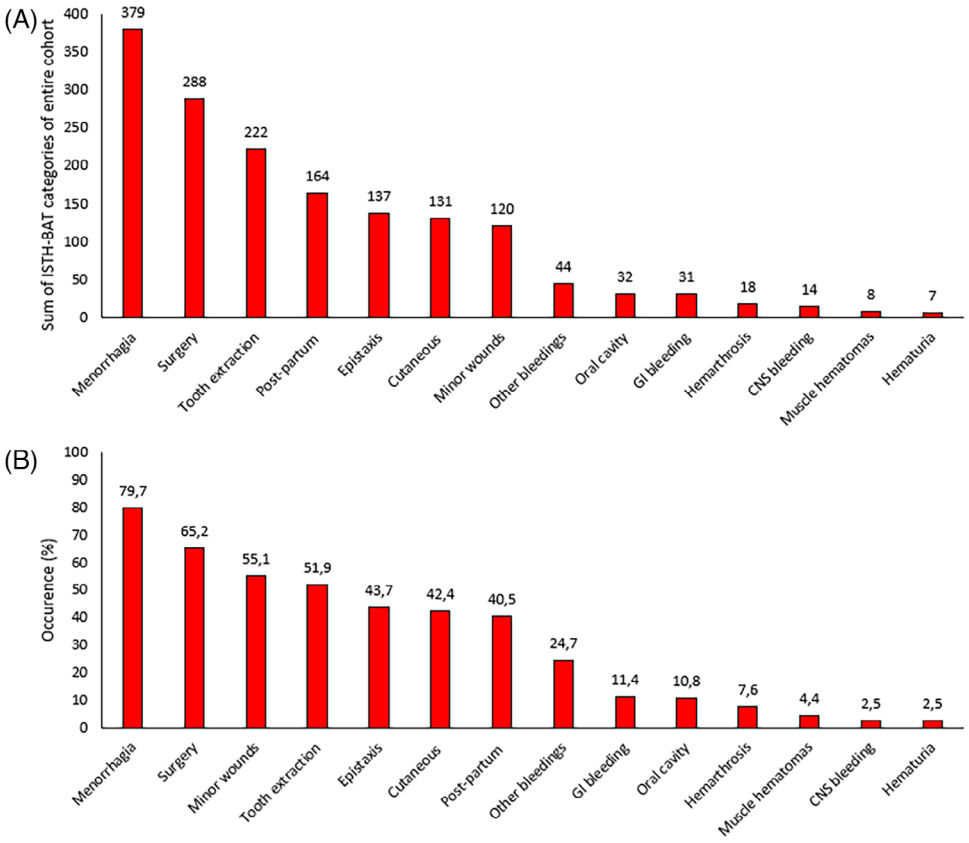

FIGURE 2 Sum of total score for each ISTH-BAT category in the entire cohort (A). Occurrence rates of bleeding symptoms (B). GI, gastrointestinal; CNS, central nervous system.

Our study has several strengths. A large number of patients have been included and they are likely representative for BDUC populations in other haemophilia treatment centres, as a wide range of ages are included and all of our included patients had completely normal tier 1 diagnostic assay results. Additionally, our study was conducted in a single centre, using the same testing protocol over the studied time period and all laboratory tests were performed using the same equipment.

Our study also has some limitations. We only included patients in whom a complete diagnostic tier 1 trajectory was followed. Patients in whom these tests were lacking, were excluded in the current cohort. It should be noted that the prevalence of FXIII and a2AP deficiency is very rare. In the Vienna BDUC bleeding biobank FXIII deficiency was found in only one (0.24%) patient.[15] Therefore, the sample size in our cohort study may be too small to detect these ultrarare bleeding disorders. However, we are a tertiary reference centre where a higher prevalence of bleeding disorders might be expected. Furthermore, we only included 11 children, aged ≥5 years. Therefore, our findings are not to be extrapolated to young children or newborns. Our data suggest that routine screening for FXIII and α2AP might not be worthwhile in cohorts similar to ours. We recommend to continue screening for these disorders in children <5 years and in selected individual cases.

In conclusion, routine testing for FXIII and α2AP deficiency is of limited value in BDUC patients aged ≥5 years. Application of this finding in clinical practices will reduce both health care costs and time investments of health care professionals, furthermore it will reduce the burden of additional blood testing for patients.

AUTHOR CONTRIBUTIONS

Sander Ariëns and Roger E.G. Schutgens designed the study, gathered the data, analysed the results and wrote the manuscript. Albert Huisman was responsible for the execution of laboratory diagnostics and contributed to the writing of the manuscript. Idske C.L. Kremer Hovinga, Rolf T. Urbanus, Karin P.M. van Galen, Lize F.D. van Vulpen

13652516, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/hae.15059 by Cochrane Netherlands, Wiley Online Library on [17/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

and Kathelijn Fischer critically reviewed the data and manuscript. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

S. Ariëns: None Declared. The institution of A. Huisman has received speaker's fees and/or research grants from Abbott diagnostics and Siemens diagnostics. I.C.L. Kremer Hovinga received speakers fee from Sanofi Genzyme. The institution of R.T. Urbanus has received speaker's fees and/or research grants from Hemab. The institution of K.P.M. van Galen has received speaker's fees and/or research grants from Octapharma and Takeda. The institution of L.F.D. van Vulpen has received speaker's fees and/or research grants from Octapharma, Griffols and NovoNordisk.

K. Fischer has received speaker's fees from Bayer, Baxter/Shire, Sobi/Biogen, CSL Behring and Novo Nordisk; has performed consultancy for Bayer, Biogen, CSL Behring, Freeline, Novo Nordisk, Roche and Sobi; and has received research support from Bayer, Baxter/Shire, Novo Nordisk, Pfizer and Biogen; all fees were paid to the institution. The institution of R.E.G. Schutgens has received speaker's fees and/or research grants from Bayer, CSL Behring, Hemab, Novartis, NovoNordisk, Octapharma, Roche, Sobi and Takeda.

FUNDING INFORMATION

The authors received no specific funding for this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

Research ethics approval was provided by the local Research Ethics Board.

ORCID

Sander Ariëns https://orcid.org/0009-0003-9675-122X
Idske C. L. Kremer Hovinga https://orcid.org/0000-0002-6244-0429
Karin P. M. van Galen https://orcid.org/0000-0003-3251-8595
Lize F. D. van Vulpen https://orcid.org/0000-0003-3242-5524
Kathelijn Fischer https://orcid.org/0000-0001-7126-6613
Roger E. G. Schutgens https://orcid.org/0000-0002-2762-6033

REFERENCES

1. Monard A, Mussert C, Henskens Y, Kruip M, Schutgens R, Schols S. Verbetering van de diagnostiek en behandelin van personen met een onverklaarde bloedingsneiging in Nederland. *NTVH*. 2023;20:150-161. al e.
2. Baker RI, O'Donnell JS. How I treat bleeding disorder of unknown cause. *Blood*. 2021;138:1795-1804. doi:10.1182/blood.2020010038
3. 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;167:304-326. doi:10.1111/bjh.13058
4. Pelcovits A, Schiffman F, Niroula R. Factor XIII deficiency: a review of clinical presentation and management. *Hematol Oncol Clin North Am*. 2021;35:1171-1180. doi:10.1016/j.hoc.2021.07.009
5. Hsieh L, Nugent D. Factor XIII deficiency. *Haemophilia*. 2008;14:1190-1200. doi:10.1111/j.1365-2516.2008.01857.x
6. Peyvandi F, Palla R, Menegatti M, et al. European network of rare bleeding disorders G. coagulation factor activity and clinical bleeding severity in rare bleeding disorders: results from the European network of rare bleeding disorders. *J Thromb Haemost*. 2012;10:615-621. doi:10.1111/j.1538-7836.2012.04653.x
7. Levy JH, Greenberg C. Biology of Factor XIII and clinical manifestations of Factor XIII deficiency. *Transfusion*. 2013;53:1120-1131. doi:10.1111/j.1537-2995.2012.03865.x
8. Foundation NBD. Factor XIII: An overview of symptoms, genetics, and treatments to help you understand factor XIII deficiency: NBDF.
9. Medscape. Alpha2-Plasmin Inhibitor Deficiency. 2022.
10. Ikematsu S, Fukutake K, Aoki N. Heterozygote for plasmin inhibitor deficiency developing hemorrhagic tendency with advancing age. *Thromb Res*. 1996;82:129-136. doi:10.1016/0049-3848(96)00059-x
11. Favier R, Aoki N, de Moerloose P. Congenital alpha(2)-plasmin inhibitor deficiencies: a review. *Br J Haematol*. 2001;114:4-10. doi:10.1046/j.1365-2141.2001.02845.x
12. Carpenter SL, Mathew P. Alpha2-antiplasmin and its deficiency: fibrinolysis out of balance. *Haemophilia*. 2008;14:1250-1254. doi:10.1111/j.1365-2516.2008.01766.x
13. Cattaneo M, Cerletti C, Harrison P, et al. Recommendations for the standardization of light transmission aggregometry: a consensus of the working party from the platelet physiology subcommittee of SSC/ISTH. *J Thromb Haemost*. 2013. doi:10.1111/jth.12231
14. Doherty D, Grabell J, Christopherson PA, et al. Zimmerman Program I. Variability in International Society on Thrombosis and Haemostasis-Scientific and Standardization Committee endorsed Bleeding Assessment Tool (ISTH-BAT) score with normal aging in healthy females: contributory factors and clinical significance. *J Thromb Haemost*. 2023;21:880-886. doi:10.1016/j.jtha.2022.11.045
15. Gebhart J, Hofer S, Panzer S, et al. High proportion of patients with bleeding of unknown cause in persons with a mild-to-moderate bleeding tendency: results from the Vienna Bleeding Biobank (VIBB). *Haemophilia*. 2018;24:405-413. doi:10.1111/hae.13422

How to cite this article: Ariëns S, Huisman A, Hovinga ICLK, et al. Limited value of testing for factor XIII and α2-antiplasmin deficiency in patients with a bleeding disorder of unknown cause. *Haemophilia*. 2024;30:998–1002. https://doi.org/10.1111/hae.15059

13652516, 2024, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/hae.15059 by Cochrane Netherlands, Wiley Online Library on [17/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License