frontiers | Research Topics

Acute and hereditary coagulation disorders

Edited by
Felix Carl Fabian Schmitt, Herbert Schöchl
and Wolfgang Miesbach

Published in
Frontiers in Medicine
Frontiers in Public Health

ISSN 1664-8714
ISBN 978-2-8325-5632-0
DOI 10.3389/978-2-8325-5632-0

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Acute and hereditary coagulation disorders

Topic editors

Felix Carl Fabian Schmitt — Heidelberg University Hospital, Germany
Herbert Schöchl — AUVA Research Centre, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Austria
Wolfgang Miesbach — University Hospital Frankfurt, Germany

Citation

Schmitt, F. C. F., Schöchl, H., Miesbach, W., eds. (2024). *Acute and hereditary coagulation disorders*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-8325-5632-0

Table of contents

frontiers | Frontiers in Medicine

TYPE Editorial
PUBLISHED 23 October 2024
DOI 10.3389/fmed.2024.1507527

OPEN ACCESS

EDITED AND REVIEWED BY
Eleni Gavriilaki,
Aristotle University of Thessaloniki, Greece

*CORRESPONDENCE
Wolfgang Miesbach
✉ miesbach@em.uni-frankfurt.de

RECEIVED 07 October 2024
ACCEPTED 09 October 2024
PUBLISHED 23 October 2024

CITATION
Schöchl H, Schmitt F and Miesbach W (2024) Editorial: Acute and hereditary coagulation disorders. *Front. Med.* 11:1507527. doi: 10.3389/fmed.2024.1507527

Editorial: Acute and hereditary coagulation disorders

Herbert Schöchl[1], Felix Schmitt[2] and Wolfgang Miesbach[3]*

[1]Ludwig Boltzmann Institute for Traumatology, Salzburg, Austria, [2]Heidelberg University, Heidelberg, Baden-Württemberg, Germany, [3]University Hospital Frankfurt, Frankfurt, Germany

KEYWORDS

editorial, advantages, coagulation, disorders, recent

Editorial on the Research Topic
Acute and hereditary coagulation disorders

We are pleased to present this edition on Research Topics about acute and hereditary coagulation disorders in "*Frontiers in Medicine*".

Coagulation disorders remain a critical area of medical research. The field of coagulation disorders continues to evolve rapidly, with new insights emerging across various conditions. In this edition, the authors of several groundbreaking studies have advanced our understanding of coagulation disorders, from rare genetic conditions to common complications of pregnancy and critical illness. Their work spans a wide range of topics, including novel diagnostic techniques, treatment strategies, and prognostic markers.

Gruneberg et al. investigated the role of fibrinolytic potential in postpartum hemorrhage (PPH) in their study "*Fibrinolytic potential as a risk factor for postpartum hemorrhage*". They found that women experiencing severe PPH showed increased fibrinolytic potential as early as hospital admission. This suggests that elevated fibrinolytic potential could be a risk factor for PPH, although additional factors are likely required to trigger the condition.

Alesci, Hecking et al. explored the utility of ACMG classification in factor VII deficiency in their study "*Utility of ACMG classification to support interpretation of molecular genetic test results in patients with factor VII deficiency*". Their findings suggest that specific combinations of ACMG-classified variants correlate with high-risk bleeding phenotypes, potentially paving the way for genotype-phenotype prediction models in the future.

Navaei et al. addressed the question "*Is it time to switch to bivalirudin for ECMO anticoagulation?*". While some studies showed the advantages of bivalirudin over heparin in preventing major bleeding and thrombosis, the majority found no significant difference. Further prospective studies are needed to reach a definitive conclusion.

Toenges et al. investigated acquired dysfibrinogenemia in septic patients in their study "*Investigation of acquired dysfibrinogenemia in adult patients with sepsis using fibrinogen function vs. concentration ratios: a cross-sectional study*". Their results suggest the presence of acquired dysfibrinogenemia in some adult septic patients, potentially contributing to a specific laboratory signature of sepsis-associated coagulation phenotype.

Huang et al. examined the "*Current status of treatment and disease burden of a cohort of hemophilia B in China*". They found that the use of prophylaxis was low, and patients faced significant financial burdens. The study highlights the need for improved access to safer and more effective drugs and efforts to reduce the financial burden on patients.

Quintana-Diaz et al. developed a mnemonic device "*COAGULATION*" for treating coagulation disorders following traumatic brain injury. This simple tool could aid clinicians in managing patients with moderate or severe traumatic brain injury on a daily basis.

Henze et al. conducted a prevalence study on "*Abdominal venous thromboses: detection of the JAK2 p.V617F mutation by next-generation ultradeep sequencing*". They found the JAK2 p.V617F mutation in 19% of patients with abdominal venous thromboses, highlighting the importance of genetic analysis in understanding the etiology of these rare thrombotic events.

Alesci, Goldmann et al. surveyed patients with mild hemophilia in Germany to gain insights into their treatment reality and quality of life. Their findings suggest that this population may underestimate bleeding complications, emphasizing the need for improved awareness and treatment strategies.

Goodarzi et al. compared two fibrinogen concentrates in their case report "*Are all fibrinogen concentrates the same?*". Their findings highlight the potential differences in clot formation and stability between different fibrinogen concentrates, emphasizing the need for careful consideration when choosing treatment options.

Finally, Zhu et al. investigated "*Combined coagulation and inflammation markers as predictors of venous thrombo-embolism and death in COVID-19*" (10). Their study suggests that combining coagulation and inflammatory markers could refine prognostication of severe outcomes in COVID-19 patients.

The results of these studies demonstrate the complexity and diversity of the research on coagulation disorders and highlight the importance of continued efforts in this field to improve diagnosis, treatment, and patient outcomes across various coagulation-related conditions.

As host editors, we extend our sincere gratitude to the journal and its editors for their invaluable support in bringing this Research Topic of research to fruition and for the authors to submit their excellent manuscript to "*Frontiers in Medicine*".

These findings not only contribute to the scientific knowledge base but also have the potential to significantly impact clinical practice, offering new hope for improved patient outcomes in the field of thrombosis and hemostasis.

We hope you enjoyed reading these ten articles as much as we did while preparing this edition. As host editors, it has been our pleasure to bring together this Research Topic of insightful works, and we trust they will inspire and inform your own endeavors in the field.

Author contributions

HS: Writing – review & editing, Writing – original draft. FS: Writing – review & editing, Writing – original draft. WM: Writing – review & editing, Writing – original draft.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 14 July 2023
DOI 10.3389/fmed.2023.1220813

OPEN ACCESS

EDITED BY
Felix Carl Fabian Schmitt,
University Hospital Heidelberg, Germany

REVIEWED BY
Daniel Gruneberg,
University Hospital Heidelberg, Germany
Johannes Zipperle,
Ludwig Boltzmann Institute for Traumatology, Austria

*CORRESPONDENCE
Rosa Sonja Alesci
✉ s.alesci@gerinnungszentrum-hochtaunus.de

RECEIVED 11 May 2023
ACCEPTED 16 June 2023
PUBLISHED 14 July 2023

CITATION
Alesci RS, Hecking C, Racké B, Janssen D and Dempfle C-E (2023) Utility of ACMG classification to support interpretation of molecular genetic test results in patients with factor VII deficiency.
Front. Med. 10:1220813.
doi: 10.3389/fmed.2023.1220813

Utility of ACMG classification to support interpretation of molecular genetic test results in patients with factor VII deficiency

Rosa Sonja Alesci[1]*, Carola Hecking[1], Benjamin Racké[2], Detlev Janssen[3] and Carl-Erik Dempfle[4]

[1]IMD Blood Coagulation Centre, Bad Homburg, Germany, [2]Institute of Immunology and Genetics, Kaiserslautern, Germany, [3]Med-i-Scene Concept, Weisendorf, Germany, [4]IMD Blood Coagulation Centre, Mannheim, Germany

Background: The American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) have introduced an internationally shared framework for variant classification in genetic disorders. FVII deficiency is a rare inherited autosomal recessive bleeding disorder with sparse data concerning ACMG classification.

Methods: To develop an approach which may improve the utility of molecular genetic test results, 129 patients with FVII deficiency were retrospectively assigned to six subgroups for exploratory analysis: *F7* gene wildtype *(group 1)*, ACMG 1 (benign variant) or ACMG 2 (likely benign variant), only *(group 2)*, ACMG 3 (variant of uncertain significance) ± ACMG 1–2 heterozygous or not classified variant *(group 3)*, ACMG 4 (likely pathogenic variant), or ACMG 5 (pathogenic variant) single heterozygous ± ACMG 1–3 single heterozygous *(group 4)*, ACMG 4–5 homozygous or ≥2 ACMG 4–5 heterozygous or ≥1 ACMG 4–5 heterozygous plus either ACMG 1 c.1238G>A modifying variant homozygous or ≥2 ACMG 1–3 *(group 5)*, FVII deficiency and another bleeding disorder *(group 6)*.

Results: Eleven of 31 patients (35.5%) in group 5 had abnormal ISTH-BS (n = 7) and/or history of substitution with recombinant factor VIIa (n = 5) versus 4 of 80 patients (5.0%, n = 1 abnormal ISTH-BS, n = 3 substitution) in groups 1 (n = 2/22), 2 (n = 1/29), 3 (n = 0/9), and 4 (n = 1/20). Four of 18 patients (22.2%) with FVII deficiency and another bleeding disorder (group 6) had an abnormal ISTH-BS (n = 2) and/or history of substitution with recombinant factor VIIa (n = 3).

Conclusion: Patients with a homozygous ACMG 4–5 variant or with specific combinations of heterozygous ACMG 4–5 ± ACMG 1–3 variants exhibited a high-risk bleeding phenotype in contrast to the remaining patients without another bleeding disorder. This result may serve as a basis to develop a genotype/phenotype prediction model in future studies.

KEYWORDS

FVII deficiency, genotype, phenotype, ACMG classification, genetic variations

Introduction

Congenital factor VII (FVII) deficiency is an autosomal recessively inherited rare bleeding disorder. The clinical phenotypes in FVII deficient patients show a wide variation from asymptomatic to severe with a poor correlation to FVII activity (1). Some patients with very low FVII activity had no bleeding symptoms whereas patients with partial FVII deficiency may have recurrent bleeding episodes (2). FVII levels above 20% are thought to be sufficient for prevention against spontaneous bleeding (1), possibly due to an enhanced effect of TF-FVII complex which activates coagulation even with small amounts of FVII (1, 3). However, previously asymptomatic patients with FVII levels above 20% may have an increased bleeding risk in situations such as major surgery or trauma. FVII deficiency may be caused by a quantitative FVII defect (type I, decreased FVII antigen) or qualitative FVII defect (type II, normal FVII antigen) (1).

Even though the prevalence of FVII deficiency was repeatedly reported to be 1:300,000–1:500,000 individuals (4–7) it is believed that mild, moderate or severe forms of FVII deficiency are by far more frequent reaching 1:59,000 individuals (8).

The coagulation factor VII gene (*F7*, HGNC ID: 3544) is located on chromosome 13 (13q34) with nine exons and eight introns, which compose a 12.8 kb gene locus near the telomeric region of the chromosome besides the gene promotor region (9). In the coagulation factor VII variant database of the European Association for Haemophilia and Allied Disorders (EAHAD) 271 unique variants of *F7* gene in 1058 individuals with FVII activity below or above the normal range were recorded until May 2022 (10). Most *F7* variants were small lesions with single nucleotide substitutions (point) in 86.7% of individuals followed by deletions (8.9%), duplications (2.6%), indel rearrangements (1.1%) and insertions (0.7%) (10). Missense variants represented 74% of single nucleotide substitutions (11).

The genotype/phenotype relationship in FVII deficient patients has extensively been studied in humans (2, 8, 12). FVII levels below 10% were seen in about 50% of patients who are homozygous or compound heterozygotes for pathogenic *F7* gene variants compared to 7% of patients with heterozygous *F7* pathogenic gene variants (6). Bleeding symptoms were discovered in 71% of patients with homozygous causative *F7* gene variants versus 50% in compound heterozygotes and 19% in heterozygotes (2).

Whereas FVII:C shows a poor correlation to severity of bleeding phenotype the type and the site of a *F7* variant may be helpful to predict hemorrhagic risks (13). For example, mutations affecting TFPI-binding exosites of FVII may markedly prolong clotting time (14). The utility of molecular genetic diagnostics depends on the validity of the pathogenicity classification for a specific variant detected. As a major step to establish an internationally shared framework for a systematic, objective and evidence-based variant classification in genetic disorders the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) published standards and guidelines for the interpretation of sequence variants in 2015 (15). However, these guidelines lacked specificity in several areas or resulted in contradictory or ambiguous interpretations. Therefore, validated "semiquantitative, hierarchical evidence-based rules for locus interpretation" (Sherloc) was developed by the ACMG-AMP (16).

The ACMG classification uses a specific standard terminology to describe gene variants that are the cause of Mendelian disorders and is based on a complex process for assigning a variant to one of the five ACMG categories (15, 16): Variants classified as pathogenic (ACMG 5) or likely pathogenic (ACMG 4) have met specific criteria and may be used by the health-care provider for clinical decision making. A variant considered benign (ACMG 1) is not causative for the patient's disorder or symptoms. ACMG 2 describes a variant which is likely benign (ACMG 2). Variants of uncertain significance (VUS; ACMG 3) should imply efforts for changing the classification to pathogenic or benign based on additional information.

In general, interpretation of gene variants classified as pathogenic is challenging (17). A pathogenic variant may be present in an individual without phenotypic correlate. In addition, classifications of specific variants may change over time or differ between databases such as Human Gene Mutation Database (HGMD) and ClinVar (17). To be useful as a contribution for therapeutic decisions the variants of the *F7* gene should primarily be analyzed with respect to abnormal bleeding phenotype. There are several *F7* gene variants associated with reduced FVII activity which are classified as benign (ACMG 1), for example the frequent missense *F7* variant p.(Arg353Gln), also known as Arg353Gln polymorphism (R353Q; rs6046) or described by the single nucleotide substitution of Arginine by Glycine residue in position 413, p.(Arg413Gln) or by the complementary DNA (cDNA) change c.1238G>A. This variant leads to a mean decrease of FVII:C activity by about 20–30% in individuals with M1M2 genotype (heterozygous) (18–21), and by 43% in homozygotes (20). The minor allele frequency (MAF) of 0.1265 means that about 12.7% of the population are carriers of this variant. The variant c.1238G>A is known to diminish FVII secretion and is thought to be not directly pathogenic but modifying, i.e., contributing to the consequence of pathogenic variants (11, 22), for example by augmentation the FVII deficiency caused by the p.(Ala354Val) (c.1061C>T) variant (23).

The EAHAD *F7* variant database provides tools to facilitate variant classification and has been prepared to list the pathogenicity of variants based on the ACMG classification (11). Unknown pathogenicity of a specific *F7* gene variant represents only one of the difficulties in conjunction with interpretation of molecular genetic test results. Heterozygosity may be of clinical relevance if there are two or more pathogenic variants. To differentiate between compound heterozygous patients (both alleles affected) and double heterozygous patients (two variants in the same allele) molecular genetic testing of the mother or the father is usually needed. However, this is a hurdle in clinical practice. In addition, benign *F7* gene variants may have a modifying effect in combination with pathogenic variants which increases the bleedings risk. Based on a large cohort of patients with FVII deficiency we explored an approach to increase the utility of ACMG classification which should facilitate interpretation of molecular

genetic test results as a basis for successive advice and decision making in daily routine.

Materials and methods

Patients

Patients with FVII deficiency and genetic analyses prompted at the Coagulation Center Hochtaunus, Bad Homburg Germany, and the Coagulation Center Mannheim, Germany, between August 2012 and December 2021 were included in this retrospective exploratory analysis. According to center's routine all patients with suspected bleeding disorder had completed the ISTH-SSC bleeding assessment tool (ISTH-BAT), a standardized questionnaire which can be used to generate a bleeding score (BS) in patients with bleedings disorders (24–26). Cut offs for an abnormal BS were ≥3 for children <12 years, ≥4 for males ≥12 years, and ≥6 for females ≥12 years. The higher score in females is due to the possibility of menorrhagia and postpartum bleeding (25). Besides standardized questionnaire the notes made by the physicians to document medical history in a non-standardized manner were used to identify and analyze bleeding symptoms. Treatment for substitution of FVII deficiency was documented.

Coagulation tests

As both centers belong to the same company the same procedures and reagents were used for coagulation tests (FVII:C, activated partial thromboplastin time, prothrombin time (PT) with Quick value) which were performed locally by routine. FVII:C was measured using Coagulation FVII deficient plasma (Siemens Healthineers, Erlangen, Germany) and the fully automated analyzer Sysmex® (Siemens Healthineers, Erlangen, Germany).

Molecular genetic analyses

Molecular genetic analyses were performed at the Office for Human Genetics, Wiesbaden, Germany, and at the Institute for Immunology and Genetics, Kaiserslautern, Germany. Before drawing peripheral blood for genetic testing all patients had to sign an informed consent form according to the German Gene Diagnostics Act. After isolation of genomic DNA the nine exons of the *F7* gene on chromosome 13q34 and their flanking introns (since January 2015: RefSeqGene: NG_009262.1, transcript: GenBank accession number NM_000131.4) were analyzed by DNA sequencing. For exclusion of large deletions and duplications a variation of the multiplex polymerase chain reaction (PCR) named MLPA (Multiplex ligation-dependent probe amplification) analysis was performed using the MLPA-kit P207 from MRC Holland, Amsterdam, The Netherlands.

Assessment of pathogenicity of variants

Reports of individual molecular genetic testing in the cohort were screened for information about ACMG classification of specific variants. If a variant has not been classified, for example due to molecular genetic testing before the ACMG–AMP standards and guidelines were introduced, databases as follows were screened for information on pathogenicity: GnomAD,[1] ClinVar,[2] VarSome,[3] Franklin by genoox,[4] EAHAD-CFDB Factor VII Gene (*F7*) Variant Database.[5] Additional information was available at the EAHAD *F7* database (MAF, Grantham Score, PolyPhen-2 Prediction, SIFT Prediction, and PROVEAN Prediction) (11), and by case specific literature searches. If a variant could not be assigned to ACMG 1, 2, 4, or 5 it was classified as unknown or – if such classification was available – as variant of uncertain significance (VUS, ACMG 3).

Statistics

The data were analyzed descriptively with categories of patients expressed as numbers and percentages. For the other variables, mean values ± standard deviation (SD) as well as median (minimum, maximum) were calculated.

A combined endpoint consisting of an abnormal bleeding score and/or substitution history for FVII deficiency was used to identify high risk phenotypes in patients without another bleeding disorder, and to support the hypothesis that heterozygous ACMG 5 variants are of clinical relevance if combined with homozygous modifying ACMG 1–3 variants.

First, a subgroup of FVII deficient patients with homozygous modifying ACMG 1 variant c.1238G>A plus heterozygous ACMG 5 variant c.1061C>T was selected and compared with a subgroup of patients who had either a single c.1238G>A variant, only, or were heterozygous for c.1238G>A variant plus heterozygous for ACMG 5 variant c.1061C>T. Thereafter, the total cohort was divided into six genetic subgroups according to *F7* genotype and presence of another bleeding disorder as shown in **Figure 1**. The combined endpoint mentioned above was used to compare group 5 (assumed high-risk phenotype) with pooled groups 1 to 4 (assumed low-risk phenotype).

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients was not required to participate in this study in accordance with the local legislation and institutional requirements. The retrospective analysis complied with the principles of the Declaration of Helsinki and the Good Clinical Practice (GCP).

Results

In total, 129 patients with diagnosis of FVII deficiency (mean FVII:C 42.4% ± 15.1%) and availability of molecular genetic report were evaluated. Eighty-nine patients were females (69.0%). Mean age at molecular genetic testing was 34.1 ± 16.7 years (median 32.5 years, range 0–84 years). Reasons for consultation

1 https://gnomad.broadinstitute.org
2 https://www.ncbi.nlm.nih.gov/clinvar/
3 https://varsome.com
4 https://franklin.genoox.com
5 https://f7-db.eahad.org

Subgroup		*F7* gene variants detected		
		ACMG 1 or 2	ACMG 3/not classified	ACMG 4 or 5
1	*F7* gene wildtype			
2	ACMG 1 (benign) or ACMG 2 (likely benign)	heterozygous [a] and/or homozygous [a]		
3	ACMG 3 (variant of uncertain significance) or not classified	*optional: heterozygous*	heterozygous [b]	
4	ACMG 4 (likely pathogenic) or ACMG 5 (pathogenic): single heterozygous	*optional: single heterozygous* [c]		single heterozygous
5	ACMG 4 (likely pathogenic) or ACMG 5 (pathogenic): homozygous or complex heterozygous	*optional*	*optional*	homozygous
		optional	*optional*	≥2 heterozygous
		c.1238G>A homozygous	*optional*	single heterozygous
		≥2 heterozygous and/or homozygous		single heterozygous
6	FVII deficiency and another bleeding disorder	*optional*		

[a] if reported; [b] no patient had a homozygous variant; [c] exception: ACMG 3 allocated to group 5 if FVII:C <30%

FIGURE 1
Genetic subgroups according to F7 genotype, ACMG classification and presence of another bleeding disorder.

of the Coagulation Center were bleeding symptoms in 23 patients (17.8%), reduced Quick value in 31 patients (24.0%), testing of a family member in 12 patients (9.3%), known FVII deficiency in 17 patients (13.2%), recurrent abortions and/or wish for a child in 38 patients (29.5%), and miscellaneous in 8 patients (6.2%).

Variants

F7 gene wildtype (WT) was diagnosed in 25 patients (19.4%). In total, the genetic reports of 104 non-WT patients contained 182 variant findings (1.8 ± 1.2 variants per patient; median 1, maximum 10). Most findings in the molecular genetic reports were ACMG 1 or 2 variants (n = 102) followed by ACMG 4 or 5 variants (n = 65). About half of ACMG 1 or 2 findings were homozygous (n = 54; 53%), whereas 62 of 65 ACMG 4 or 5 findings (95.4%) were heterozygous. Further details of the variants are listed in the Supplementary Tables 1–4.

About half of the 53 different *F7* gene variants identified were pathogenic (ACMG 5 n = 17; 32.1%) or likely pathogenic (ACMG 4 n = 11; 20.8%) (Supplementary Tables 1–3). Only 13 variants were benign (ACMG 1 n = 10; 18.9%) or likely benign (ACMG 2 n = 3; 5.7%). Ten variants were of uncertain significance (ACMG 3; 18.9%), and 2 variants remained unclassified (3.8%). Twenty variants (37.7%) seen in one (n = 16), two (n = 2) or three (n = 2) patients are not listed in the EAHAD database for *F7* gene variants, mostly ACMG 1 or 2 variants (n = 8), and ACMG 3 or not classified variants (n = 8) (Supplementary Tables 1–3). For many variants with single nucleotide substitutions the EAHAD *F7* database contains information concerning MAF, Grantham Score, PolyPhen-2 Prediction, SIFT Prediction, and PROVEAN Prediction (Supplementary Tables 1–3).

Frequent variants c.1238G>A ± c.1061C>T

The missense *F7* gene variant c.1238G>A was detected in 81 of 104 non-WT patients (77.9%), 47 of 81 (58.0%) were homozygous (Supplementary Table 1). In 35 of 81 carriers of c.1238G>A variant (43.2%) no other *F7* gene variant was found (Table 1). Mean FVII:C in 12 patients with single heterozygous c.1238G>A variant was 53.8 ± 14.9% with FVII:C below 50% in two patients (18 and 30%, respectively). Twenty-three patients with single homozygous c.1238G>A variant had mean FVII:C of 44.5 ± 12.1%.

In 18 patients with c.1238G>A variant the ACMG 5 variant c.1061C>T was present (all heterozygous). One of these had *F7* deficiency together with another bleeding disorder (von Willebrand syndrome). The c.1061C>T variant was not accompanied by the c.1238G>A variant in one patient, only. However, this male patient with FVII:C 33% and ISTH BS 5 had the c.1388delC variant (heterozygous, ACMG 4) which was also present in six other patients together with the c.1061C>T variant. As shown in Table 1, six of 12 patients (50.0%) with homozygous c.1238G>A variant and heterozygous c.1061C>T variant had an abnormal ISTH bleeding score (n = 4) and/or history of rFVIIa substitution (n = 2) versus only 1 of 41 patients (2.4%) in the other three subgroups of patients with c.1238G>A variant. This finding supports allocation of patients with a single heterozygous ACMG 4–5-variant plus the homozygous ACMG 1 c.1238G>A variant to group 5. However, 5 of 12 patients with homozygous c.1238G>A variant and c.1061C>T variant also had heterozygous c.1391delC variant. Of these, one patient had an abnormal ISTH BS and another patient needed substitution with rFVIIa.

TABLE 1 Characteristics, ISTH BS, and substitution in FVII deficient patients with c.1238G>A variant ± heterozygous c.1061C>T variant.

	Single c.1238G>A, only		c.1238G>A plus heterozygous c.1061C>T[a]	
	Heterozygous c.1238G>A	Homozygous c.1238G>A	Heterozygous c.1238G>A	Homozygous c.1238G>A
All patients	*n* = 12	*n* = 23	*n* = 6	*n* = 12
Age[c]	35.1 ± 18.6	30.7 ± 16.7	24.5 ± 8.7	32.1 ± 16.8
Female sex	8 (66.7%)	15 (65.2%)	3 (50.0%)	6 (50.0%)
FVII activity (%)[b]	53.8 ± 14.9 57.5 (18–66)	44.5 ± 12.1 45 (8–65)	38.3 ± 11.1 41 (19–53)	32.6 ± 5.8 30 (27–44)
Quick value (%)[b]	75.5 ± 13.5 79.5 (48–87)	67.6 ± 13.0 69 (21–85)	65.0 ± 11.6 60.5 (53–81)	59.3 ± 3.1 58.5 (56–66)
Patients with no other bleeding disorder, only	*n* = 8	*n* = 19	*n* = 5	*n* = 12
Female sex	6 (75.0%)	11 (57.9%)	3 (60.0%)	6 (50.0%)
Reason for consultation				
- Bleeding symptoms	3/8	1/19	1/5	3/12
- Laboratory findings	2/8	3/19	2/5	6/12
- Family screening	1/8	5/19	1/5	0/12
- FVII deficiency known	0	4/19	0/5	2/12
- Abortions/wish for child	1/6	4/11	1/2	1/6
- Others	1/8	2/19	0/5	0/12
ISTH BS[c]				
- Females	1.3 ± 1.0	1.6 ± 1.8	1.7 ± 1.5	3.7 ± 2.8
- Males	1.0 ± 1.4	1.3 ± 1.6	2.5 ± 0.7	1.3 ± 2.0
ISTH BS ≥ cut-off	0/8 (0%)	0/19 (0%)	0/5 (0%)	4/12 (33.3%)
- Children ≥ 3	0/0	0/1	0/0	2/2
- Males ≥ 4	0/2	0/7	0/2	0/5
- Females ≥ 6	0/6	0/11	0/3	2/5
rFVIIa substitution	1 (15.5%)[d]	0 (0%)	0 (0%)	2 (16.7%)[d]

BS, bleeding score; rFVIIa, recombinant factor VIIa; SD, standard deviation.
[a]Up to 8 further *F7* gene variants present (mostly c.1391Cdel, *n* = 6) in 3 patients heterozygous for c.1238G>A and in 5 patients homozygous for c.1238G>A.
[b]First row: mean ± SD; second row: median (range).
[c]Mean ± SD.
[d]All patients with rFVIIa substitution had ISTH BS below cut-off.
The grey color differentiates two groups with single c.1238G>A variants, only, from two groups with c.1238G>A variants plus c.1061C>T variant.

Genotype/phenotype analyses

For genotype/phenotype analyses, 18 patients (14.7%) with another bleeding disorder besides FVII deficiency (WT *n* = 3, ACMG 1–3 variants, only, *n* = 9, ACMG 4 or 5 variants *n* = 6) were excluded from the assignment to the groups 1 to 5. Of the remaining 111 patients, 22 (19.8%) were *F7* gene wildtype, 29 (26.1%) had ACMG 1 or 2 variants, only, 51 (45.9%) had at least one ACMG 4 or 5 variant, and 9 (8.1%) had to be assigned to group 3 due to ACMG 3 or unclassified variants (Table 2). Only one patient with at least two heterozygote ACMG 4 or 5 variants was known to be compound heterozygote.

Median number of *F7* gene variants per patient was 2 in group 4 (range 1–2) as well as in group 5 (range 1–10) whereas median number was 1 in groups 2 (range 1–3), 3 (range 1 – 5) and 6 (range 0–3) (Table 2). Mean age ranged from 30.2 ± 15.4 years in group 3 (ACMG 3 or not classified) to 36.9 ± 14.1 years in group 1 (*F7* gene wildtype).

The groups differed with respect to the proportion of patients with female sex between 54.8% in group 5 and 83.3% in group 6. Reasons for consultation of the Coagulation Center were also different. Group 6 reached the numerically highest rates in the consultation categories bleeding symptoms (44.4%), and abortions and/or wish for a child (50.0%), the latter together with group 1 (54.5%). Most patients in group 5 were admitted due to abnormal laboratory findings (45.2%) or known FVII deficiency (29.0%) (Table 2).

Mean FVII:C and Quick values were lowest in group 5 (29.5 and 54.4%, respectively), and ranged from 43.4 to 49.7%, and 68.2 to 76.2% in the other groups, respectively (Figure 2 and Table 2). In group 5, FVII:C was 5–6% in three patients with homozygous ACMG 4 or 5 variants, 26.4 ± 10.7% in 10 patients with ≥2 heterozygous ACMG 4 or 5 variants (*n* = 8) or ACMG 3–5 variants (*n* = 2), and 35.3 ± 11.1% in 18 patients with one heterozygous ACMG 4 or 5 variant combined with homozygous c.1238G>A or ≥2 ACMG 1–3 variants.

TABLE 2 Characteristics, ISTH BS, and substitution in genetic subgroups of patients with FVII deficiency according to Figure 1.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
	F7 gene wildtype	ACMG 1 or ACMG 2	ACMG 3 or not classified ± ACMG 1–2 heterozygous	ACMG 4 or ACMG 5 single heterozygous ± ACMG 1–3 single heterozygous	ACMG 4 or ACMG 5 homozygous or complex heterozygous[a]	FVII deficiency and another bleeding disorder[b]
Number of patients	*n* = 22	*n* = 29	*n* = 9	*n* = 20	*n* = 31	*n* = 18
Number of *F7* gene variants per patient[c]	0	1.1 ± 0.4 1 (1–3)	1.7 ± 1.3 1 (1–5)	1.6 ± 0.5 2 (1–2)	2.7 ± 1.7 2 (1–10)	1.2 ± 0.8 1 (0–3)
Age[d]	36.9 ± 14.1 34 (16–69)	31.9 ± 19.0 25 (1–84)	30.2 ± 15.4 37 (0–48)	34.0 ± 17.5 34.5 (2–82)	36.5 ± 19.3 30 (6–83)	32.4 ± 10.1 32 (6–51)
Female sex	17 (77.3%)	18 (62.1%)	6 (66.7%)	16 (80.0%)	17 (54.8%)	15 (83.3%)
Reason for consultation						
- Bleeding symptoms	1 (4.5%)	5 (17.2%)	0	5 (25.0%)	4 (12.9%)	8 (44.4%)
- Laboratory findings	4 (18.1%)	6 (20.7%)	2 (22.2%)	5 (25.0%)	14 (45.2%)	0
- Family screening	3 (13.6%)	6 (20.7%)	1 (11.1%)	2 (10.0%)	0	0
- FVII deficiency known	1 (4.5%)	5 (17.2%)	2 (22.2%)	0	9 (29.0%)	0
- Abortions/wish for child	12 (54.5%)	4 (13.8%)	3 (33.3%)	6 (30.0%)	4 (12.9%)	9 (50.0%)
- Others	1 (4.5%)	3 (10.3%)	1 (11.1%)	2 (10.0%)	0	1 (5.6%)
FVII activity (%)[c]	48.6 ± 14.0 48.5 (8–67)	45.7 ± 14.5 48.0 (8–66)	44.1 ± 11.0 42 (28–63)	43.4 ± 13.6 46 (19–65)	29.5 ± 13.6 30.0 (2–61)	49.7 ± 10.2 49.5 (34–65)
Quick value (%)[c]	74.7 ± 15.0 76 (27–99)	68.2 ± 15.6 72 (21–87)	76.2 ± 12.5 78 (54–98)	70.3 ± 14.1 71.5 (46–90)	54.4 ± 16.4 57 (16–92)	72.3 ± 10.9 72 (53–92)
ISTH BS[c]						
- Females	1.5 ± 1.7	1.7 ± 1.5	2.2 ± 1.9	2.3 ± 1.4	3.5 ± 2.7	2.4 ± 1.8
	1 (0–6)	1 (0–5)	1.5 (0–5)	2 (0–5)	3 (0–9)	3 (0–6)
- Males	0.6 ± 0.9	1.3 ± 1.1	1 and 1	0, 0, and 2	1.6 ± 1.4	2 and 8
	0 (0–2)	2 (0–3)			2 (0–5)	
- Children	-	0 and 2	2	0	3 and 5	2
ISTH BS ≥ cut-off	1 (4.5%)	0 (0%)	0 (0%)	0 (0%)	7 (22.6%)	2 (11.1%)
- Females ≥ 6	1/17	0/18	0/6	0/16	4/16	1/15
- Males ≥ 4	0/5	0/9	0/2	0/3	1/13	1/2
- Children ≥ 3	0/0	0/2	0/1	0/1	2/2	0/1
rFVIIa substitution	1 (4.5%)	1 (3.4%)	0 (0%)	1 (5.0%)	5 (16.1%)[e]	3 (16.7%)[f]

BS, bleeding score; rFVIIa, recombinant factor VIIa; SD, standard deviation.

[a]Two patients with heterozygous single ACMG 4 or 5 variant and single ACMG 3 variant included in group 5 due to FVII:C <30%.

[b]The grey color indicates that patients in group 6 were excluded from the assignment to groups 1 to 5 due to another bleeding disorder besides FVII deficiency: von Willebrand syndrome (vWS) (*n* = 10), FV deficiency (*n* = 1), FX deficiency (*n* = 1), FXI deficiency (*n* = 4), PAI-1 deficiency (*n* = 1), FXIII deficiency (*n* = 1).

[c]First row: mean ± SD or numbers in single patients; second row: median (range).

[d]Mean ± SD.

[e]One female with ISTH BS 9; 4 patients with ISTH BS < cut-off.

[f]One female with ISTH BS 6; 2 patients with ISTH BS < cut-off.

10.3389/fmed.2023.1220813

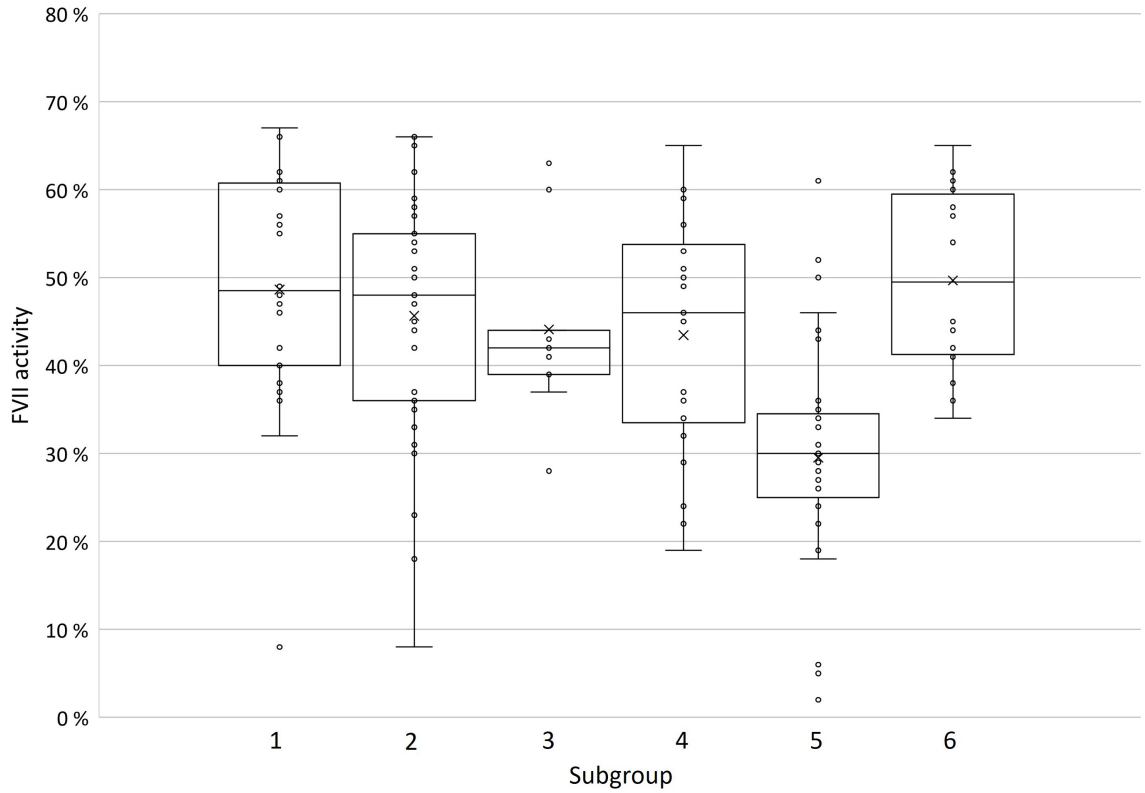


FIGURE 2
FVII activities stratified by six subgroups according to Figure 1.

In females ≥12 years, mean ISTH BS was numerically higher in group 5 (3.5 ± 2.7; median 3; range 0–9) versus 1.9 ± 1.6 (median 1, range 0–6) in groups 1 to 4 (pooled data of 57 females). In males ≥12 years, 13 patients of group 5 had mean ISTH BS of 1.6 ± 1.4 (median 2, range 0–5) versus 1.0 ± 1.0 (median 1, range 0–3) in 19 males ≥12 years included in groups 1 to 4. Both children included in group 5 had an abnormal ISTH BS versus none of 4 children in groups 1 to 4 (Table 2).

Eleven of 31 pts. (35.5%) in group 5 had an abnormal ISTH-BS (n = 7) and/or history of substitution with recombinant factor VIIa (n = 5) versus 4 of 80 pts. (5.0%, n = 1 abnormal ISTH-BS, n = 3 substitution) in groups 1 to 4 (Table 2). In group 5, the percentages of patients with abnormal ISTH-BS and/or history of substitution were similar in patients with homozygous ACMG 4 or 5 variants (1 of 3 patients, 33%), ≥2 heterozygous ACMG 4 or 5 variants (3 of 10 patients, 30%), one heterozygous ACMG 4 or 5 variant plus homozygous c.1238G>A variant (6 of 15 patients, 40%) and one heterozygous ACMG 4 or 5 variant plus ≥2 heterozygous ACMG 1 to 3 variants (1 of 3 patients, 33%). In group 6 (FVII deficiency and another bleeding disorder), 4 of 18 patients (22.2%) had an abnormal ISTH-BS (n = 2) and/or history of substitution with recombinant factor VIIa (n = 3).

Discussion

To the best of our knowledge, this exploratory analysis is the first investigation focusing on the usefulness of ACMG classification for interpretation of molecular genetic test results in patients with FVII deficiency. On one site, an abnormal ISTH-BS and/or a history of substitution was observed in few patients (4/80; 5.0%) with *F7* gene wildtype or ACMG 1–3 variants or single heterozygous ACMG 4–5 variant with/without single heterozygous ACMG 1–3 variant. On the other site, 11 of 31 patients (35.5%) with four different scenarios of ACMG 4–5 ± ACMG 1–3 variant findings reached the combined endpoint of this exploratory analysis. Pooling patients with different constellations of zygosity and ACMG classifications in two genotypic categories was based on our preceding analysis of 44 patients with co-existence of heterozygous or homozygous c.1238G>A variant and heterozygous ACMG 5 c.1061C>T variant which was not biased by possible differences between variants of the same ACMG class such as truncating versus not-truncating variations (6) or large deletions versus others (27). This preceding analysis supports the hypothesis, that heterozygous ACMG 4–5 variants may become clinically relevant if combined with a modifying homozygous ACMG 1–3 variant or ≥2 heterozygous ACMG 1–3 variants. In the total cohort, 7 of 18 patients (38.9%) with this kind of molecular genetic finding reached the combined end point which is similar to 4 of 13 patients (30.8%) with homozygous or compound/double heterozygous ACMG 4 or 5 variants. Homozygous and compound heterozygous pathogenic *F7* variants are known to be associated with an increased bleeding risk (2, 6, 28). Of note, differentiation between double heterozygosity affecting one allele, only, and compound heterozygosity was not

available in 9 of 10 patients with two heterozygous ACMG 3–5 variants in our cohort.

Since 2015, the ACMG classification has been introduced as the internationally accepted standard for the interpretation of gene variants in heritable diseases (15, 16). For example, novel *F8* and *F9* gene variants in patients with hemophilia A or B included in the PedNet Registry were classified according to ACMG/AMP guidelines (29). In children with suspected inherited bleeding disorder genetic screening based on the ACMG guidelines may serve as a tool for early diagnosis, especially in patients with inherited thrombocytopenia (30). In the EAHAD blood coagulation factor VII variant database the assignment of *F7* gene variants to ACMG classes is already foreseen (11). Based on the ACMG classification a simplification may be possible by assigning patients with FVII deficiency and no other bleeding disorder to the categories "low risk" and "high risk" for abnormal bleeding tendency.

Our investigation may serve as a kick-off to promote the use of ACMG classification in FVII deficiency. One major question to be answered in patients with FVII deficiency refers to prophylaxis with procoagulants for surgery (1) or for delivery (31). In the past, the prediction of bleeding risk to decide on substitution therapy has been mainly based on FVII:C levels, the personal clinical history and the first bleeding symptom (1, 32). In a larger cohort and preferably prospective study, the hypothesis may be tested that the two categories identified in our investigation ("low risk" versus "high risk" for abnormal bleeding tendency) are appropriate to support ACMG classification-based interpretation of molecular genetic test results in FVII deficient patients as a contribution to successive advice and decision making in daily routine.

Even in the "high risk" group the median FVII:C was rather high (30.0%, range 2–61%) if compared to an Italian investigation which showed FVII:C > 25% in 7.1% of homozygous or compound heterozygous patients, only (6). Whereas the Italian study included patients with FVII:C < 50% diagnosed because of bleeding tendency or prolonged prothrombin time, only, maximum FVII:C was higher in our retrospective study and other reasons for consultation such as family screening or abortions/wish for a child were allowed.

As already shown by the Greifswald FVII deficiency study, the most frequent pathogenic variant in the German population is the ACMG 5 missense mutation p.(Ala294Val) (c.1061C>T) (33), also frequently combined with the ACMG 5 variant c.1391delC p.(Pro464Hisfs*32) (34) which was the same in Polish and Italian cohorts (35, 36). In our cohort the c.1061C>T variant was present in 19 of 129 patients (14.7%). Six of these patients had a combination with the c.1391delC variant and 18 patients with the c.1238G>A modifying variant. As published by Fromovich-Amit et al. (23) FVII secretion was not further reduced by the variant c.1238G>A in the presence of the c.1061C>T variant. In our cohort there was a small difference with respect to FVII activity in patients heterozygous for c.1061C>T variant together with homozygous c.1238G>A variant (FVII:C 32.6 ± 5.8%, n = 12) versus combination with heterozygote c.1238G>A variant (FVII:C 38.3 ± 11.1%, n = 6) (**Table 1**).

We observed a difference in zygosity of ACMG 1 or 2 variants versus ACMG 4 or 5 variants (homozygous: 52.9% versus 4.6%). This may reflect the typically higher minor allele frequency (MAF) of ACMG 1 or 2 variants versus ACMG 4 or 5 variants in conjunction with the higher probability of decreased FVII:C in homozygous versus heterozygous variants.

Key limitations of this investigation are the retrospective design based on patient datasets and some ACMG findings derived from partially outdated rules for classification of pathogenicity and older literature searches. It should be noted that the ACMG classification is a learning system with recent or upcoming new recommendations (37). A disease specific expert panel has been founded for coagulation factor deficiencies (38). However, until now, published experiences to adapt criteria for ACMG classification are available for other diseases such as recessively inherited hemoglobinopathies, only (39). Further limitations of our analyses are the exclusion of patients without molecular genetic testing, the baseline differences between groups, no FVII antigen testing to evaluate possible FVII:C discrepancies (40), and restriction to descriptive statistics without p-values as no formal hypothesis was defined in advance.

In conclusion, ACMG classification is a promising tool to improve interpretation of molecular genetic test results in patients with FVII deficiency. The results of this exploratory analysis suggest phenotype differences between patients with a homozygous ACMG 4–5 variant or specific combinations of heterozygous ACMG 4–5 ± ACMG 1–3 variants, on one side, and patients with *F7* gene wildtype, ACMG 1–3 variants, only, or single heterozygous ACMG 4–5 ± single heterozygous ACMG 1–3 variants, on the other side. This finding may serve as a basis to develop a genotype/phenotype prediction model in future studies.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article and **Supplementary Tables 1–3**.

Author contributions

RA had the idea and concept for the study and did the preparing work as well as manuscript outline. CH and C-ED helped to detect the patients, gave their input to the idea, and did proofreading. BR worked in the lab and did essential work to find out the ACMG classification. DJ performed the statistical analysis, literature and *F7* variant database reviews, and was involved in the preparation of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study received funding from Novo Nordisk Pharma GmbH, Mainz, Germany. The funder was not involved in the

study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest

DJ is the owner of Med-i-Scene Concept GmbH, Weisendorf, Germany, which received the funding for this study from Novo Nordisk Pharma GmbH, Mainz, Germany, in conjunction with an existing basic agreement for scientific services.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor FS declared a past co-authorship with the author RA.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1220813/full#supplementary-material

References

1. Napolitano M, Siragusa S, Mariani G. Factor FVII deficiency: clinical phenotype, genotype and therapy. *J Clin Med.* (2017) 6:38. doi: 10.3390/jcm6040038

2. Herrmann FH, Wulff K, Auerswald G, Schulman S, Astermark J, Batorova A, et al. Greifswald Factor FVII Deficiency Study Group. Factor VII deficiency: clinical manifestations of 717 subjects from Europe and Latin America with mutations in the factor 7 gene. *Haemophilia.* (2009) 15:267–80. doi: 10.1111/j.1365-2516.2008.01910.x

3. Monroe DM, Key NS. The tissue-factor VIIa complex: procoagulant activity, regulation, and multitasking. *J Thromb Haemost.* (2007) 5:1097–105. doi: 10.1111/j.1538-7836.2007.02435.x

4. Franchini M, Marano G, Pupella S, Vaglio S, Masiello F, Veropalumbo E, et al. Rare congenital bleeding disorders. *Ann Transl Med.* (2018) 6:331.

5. Peyvandi F, Menegatti M. Treatment of rare factor deficiency in 2016. *Hematol Am Soc Hematol Educ Prog.* (2016) 2016:663–9. doi: 10.1182/asheducation-2016.1.663

6. Quintavalle G, Riccardi F, Rivolta GF, Martorana D, Di Perna C, Percesepe A, et al. F7 gene variants modulate protein levels in a large cohort of patients with factor VII deficiency. Results from a genotype–phenotype study. *Thromb Haemost.* (2017) 117:1455–64. doi: 10.1160/TH17-02-0085

7. Sevenet PO, Kaczor DA, Depasse F. Factor VII deficiency: from basics ot clinical laboratory diagnosis in patient management. *Clin Appl Thromb Haemostasis.* (2017) 23:703–10. doi: 10.1177/1076029616670257

8. Bernardi F, Mariani G. Biochemical, molecular and clinical aspects of coagulation factor VII and its role in hemostasis and thrombosis. *Haematologica.* (2021) 106:351–62. doi: 10.3324/haematol.2020.248542

9. O'Hara PJ, Grant FJ, Haldeman BA, Gray CL, Insley MY, Hagen FS, et al. Nucleotide sequence of the gene coding for human factor VII, a vitamin K-dependent protein participating in blood coagulation. *Proc Natl Acad Sci USA.* (1987) 84:5158–62. doi: 10.1073/pnas.84.15.5158

10. EAHAD. *EAHAD Coagulation Factor Variant Databases. Factor FVII Gene (F7) Variant Database.* (2022). Available online at: https://f7-db.eahad.org (accessed December 20, 2022).

11. Giansily Blaizot M, Rallapalli PM, Perkins SJ, Kemball-Cook G, Hampshire DJ, Gomez K, et al. The EAHAD blood coagulation factor VII variant database. *Hum Mut.* (2020) 41:1209–19.

12. Giansily Blaizot M, Aguilar-Martinez P, Biron-Andreani C, Jeanjean P, Igual H, Schved J-F. Analysis of the genotypes and phenotypes of 37 unrelated patients with inherited factor VII deficiency. *Eur J Hum Genet.* (2001) 9:105–12. doi: 10.1038/sj.ejhg.5200593

13. Zhang X, Wang S, Leng S, Feng Q, Zhang Y, Xu S, et al. Novel factor VII gene mutations in six families with hereditary coagulation factor VII deficiency. *J Clin Lab Anal.* (2021) 35:e23905. doi: 10.1002/jcla.23905

14. Seanoon K, Payongsri P, Vivithanaporn P, Sirachainan N, Chuansumrit A, Hongeng S, et al. Mutations of TFPI-binding exosites on factor VII cause bleeding phenotypes in factor VII deficiency. *Blood Adv.* (2022) 6:5887–97. doi: 10.1182/bloodadvances.2022007560

15. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association of Molecular Pathology. *Genet Med.* (2015) 17:405–24.

16. Nykamp K, Anderson M, Powers M, Garcia J, Herrera B, Yuan-Yuan H, et al. Sherloc: a comprehensive refinement of the ACMG-AMP variant classification criteria. *Genet Med.* (2017) 19:1105–17.

17. Van Rooij J, Arp P, Broer L, Verlouw J, van Rooij F, Kraaij R, et al. Reduced penetrance of pathogenic ACMG variants in a deeply phenotyped cohort study and evaluation of ClinVar classification. *Genet Med.* (2020) 22:1812–20. doi: 10.1038/s41436-020-0900-8

18. Green F, Kelleher C, Wilkes H, Temple A, Meade T, Humphries S. A common genetic polymorphism associated with lower coagulation factor VII levels in healthy individuals. *Ateriosclerosis Thromb.* (1991) 11:540–6.

19. Humphries S, Temple A, Lane A, Green J, Cooper J, Miller G. Low plasma levels of factor VIIc and antigen are more strongly associated with the 10 base pair promoter (-323) insertion than the glutamine 353 variant. *Thromb Haemost.* (1996) 75:567–72.

20. Mtiraoui N, Aboud N, Bouraoui H, Haizem S, Gris JC, Busson M, et al. Reduction of coagulation factor VII plasma levels by R353Q but not the -323P0/10 promotor polymorphism in healthy Tunisians. *Am J Hematol.* (2005) 79:11–6.

21. Girolami A, Paoletti M, Ferrari S, Garcia D. Peculiar congenital factor VII defect with the proposita and her mother showing the same compound heterozygosity for Thr384Met and Arg413Gln. *Acta Haematol.* (2021) 144:100–4. doi: 10.1159/000507071

22. Miolo G, Caggiari L, De Zorzi M, Tedeschi M, Tessitori G, Percesepe A, et al. Impact of a new genetic variant on FVII:C activity. *Integr Mol Med.* (2019) 6:2–4. doi: 10.15761/IMM.1000381

23. Fromovich-Amit Y, Zivelin A, Rosenberg N, Tamary H, Landau M, Seligsohn U. Characterization of mutations causing factor VII deficiency in 61 unrelated Israeli patients. *J Thromb Haemost.* (2004) 2:1774–81. doi: 10.1111/j.1538-7836.2004.00921.x

24. Rodeghiero F, Tosetto A, Abshire T, Arnold DM, Coller B, James P, et al. ISTH/SSC bleeding assessment tool: a standardized questionnaire and a proposal for a new bleeding score for inherited bleeding disorders. *J Thromb Haemost.* (2010) 8:2063–5. doi: 10.1111/j.1538-7836.2010.03975.x

25. Elbatarny M, Mollah S, Grabell J, Bae S, Deforest M, Tuttle A, et al. Normal range of bleeding scores for the ISTH–BAT: adult and pediatric data from the merging project. *Haemophilia.* (2014) 20:831–5. doi: 10.1111/hae.12503

26. ISTH-SSC. *ISTH-SSC Bleeding Assessment Tool.* (2022). Available online at: https://bleedingscore.certe.nl (accessed December 20, 2022).

27. Rath M, Najm J, Sirb H, Kentouche K, Dufke A, Pauli S, et al. Large deletions play a minor but essential role in congenital coagulation factor VII and X deficiencies. *Hamostaseologie.* (2015) 35(Suppl. 1):S36–42.

28. Mariani G, Herrmann FH, Dolce A, Batorova A, Etro D, Peyvandi F, et al. Clinical phenotypes and factor VII genotype in congenital factor VII deficiency. *Thromb Haemost.* (2005) 93:481–7.

29. Andersson NG, Labarque V, Letelier A, Mancuso ME, Bührlen M, Fischer K, et al. Novel F8 and F9 gene variants from the PedNet hemophilia registry classified according to ACMG/AMP guidelines. *Hum Mutat.* (2020) 41:2058–72. doi: 10.1002/humu.24117

30. Andersson NG, Rossing M, Ferrari MF, Gabrielaite M, Leinoe E, Ljung R, et al. Genetic screening of children with suspected inherited bleeding disorders. *Haemophilia.* (2020) 26:314–24.

31. Baumann Kreuziger LM, Morton CT, Reding MT. Is prophylaxis required for delivery in women with factor VII deficiency? *Haemophilia.* (2013) 19:827–32.

32. Di Minno MN, Dolce A, Mariani G, Ster Study Group. Bleeding symptoms at disease presentation and prediction of ensuing bleeding in inherited FVII deficiency. *Thromb Haemost.* (2013) 109:1051–9.

33. Herrmann FH, Wulff K, Strey R, Siegemund A, Astermark J, Schulmann S. Variability of clinical manifestations of factor VII-deficiency in homozygous and heterozygous subjects of the European F7 gene mutation A294V. *Haematologica.* (2008) 93:1273–5. doi: 10.3324/haematol.12567

34. Wulff K, Herrmann FH. Twenty two novel mutations of the factor VII gene in factor VII deficicency. *Hum Mutat.* (2000) 15:489–96. doi: 10.1002/1098-1004(200006)15:6<489::AID-HUMU1>3.0.CO;2-J

35. Arbini AA, Bodkin D, Lopaciuk S, Bauer KA. Molecular analysis of Polish patients with factor VII deficiency. *Blood.* (1994) 84:2214–20.

36. Bernardi F, Liney DL, Patracchini P, Gemmati D, Legnani D, Arcieri P, et al. Molecular defects in CRM+ factor VII deficiencies: modelling of missense mutations in the catalytic domain of FVII. *Br J Haematol.* (1994) 86:610–8. doi: 10.1111/j.1365-2141.1994.tb04793.x

37. Pejaver V, Byrne AB, Feng B-J, Pagel KA, Mooney SD, Karchin R, et al. Calibration of computational tools for missense variant pathogenicity classification and ClinGen recommendations for PP3/BP4 criteria. *Am J Hum Genet.* (2022) 109:2163–77. doi: 10.1016/j.ajhg.2022.10.013

38. Mohan S, Lee K, Carcao M, Doshi BS, Downes K, Kemball-Cook G, et al. ClinGen coagulation factor deficiency variant curation expert panel: meeting the need for recommendations to curate variants in the coagulation factor genes. *Blood.* (2019) 134(Suppl 1):5794.

39. Kountouris P, Stephanou C, Lederer CW, Traeger-Synodinos J, Bento C, Harteveld CL, et al. Adapting the ACMG/AMP variant classification framework: a perspective from the ClinGen hemoglobinopathy variant curation expert panel. *Hum Mutat.* (2022) 43:108. doi: 10.1002/humu.24280

40. Triplett DA, Brandt JT, Batard MA, Dixon JL, Fair DS. Hereditary factor VII deficiency: heterogeneity defined by combined functional and immunochemical analysis. *Blood.* (1985) 66:1284–7.

frontiers | Frontiers in Medicine

TYPE Review
PUBLISHED 21 August 2023
DOI 10.3389/fmed.2023.1237601

OPEN ACCESS

EDITED BY
Felix Carl Fabian Schmitt,
Heidelberg University Hospital, Germany

REVIEWED BY
Stephan Katzenschlager,
Heidelberg University, Germany
Simon Dubler,
Essen University Hospital, Germany

*CORRESPONDENCE
Jun Teruya
✉ jteruya@bcm.edu

RECEIVED 09 June 2023
ACCEPTED 31 July 2023
PUBLISHED 21 August 2023

CITATION
Navaei A, Kostousov V and Teruya J (2023) Is it time to switch to bivalirudin for ECMO anticoagulation?
Front. Med. 10:1237601.
doi: 10.3389/fmed.2023.1237601

Is it time to switch to bivalirudin for ECMO anticoagulation?

Amir Navaei[1,2], Vadim Kostousov[2] and Jun Teruya[1,2,3]*

[1]Department of Pediatrics, Texas Children's Hospital, Baylor College of Medicine, Houston, TX, United States, [2]Department of Pathology & Immunology, Baylor College of Medicine, Houston, TX, United States, [3]Department of Medicine, Texas Children's Hospital, Baylor College of Medicine, Houston, TX, United States

For decades, unfractionated heparin (hereafter, heparin) has been the primary anticoagulant used for extracorporeal membrane oxygenation (ECMO) support. More recently, however, bivalirudin, a direct thrombin inhibitor, has emerged as an alternative. This systematic review based on PRISMA guidelines, aims to summarize 16 comparative studies and 8 meta-analysis and review articles published from January, 2011 till May, 2023 which directly compares ECMO courses using heparin versus bivalirudin as the anticoagulant. While this comparison is complicated by the lack of a standardized definition of major bleeding or thrombosis, our overall findings suggest there is no statistical difference between heparin and bivalirudin in incidence of bleeding and thrombosis. That said, some studies found a statistical significance favoring bivalirudin in reducing major bleeding, thrombosis, and the need for transfusions. We also offer essential guidance for appropriately selecting an anticoagulant and monitoring its effect in ECMO settings.

KEYWORDS

heparin, bivalirudin, review, ECMO, monitor

Introduction

Extracorporeal membrane oxygenation (ECMO) is widely used to support the heart and/or lungs, and the conditions for which it is indicated have recently expanded to include sepsis, extracorporeal cardiopulmonary resuscitation (ECPR), and coronavirus disease 2019 (COVID-19). It is also now used as a bridge to lung transplantation or implantation of a ventricular assist device (VAD) (1, 2). For many years, unfractionated heparin (hereafter, heparin) has been the primary anticoagulant used with ECMO. Here, we review the literature comparing bleeding, thrombosis, hospital course, and outcome among patients on ECMO using either heparin or bivalirudin as the anticoagulant, and we offer recommendations for the selection and monitoring of anticoagulants in this context.

Choice of anticoagulant

Heparin is currently the primary anticoagulant used by the vast majority of institutions [94%, according to a recent survey (3)] in pediatric and adult ECMO management. Typically, once two units of red blood cells are in the blood reservoir, 200 units of heparin are added to the pediatric circuit and 1,000 units are added to the adult circuit. A bolus of heparin (50–100 units/kg) is also administered during cannulation (4) and, depending on the risk of bleeding, it is continuously infused post-cannulation. More recently, however, a small percentage of centers (6%) reported using bivalirudin as the primary anticoagulant for ECMO, 60% of which were primary neonatal and pediatric centers (3, 5).

Heparin vs. bivalirudin

Heparin has several advantages: it is easy to use, relatively inexpensive, and in case of significant bleeding or overdose, it can be neutralized by administering protamine. On the other hand, heparin alone has no anticoagulant effect. To become an anticoagulant, it must form a complex with antithrombin (AT) and heparin cofactor II and release tissue factor pathway inhibitor (TFPI) from endothelial cells to express its entire anticoagulant action. AT plays the most important role in this process, and in 2014, the Extracorporeal Life Support Organization (ELSO) guidelines first suggested AT monitoring and replenishment during ECMO. However, the need to monitor and replenish AT levels, especially among newborns, remains controversial (6). In general, young children have lower physiologic concentrations of natural anticoagulants, including AT, and this is most true of children under 1 year of age. However, heparin, which is negatively charged, binds to more than 100 positively charged plasma proteins, including von Willebrand factor and C-reactive protein non-specifically (7, 8). When the patient on ECMO is in acute phase reaction, all acute phase reactant levels are increased, and overall heparin anticoagulant effect may be reduced. Due to its binding with many plasma proteins, the anticoagulant effect of heparin thus needs to be constantly monitored by activated partial thromboplastin time (aPTT) and/or anti-factor Xa assay (anti-Xa).

Bivalirudin, on the other hand, binds directly to thrombin, which is both circulating and clot-bound. Therefore, it does not require binding to other proteins to exhibit its anticoagulant effect. Approximately 20% of bivalirudin is cleared from the kidneys and the remainder is proteolyzed. While there are no reversal agents for bivalirudin, it has a much shorter half-life of 25 min in adults and 15 min in newborns, compared to heparin's half-life of 90 min (9). Therefore, it is not a significant problem using bivalirudin in cases of active bleeding during ECMO support. A summary of this comparison can be found in Table 1. A growing number of studies have evaluated the use of bivalirudin versus heparin for ECMO.

TABLE 1 Heparin and bivalirudin comparison.

Drug	Heparin	Bivalirudin
Action	• Binding to antithrombin and heparin cofactor II → anti-Xa and anti-IIa • Release tissue factor pathway inhibitor	• Binding to circulating and clot bound thrombin
Half-life	90 min	15–25 min
Clearance	Mainly reticulo-endothelial system (RES), some renal clearance	Mainly extravascular proteolysis, 20% renal clearance
Binding protein	>100 plasma proteins including antithrombin and heparin cofactor II	Only thrombin
Bioavailability	Poor	Good
Monitoring	aPTT and anti-Xa	aPTT, HPTT, dTT, ECT, ECA, anti-factor IIa
Antidote	Protamine (Heparin rebound may happen)	None

dTT, diluted thrombin time; HPTT, heparinase treated activated partial thromboplastin time; aPTT, activated partial thromboplastin time; ECT, ecarin clotting time; ECA, ecarin chromogenic assay; AT, antithrombin.

Methods

We conducted a systematic review of the literature for anticoagulation comparison between bivalirudin and heparin in ECMO patients. Systematic review was performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) by using Embase, PubMed and Cochrane Libraries. Literature review performed by following keywords: (ECMO, extracorporeal membrane oxygenator, ECLS, extracorporeal) AND (heparin OR unfractionated heparin) AND (bivalirudin OR angiomax). Titles were screened by two independent reviewers. Full text articles were reviewed with following inclusion criteria: (1) including ECMO patients, (2) comparing bivalirudin and heparin, (3) have outcome measurements, and (4) January, 2011- May, 2023 (Figure 1).

In our literature review we identified 16 such studies, all of which were retrospective cohort studies published from 2011 to 2023. The majority included adult patients, while only 5 focused exclusively on pediatric patients. There is no universal scoring system for bleeding severity, so to compare these studies we rated bleeding severity using Bleeding Academic Research Consortium (BARC) scoring (10).

Results

A total of 1,365 adult and 312 pediatric ECMO runs were collectively included in these studies, of which 558 adults and 116 pediatric patients received bivalirudin for anticoagulation (Tables 2A,B) (11–26). Six studies evaluated time to achieve therapeutic target with three showing significantly shorter time for bivalirudin and three finding no difference (12, 13, 17, 20, 22, 24). Seven studies assessed the percentage of aPTT being at therapeutic range, of which four (57%) showed bivalirudin having a significantly higher percentage of therapeutic laboratory targets versus heparin (12, 13, 20–24).

Bleeding and thrombotic complications are the most clinically significant concerns associated with ECMO. A majority of the studies (13/15) evaluated major bleeding—i.e., bleeding associated with a hemoglobin drop of at least 2 g/dL, or a transfusion requirement of one or more doses of 10 mL/kg red blood cells (RBC) transfusion in a 24-h period. The bleeding included retroperitoneal, pulmonary, gastrointestinal, and central nervous system bleeding; bleeding that required surgical intervention; central cannulation site bleeding that required re-exploration; and fatal bleeding (type 3–5 according to BARC scale) (10). Overall, these studies suggest there is no significant difference between bivalirudin and heparin in the incidence of major bleeding (13, 16, 17, 19–21, 23–26). Four studies showed a significant decrease in the frequency of major bleeding in the bivalirudin group (11, 12, 15, 22). One study found a significant decrease in the number of laboratory tests in the bivalirudin group, which was associated with significantly lower blood loss. This latter finding is consistent with the fact that aPTT was more often at target, so use of bivalirudin required less frequent blood draws and adjustments (20). Two studies also showed that the bivalirudin group required significantly fewer RBC transfusions (15, 16). Thus, overall findings suggest that the risk of bleeding is the same with bivalirudin and heparin, but the former may

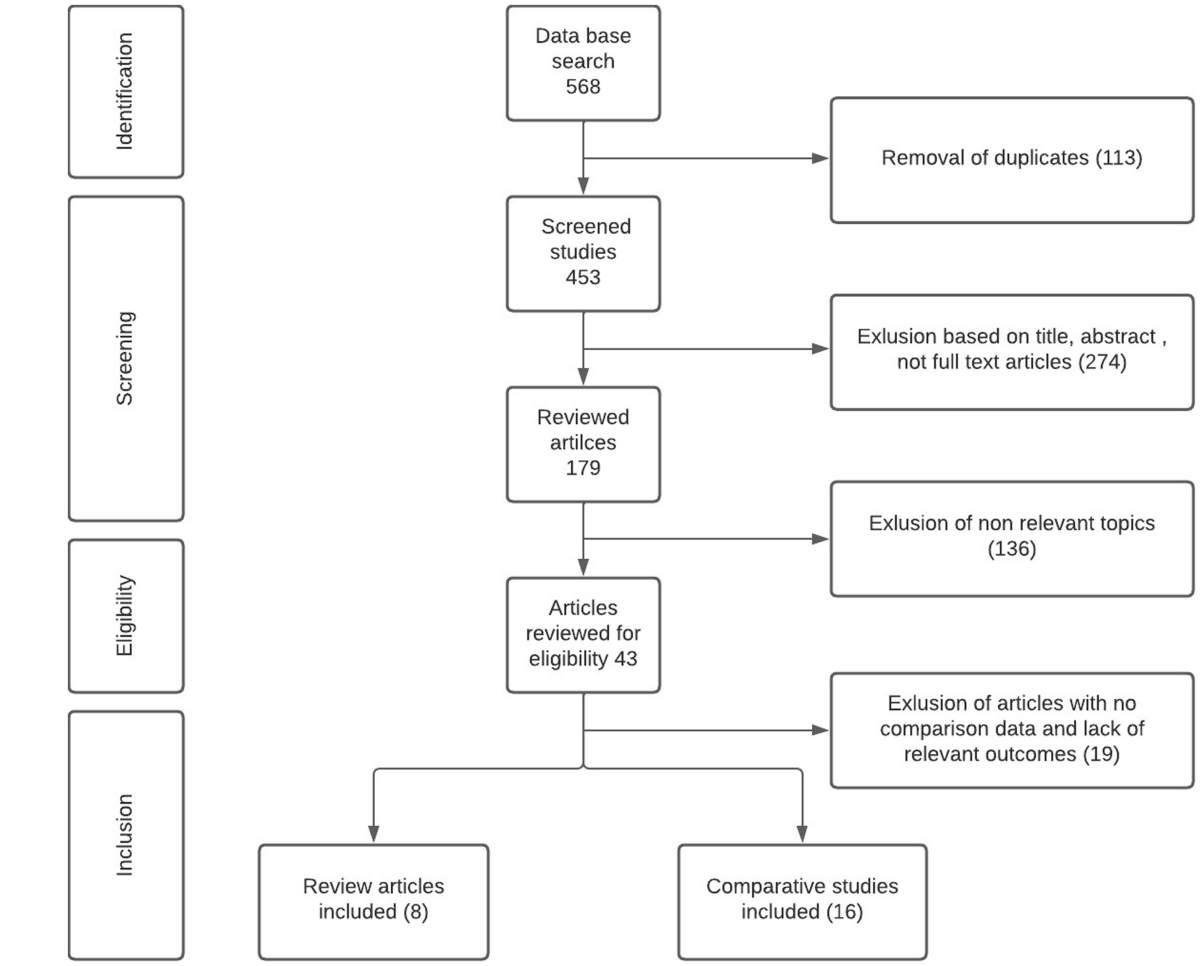


FIGURE 1
Flow diagram according to PRISMA guidelines.

have some benefit in reducing the need for phlebotomy and potentially decreasing the risk of bleeding.

Thrombotic complications can be a clinical marker of inadequate anticoagulation during an ECMO course, but they are challenging to assess retrospectively due to a lack of standardized reporting. The majority of studies (11/15) evaluating thrombotic complications of ECMO circuit reported no significant difference between the use of bivalirudin and heparin (11, 13, 14, 16, 19–25). Two studies showed significantly fewer ECMO-associated thrombotic events per day and an increased time to thrombosis among those receiving bivalirudin versus heparin (12, 15). One study showed a longer duration of freedom to circuit intervention (19) Thus, overall findings suggest that bivalirudin does not increase thrombotic complications in ECMO and may instead decrease them due to more stable anticoagulation.

One major concern about bivalirudin is that it costs more than heparin. Some studies— mainly in patients using a ventricular assist device (VAD)—evaluated the cost of each medication as well as the cost of associated laboratory tests and heparin's additional requirement of AT replacement, especially in pediatric patients. While Hamza, et al. found a significantly lower overall cost of therapy with bivalirudin compared to heparin ($494 vs. $1,184 per day; $p = 0.03$) (24), other studies found that, overall, the costs are equal. When using bivalirudin, the cost of the drug itself accounted for a higher percentage of overall costs, whereas the larger percentage of heparin's costs were attributable to laboratory diagnostic studies and AT replacement (27).

Once a clot is formed, heparin cannot inhibit clot-bound thrombin and slow down the process of clot propagation. In contrast, the relatively small molecular size of bivalirudin allows it to bind to both plasma-free thrombin and clot-bound thrombin. This is a potential advantage over both heparin and low molecular weight heparin (LMWH), as clot-bound thrombin remains active during amplification of the coagulation cascade (9).

In sum, bivalirudin's potential benefits are that it: offers more stable anticoagulation; provides a faster response; requires less titration; and potentially decreases circuit clotting and major bleeding complications. Moreover, bivalirudin is no longer considered a novel anticoagulant as many centers, particularly adult hospitals, have become more familiar with it. Therefore, we suggest transitioning from heparin to bivalirudin as the primary agent for ECMO anticoagulation, keeping in mind that heparin may continue to be needed for new circuit priming and bolus administration until use of bivalirudin is established for these purposes.

TABLE 2A Review of studies comparing heparin and bivalirudin for ECMO anticoagulation. Comparison of heparin vs bivalirudin.

Author, year	Adults (Children)	VV or VA	Heparin	Bivalirudin	Thrombosis, %	Circuit Exchange, %	Major Bleed, %	Minor Bleed, %	ECMO Mortality, %	Transfusion reduction	Time to Target hours	TTR, %	Other findings
Tong et al., 2023 (11)	34	VV	20	14	35 vs 14, ns	—	30 vs 0, p=0.02	55 vs 57, ns	40 vs 7, p=0.033	OR 0.51, p=0.04 (RBC)	—	—	Platelet transfusion volume (0 vs 300 mL) and the incidence of major bleeding were significantly lower in bivalirudin group.
Uricchio et al., 2023 (12)	143	VA	89	54	0.1 vs 0.04 per day, p<0.001	—	49 vs 29, p=0.02	15 vs 13, ns	58 vs 74, p=0.04	OR 0.45, p=0.004 (RBC/day)	28 vs 21, p=0.001	40 vs 50, p<0.001	Bivalirudin was independently associated with an increased time to thrombosis. Patients receiving bivalirudin required less total RBC (p=0.04) and plasma administration (p=0.03).
Sheridan et al., 2022 (13)	150	VA	50	100	0 vs 1, ns	4 vs 4, ns	4 vs 6, ns	8 vs 4, ns	50 vs 57, ns	—	18 vs 2, p<0.001	33 vs 86, p<0.001	No difference was found in rates of bleeding between 2 groups. Bivalirudin yielded higher percent time in therapeutic range, faster time to therapeutic range and required fewer dose adjustments to maintain therapeutic range (2 vs. 11, P <0.001) compared to heparin.
Seelhammer et al., 2021 (14)	333 and (89)	VA 84%	223 and (65)	110 and (24)	17 vs 12, ns; (22 vs 13, ns)	5 vs 4, ns; (6 vs 13, ns)	—	—	53 vs 38, p=0.01 (57 vs 42, ns)	OR 0.28, p=0.02 (24 h, peds)	—	—	The reduced mortality in the adult bivalirudin group and reduction in the composite transfusion requirement in the first 24 hours was noted in the pediatric bivalirudin group.
Rivosecchi et al., 2021 (15)	295	VV	162	133	33 vs 17, p=0.003	—	41 vs 12, p<0.001	—	38 vs 27, p=0.054	OR 0.38, p<0.001 (RBC)	—	—	Overall decrease in the number of device thrombotic complications, significant increase in time to circuit thrombosis in bivalirudin group. Heparin was associated with significantly higher risk of clot formation. Significantly less volume of RBC, plasma and platelet transfusion and decreased number of major bleeding events in patients receiving bivalirudin.
Giuliano et al., 2021 (16)	144	VA 81%	131	13	0.05 vs 0.03 per day, ns	—	0.08 vs 0.03 per day, ns	—	62 vs 69, ns	OR 0.34 p<0.001 (RBC)	—	—	The rate of bleeding or thrombotic complications did not differ between heparin and bivalirudin, but patients on bivalirudin received significantly fewer blood transfusions (1.0 units of RBC/day vs 2.9 units/day on heparin; p<0.001).
Kaushik et al., 2021 (17)	(35)	VA 86%	27	8	—	11 vs 25, ns	44 vs 13, ns	4 vs 13, ns	26 vs 62, ns	—	12 vs 14.5, ns	44 vs 65, p=0.014	3/8 patients switched to bivalirudin due to concern for HIT and 1/8 for heparin resistance. Patients in the primary bivalirudin group had more stable aPTT values with only 9% of aPTT values having more than 30% variability from goal aPTT compared to 30% in the heparin group (p=0.003). Patients with primary bivalirudin use had a significantly lower dose requirement at 12 (p=0.003), 36 (p=0.007), and 48 (p=0.0002) hours compared to patients with secondary use of bivalirudin.
Rabinowitz et al. 2021 (18)	(67)	VA 61%	35	32	—	—	—	—	34 vs 37, ns	—	—	—	No correlation between anticoagulant dosing and any laboratory test variables in 37% of the heparin and 44% of the bivalirudin group.

(Continued)

10.3389/fmed.2023.1237601

TABLE 2 (Continued)

Author, year	Adults (Children)	VV or VA	Heparin	Bivalirudin	Thrombosis, %	Circuit Exchange, %	Major Bleed, %	Minor Bleed, %	ECMO Mortality, %	Transfusion reduction	Time to Target hours	TTR, %	Other findings
Schill et al, 2020 (19)	(48)	VA 67%	34	14	21 vs 7, ns	20 vs 57, ns	12 vs 7, ns	—	27 vs 36, ns	OR 1.03, ns (RBC)	—	—	The bivalirudin group had longer support duration compared to the heparin (11 vs 3.3 days, p<0.001), but the freedom to first circuit intervention was longer in bivalirudin group (P = 0.02).
Machado et al., 2020 (20)	(32)	VA	14	18	6 vs 29, ns	35 vs 22, ns	11 vs 36, ns	—	43 vs 11, p=0.04	OR 0.4, ns (total)	13 vs 21, p=0.06	54 vs 57, ns	Mean percentage of time with therapeutic aPTT and ACT was not different. Phlebotomy- associated blood loss per ECMO hour was double in heparin 1.08 mL/hour compared to bivalirudin 0.51 mL/hour (p<0.001) as well as interventions to control bleeding.
Kaseer et al., 2020 (21)	52	VA 54%	33	19	33 vs 26, ns	27 vs 26, ns	18 vs 5, ns	—	45 vs 37, ns	—	—	50 vs 86,p=0.007	No difference in composite thrombotic or bleeding complications. Higher percent time to be in therapeutic aPTT range with bivalirudin.
Hamzah et al., 2020 (22)	(32)	VA 91%	16	16	19 vs 0, ns	6 vs 0, ns	75 vs 19; p=0.001	—	44 vs 38, ns	—	29 vs 11, p=0.001	—	Shorter time to therapeutic target time with bivalirudin. Less bleeding events per 10 days of ECMO support with bivalirudin (p=0.002). Lower rates of RBC and plasma transfusion with bivalirudin. Total cost of therapy less with bivalirudin (US $1184 vs. $494 per day; p=0.03)
Macielak et al., 2019 (23)	110	VA 67%	100	10	0.21 vs 0.09 per day; ns	—	0.22 vs 0.14 per day, ns	—	—	OR 1.17 (RBC)	—	38 vs 53, p<0.001	There were no differences in the rate of bleeding events. An increased number of adjustments to either anticoagulant was associated with a higher rate of bleeding events per day (p = 0.006).
Berei et al., 2017 (24)	72	VA 92%	28	44	25 vs 23, ns	—	25 vs 45, ns	25 vs 23, ns	32 vs 36, ns	—	56 vs 25, ns	83 vs 88, ns	No significant difference between heparin and bivalirudin for bleeding and thrombotic complications.
Pieri et al., 2014 (25)	20	VA 50%	10	10	30 vs 10, ns	—	40 vs 30, ns	20 vs 0, ns	50 vs 40, ns	—	—	—	No significant difference between heparin and bivalirudin for bleeding and thrombotic complications.
Ranucci et al., 2011 (26)	12 and (9)	VA	3 and (5)	9 and (4)	25 vs 8, ns	—	25 vs 0, ns	—	63 vs 31, ns	OR 0.6, ns (RBC)	—	—	No significant difference between heparin and bivalirudin for bleeding and thrombotic complications.

ns = not significant, TR = transfusion reduction, VV = veno-venous ECMO, VA = veno-arterial ECMO, RBC = red blood cells, TTR = therapeutic target range, HIT = heparin induced thrombocytopenia.

10.3389/fmed.2023.1237601

10.3389/fmed.2023.1237601

TABLE 2B Number of publications with outcomes favoring bivalirudin or heparin (11–26).

	Favoring bivalirudin	Favoring heparin	No difference
Thrombosis	2	0	12
Major bleeding	4	0	10
Minor bleeding	0	0	6
Mortality	3	1	11
Transfusion reduction	5	0	4

Heparin monitoring

In 90% of centers across the US, heparin monitoring is performed via anti-factor Xa (anti-Xa) activity assay. The common target level for thrombosis is 0.3—0.7 units/mL (7). (In our institution, we use the default target of 0.3–0.5 units/mL for ECMO). In 75% of centers, activated clotting time (ACT) is also used as an adjunct test, with 180–200 s the most common target but ranging from 180–240 s in centers using different ACT machines. Although aPTT and anti-Xa are recommended as the most reliable combination for monitoring heparin anticoagulation, the combination of ACT and anti-Xa is still most often (68%) used for this purpose (4). Viscoelastometric studies such as thromboelastography (TEG™) or rotational thromboelastometry (ROTEM™) are used in 41% of centers. Antithrombin level is now more frequently monitored, using targets that range widely from 60–100%. Institutions are improving in establishing protocols, but almost 50% still manage ECMO anticoagulation without a standard protocol, especially in hospitals with mixed adult/pediatric patient populations. Along with anti-Xa, aPTT is commonly targeted for heparin anticoagulation monitoring with a wide range of 60–100 s, depending upon patient age (4). aPTT results can be affected significantly by heparin contamination and underlying coagulopathy, such as disseminated intravascular coagulation (DIC) or dilutional coagulopathy, depending on whether the sample is drawn from a central line or directly from the ECMO circuit. Heparinase-treated aPTT (HPTT) helps to eliminate the heparin effect and reveal the underlying coagulable state, yet HPTT is still rarely used in most institutions due to its cost. It is also important to note that phospholipid-binding proteins such as lupus anticoagulant and C-reactive protein (CRP) may also prolong aPTT and HPTT (7, 28) and this effect must be considered when assessing aPTT and anti-Xa in gaging anticoagulation with heparin.

Anti-Xa assay is the most reliable test for measuring heparin's effect because it directly measures heparin anticoagulant activity (29). There are various types of anti-Xa assays and the lack of standardization can cause a discrepancy in results. Anti-Xa assays that do not contain exogenous AT and rely solely on endogenous AT activity are preferred for evaluating heparin anticoagulation (30). They are helpful in cases of heparin resistance due to low AT levels or polymorphism of the binding site. Significantly elevated factor VIII may shorten aPTT and artificially present as heparin resistance. Therefore, the best practice is using both aPTT and anti Xa to target heparin's effect (28).

Bivalirudin monitoring

Several laboratory tests are available for monitoring the anticoagulant effect of bivalirudin, of which aPTT and ACT are the most commonly used. aPTT is the most common monitoring tool, with the standard target of 60–80 s (4). ACT is also used in some centers as an adjunct to aPTT (31).

Other coagulation assays include diluted thrombin time (dTT), chromogenic anti-factor IIa (anti-IIa) assay, ecarin clotting time (ECT), and the ecarin chromogenic assay (ECA) (9). Preanalytical errors such as heparin contamination can significantly affect aPTT, especially when the sample is collected through indwelling lines with a heparin lock. Therefore, HPTT should be used since heparinase is able to neutralize up to two units/mL of heparin in 500 µL of citrated plasma (32). The main disadvantages of using HPTT are its cost, incubation time, and lack of automatization; for these reasons, most centers do not use it as the standard test. Appropriately collecting samples from the ECMO circuit, not from an indwelling catheter, would decrease heparin contamination and is advised for institutions that do not have HPTT available. In our institution, we routinely use HPTT as a reflex test for possible heparin-contaminated aPTT samples screened by prolonged thrombin time and as part of a comprehensive ECMO panel.

Prolonged aPTT and HPTT may also be caused by other factors, such as lupus anticoagulant, high CRP, deficiency of intrinsic factors, or the presence of factor-specific inhibitors. Some studies therefore found that aPTT does not correlate well with bivalirudin level. Bivalirudin level measured by dTT has been reported to better correlate with bivalirudin administration dose (33, 34). Using dTT from spiked pooled normal plasma, *in vitro* studies reported concentrations anywhere from 0.2 to 1.5 µg/mL for bivalirudin, correlating with 1.5 to 2.5 times prolongation of normal aPTT values (33–35). Our institutional target range using dTT is 0.8 to 2.6 µg/mL as equivalent to 1.7–2.3 prolongation of upper normal aPTT value and is similar to previously described ranges—i.e., 0.5–2.5 µg/mL, as reported by Hasan, et al., using the same assays and reagents (36).

Special considerations

ECMO cannulation requires intraoperative anticoagulation to prevent thrombotic complications due to hypercoagulable state secondary to endothelial cell injury, interruption of blood flow, and exposure to the cannula and foreign surfaces. The current practice is to administer 50–100 units/kg of heparin as a bolus dosing during cannulation with ACT monitoring intraoperatively. Bolus dosing of bivalirudin has been used mainly in cardiac catheterization and in interventional radiology procedures in adults. Due to the lack of pediatric data regarding bivalirudin bolus dosing, it is more difficult to standardize it in a pediatric population. The only contraindication for a heparin bolus would be suspicion of heparin-induced thrombocytopenia (HIT), which is uncommon in patients younger than 10 years old. Therefore, it is preferable to continue using a heparin bolus during ECMO cannulation.

Due to the ratio of circuit volume to total blood volume, pediatric patients—especially infants—are at higher risk of developing post-cannulation dilutional coagulopathy. This issue has been addressed by our institution and others by priming the circuit with plasma and RBC. Prior to starting bivalirudin, it is important to make sure

dilutional coagulopathy is resolved and have a baseline aPTT level. In this way, appropriate anticoagulation can be achieved by targeting aPTT or HPTT. Also, aPTT can be prolonged due to lupus anticoagulant or C-reactive protein, so in patients with a high aPTT at baseline, it is important to target bivalirudin anticoagulation by targeting 1.5-2X increased in aPTT from the baseline.

Due to the short half-life of bivalirudin, its levels may be reduced in areas where blood is static. The ECMO circuit, which is different from cardiopulmonary bypass, does not contain a reservoir or areas of significant concern for stasis, so this risk is more likely in patients with anatomical etiologies for stasis, such as cardiac stunning, which may increase thrombotic complications. There is a paucity of data on this topic and each patient needs to be assessed individually. If prolonged stasis in a patient is of concern, heparin could be the preferred anticoagulation of choice. Clamping of cannulas during weaning trials from veno-arterial (VA) ECMO may potentially cause stagnation. Cannulas are unclamped periodically to prevent this outcome, but there is no standard methodology for doing so. (In our institution we unclamp every five minutes to avoid stagnation.) If prolonged stasis is a concern, practitioners may consider administering a heparin bolus of 25 units/kg prior to the clamping trial. Also, when bivalirudin is administered to the circuit during clamping trials, the patient may not receive adequate anticoagulation and therefore may need additional bivalirudin during them.

Bivalirudin clearance depends on renal function but is independent of dose and gender. Approximately 20% of the unchanged drug is cleared via the kidney, and the remainder presumably undergoes proteolysis intracellularly (37). Dialysis and increased urine output will reduce bivalirudin's effect. In a retrospective multicenter study, it was reported that in pediatric ECMO population a decreased bivalirudin dose requirement of about 28% among patients with estimated glomerular filtration rate (eGFR) of >60, 30–60 and < 30 mL/min/1.73m^2. Also, patients with lower eGFR had longer duration of supratherapeutic anticoagulation targets, higher bleeding tendency and transfusion of blood product requirement, close monitoring and dose adjustment (38). In the setting of accidental overdose or active bleeding that requires reversal of the bivalirudin effect, typically discontinuation of bivalirudin infusion is sufficient due to short half life, however, administration of plasma, prothrombin complex concentrate and hemodialysis can be considered in severe cases.

Severe acute respiratory syndrome coronavirus 2 (SARS-COV2) infection showed unique coagulation challenges during COVID-19 pandemic. Patients with severe SARS-COV2 infection demonstrated diffuse endothelial injury, micro and macrovascular thrombosis. Heparin was the standard anticoagulation of choice for thromboprophylaxis in COVID-19 infection. In severe SARS-COV2 patients with acute respiratory distress syndrome (ARDS) many patients required ECMO support for prolonged durations. In a retrospective case control study patients with SARS-COV2 ARDS on ECMO required higher doses of bivalirudin to achieve therapeutic aPTT targets compared to non COVID 19 group. However, there was no difference between the bleeding and thrombotic complications among two groups (39).

Prevalence of obesity among children is increasing in the United States. Obese patients not only have higher risk of thrombosis but also require special considerations for drug dosing. In a retrospective study conducted in patients with high body mass index (BMI) who had HIT, bivalirudin dosing based on total body weight was found to have most accurate predictor of achieving aPTT goal (40).

Management of ECMO anticoagulation in patients with underlying hepatic synthetic dysfunction, shock liver and DIC in the setting of septic shock can be challenging. Safety of using bivalirudin in adult patients with hepatic and renal dysfunction has been demonstrated in the literature (41). However, there is scarcity of pediatric and adult ECMO data. For management of anticoagulation with bivalirudin in patients with underlying coagulopathy, dTT and viscoelastometric studies would be instrumental. Consultation with coagulation expert and individual based risk assessment is essential in this population.

Coronary artery disease (CAD) is more common in adult population, however, coronary and other vascular stent placements occasionally required in pediatric patient as well. Using antiplatelet agents along with anticoagulants such as heparin or bivalirudin are the routine management to prevent thrombosis of the stent. ECMO patients due to decreased platelet function and acquired von Willebrand syndrome, potentially have a higher risk of bleeding when antiplatelet agents are utilized. A retrospective single center study showed no difference in bleeding complications in patients on VA ECMO who received dual antiplatelet therapy along with heparin for anticoagulation compared to heparin monotherapy (42). In as sub study of a randomized control trial conducted in Sweden, in patients with ST elevated myocardial infarction (STEMI) who are not on ECMO and undergoing primary percutaneous coronary intervention (PCI) and treated with aspirin and potent P2Y12-inhibitors, anticoagulation with bivalirudin was not superior to heparin regarding the composite end point of all-cause death, myocardial infarction, or major bleeding (43). A recent published randomized controlled trial in patients with STEMI undergoing primary PCI who had not received previous glycoprotein IIb/IIIa inhibitors compared bivalirudin with a post-PCI high-dose infusion vs. heparin monotherapy, showed significantly reduced the 30-day composite rate of all-cause mortality or BARC types 3–5 major bleeding in bivalirudin group (44). Due to scarcity of data in using antiplatelet agents along with bivalirudin vs. heparin in ECMO patients, decision needs to be made based on individual risk factors.

Institutional experience and proposed ECMO anticoagulation protocol with bivalirudin as the primary anticoagulant

Historical aspect and current practice

Bivalirudin was introduced to our practice in 2016. Initially utilized mainly for ECMO and VAD anticoagulation when encountered heparin resistance. Currently, in our institution, the pediatric intensive care unit (PICU) and cardiac intensive care unit (CICU) both use bivalirudin as the primary anticoagulant for all ECMO runs. Our neonatal intensive care unit (NICU) continues to use heparin as the primary anticoagulant, while bivalirudin is the secondary agent and used mainly for heparin resistance. However, based on the growing experience of our primary care providers and the cost–benefit ratio, we are considering transitioning to bivalirudin as the primary anticoagulant for all ECMO runs, including for newborns in the NICU. Multimodal ECMO coagulation monitoring with standard coagulation assays and ROTEM has been utilized to manage complex ECMO coagulation management. Initially, HPTT has been the main targeted coagulation assay for bivalirudin management and recently added dTT alongside HPTT to improve accuracy.

Proposed anticoagulation protocol

Pre-cannulation

Before ECMO cannulation, it is important to evaluate baseline coagulable state to assess underlying coagulopathy, which will guide the choice of circuit prime and transfusion requirements. A comprehensive multimodal approach is advised, including complete blood count (CBC), prothrombin time (PT)/international normalized ratio (INR), aPTT/HPTT, fibrinogen, D-dimer, ACT, AT, plasma hemoglobin (PHb) and TEG or ROTEM. The baseline evaluation should include von Willebrand factor (VWF) activity, VWF antigen, and VWF activity/VWF antigen ratio (45).

Circuit prime

Heparin remains the main anticoagulant of choice for priming the circuit when an RBC prime is used, mainly because more practitioners have experience with it and due to the lack of standardized dosing for other anticoagulants. Following ELSO recommendations, RBC priming should be used for the majority of circuits if time allows. In an emergency setting, crystalloids can be used for patients weighing >30 kg. As there are no standardized recommendations for circuit priming, it is important to consider adding plasma to the priming if the circuit volume is >50% of the total blood volume.

Heparin bolus during cannulation

Heparin remains the preferred anticoagulant during cannulation given: the negligible incidence of HIT in pediatric patients; institutions' significant experience with the effects of heparin in cardiopulmonary bypass; and its reversibility with protamine. Depending on bleeding risk, a bolus of 50–100 units/kg should be administered during cannulation.

Post-cannulation

After cannulation, it is important to repeat coagulation tests, including PT/INR, aPTT/HPTT, D-dimer, fibrinogen, hemoglobin, and platelet count. Prompt correction of coagulopathy with plasma, cryoprecipitate, and platelet transfusions is essential to decrease bleeding complications.

Bivalirudin can be started if ACT <200 s, INR <2.0, HPTT is within 15 s of the baseline, fibrinogen level is at least >100 mg/dL, and

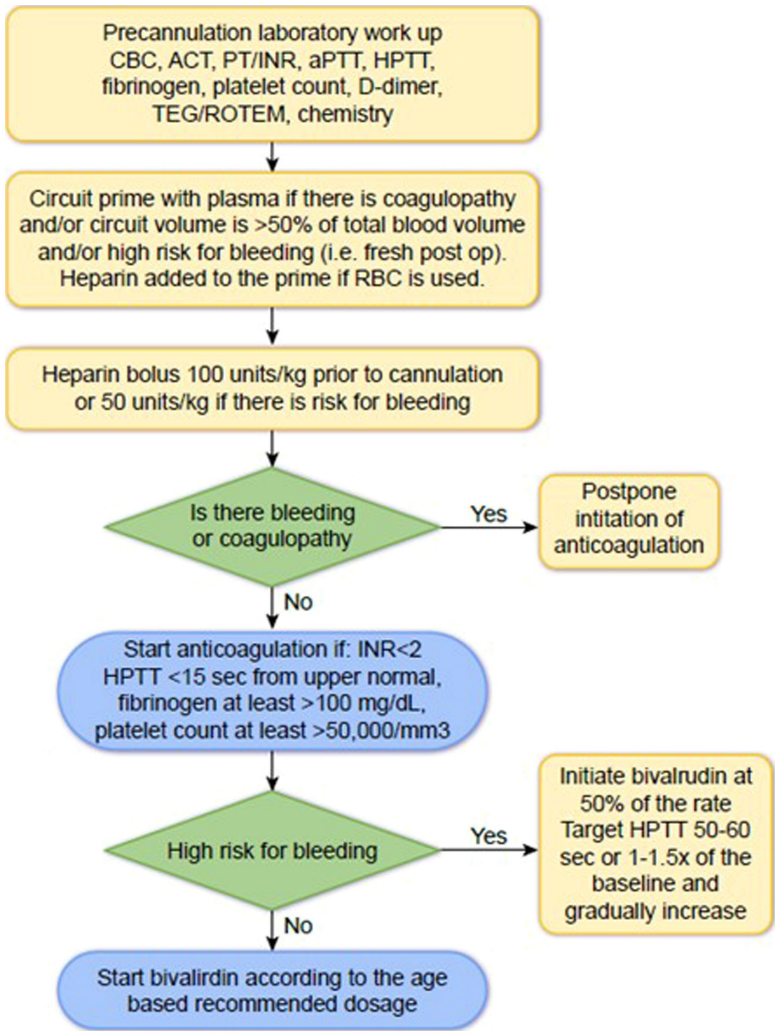


FIGURE 2
How to start bivalirudin at the initiation of ECMO CBC, complete blood count; ACT, activated clotting time; PT, prothrombin time; INR, international normalized ratio; aPTT, activated partial thromboplastin time; HPTT, aPTT with Hepzyme.

platelet count is at least >50,000/mm^3 (Figure 2). Since ACT value depends on the instrument, it must be established at each hospital. If there is active bleeding or coagulopathy, practitioners should delay initiation of bivalirudin until bleeding is controlled and coagulopathy is resolved. The initial bivalirudin dosage can be adjusted based on creatinine clearance and patient age (Table 3).

Monitoring

The target HPTT is 2-3x baseline if there is no risk for bleeding, and 1.5-2x baseline if there is high risk for bleeding. HPTT should be repeated two hours after the initiation of bivalirudin and two hours after every titration (Table 4).

If dTT is available, the standard target is 0.8–2.6 μg/mL (46). Since a dTT assay is not standardized for ECMO anticoagulation, combining HPTT with dTT will help optimize the anticoagulation (Table 5). The target range for dTT may vary based on the assay and reagents, so each institution should adjust its protocol based on validation results. Target ranges reported in the literature are summarized in Table 6.

Follow-up laboratory studies, including PT/INR, aPTT/HPTT, dTT (if available), fibrinogen, platelet count, and D-dimer, should be repeated every six hours or four times a day. Frequency can be reduced once coagulation markers are stable and stable anticoagulation is achieved. ROTEM/TEG and PHb should be repeated daily (Table 7).

TABLE 3 Bivalirudin initiation dose based on creatinine clearance and age.

Age-based initial dosage adjustment	Normal creatinine clearance for age (mg/kg/ h)	Mild–moderate decrease in creatinine clearance by age (mg/kg/ h)	Severely decreased creatinine clearance by age (mg/kg/ h)
Infants <1 year	0.3	0.2	0.1
Children 2–10 years	0.2	0.1	0.05
>10 years	0.15	0.1	0.05

TABLE 4 Dose adjustment based on heparinase-treated aPTT.

Dose adjustment of bivalirudin using HPTT	Dose adjustment	Time to repeat HPTT
<60 s and/or < 1.5x from baseline	Increase infusion rate by 20%	2 h after dose adjustment
60–70 s and < 1.5x baseline	Increase infusion rate by 10%	2 h after dose adjustment
70–80 s and at least 1.5x baseline	No change	Repeat HPTT as per ECMO maintenance protocol
80–100 s and/or > 3x increase from baseline	Decrease infusion rate by 20%	2 h after dose adjustment
>100 s	Hold infusion for 1 h then restart at 50% less than previous rate	2 h after dose adjustment

HPTT, heparinase treated activated partial thromboplastin time; aPTT, activated partial thromboplastin time.

Recently published review articles

There are several recent review articles comparing heparin to bivalirudin for ECMO anticoagulation (48–55), and all of them favor bivalirudin (Table 8). It should be noted, however, that all but one of these studies are meta-analyses and are compromised by the lack of standard definitions for bleeding and thrombosis. With this significant limitation in mind, the overall consensus favors bivalirudin for ECMO

TABLE 5 Dose adjustment based on heparinase-treated aPTT and diluted thrombin time.

Dose adjustment of bivalirudin using dTT (bivalirudin level)	Dose adjustment	Time to repeat dTT and HPTT
<0.8 μg/mL and HPTT <60 s	Increase infusion rate by 20%	2 h after dose adjustment
1–2 μg/mL and HPTT <1.5x of baseline	Increase infusion rate by 10%	2 h after dose adjustment
1–2 μg/mL and HPTT >1.5x of baseline	No change	Repeat dTT as per ECMO maintenance protocol
>2.6 μg/mL	Decrease infusion rate by 20%	2 h after dose adjustment

dTT, diluted thrombin time; HPTT, heparinase-treated activated partial thromboplastin time; aPTT, activated partial thromboplastin time.

TABLE 6 Summary of reported dTT and aPTT target ranges in the literature.

	dTT target	aPTT target
Hasan et al., 2023 (36)	0.5–2.5 μg/mL	60–120 s
Teruya et al., 2021 (46)	0.8–2.6 μg/mL	1.7–2.3 x normal (60–80 s)
Beyer et al., 2020 (34)	0.5–1.5 μg/mL (dTT 40–80 s)	1.5–2 x normal (50–80 s)
Colucci et al., 2015 (47)	0.8–1.4 μg/mL	2.5–3.0 x normal (74–90 s)

dTT, diluted thrombin time; aPTT, activated partial thromboplastin time.

TABLE 7 ECMO coagulation panel and usual targets.

Test	Desired target/range	Purpose
INR	<2.5	To assess underlying coagulopathy
HPTT	60–80 s	Targeting bivalirudin anticoagulation
dTT (bivalirudin level)	0.8–2.6 μg/mL	
Fibrinogen	>200 mg/dL FEU	To monitor clot firmness
Platelet count	>100,000/mm^3	
Plasma hemoglobin	<150 mg/dL	To monitor for hemolysis
D-dimer	Not established	To monitor fibrin formation and fibrinolysis in the circuit and patient's circulation

INR, international normalized ratio; HPTT, heparinase-treated activated partial thromboplastin time; dTT, diluted thrombin time, FEU, fibrinogen equivalent unit.

TABLE 8 Review articles about heparin versus bivalirudin for ECMO anticoagulation.

Author, year	# of studies reviewed	Major findings
Li DH et al., 2022 (48)	9	Hospital mortality and thrombosis events decreased in adult patients on bivalirudin
Liu L et al., 2022 (49)	14	Risks of in-circuit and any thrombosis, hospital mortality, and bleeding are decreased on bivalirudin with positive impact on ECMO survival
Ma M et al., 2022 (50)	10	Major bleeding in children, adult patients' thrombosis, in-circuit thrombosis/interventions and in-hospital mortality are reduced on bivalirudin
Li MJ, et al., 2022 (51)	9	Lower risks of major bleeding, ECMO in-circuit thrombosis, stroke and in-hospital mortality, and higher rates of survival to ECMO decannulation in patients on bivalirudin
Gu J et al., 2023 (52)	10	Reduction in thrombotic events, major bleeding events and in-hospital mortality in patients treated with bivalirudin
Wieruszewski PM et al., 2023 (53)	5	Higher risk of circuit-related thrombosis and to die in adult patients on heparin
Hasegawa D et al., 2023 (54)	11	Bivalirudin was associated with significantly lower short-term mortality
Valdes et al., 2023 (55)	7	Trends in lower rates of bleeding, transfusion requirements, and thrombosis with no difference in mortality in pediatric patients

All are meta-analysis except for Valdes et al. (55).

anticoagulation due to its potential ability to lower major bleeding risk, lower circuit and patient related thrombosis, and increase survival in both adult and pediatric patients.

Conclusion

According to several studies, bivalirudin shows a statistically significant advantage over heparin in preventing major bleeding and thrombosis, and in reducing transfusions. However, the majority of other studies showed no statistical difference between bivalirudin and heparin, likely due to the studies' retrospective nature. Further prospective studies are needed to make a definitive determination.

Author contributions

AN, VK, and JT contributed to conception of the review. AN and JT wrote the first draft of the review. VK made literature search, created tables, and edited final version. JT edited final version. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Sakurai K, Singhal N. Extracorporeal membrane oxygenation in children: a brief review. *J Paediatr Child Health*. (2022) 58:1525–31. doi: 10.1111/jpc.16151

2. Di Nardo M, Hoskote A, Thiruchelvam T, Lillie J, Horan M, Belda Hofheinz S, et al. Extracorporeal membrane oxygenation in children with coronavirus disease 2019: preliminary report from the collaborative european chapter of the extracorporeal life support organization prospective survey. *ASAIO J*. (2021) 67:121–4. doi: 10.1097/MAT.0000000000001309

3. Ozment CP, Scott BL, Bembea MM, Spinella PC. Anticoagulation and transfusion management during neonatal and pediatric extracorporeal membrane oxygenation: a survey of medical directors in the United States. *Pediatr Crit Care Med*. (2021) 22:530–41. doi: 10.1097/PCC.0000000000002696

4. Gajkowski EF, Herrera G, Hatton L, Velia Antonini M, Vercaemst L, Cooley E. ELSO guidelines for adult and pediatric extracorporeal membrane oxygenation circuits. *ASAIO J*. (2022) 68:133–52. doi: 10.1097/MAT.0000000000001630

5. Alexander PMA, Muszynski JA. Ongoing variability in pediatric extracorporeal membrane oxygenation anticoagulation practices – could consensus change the next survey results? *Pediatr Crit Care Med*. (2021) 22:581–4. doi: 10.1097/PCC.0000000000002762

6. Chlebowski MM, Baltagi S, Carlson M, Levy JH, Spinella PC. Clinical controversies in anticoagulation monitoring and antithrombin supplementation for ECMO. *Crit Care*. (2020) 24:19–2. doi: 10.1186/s13054-020-2726-9

7. Potempa LA, Gewurz H. Influence of heparin on interactions between C-reactive protein and polycations. *Mol Immunol*. (1983) 20:501–9. doi: 10.1016/0161-5890(83)90088-3

8. Poletti LF, Bird KE, Marques D, Harris RB, Suda Y, Sobel M. Structural aspects of heparin responsible for interactions with von Willebrand factor. *Arterioscler Thromb Vasc Biol*. (1997) 17:925–31. doi: 10.1161/01.atv.17.5.925

9. Warkentin TE, Greinacher A, Koster A. Bivalirudin. *Thromb Haemost*. (2008) 99:830–9. doi: 10.1160/TH07-10-0644

10. Mehran R, Rao SV, Bhatt DL, Gibson CM, Caixeta A, Eikelboom J, et al. Special report standardized bleeding definitions for cardiovascular clinical trials a consensus report from the bleeding academic research consortium. *Circulation*. (2011) 123:2736–47. doi: 10.1161/CIRCULATIONAHA.110.009449

11. Tong Y, Rouzhahong J, Zhou W, Wang R, Wang Y, Ren Y, et al. Comparison of bivalirudin versus heparin in adult extracorporeal membrane oxygenation anticoagulant therapy: a retrospective case-control study. *Int J Artif Organs*. (2023) 46:162–70. doi: 10.1177/03913988221148763

12. Uricchio MN, Ramanan R, Esper SA, Murray H, Kaczorowski DJ, D'Aloiso B, et al. Bivalirudin versus unfractionated heparin in patients with cardiogenic shock requiring venoarterial extracorporeal membrane oxygenation. *ASAIO J*. (2023) 69:107–13. doi: 10.1097/MAT.0000000000001723

13. Sheridan EA, Sekela ME, Pandya KA, Schadler A, Ather A. Comparison of bivalirudin versus unfractionated heparin for anticoagulation in adult patients on extracorporeal membrane oxygenation. *ASAIO J*. (2022) 68:920–4. doi: 10.1097/MAT.0000000000001598

14. Seelhammer TG, Bohman JK, Schulte PJ, Hanson AC, Aganga DO. Comparison of bivalirudin versus heparin for maintenance systemic anticoagulation during adult and

pediatric extracorporeal membrane oxygenation. *Crit Care Med*. (2021) 49:1481–92. doi: 10.1097/CCM.0000000000005033

15. Rivosecchi RM, Arakelians AR, Ryan J, Murray H, Ramanan R, Gomez H, et al. Comparison of anticoagulation strategies in patients requiring venovenous extracorporeal membrane oxygenation: heparin versus bivalirudin. *Crit Care Med*. (2021) 49:1129–36. doi: 10.1097/CCM.0000000000004944

16. Giuliano K, Bigelow BF, Etchill EW, Velez AK, Ong CS, Choi CW, et al. Extracorporeal membrane oxygenation complications in heparin- and bivalirudin-treated patients. *Crit Care Explor*. (2021) 3:e0485. doi: 10.1097/CCE.0000000000000485

17. Kaushik S, Derespina KR, Chandhoke S, Shah DD, Cohen T, Shlomovich M, et al. Use of bivalirudin for anticoagulation in pediatric extracorporeal membrane oxygenation (ECMO). *Perfusion*. (2023) 38:58–65. doi: 10.1177/02676591211034314

18. Rabinowitz EJ, Ouyang A, Armstrong DR, Wallendorf M, Said AS. Poor reliability of common measures of anticoagulation in pediatric extracorporeal membrane oxygenation. *ASAIO J*. (2022) 68:850–8. doi: 10.1097/MAT.0000000000001582

19. Schill MR, Douds MT, Burns EL, Lahart MA, Said AS, Abarbanell AM. Is anticoagulation with bivalirudin comparable to heparin for pediatric extracorporeal life support? Results from a high-volume center. *Artif Organs*. (2021) 45:15–21. doi: 10.1111/aor.13758

20. Machado DS, Garvan C, Philip J, Harrington D, Spiess B, Kelly B, et al. Bivalirudin may reduce the need for red blood cell transfusion in pediatric cardiac patients on extracorporeal membrane oxygenation. *ASAIO J*. (2021) 67:688–96. doi: 10.1097/MAT.0000000000001291

21. Kaseer H, Soto-Arenall M, Sanghavi D, Moss J, Ratzlaff R, Pham S, et al. Heparin vs bivalirudin anticoagulation for extracorporeal membrane oxygenation. *J Card Surg*. (2020) 35:779–86. doi: 10.1111/jocs.14458

22. Hamzah M, Jarden AM, Ezetendu C, Stewart R. Evaluation of bivalirudin as an alternative to heparin for systemic anticoagulation in pediatric extracorporeal membrane oxygenation. *Pediatr Crit Care Med*. (2020) 21:827–34. doi: 10.1097/PCC.0000000000002384

23. Macielak S, Burcham P, Whitson B, Abdel-Rasoul M, Rozycki A. Impact of anticoagulation strategy and agents on extracorporeal membrane oxygenation therapy. *Perfusion*. (2019) 34:671–8. doi: 10.1177/0267659119842809

24. Berei TJ, Lillyblad MP, Wilson KJ, Garberich RF, Hryniewicz KM. Evaluation of systemic heparin versus bivalirudin in adult patients supported by extracorporeal membrane oxygenation. *ASAIO J*. (2018) 64:623–9. doi: 10.1097/MAT.0000000000000691

25. Pieri M, Agracheva N, Bonaveglio E, Greco T, De Bonis M, Covello RD, et al. Bivalirudin versus heparin as an anticoagulant during extracorporeal membrane oxygenation: a case-control study. *J Cardiothorac Vasc Anesth*. (2013) 27:30–4. doi: 10.1053/j.jvca.2012.07.019

26. Ranucci M, Ballotta A, Kandil H, Isgrò G, Carlucci C, Baryshnikova E, et al. Bivalirudin-based versus conventional heparin anticoagulation for postcardiotomy extracorporeal membrane oxygenation. *Crit Care*. (2011) 15:R275. doi: 10.1186/cc10556

27. Seelhammer TG, Brokmeier HM, Hamzah M, Wieruszewski PM. Analysis of wholesale drug acquisition and laboratory assessment costs between heparin compared with Bivalirudin-based systemic anticoagulation strategies in adult extracorporeal membrane oxygenation. *Crit Care Med*. (2023) 51:e115–21. doi: 10.1097/CCM.0000000000005821

28. Kostousov V, Devaraj S, Bruzdoski K, Hensch L, Hui SK, Teruya J. C-reactive protein-induced activated partial thromboplastin time prolongation in heparinized samples is attenuated by elevated factor VIII. *Int J Lab Hematol*. (2021) 43:139–42. doi: 10.1111/ijlh.13314

29. Hirsh J, Anand SS, Halperin JL, Fuster V. Guide to anticoagulant therapy: heparin. *Circulation*. (2001) 103:2994–3018. doi: 10.1161/01.cir.103.24.2994

30. Lehman CM, Rettmann JA, Wilson LW, Markewitz BA. Comparative performance of three anti-factor Xa heparin assays in patients in a medical intensive care unit receiving intravenous, unfractionated heparin. *Am J Clin Pathol*. (2006) 126:416–21. doi: 10.1309/8E3U7RXEPXNP27R7

31. Snyder CW, Goldenberg NA, Nguyen ATH, Smithers CJ, Kays DW. A perioperative bivalirudin anticoagulation protocol for neonates with congenital diaphragmatic hernia on extracorporeal membrane oxygenation. *Thromb Res*. (2020) 193:198–203. doi: 10.1016/j.thromres.2020.07.043

32. Teruya J, Hensch L, Bruzdoski K, Adachi I, Hui SR, Kostousov V. Monitoring bivalirudin therapy in children on extracorporeal circulatory support devices: Thromboelastometry versus routine coagulation testing. *Thromb Res*. (2020) 186:54–7. doi: 10.1016/j.thromres.2019.12.007

33. Lind SE, Boyle ME, Fisher S, Ishimoto J, Trujillo TC, Kiser TH. Comparison of the aPTT with alternative tests for monitoring direct thrombin inhibitors in patient samples. *Am J Clin Pathol*. (2014) 141:665–74. doi: 10.1309/AJCPGTCEX7K4GXQO

34. Beyer JT, Lind SE, Fisher S, Trujillo TC, Wempe MF, Kiser TH. Evaluation of intravenous direct thrombin inhibitor monitoring tests: correlation with plasma concentrations and clinical outcomes in hospitalized patients. *J Thromb Thrombolysis*. (2020) 49:259–67. doi: 10.1007/s11239-019-01961-3

35. Love JE, Ferrell C, Chandler WL. Monitoring direct thrombin inhibitors with a plasma diluted thrombin time. *Thromb Haemost*. (2007) 98:234–42. doi: 10.1160/TH06-10-0607

36. Hasan RA, Pak J, Kirk CJ, Friedland-Little JM, Chandler WL. Monitoring direct thrombin inhibitors with calibrated diluted thrombin time vs activated partial thromboplastin time in pediatric patients. *Am J Clin Pathol*. (2023) 159:60–8. doi: 10.1093/ajcp/aqac131

37. Robson R, White H, Aylward P, Frampton C. Bivalirudin pharmacokinetics and pharmacodynamics: effect of renal function, dose, and gender. *Clin Pharmacol Ther*. (2002) 71:433–9. doi: 10.1067/mcp.2002.124522

38. Hamzah M, Seelhammer TG, Yabrodi M, Beshish AG, Byrnes JW, Hall J, et al. Evaluation of bivalirudin dosing in pediatric extracorporeal membrane oxygenation with renal insufficiency or renal replacement therapy. *ASAIO J*. (2022) 68:E196–203. doi: 10.1097/MAT.0000000000001818

39. Trigonis R, Smith N, Porter S, Anderson E, Jennings M, Kapoor R, et al. Efficacy of bivalirudin for therapeutic anticoagulation in COVID-19 patients requiring ECMO support. *J Cardiothorac Vasc Anesth*. (2022) 36:414–8. doi: 10.1053/j.jvca.2021.10.026

40. Tsu LV, Dager WE. Comparison of bivalirudin dosing strategies using total, adjusted, and ideal body weights in obese patients with heparin-induced thrombocytopenia. *Pharmacotherapy*. (2012) 32:20–6. doi: 10.1002/PHAR.1016

41. Kiser TH, Fish DN. Evaluation of bivalirudin treatment for heparin-induced thrombocytopenia in critically ill patients with hepatic and/or renal dysfunction. *Pharmacotherapy*. (2006) 26:452–60. doi: 10.1592/phco.26.4.452

42. Staudacher DL, Biever PM, Benk C, Ahrens I, Bode C, Wengenmayer T. Dual antiplatelet therapy (DAPT) versus no antiplatelet therapy and incidence of major bleeding in patients on venoarterial extracorporeal membrane oxygenation. *PLoS One*. (2016) 11:e0159973. doi: 10.1371/journal.pone.0159973

43. James S, Koul S, Andersson J, Angerås O, Bhiladvala P, Calais F, et al. Bivalirudin versus heparin monotherapy in ST-segment-elevation myocardial infarction. *Circ Cardiovasc Interv*. (2021) 14:e008969. doi: 10.1161/CIRCINTERVENTIONS.120.008969

44. Li Y, Liang Z, Qin L, Wang M, Wang X, Zhang H, et al. Bivalirudin plus a high-dose infusion versus heparin monotherapy in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention: a randomised trial. *Lancet*. (2022) 400:1847–57. doi: 10.1016/S0140-6736(22)01999-7

45. Ruth A, Meador M, Hui R, Loftis L, Teruya J. Acquired von Willebrand syndrome in pediatric extracorporeal membrane oxygenation patients: a single institution's experience. *Pediatr Crit Care Med*. (2019) 20:980–5. doi: 10.1097/PCC.0000000000002009

46. Teruya J, Bruzdoski K, Hensch L, Hui SKR, Kostousov V. How to best monitor bivalirudin anticoagulant effect for ECMO and VAD—comparison of four assay methods. *Int J Lab Hematol*. (2022) 44:589–94. doi: 10.1111/ijlh.13780

47. Colucci G, Nagler M, Klaus N, Conte T, Giabbani E, Alberio L. Practical guidelines for argatroban and bivalirudin in patients with heparin-induced thrombocytopenia. *J Transl Sci*. (2015) 1:37–42. doi: 10.15761/JTS.1000109

48. Li DH, Sun MW, Zhang JC, Zhang C, Deng L, Jiang H. Is bivalirudin an alternative anticoagulant for extracorporeal membrane oxygenation (ECMO) patients? A systematic review and meta-analysis. *Thromb Res*. (2022) 210:53–62. doi: 10.1016/j.thromres.2021.12.024

49. Liu L, Liu F, Tan J, Zhao L. Bivalirudin versus heparin in adult and pediatric patients with extracorporeal membrane oxygenation therapy: a systematic review and meta-analysis. *Pharmacol Res*. (2022) 177:106089. doi: 10.1016/j.phrs.2022.106089

50. Ma M, Liang S, Zhu J, Dai M, Jia Z, Huang H, et al. The efficacy and safety of bivalirudin versus heparin in the anticoagulation therapy of extracorporeal membrane oxygenation: a systematic review and meta-analysis. *Front Pharmacol*. (2022) 13:771563. doi: 10.3389/fphar.2022.771563

51. Li MJ, Shi JY, Zhang JH. Bivalirudin vs. heparin in paediatric and adult patients on extracorporeal membrane oxygenation: a meta-analysis. *Br J Clin Pharmacol*. (2022) 88:2605–16. doi: 10.1111/bcp.15251

52. Gu J, Yu H, Lin D. Superiority of bivalirudin over heparin anticoagulation therapy for extracorporeal membrane oxygenation? Too early to draw conclusions. *Heliyon*. (2023) 9:e13530. doi: 10.1016/j.heliyon.2023.e13530

53. Wieruszewski PM, Macielak SA, Nei SD, Moman RN, Seelhammer TG, Nabzdyk CGS, et al. Heparin versus bivalirudin for anticoagulation in adult extracorporeal membrane oxygenation: a systematic review and meta-analysis. *ASAIO J*. (2023) 69:137–44. doi: 10.1097/MAT.0000000000001808

54. Hasegawa D, Sato R, Prasitlumkum N, Nishida K, Keaton B, Acquah SO, et al. Comparison of bivalirudin versus heparin for anticoagulation during extracorporeal membrane oxygenation. *ASAIO J*. (2023) 69:396–401. doi: 10.1097/MAT.0000000000001814

55. Valdes CA, Sharaf OM, Bleiweis MS, Jacobs JP, Mumtaz M, Sharaf RM, et al. Heparin-based versus bivalirudin-based anticoagulation in pediatric extracorporeal membrane oxygenation: a systematic review. *Front Med (Lausanne)*. (2023) 10:1137134. doi: 10.3389/fmed.2023.1137134

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 08 September 2023
DOI 10.3389/fmed.2023.1208103

OPEN ACCESS

EDITED BY
Thomas Renne,
University Medical Center
Hamburg-Eppendorf, Germany

REVIEWED BY
Agneta Wikman,
Karolinska University Laboratory, Sweden
Jovan P. Antovic,
Karolinska Institutet (KI), Sweden
Riitta Lassila,
University of Helsinki, Finland

*CORRESPONDENCE
Felix Carl Fabian Schmitt
✉ felix.schmitt@med.uni-heidelberg.de

†These authors have contributed equally to this work and share senior authorship

RECEIVED 18 April 2023
ACCEPTED 27 July 2023
PUBLISHED 08 September 2023

CITATION
Gruneberg D, Braun P, Schöchl H, Nachtigall-Schmitt T, von der Forst M, Tourelle K, Dietrich M, Wallwiener M, Wallwiener S, Weigand MA, Fluhr H, Spratte J, Hofer S and Schmitt FCF (2023) Fibrinolytic potential as a risk factor for postpartum hemorrhage. *Front. Med.* 10:1208103. doi: 10.3389/fmed.2023.1208103

Fibrinolytic potential as a risk factor for postpartum hemorrhage

Daniel Gruneberg[1], Paula Braun[1], Herbert Schöchl[2], Tereza Nachtigall-Schmitt[3], Maik von der Forst[1], Kevin Tourelle[1], Maximilian Dietrich[1], Markus Wallwiener[3], Stephanie Wallwiener[3], Markus A. Weigand[1], Herbert Fluhr[4], Julia Spratte[3], Stefan Hofer[5†] and Felix Carl Fabian Schmitt [1*†]

[1]Department of Anesthesiology, Heidelberg University Hospital, Heidelberg, Germany, [2]Ludwig Boltzmann Institute for Traumatology, The Research Center in Cooperation with Allgemeine Unfallversicherungsanstalt, Vienna, Austria, [3]Department of Obstetrics and Gynecology, University of Heidelberg, Heidelberg, Germany, [4]Division of Obstetrics, Department of Obstetrics and Gynecology, Medical University of Graz, Graz, Austria, [5]Department of Anesthesiology, Kaiserslautern Westpfalz Hospital, Kaiserslautern, Germany

Background: Postpartum hemorrhage (PPH) is still the leading cause of maternal morbidity and mortality worldwide. While impaired fibrin polymerization plays a crucial role in the development and progress of PPH, recent approaches using viscoelastic measurements have failed to sensitively detect early changes in fibrinolysis in PPH. This study aimed to evaluate whether women experiencing PPH show alterations in POC-VET fibrinolytic potential during childbirth and whether fibrinolytic potential offers benefits in the prediction and treatment of PPH.

Methods: Blood samples were collected at three different timepoints: T0 = hospital admission (19 h ± 18 h prepartum), T1 = 30–60 min after placental separation, and T2 = first day postpartum (19 h ± 6 h postpartum). In addition to standard laboratory tests, whole-blood impedance aggregometry (Multiplate) and viscoelastic testing (VET) were performed using the ClotPro system, which included the TPA-test lysis time, to assess the POC-VET fibrinolytic potential, and selected coagulation factors were measured. The results were correlated with blood loss and clinical outcome markers. Severe PPH was defined as a hemoglobin drop > 4g/dl and/or the occurrence of shock or the need for red blood cell transfusion.

Results: Blood samples of 217 parturient women were analyzed between June 2020 and December 2020 at Heidelberg University Women's Hospital, and 206 measurements were eligible for the final analysis. Women experiencing severe PPH showed increased fibrinolytic potential already at the time of hospital admission. When compared to non-PPH, the difference persisted 30–60 min after placental separation. A higher fibrinolytic potential was accompanied by a greater drop in fibrinogen and higher d-dimer values after placental separation. While 70% of women experiencing severe PPH showed fibrinolytic potential, 54% of those without PPH showed increased fibrinolytic potential as well.

Conclusion: We were able to show that antepartal and peripartal fibrinolytic potential was elevated in women experiencing severe PPH. However, several women showed high fibrinolytic potential but lacked clinical signs of PPH. The findings indicate that high fibrinolytic potential is a risk factor for the development of coagulopathy, but further conditions are required to cause PPH.

KEYWORDS

fibrinolytic potential, PPH, ClotPro, TPA-test, viscoelastic hemostatic assay, point-of-care, VET

1. Introduction

Postpartum hemorrhage (PPH) is defined as excessive bleeding in the context of childbirth. Accounting for one death every 7 min, PPH is still the leading cause of maternal morbidity and mortality worldwide (1). Despite all the efforts to improve the prevention and treatment of PPH, healthcare providers are facing an increasing number of cases (2, 3). The reasons behind this are the growing incidences of high-risk pregnancies and atony-related PPH (2). Furthermore, little progress has been made in the early recognition of women at increased risk (4).

To facilitate prevention, efficient tools to identify women at risk are needed (4). Unfortunately, to this date, the ability to predict PPH remains low (5). Typically, an interplay of uterine atony, trauma, and placental abnormalities leads to bleeding (1). PPH further progresses if disturbances in hemostasis occur and aggravate bleeding, whereby the fibrinolytic system plays a crucial role (6). Roberts et al. showed that hyperfibrinolysis is common in PPH, and malfunctioning of this delicate system determines maternal mortality and morbidity (7). Despite the beneficial effects of tranexamic acid (TXA) in PPH (8–10), prophylactic use of antifibrinolytic agents during childbirth is still under debate (11). Thus, Arnolds et al. concluded in 2020 that further research is needed to determine optimal methods to assess fibrinolytic activity in PPH (12). Assessment of fibrinolytic potential might facilitate the identification of women at elevated risk for excessive bleeding antepartum and help in the timely recognition of coagulopathy and the progress of PPH.

The ClotPro TPA-test is a new viscoelastic point-of-care test assay to assess fibrinolytic potential. TPA-test stands for "tissue plasminogen activator-test." The assay is, like the ex-test, a recombinant tissue factor-activated whole blood test. However, unlike the ex-test, the TPA-test contains a 650 ng/ml recombinant tissue plasminogen activator. The assay results in an extrinsically stimulated coagulation with r-tPA-stimulated hyperfibrinolysis. Typically, the viscoelastic trace shows the initiation of the clot comparable to the ex-test but is followed by fast lysis. Typical TPA-test viscoelastic traces are shown in Figures 1B, C. The fibrinolytic potential is measured as "lysis time," which is defined as the timespan between clotting time and 50% clot lysis (Figure 1A). Typical TPA-test lysis time was reported to be ~220s in a patient population scheduled for elective orthopedic surgery (13). A higher fibrinolytic potential is expressed as shorter TPA-test lysis time (LT). To date, evidence of the clinical use of the ClotPro TPA-test is limited to a few reports. There are three retrospective analyses and one small case report showing prolonged TPA-test lysis time in patients with COVID-19 (14–17). One study and one case report utilized the TPA-test for detecting the TXA effect in cardiac surgery and elective orthopedic surgery (13, 18). Coupland et al. showed that the TPA-test LT indicates fibrinolytic resistance in critically ill patients, independent of COVID-19 (19). Finally, Nitsche et al. found in an experimental study that like other viscoelastic test parameters, TPA-test lysis time is temperature dependent and is prolonged in hypothermia (20).

To the best of our knowledge, fibrinolytic potential during pregnancy and childbirth has not yet been characterized.

To fill this knowledge gap, we evaluated whether women experiencing PPH show alterations in fibrinolytic potential during childbirth and whether ClotPro TPA-test lysis time may have a clinical value in the prediction and treatment of PPH. To answer these questions, blood samples of 217 women were taken at three different timepoints peripartum. TPA-test lysis time, laboratory coagulation status, clinical parameters, and short-term outcome were measured.

2. Materials and methods

2.1. Study design

The study was approved by the Ethics Committee of the Medical Faculty of Heidelberg (trial code no. S-759/2019) and is registered at the German clinical trials register (DRKS00021531). Data were collected prospectively in an observational single-center study at Heidelberg University Women's Hospital, a level-one perinatal center. Data were collected between June 2020 and December 2020.

Blood samples and clinical data of 217 women were collected peripartum. The inclusion criteria were age ≥18 years and written informed consent. The exclusion criteria were refusal of informed consent and age <18 years. To avoid potential bias, women with a medical history of coagulopathy were excluded from the final analysis. Medical history was obtained by the gynecologist and anesthetist. In addition, individuals were excluded from further analysis of the fibrinolytic potential after they received TXA. TXA leads to a strong inhibition of fibrinolysis, resulting in an artificial prolongation of TPA-test lysis time.

2.2. Study groups

As described in previous reports and international guidelines, mild PPH was defined as estimated blood loss >500 ml and hemoglobin drop >3 g/dl (21–23). Severe PPH was defined as the appearance of one of the following criteria: peripartum hemoglobin drop of ≥4 g/dl and transfusion of red blood cell concentrates or shock (24–29). Non-PPH was defined as a hemoglobin drop of ≤3 g/dl and estimated blood loss ≤500 ml (21–23).

2.3. Measurements

Blood samples were collected at three different timepoints. The first sample (T0) was taken at the time of hospital admission (19 h ± 18 h prepartum). The second (T1) was taken 30–60 min after placental separation and the third (T2) on the first day postpartum (19 h ± 6 h after placental separation). At each timepoint, blood samples were taken for viscoelastic testing (ClotPro), whole-blood impedance aggregometry (Multiplate), enzyme-linked immunosorbent assay (ELISA), and laboratory coagulation tests. All POC measurements were performed by

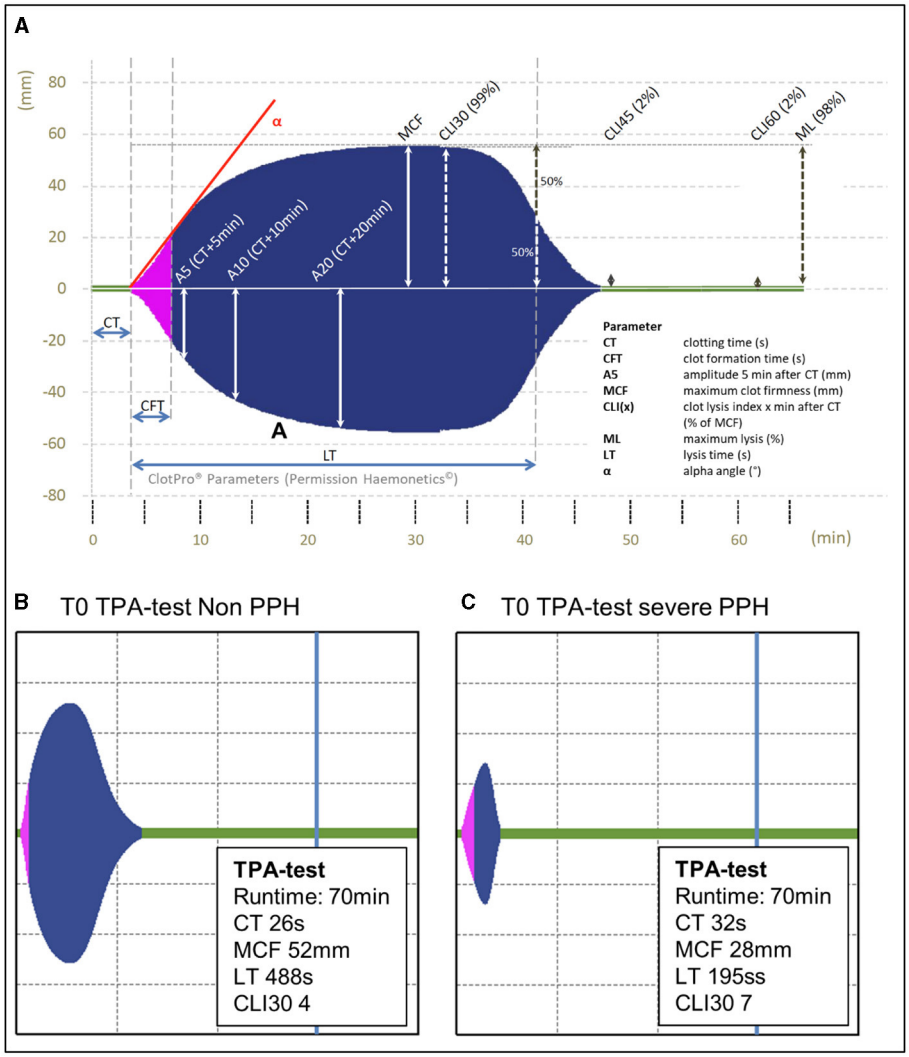


FIGURE 1
(A) ClotPro viscoelastic trace including the description of parameters. **(B, C)** Exemplary viscoelastic traces at T0 (prepartum) obtained from two women included in the study. **(B)** Shows the TPA-test trace of a woman with relatively low fibrinolytic potential and blood loss outside the normal range and **(C)** shows the TPA-test trace of a woman presenting with relatively high fibrinolytic potential at hospital admission and developing severe PPH in the course of delivery. ClotPro® hemostasis analyzer tracing image used by permission of Haemonetics Corporation. ClotPro® is a registered trademark of Haemonetics Corporation in the US, other countries or both.

the same experimenter. Citrate-anticoagulated whole blood was used for ClotPro analysis, and hirudin-anticoagulated whole blood was used for Multiplate analysis. All samples were transported from the delivery room or operation room to the research lab (5–10 min transportation time) at room temperature. Samples for POC VET and Multiplate were analyzed immediately in the research lab. Samples for routine coagulation testing were sent to the laboratory facility via tube mail and analyzed immediately after. Measurements of single coagulation factors were performed with frozen plasma samples. Therefore, blood samples were transported to the research lab, centrifuged at 3,000 rpm for 10 min, and plasma was aliquoted in 100 µl tubes. Samples were deep-frozen at −80°C and stored for 9 months. They were transported on dry ice to the laboratory facility for analysis and analyzed immediately after defreeze. At each timepoint, the following coagulation tests were performed for every patient.

2.4. Laboratory coagulation tests

Full blood count, hemoglobin, erythrocytes, hematocrit (Hct), prothrombin time, activated partial thromboplastin time, fibrinogen (Clauss fibrinogen assay, analyzed with the Sysmex CS 5100 coagulation analyzer), d-dimers, alpha-2-antiplasmin, factors V, VIII, IX, and XIII, protein C, protein S, von Willebrand factor (vWF) antigen, and activity were measured in the laboratory facility at Heidelberg University Clinic.

2.5. Point-of-care viscoelastic testing

For ClotPro (Enicor GmbH, Munich, Germany), we ran five channels for each blood sample (EX-test, IN-test, FIB-test, AP-test, and TPA-test). A detailed description of the TPA-test assay is provided in the introduction section of the present study. Based

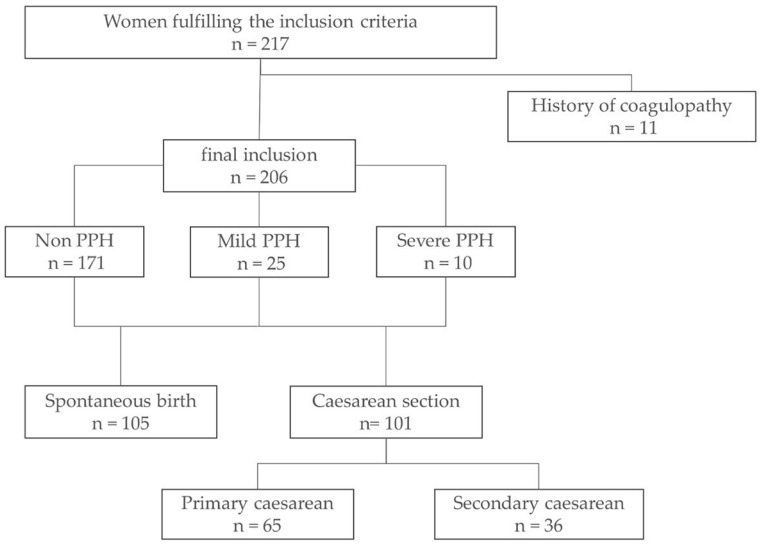


FIGURE 2
Data collection and inclusion. Flowchart of the data collection process and data exclusion. Definition of mild and severe PPH according to estimated blood loss, ΔHb, and shock, as described above. PPH, postpartum hemorrhage.

on the study by Tahitu et al. (30), the enzymatic fibrinolysis index was defined as Clotpro EX-test maximum clot lysis (ML) minus AP-test ML.

Whole-blood impedance aggregometry was performed with the Multiplate analyzer (Roche Diagnostics Deutschland GmbH, Mannheim, Germany). ADP-test, TRAP-test, and ASPI-test were performed for each sample at each timepoint.

All measurements were performed according to the manufacturer's guidelines.

2.6. Clinical data

Clinical data and patient characteristics were obtained from electronic medical reports. We documented maternal age, date and time of birth, gestational age, birth weight, gravida and parity, history of miscarriage, gemini status, mode of delivery, presence of placental pathologies, presence and degree of birth injury, presence of preeclampsia or HELLP syndrome, occurrence for postpartum uterine atony, clinical outcome (death, shock, hospital and intensive-care-unit length of stay, and need for re-operation), transfusion of red blood cell concentrate or fresh-frozen plasma, any medication given in the peripartum period (including uterotonics, TXA, fibrinogen concentrate, and vasopressors), and prescription of coagulant drugs). Estimated blood loss was documented according to the anesthesia protocol and/or birth report by midwives. The common causes of PPH were assessed according to the Four Ts mnemonic device for PPH (31).

2.7. Statistical analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina, USA). For continuous data, ANOVA was performed for equal variances and a mixed linear model with Tukey–Kramer *post-hoc* test for unequal variances. A chi-squared test was performed for categorical data. For multiple comparisons of categorical data, *p*-value correction was made by performing a Bonferroni post-test. For ROC analysis, simple and multiple linear regression models were used. Probability values of $p < 0.05$ were generally considered statistically significant.

3. Results

3.1. Study groups

From 217 women initially recruited, 206 participants were eligible for final analysis (Figure 2). Eleven women had a medical history of coagulopathy (factor V Leiden mutation, von Willebrand disease, F XII deficiency, MTHFR mutation, and autoimmune thrombocytopenia either alone or in combination) and were subsequently excluded from further analysis. Three of 11 women with preexisting coagulopathy had a prescription for coagulation modifiers, which potentially influence coagulation and fibrinolysis. Patients were grouped based on the presence and severity of PPH: 25 (12.1%) women showed mild and 10 (4.9%) women showed severe PPH according to the aforementioned criteria of estimated blood loss, hemoglobin drop, red blood cell transfusion, and the presence of shock. The remaining 171 women revealed no evidence for PPH with estimated blood loss $\leq$500 ml and ΔHb $\leq$ 3g/dl. The mode of delivery did not differ significantly between groups.

3.2. Baseline characteristics

Before POC-VET fibrinolytic potential was evaluated, baseline characteristics, including sociodemographic data, parameters of

TABLE 1 Demographic data and baseline characteristics based on the PPH group.

	Total $n = 206$	Non-PPH $n = 171$	Mild PPH $n = 25$	Severe PPH $n = 10$	p-value non vs. mild	p-value non vs. severe	p-value mild vs. severe
Age (year)	33.4 ± 5.0	33.2 ± 4.9	33.4 ± 4.8	36.5 ± 5.7	0.80	**0.04**	0.10
Gestation age (week)	38.9 ± 1.4	38.9 ± 1.4	38.8 ± 1.9	39.1 ± 1.5	0.85	0.57	0.55
Obesity	53 (25.7%)	43 (25.2%)	7 (28.0%)	3 (30.0%)	0.76	0.73	0.91
Gravidity	2 (1–3)	2 (1–3)	2 (1–3)	2 (1–3)	0.42	0.86	0.54
Parity	1 (0–1)	1 (0–1)	0 (0–1)	0 (0–1)	0.65	0.82	0.95
Nullipara	97 (47.1%)	76 (44.4%)	14 (56.0%)	7 (70.0%)	0.28	0.11	0.45
Gemini	10 (4.9%)	6 (3.5%)	2 (8.0%)	2 (20.0%)	0.29	**0.01**	0.31
Preterm birth	14 (6.8%)	9 (5.3%)	4 (16.0%)	1 (10%)	**0.04**	0.52	0.65
Birthweight (g)	3,322 ± 502	3,307 ± 501	3,446 ± 467	3,268 ± 611.3	0.20	0.81	0.34
Spontaneous birth (n)	105	90 (53%)	8 (32%)	7 (70%)	0.59	0.48	0.17
Primary cesarian (n)	65	53 (31%)	11 (44%)	1 (10%)	0.16	0.85	0.12
Secondary cesarian (n)	36	28 (16%)	6 (24%)	2 (20%)	1.0	1.0	1.0

Data are presented as mean ± SD, median and IQR, or absolute and relative counts as appropriate; obesity was defined as BMI > 35, and preterm birth was defined as birth before 37+0 weeks of pregnancy.
PPH, postpartum hemorrhage.
The bold values indicate significant values of $p < 0.05$.

pregnancy, and mode of delivery, were obtained. The data are outlined in Table 1.

Women in the severe PPH group were older, with a mean difference of 3 years, and the proportion of gemini pregnancies was higher in this group than in the non-PPH group. The frequency of preterm birth was higher in the mild PPH group than in the non-PPH group. Other baseline characteristics were similar between the groups.

3.3. Follow-up

In the follow-up, maternal outcomes were negatively influenced by the severity of PPH. Increased blood loss in severe PPH was accompanied by a higher rate of shock, red blood cell transfusion, and the need for revision surgery. Women in the severe PPH group had a longer hospital length of stay and a higher ICU admission rate than those in the non-PPH and mild PPH groups. Data are presented in Supplementary Table 1.

At the time of T1 blood sampling, none of the parturient women presented with ongoing bleeding. One woman developed bleeding after T1 blood sampling. In all other cases, the bleeding had already stopped at timepoint T1.

3.4. Antepartum coagulation status

To determine antepartum differences in fibrinolytic potential, TPA-test lysis time and other indicators of clot initiation, firmness, and clot lysis were measured at the time of inclusion (T0).

Antepartum TPA-test lysis time was lower in the severe PPH group than in the non-PPH and mild PPH groups. Statistical significance was reached only in the comparison between the non-PPH and severe PPH groups (mean difference 21.7s; 95% CI −3.2 to 40.4; $p = 0.04$; Table 2). Shortened TPA-test lysis time indicates an increased fibrinolytic potential. However, 93 women (45% of the entire study population) showed low TPA-test lysis time <250 s but blood loss within the normal range of delivery. The proportion of low TPA-test lysis time in the non-PPH group was 54%, which increased to 70% in the severe PPH group. Women with low TPA-test lysis time and severe PPH showed significantly more risk factors for bleeding, according to the Four Ts mnemonic device (0.4 in the non-PPH vs. 1.0 in the severe PPH group, $p = 0.005$). Antepartum hemoglobin, Hct, and quantitative and qualitative platelet analyses, as well as classical POC-VET lysis parameters, measured as EX-test lysis index 30 and 60 did not differ between the groups and were within the normal range. In-test clotting time was prolonged in the mild PPH group, but the values were still within the normal range defined for pregnancy.

3.5. Evaluation of the fibrinolytic system over the course of delivery

In the following section, POC-VET fibrinolytic potential characterized over the course of delivery has been described. Figure 3 shows the TPA-test lysis time at three different timepoints during birth. At the time of hospital admission and 30–60 min after placental separation, the TPA-test lysis time was significantly reduced in the severe PPH group compared to the non-PPH group. On the first day postpartum, the TPA-test lysis time increased and exceeded the antepartum values, independent of the presence of PPH. Hence, there were no significant differences in fibrinolytic potential on this day.

Three individuals in the mild PPH group and two individuals in the severe PPH group received TXA over the course of delivery. While both women in the severe PPH group received TXA after

10.3389/fmed.2023.1208103

TABLE 2 Measurements of coagulation parameters antepartum (T0).

	Non-PPH $n = 171$	Mild PPH $n = 25$	Severe PPH $n = 10$	p-value non vs. mild	p-value non vs. severe	p-value mild vs. severe
Red blood cells						
Hb (g/dl)	12.2 ± 1.0	12.4 ± 0.94	12.7 ± 1.2	0.40	0.13	0.39
Hct (%)	0.36 ± 0.03	0.36 ± 0.03	0.37 ± 0.04	0.56	0.13	0.32
Clot initiation						
CT Ex-test (s)	44.0 ± 7.6	46.6 ± 8.13	47.1 ± 8.6	0.11	0.21	0.86
CT In-test (s)	140.8 ± 10.4	145.44 ± 12.44	141.5 ± 11	0.04	0.83	0.32
Clot firmness						
Ex-test A10 (mm)	59.6 ± 3.8	60.4 ± 3.69	59.8 ± 3.9	0.31	0.85	0.67
Fib test A10 (mm)	24.2 ± 3.4	25.2 ± 3.93	25.1 ± 2.8	0.19	0.44	0.94
Clot lysis						
Ex-test CLI30	99.3 ± 0.8	99.16 ± 0.69	99.1 ± 0.6	0.55	0.53	0.83
Ex-test CLI60	94.4 ± 2.3	94.56 ± 2.47	94.9 ± 2.1	0.79	0.54	0.70
Ex-test ML	6.4 ± 2.6	6.5 ± 2.4	5.8 ± 2.2	0.80	0.49	0.45
AP test ML	6.1 ± 2.4	5.5 ± 2.0	5.0 ± 2.3	0.26	0.16	0.55
Platelets						
Platelet count (nl^{-1})	224.1 ± 63.7	217.2 ± 55.19	222.2 ± 64.1	0.61	0.93	0.83
TRAP AUC	103.7 ± 27.8	105.1 ± 29.5	105.1 ± 28.7	0.81	0.88	1.0
ASPI AUC	96.1 ± 27.3	95.32 ± 23.7	94.9 ± 21.2	0.89	0.89	0.97
ADP AUC	69.5 ± 24.9	71.4 ± 30.23	74.1 ± 24.9	0.74	0.58	0.76
Fibrinolytic potential						
TPA-test lysis time (s)	253.6 ± 50.4	258.5 ± 46.7	231.9 ± 24	0.89	**0.04**	0.11
D-dimers (mg/L)	2.2 ± 1.7	2.6 ± 2.3	3.0 ± 1.8	0.28	0.14	0.52

Measurements of coagulation parameters antepartum (T0). Data are presented as mean ± SD.
Hb, hemoglobin; Hct, hematocrit; CT, clotting time; A10, clot firmness after 10 min runtime; CLI30, clot lysis index after 30 min runtime; CLI60, clot lysis index after 60 min runtime; AUC, area under the curve; P1, non-PPH vs. mild PPH; P2, non-PPH vs. severe PPH; P3, mild PPH vs. severe PPH.
The bold values indicate significant values of $p < 0.05$.

T1 blood sampling, the three women in the mild PPH group received TXA between T0 and T1. After receiving TXA, the TPA-test lysis time values of these individuals were excluded from further analysis.

As shown in Figure 4, increased fibrinolytic potential in severe PPH was accompanied by a significant drop in fibrinogen concentration and elevation in d-dimer levels 30–60 min after placental separation. Fibrinogen levels remained low on the first day postpartum. D-dimer-fibrinogen ratios were calculated for every timepoint and are shown in Supplementary Figure 1.

While the main inhibitors of the fibrinolytic system [alpha-2-antiplasmin and plasminogen activator inhibitor 1 (PAI-1)] did not differ antepartum (at the time when the fibrinolytic potential was already altered), they were reduced after placental separation, indicating a disinhibition of fibrinolysis. The data are shown in Table 3. PAI-1 drop after placental separation was more pronounced in the severe PPH group than in the mild PPH group. Overall, a greater extent of fibrinolysis disinhibition 30–60 min after placental separation was observed in the severe PPH group than in the other two groups. Correlations of TPA-test lysis time with fibrinogen, d-dimer, alpha-2-antiplasmin, and PAI-1 levels were calculated as measures of increased fibrinolysis. TPA-test lysis time showed low to moderate correlation with fibrinogen, d-dimer, alpha-2-antiplasmin, and PAI-1 levels. The data are shown in Supplementary Table 2.

Parameters with potential impact on the hemostatic process, including Hct, Hb, thrombocyte counts, vWF, and factor XIII were measured at all timepoints, and the data are given in Supplementary Table 4. In summary, Hb and Hct were significantly decreased at timepoints T1 and T2 in the severe PPH group compared with the non-PPH group, while thrombocyte counts remained stable. vWF activity was increased at hospital admission in the severe PPH group (Supplementary Table 4).

Finally, TPA-test lysis time was compared to classic POC-VET fibrinolysis parameters. Enzymatic fibrinolytic index (EFI) was calculated as a marker of fibrinolysis insensitive to clot retraction. None of the 206 individuals in this study reached the common cut-off of EFI >15% for hyperfibrinolysis.

3.6. Incidence of additional risk factors for PPH

It was observed that insufficient uterine tone, placental retention, and birth injuries were more common in the severe PPH group. The data are presented in Table 4.

To evaluate the role of uterine atony as a potential confounder of the influence of fibrinolytic potential on bleeding severity, we performed a subgroup analysis of fibrinolytic potential for women with and without uterine atony. Since only two individuals had documented uterine atony in the PPH groups, it was not possible to calculate meaningful statistics. However, TPA-test lysis time was shorter in the severe PPH group than in the non-PPH and mild PPH groups at timepoints T0 and T1, both in the subset with and without uterine atony. The data are presented in Supplementary Table 3.

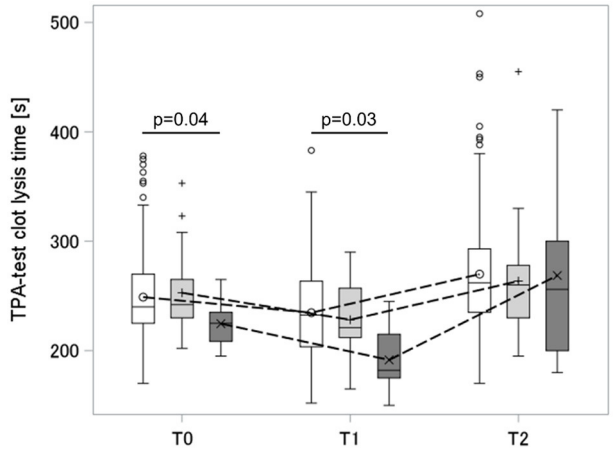


FIGURE 3
Dynamic of TPA-test lysis time peripartum. Box and whisker plot: The box indicates the Q1–Q3 inter-quartile range, the horizontal line inside the box shows the median value, the marker inside the box displays the mean, the whiskers indicate observation nearest to the fences (= 1.5 IQR), the markers below and above the fences demonstrate observations above and below the fences; T0, antepartum; T1, 30–60 min after placental separation; T2, first day postdelivery; groups: white, non-PPH; light gray, mild PPH; dark gray, severe PPH. PPH, postpartum hemorrhage.

3.7. ROC analysis–clinical value of antepartum TPA-test lysis time in the prediction of PPH

ROC analysis revealed a low predictive value of antepartum TPA-test lysis time as a single parameter. For the prediction of severe PPH, AUC was 0.634 ($p = 0.053$). When combined with fibrinogen, the predictive power increased (AUC 0.732; $p = 0.002$). The best prediction of severe PPH was achieved in a multiple linear regression model using TPA-test lysis time and the number of risk factors, according to the 4Ts (AUC 0.75; $p = 0.0003$).

4. Discussion

In the current study, we evaluated alterations of the POC-VET fibrinolytic potential in PPH. We demonstrated that women who developed severe PPH in the course of delivery show increased fibrinolytic potential already at the time of hospital admission. We also demonstrated that the difference in fibrinolytic potential persists 30–60 min after placental separation. Furthermore, we revealed that childbirth is accompanied by changes in fibrinolytic potential, independent of the occurrence of PPH. Parturient women showed an increase in fibrinolytic potential within the first hour after placental separation, followed by a decrease that exceeded the baseline level on the first day postdelivery.

The fact that fibrinolytic potential in the severe PPH group was already increased at the time of hospital admission indicates that

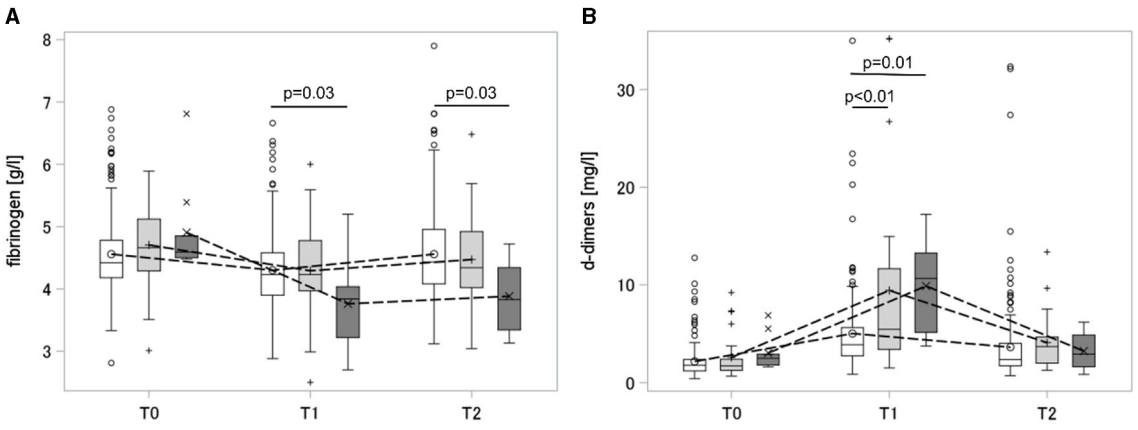


FIGURE 4
Dynamic of fibrinogen **(A)** and d-dimers **(B)** peripartum. Box and whisker plot: The box indicates the Q1–Q3 inter-quartile range, the horizontal line inside the box shows the median value, the marker inside the box displays the mean, the whiskers indicate observation nearest to the fences (=1.5 IQR), the markers below and above the fences demonstrate observations above and below the fences; T0, antepartum; T1, 30–60 min after placental separation; T2, first day postdelivery; groups: white, non-PPH; light gray, mild PPH; dark gray, severe PPH. PPH, postpartum hemorrhage.

TABLE 3 Measurements of fibrinolytic inhibitors over time.

	Non-PPH $n = 171$	Mild PPH $n = 25$	Severe PPH $n = 10$	p-value non vs. mild	p-value non vs. severe	p-value mild vs. severe
Alpha-2-antiplasmin						
T0	96.7 ± 18.5	95 ± 12.6	87.7 ± 33.3	0.68	0.14	0.30
T1	87.4 ± 20	86.6 ± 16.1	68.3 ± 17	0.85	**<0.01**	**0.02**
T2	98 ± 17.9	93.4 ± 11.8	82.1 ± 11.4	0.21	**0.01**	0.11
PAI-1						
T0	67.1 ± 28.6	68.8 ± 33.2	74.7 ± 48.3	0.79	0.44	0.60
T1	42.7 ± 23.3	58.2 ± 55.6	41.7 ± 42	**0.02**	0.93	0.18
T2	26.8 ± 14.1	24.1 ± 13.7	72.7 ± 85	0.53	**<0.01**	**<0.01**

PAI-1, plasminogen activator inhibitor 1; T0, at hospital admission; T1, 30–60 min after placental separation; T2, first day postpartum; P1, non-PPH vs. mild PPH; P2, non-PPH vs. severe PPH; P3, mild PPH vs. severe PPH.
The bold values indicate significant values of $p < 0.05$.

TABLE 4 Risk factors for the development of PPH and the use of uterotonics.

	Non-PPH $n = 171$	Mild PPH $n = 25$	Severe PPH $n = 10$
Tone			
Insufficient uterine contraction *n* (%)	0 (0%)	2 (8.0%)	2 (20.0%)
Oxytocin usage *n* (%)	143 (83.6 %)	24 (96.0%)	9 (90.0%)
Sulprostone usage *n* (%)	0	1 (4%)	0
Tissue			
Retention of placenta *n* (%)	0	0	3 (30.0%)
Placenta previa	1 (0.5%)	2 (8%)	0
PAS disorder *n* (%)	2 (1.1%)	0	0
Trauma			
Birth injury *n* (%)	68 (39.8%)	8 (32.0%)	7 (70.0%)

Additional risk factors for PPH according to the Four Ts mnemonic device. Values are extracted from electronic medical reports and presented as absolute counts and relative proportions.
Insufficient uterine contraction clinically diagnosed by obstetricians.
PAS disorder, placenta accrete spectrum disorder.

the ClotPro TPA-test lysis time could identify altered fibrinolysis earlier than classical POC-VET fibrinolysis markers. This offers potential clinical use in the prevention and timely treatment of PPH. The fact that TPA-test lysis time indeed measures amplified fibrinolysis is supported by correlations between increased fibrinolytic potential, disinhibition of fibrinolysis (reduced PAI-1 and alpha-2-antiplasmin), and fibrinogen consumption markers (drop in fibrinogen and rise in d-dimers).

ROC analysis revealed that TPA-test lysis time alone is not specific enough to predict PPH. However, a combination of antepartum TPA-test lysis time with either fibrinogen levels or the number of risk factors according to the 4Ts improves the prediction of severe PPH. Nevertheless, we found important restrictions in the clinical applicability of TPA-test lysis time. A high fibrinolytic potential was found in 54% of individuals in the non-PPH group, leading to an AUC of only 0.6434 for TPA-test lysis time to predict severe PPH. Women presenting with high fibrinolytic potential but without PPH showed significantly fewer risk factors according to the Four Ts mnemonic device for PPH than women with severe PPH. Our interpretation of the results is that increased fibrinolytic potential should be understood as a moderating variable that increases the risk of developing coagulopathy, while high fibrinolytic potential alone is not sufficient for causing excessive bleeding. Additional factors such as uterine atony, soft tissue trauma, and placental pathologies are needed to cause severe PPH.

While our data provide new insights into the measurement of POC-VET fibrinolytic potential during childbirth, our study also has some limitations. First, blood loss was estimated by the obstetric team. However, no exact blood loss measurements were performed using calibrated blood bags because such systems were not established at the site of investigation, and implementation in a multidisciplinary setting would have surpassed the capabilities of this study. Since technical aids for blood loss measurement are not established to a large extent, hemoglobin and hematocrit are established as common parameters to objectify blood loss in clinical studies (24, 26, 32). Hemoglobin drop has been shown to correlate with PPH severity, and the most significant decline occurs within the first 24 h postdelivery (29). Second, there was a significant difference in age between the non-PPH and severe PPH groups. As fibrinolysis might be influenced by age and

complications might increase in high-risk pregnancies in older women, age might have acted as a confounder in the present study. Since the size of the severe PPH group was extremely small, it was not possible to perform age-matched comparisons of the results. However, no significant correlation was found between age and TPA-test lysis time in our study population of parturient women between 20 and 45 years of age. Therefore, it seems unlikely that age differences would have exerted significant bias on our measurements. Nevertheless, age should be considered a potential confounder in future studies, especially if individuals of older age are studied. Furthermore, it must be mentioned that POC-VET fibrinolytic potential, measured by performing the ClotPro TPA-test, does not allow for statements about local fibrinolysis at the uteroplacental unit level, as it measures fibrinolytic potential in the systemic circulation. Although early administration of TXA was already recommended by international guidelines (22, 33) at the time of data collection, the recommendations were based on moderate evidence. During that time, the local standard was to administer TXA more restrictively than it is done today. Only a few patients included in the PPH group received TXA.

5. Conclusion

In the present study, we demonstrated that women who developed severe PPH presented with increased POC-VET fibrinolytic potential before experiencing overt bleeding, but this increased fibrinolytic potential alone was not sufficient for predicting PPH. We interpret fibrinolytic potential as a moderating variable that increases the risk of developing coagulopathy, although high fibrinolytic potential alone is not sufficient for causing excessive bleeding. Further research is needed to integrate fibrinolytic potential into established risk scores to refine their predictive ability and evaluate its use to guide targeted antifibrinolytic therapy in PPH and other fields.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Medical Faculty of Heidelberg, Heidelberg, Germany. The patients/participants provided their written informed consent to participate in this study.

Author contributions

DG took responsibility for the content of the manuscript and acted as the guarantor for the article. FS, DG, HF, MF, KT, and MD were responsible for the planning and design of the study. PB was responsible for data acquisition and data integrity. TN-S, JS, MW, and HF helped recruit the patients. DG and FS were responsible for data analysis, interpretation, and wrote the manuscript with input from all other authors. HS, MAW, and SH gave substantial advice for manuscript writing and critically reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

The authors thank the obstetricians, midwives, and nurses for their great work in clinical practice and Ute Krauser for her support in handling and measurement of blood samples.

Conflict of interest

HS has received honoraria for participation in advisory board meetings for Alexion, Bayer Healthcare, Boehringer Ingelheim, Haemonetics and Vifor, and has received study grants and speaker's honorarium from CSL Behring. FS has received research support from CSL Behring and Philips, as well as speaker honorarium from CSL Behring and AstraZeneca and he is a consultant in an advisory board for CSL Behring.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The authors declare that this study received funding from Enicor (consumables). The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1208103/full#supplementary-material

References

1. Bienstock JL, Eke AC, Hueppchen NA. Postpartum hemorrhage. *N Engl J Med.* (2021) 384:1635–45. doi: 10.1056/NEJMra1513247

2. Callaghan WM, Kuklina EV, Berg CJ. Trends in postpartum hemorrhage: United States, 1994-2006. *Am J Obstet Gynecol.* (2010) 202:353.e1–6. doi: 10.1016/j.ajog.2010.01.011

3. Knight M, Callaghan WM, Berg C, Alexander S, Bouvier-Colle MH, Ford JB, et al. Trends in postpartum hemorrhage in high resource countries: a review and recommendations from the International Postpartum Hemorrhage Collaborative Group. *BMC Pregnancy Childbirth.* (2009) 9:55. doi: 10.1186/1471-2393-9-55

4. Neary C, Naheed S, McLernon DJ, Black M. Predicting risk of postpartum haemorrhage: a systematic review. *BJOG.* (2021) 128:46–53. doi: 10.1111/1471-0528.16379

5. Prata N, Hamza S, Bell S, Karasek D, Vahidnia F, Holston M. Inability to predict postpartum hemorrhage: insights from Egyptian intervention data. *BMC Pregnancy Childbirth.* (2011) 11:97. doi: 10.1186/1471-2393-11-97

6. Oh KJ, Hong JS, Youm J, Cho SH, Jung EY. Can coagulopathy in post-partum hemorrhage predict maternal morbidity? *J Obstet Gynaecol Res.* (2016) 42:1509–18. doi: 10.1111/jog.13098

7. Roberts I, Shakur H, Fawole B, Kuti M, Olayemi O, Bello A, et al. Haematological and fibrinolytic status of Nigerian women with post-partum haemorrhage. *BMC Pregnancy Childbirth.* (2018) 18:143. doi: 10.1186/s12884-018-1794-1

8. Binyamin Y, Orbach-Zinger S, Gruzman I, Frenkel A, Lerman S, Zlotnik A, et al. The effect of prophylactic use of tranexamic acid for cesarean section. *J Matern Fetal Neonatal Med.* (2022) 35:9157–62. doi: 10.1080/14767058.2021.2019215

9. Ilich A, Nouboussie DF, Henderson M, Ellsworth P, Betbadal KF, Campello E, et al. Development and application of global assays of hyper- and hypofibrinolysis. *Res Pract Thromb Haemost.* (2020) 4:46–53. doi: 10.1002/rth2.12275

10. Picetti R, Miller L, Shakur-Still H, Pepple T, Beaumont D, Balogun E, et al. The WOMAN trial: clinical and contextual factors surrounding the deaths of 483 women following post-partum haemorrhage in developing countries. *BMC Pregnancy Childbirth.* (2020) 20:409. doi: 10.1186/s12884-020-03091-8

11. Higgins N, Patel SK, Toledo P. Postpartum hemorrhage revisited: new challenges and solutions. *Curr Opin Anaesthesiol.* (2019) 32:278–84. doi: 10.1097/ACO.0000000000000717

12. Arnolds DE, Scavone BM. Thromboelastographic assessment of fibrinolytic activity in postpartum hemorrhage: a retrospective single-center observational study. *Anesth Analg.* (2020) 131:1373–9. doi: 10.1213/ANE.0000000000004796

13. Groene P, Sappel SR, Saller T, Nitschke T, Sa PA, Paulus A, et al. Functional testing of tranexamic acid effects in patients undergoing elective orthopaedic surgery. *J Thromb Thrombolysis.* (2021) 51:989–96. doi: 10.1007/s11239-020-02272-8

14. Bachler M, Bosch J, Sturzel DP, Hell T, Giebl A, Strohle M, et al. Impaired fibrinolysis in critically ill COVID-19 patients. *Br J Anaesth.* (2021) 126:590–8. doi: 10.1016/j.bja.2020.12.010

15. Duque P, Chasco-Ganuza M, Burgos-Santamaria A, Terradillos E. Increased resistance to fibrinolysis activation in patients with coronavirus disease 2019: a case series. *Blood Coagul Fibrinolysis.* (2021) 32:298–301. doi: 10.1097/MBC.0000000000001017

16. Heubner L, Greiner M, Vicent O, Beyer-Westendorf J, Tiebel O, Scholz U, et al. Predictive ability of viscoelastic testing using ClotPro(R) for short-term outcome in patients with severe COVID-19 ARDS with or without ECMO therapy: a retrospective study. *Thromb J.* (2022) 20:48. doi: 10.1186/s12959-022-00403-0

17. Zatroch I, Smudla A, Babik B, Tanczos K, Kobori L, Szabo Z, et al. Procoagulation, hypercoagulation and fibrinolytic "shut down" detected with ClotPro® viscoelastic tests in COVID-19 patients. *Orv Hetil.* (2020) 161:899–907. doi: 10.1556/650.2020.31870

18. Yoshii R, Takahashi Y, Sawa T, Amaya F, Ogawa S. Long duration of action of tranexamic acid after cardiac surgery in a hemodialysis patient: a case report. *A & A Pract.* (2023) 17:e01676. doi: 10.1213/XAA.0000000000001676

19. Coupland LA, Rabbolini DJ, Schoenecker JG, Crispin PJ, Miller JJ, Ghent T, et al. Point-of-care diagnosis and monitoring of fibrinolysis resistance in the critically ill: results from a feasibility study. *Crit Care.* (2023) 27:55. doi: 10.1186/s13054-023-04329-5

20. Nitschke T, Groene P, Acevedo AC, Kammerer T, Schafer ST. Coagulation under mild hypothermia assessed by thromboelastometry. *Transfus Med Hemother.* (2021) 48:203–9. doi: 10.1159/000513922

21. Ashwal E, Bergel Bson R, Aviram A, Hadar E, Yogev Y, Hiersch L. Risk factors for postpartum hemorrhage following cesarean delivery. *J Matern Fetal Neonatal Med.* (2022) 35:3626–30. doi: 10.1080/14767058.2020.1834533

22. World Health Organization. *WHO Recommendations for the Prevention and Treatment of Postpartum Haemorrhage*. Geneva: World Health Organization (2012).

23. Rottenstreich M, Rotem R, Glick I, Shen O, Grisaru-Granovsky S, Sela HY. Mild gestational thrombocytopenia in primiparous women, does it affect risk of early postpartum hemorrhage? A retrospective cohort study. *J Matern Fetal Neonatal Med.* (2021) 35:8426–33. doi: 10.1080/14767058.2021.1977793

24. Delorme P, Kayem G, Legardeur H, Roux-Dessarps LA, Girard G, Meunier G, et al. Carbetocin versus oxytocin for the prevention of postpartum hemorrhage in cesarean deliveries: a retrospective study of two consecutive periods. *AJP Rep.* (2020) 10:e241–e6. doi: 10.1055/s-0040-1715163

25. Drukker L, Srebnik N, Elstein D, Levitt L, Samueloff A, Farkash R, et al. The association between ABO blood group and obstetric hemorrhage. *J Thromb Thrombolysis.* (2016) 42:340–5. doi: 10.1007/s11239-016-1360-5

26. Ducloy-Bouthors AS, Mignon A, Huissoud C, Grouin JM, Mercier FJ. Fibrinogen concentrate as a treatment for postpartum haemorrhage-induced coagulopathy: a study protocol for a randomised multicentre controlled trial. The fibrinogen in haemorrhage of DELivery (FIDEL) trial. *Anaesth Crit Care Pain Med.* (2016) 35:293–8. doi: 10.1016/j.accpm.2015.10.011

27. Lockhart E. Postpartum hemorrhage: a continuing challenge. *Hematology Am Soc Hematol Educ Program.* (2015) 2015:132–7. doi: 10.1182/asheducation-2015.1.132

28. Wikkelso AJ, Edwards HM, Afshari A, Stensballe J, Langhoff-Roos J, Albrechtsen C, et al. Pre-emptive treatment with fibrinogen concentrate for postpartum haemorrhage: randomized controlled trial. *Br J Anaesth.* (2015) 114:623–33. doi: 10.1093/bja/aeu444

29. Yefet E, Yossef A, Suleiman A, Hatokay A, Nachum Z. Hemoglobin drop following postpartum hemorrhage. *Sci Rep.* (2020) 10:21546. doi: 10.1038/s41598-020-77799-0

30. Tahitu M, Ramler PI, Gillissen A, Caram-Deelder C, Henriquez D, de Maat MPM, et al. Clinical value of early assessment of hyperfibrinolysis by rotational thromboelastometry during postpartum hemorrhage for the prediction of severity of bleeding: a multicenter prospective cohort study in the Netherlands. *Acta Obstet Gynecol Scand.* (2022) 101:145–52. doi: 10.1111/aogs.14279

31. Anderson JM, Etches D. Prevention and management of postpartum hemorrhage. *Am Fam Physician.* (2007) 75:875–82.

32. Charbit B, Mandelbrot L, Samain E, Baron G, Haddaoui B, Keita H, et al. The decrease of fibrinogen is an early predictor of the severity of postpartum hemorrhage. *J Thromb Haemost.* (2007) 5:266–73. doi: 10.1111/j.1538-7836.2007.02297.x

33. Obstetrics GSoGa. *Peripartal Haemorrhage, Diagnosis and Therapy Guideline of The [S2k-Level, AWMF Registry No. 015/063]*. Berlin: AWMF (2022).

frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 06 December 2023
DOI 10.3389/fpubh.2023.1309094

OPEN ACCESS

EDITED BY
Felix Carl Fabian Schmitt,
Heidelberg University Hospital, Germany

REVIEWED BY
Laura Uccella,
Lugano Regional Hospital, Switzerland
Aman Mamo,
Madda Walabu University, Ethiopia

*CORRESPONDENCE
Raúl Juárez-Vela
✉ raul.juarez@unirioja.es

RECEIVED 07 October 2023
ACCEPTED 21 November 2023
PUBLISHED 06 December 2023

CITATION
Quintana-Diaz M, Anania P, Juárez-Vela R, Echaniz-Serrano E, Tejada-Garrido CI, Sanchez-Conde P, Nanwani-Nanwani K, Serrano-Lázaro A, Marcos-Neira P, Gero-Escapa M, García-Criado J and Godoy DA (2023) "COAGULATION": a mnemonic device for treating coagulation disorders following traumatic brain injury—a narrative-based method in the intensive care unit.
Front. Public Health 11:1309094.
doi: 10.3389/fpubh.2023.1309094

"COAGULATION": a mnemonic device for treating coagulation disorders following traumatic brain injury—a narrative-based method in the intensive care unit

Manuel Quintana-Diaz[1,2,3], Pasquale Anania[4], Raúl Juárez-Vela[3,5,6]*, Emmanuel Echaniz-Serrano[7,8], Clara Isabel Tejada-Garrido[5,6], Pilar Sanchez-Conde[9], Kapil Nanwani-Nanwani[2,3], Ainhoa Serrano-Lázaro[3,10], Pilar Marcos-Neira[11], María Gero-Escapa[12], Jorge García-Criado[13] and Daniel Agustín Godoy[14]

[1]Department of Medicine, Faculty of Medicine, Autonomous University of Madrid, Madrid, Spain, [2]Intensive Care Unit, La Paz University Hospital, Madrid, Spain, [3]Institute for Health Research (idiPAZ), La Paz University Hospital, Madrid, Spain, [4]Department of Neurosurgery, Ospedale Policlinico San Martino, Istituto di Ricovero eCura a Carattere Scientifico (IRCCS) for Oncology and Neuroscience, Genoa, Italy, [5]Department of Nursing, University of La Rioja, Logroño, Spain, [6]Health and Healthcare Research Group (GRUPAC), Faculty of Health Sciences, University of La Rioja, Logroño, Spain, [7]Department of Nursing and Physiatry, Faculty of Health Sciences, University of Zaragoza, Zaragoza, Spain, [8]Aragon Healthcare Service, Aragon, Zaragoza, Spain, [9]Faculty of Medicine, University of Salamanca, Salamanca, Spain, [10]Intensive Care Unit, Valencia University Clinical Hospital, Valencia, Spain, [11]Intensive Care Unit, Germans Trias i Pujol University Hospital, Badalona, Spain, [12]Intensive Care Unit, Burgos University Hospital, Burgos, Spain, [13]Emergency Department, University Hospital of Salamanca, Salamanca, Spain, [14]Critical Care Department, Neurointensive Care Unit, Sanatorio Pasteur, Catamarca, Argentina

Introduction: Coagulopathy associated with isolated traumatic brain injury (C-iTBI) is a frequent complication associated with poor outcomes, primarily due to its role in the development or progression of haemorrhagic brain lesions. The independent risk factors for its onset are age, severity of traumatic brain injury (TBI), volume of fluids administered during resuscitation, and pre-injury use of antithrombotic drugs. Although the pathophysiology of C-iTBI has not been fully elucidated, two distinct stages have been identified: an initial hypocoagulable phase that begins within the first 24 h, dominated by platelet dysfunction and hyperfibrinolysis, followed by a hypercoagulable state that generally starts 72 h after the trauma. The aim of this study was to design an acronym as a mnemonic device to provide clinicians with an auxiliary tool in the treatment of this complication.

Methods: A narrative analysis was performed in which intensive care physicians were asked to list the key factors related to C-iTBI. The initial sample was comprised of 33 respondents. Respondents who were not physicians, not currently working in or with experience in coagulopathy were excluded. Interviews were conducted for a month until the sample was saturated. Each participant was asked a single question: Can you identify a factor associated with coagulopathy in patients with TBI? Factors identified by respondents were then submitted to a quality check based on published studies and proven evidence. Because all the factors identified had strong support in the literature, none was eliminated. An acronym was then developed to create the mnemonic device.

Results and conclusion: Eleven factors were identified: cerebral computed tomography, oral anticoagulant & antiplatelet use, arterial blood pressure (Hypotension), goal-directed haemostatic therapy, use fluids cautiously, low calcium levels, anaemia-transfusion, temperature, international normalised ratio (INR), oral antithrombotic reversal, normal acid–base status, forming the acronym "Coagulation." This acronym is a simple mnemonic device, easy to apply for anyone facing the challenge of treating patients of moderate or severe TBI on a daily basis.

KEYWORDS

coagulopathy, diagnosis, traumatic brain injury, haemostasis, blood coagulation, anticoagulants, antithrombotic drugs

1 Specific aims and theoretical grounds of the study

A narrative approach involves pluralism, relativism, and subjectivity. Despite advances in the care of neurocritically ill patients from the prehospital stage through rehabilitation, traumatic brain injury (TBI) continues to be a pathological entity associated with significant rates of functional disability and mortality (1). Narratives have classically been a mode of transmitting culture on macro-societal levels, and a way to create evidence. Irrespective of the mechanism of injury, TBI is understood to consist of two clearly defined processes (1–4). The primary injury depends on the type, location, and amount of energy absorbed by the structures that comprise the cranial cavity and the results of focal (intra or extra-axial haemorrhages) or diffuse (axonal damage) lesions. These lesions, in turn, trigger different and multiple neurotoxic cascades, most notably, the inflammatory and coagulation cascades that negatively influence haemodynamics, oxygenation, and cerebral energy metabolism (1–4). Secondary injury involves a series of phenomena that can originate within the skull (intracranial hypertension, cerebral hypoxia) or systemically (hypotension, hypoxemia, sodium, and glycaemic alterations) which combine to perpetuate or exacerbate the primary injury (1–4). While primary injuries are irreversible, secondary injuries can be prevented and corrected, and this is precisely one of the pillars upon which the modern management of severe TBI is based (1–4).

Normal haemostasis involves a delicate balance between mechanisms that promote bleeding and those that try to prevent it, a balance that can be disrupted following TBI. Coagulation disorders in severe isolated TBI (C-iTBI) are common and contribute to secondary damage, mainly due to their role in facilitating the development or progression of both ischemic and haemorrhagic lesions (5–10). TBI has increasingly come to affect older patients who often present with polypharmacy including antithrombotics and anticoagulants due to multiple comorbidities (11). The incidence of C-iTBI as reported in the literature averages 33%, with figures ranging from 7 to 90%. The disparities in results may be due to differences in study design, lack of uniform definition, varied cohorts, points of the parameters used to define it, concomitant presence of polytrauma, or the time it was analysed (5–10, 12). Nonetheless, based on these figures, we can posit that approximately one of three patients will develop C-iTBI, strongly suggesting its origin in the brain itself (7–10, 12).

The pathophysiology of C-iTBI has not yet been fully and accurately elucidated; however, current evidence indicates that it is a primary haemostasis disorder broadly characterised by an early hypocoagulable period, where platelet dysfunction and hyperfibrinolysis predominate, and a later prothrombotic stage, where the described mechanisms reverse their role (12). By platelet dysfunction, we understand the generic and non-specific term conventionally used to denote alterations in platelet physiology. In the context of trauma, this may either involve a decrease in number (thrombocytopenia) or function, i.e., alteration of one of the steps in which platelets are actively involved (adhesion, aggregation, and secretion). Such alterations may occur directly secondary to traumatic injury or as a consequence of pre-existing disease or prior use of antithrombotic medications. The incidence of platelet dysfunction ranges from less than 1% in mild TBI to >60% in severe TBI.

Predisposing factors identified for the development of C-iTBI include the severity of the trauma (GCS <9), age (> 75 years), previous fluid therapy (> 2 L), base excess (< −6), hypothermia, arterial hypotension, and prior use of anticoagulant or antiplatelet agents (12, 13). The presence of C-iTBI adds predictive power to validated prognostic scales (14, 15). C-iTBI is associated with poor outcomes in terms of functionality and mortality, which explains why this factor is a key therapeutic target to consider (7–10, 12–16). The real implications that early detection and correction of coagulation disorders have in the context of severe TBI remain unknown, however, they open up a wide range of future research possibilities.

Healthcare providers communicate the events of treating a difficult case through narratives. Narratives are also used to concretize a body of knowledge in specific contexts, as in the case of intensive care medicine. Stories encapsulate professional experiences to be shared with students in practise, or advanced professionals. In this study, we used the narrative experience of intensive care physicians to design a mnemonic device we called "Coagulation" to provide clinicians with an auxiliary tool in the treatment of C-iTBI.

2 Methods

To carry out this study based on narrative, the authors of the manuscript have selected the best possible evidence on the topic under study. Once selected, it was debated until the variables that were considered most important were obtained. Three rounds were carried

out until the selection of variables began to be repeated and we considered that the selection was saturated, something that in qualitative methodology indicates the end of the selection process. The initial sample was comprised of 33 respondents. Respondents who were not physicians, not currently working in or with experience in coagulopathy were excluded. The process and the meeting to select variables were conducted for a month until the sample was saturated. In this meeting, all participants have been arranged to make the mnemonic rule more easily understandable.

Each participant was asked a single question: Can you identify a factor associated with coagulopathy in patients with TBI? The respondents identified specific concepts during the interviews which were then collected and mapped. Following the initial reading and validation, we consulted the literature to justify the factors identified.

Ultimately, we identified 11 factors.

The factors identified by the respondents were submitted to a quality check, based on the published literature and proven evidence. Because all the factors identified had strong support in the literature, none was eliminated.

3 Results

3.1 Factor 1: cerebral computed tomography was identified by participants as "Essential to the classification of severity and monitoring"

Cerebral computed tomography (CT) is the neuroimaging of choice during the acute phase of severe TBI (17–19). In addition to being widely available, it is cost-effective, has a short radiation exposure time and can even be performed on patients with prostheses or on mechanical ventilation (17–19). The CT scan allows for the simultaneous evaluation of other body regions and is an essential complementary tool to categorize TBI (17–19). It provides an objective assessment of the type and extension of lesions, their volume, location, and impact on intracranial anatomical structures (18–20). It is highly sensitive for the detection of blood collections (17, 18, 21). Finally, it yields pathophysiological information and has a proven predictive capacity for both intracranial hypertension and outcome (20, 22, 23).

Different CT scales have been developed and validated in the clinical field (20, 23–26). Its serial determination allows clinicians to monitor the progression of the broad spectrum of lesions following TBI (17–20, 22–26). Therefore, CT plays an important role in diagnosis, evaluation of lesion progression, and therapeutic decision-making in the context of C-iTBI (Figure 1).

3.2 Factor 2: oral anticoagulant and antiplatelet use was identified by participants as "High Alert"

The increase in life expectancy over the last decades has led to an increasingly older adult population with associated pathologies, mainly cardiac and cerebrovascular, that require the use of oral anticoagulants (OAC) or antiplatelet agents (APA). The global incidence of cerebral bleeding secondary to OAC/APA utilisation is 20% (12). Specifically, clinical studies have shown that the use of warfarin before iTBI doubles the possibility of poor outcomes (27), while pre-injury use of APAs increases the risk of presenting post-traumatic cerebral haemorrhage (28). This risk is increased even further with the use of second-generation APAs (clopidogrel) in individuals with mild TBI (29). Regarding the new oral anticoagulants (NOAC), the few prospective clinical studies conducted on iTBI have had conflicting results, making it difficult to draw valid conclusions (30, 31), however a retrospective study found increased hematoma expansion in individuals with pre-injury use of NOACs compared to warfarin (32). Recently published data from the Collaborative European NeuroTrauma Effectiveness Research in Traumatic Brain Injury (CENTER-TBI) corroborate the aforementioned epidemiological data, indicating that prior use of OACs/APAs increases the risk of development or progression of haemorrhagic lesions, with a 3-fold higher mortality rate and greater frequency of poor outcomes at 6 months after iTBI, especially in individuals taking vitamin K antagonists (12, 13). Mathieu et al. (33) serially and comparatively analysed CT scans in iTBI patients who used antithrombotic agents (APAs or OACs) pre-injury vs. those who did not. In the group with prior antithrombotic use, an increase in the volume of extra-axial hematomas was observed, as well as an increase in the rate of haematoma expansion and the risk of developing delayed traumatic intracerebral haemorrhages (33). A Swedish study evaluating the risk of bleeding in individuals with prior use of OACs vs. APAs and iTBI secondary to ground-level falls found an increased risk of bleeding in the group that had used APAs (34). Finally, a recent meta-analysis (low quality of the included studies) that sought to establish the adverse effects of pre-iTBI APA use, found associations between dual therapy and the progression of lesions (OR 2.81; 95% CI 1.19–6.65; $p = 002$) and need for neurosurgery (OR 1.61; 95% CI 1.15–2.28) but no related impact on hospital mortality (35).

3.3 Factor 3: arterial blood pressure (hypotension) was defined by participants as "the most dreaded latent threat for the traumatised brain"

Maintaining cerebral blood flow (CBF), one of the primary goals in the management of TBI, is usually associated with the metabolic rate of oxygen ($CMRO_2$) (1, 4, 17). Its key determinants are cerebral perfusion pressure (CPP) and the diameter of resistance arterioles (50–150 µm) (1, 4). CPP is the result of the difference between mean arterial pressure (MAP) and intracranial pressure (ICP) (1, 4).

$$CPP = MAP - ICP$$

The CBF remains normal and stable through the intrinsic capacity of its resistance vessels to modify their diameter, a phenomenon called "cerebral autoregulation" (CAR). The change in diameter is due to different stimuli, including MAP, arterial partial pressure of oxygen (PaO_2), arterial partial pressure of carbon dioxide ($PaCO_2$), and others of neural origin (1). CAR is a natural survival mechanism that is not infinite; on the contrary, it typically functions within certain CPP levels ranging from 50 to 150 mmHg. Above or below these limits, the CBF passively follows changes in MAP (1, 17).

During TBI, however, this mechanism is altered, and the limits narrow and shift to the right. Therefore, during injury, higher levels

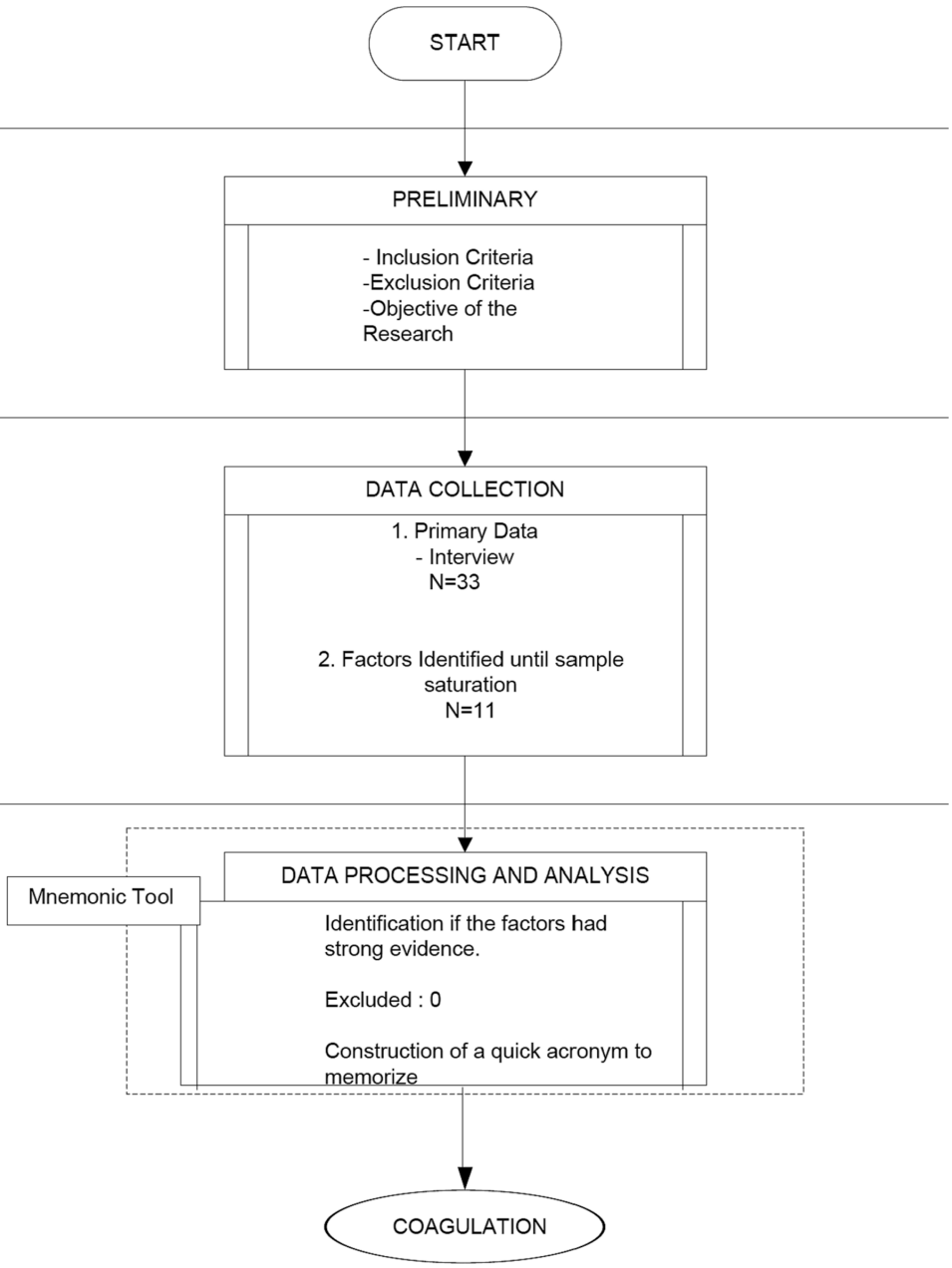


FIGURE 1
Flow chart.

of CPP are necessary to maintain adequate levels of CBF (17, 21). When CAR is disrupted or completely lost, even temporarily, arterial hypotension is deleterious as it causes a decrease in CBF and ischemic hypoxia with irreversible and devastating consequences (1, 17).

The traumatised brain does not cause arterial hypotension *per se* and it should not always be attributed to hypovolaemia, which is why its cause should be thoroughly investigated (36). Arterial hypotension independently increases the possibility of poor outcomes (37, 38) and is a risk factor for the development of C-iTBI (12, 13).

3.4 Factor 4: goal-directed haemostatic therapy was defined by participants as a factor in which "Knowledge of pathophysiology is essential"

Goal-directed haemostatic therapy (GDHT) is an element of personalised precision medicine. This approach focuses on the specific point problem a given pathology can cause, considering the overall context at all times. In order to do so, monitoring systems capable of promptly alerting healthcare professionals to any alterations that

threaten physiological homeostasis are essential. At the same time, in-depth knowledge of the pathophysiology of the disorder is necessary for the correct interpretation, analysis, and decision-making based on the information such monitoring provides.

The coagulation system is finely tuned process, continuously balancing the mechanisms that promote and prevent clot formation. C-iTBI, a multifactorial entity different from coagulopathy secondary to multisystem trauma without TBI, occurs when these mechanisms are disrupted (6–10, 39). While its pathophysiology has yet to be accurately defined, C-iTBI generally begins within 24 h of admission; the more severe the TBI, the earlier it manifests (40). Its duration is variable, averaging 72 h, although this period is sometimes prolonged (40). C-iTBI is characterised by an initial hypocoagulable state consisting of platelet dysfunction, increased consumption of platelets and coagulation factors, disseminated intravascular coagulation (DIC), and hyperfibrinolysis, followed by a hypercoagulable, prothrombotic state, both local (cerebral microcirculation-ischemic lesions) and systemic (deep vein thrombosis). However, these states are linked and often it is difficult to differentiate one from the other (6–10, 39).

Although discussion of these mechanisms is beyond the scope of this manuscript, it is worth noting that multiple mediators and cascades are involved in the genesis of C-iTBI, including protein C, protein S, thromboxane, prostaglandins, adenosine, and brain tissue factor (6, 10, 39).

3.5 Factor 5: use fluids cautiously was defined by participants as a factor in which "Type and volume matter"

Fluid administration is one of the key elements in the initial resuscitation of TBI, especially when necessary to correct arterial hypotension and maintain adequate CPP values (41). Recent guidelines recommend 0.9% normal saline as the fluid of choice (41). Some considerations are necessary in this context such as avoiding hypotonic solutions (dextrose 5%, lactated ringer, albumin 4%) and the use of colloids (41). In terms of the implications that fluids have on coagulation, both isotonic crystalloids and colloid solutions provoke haemodilution, causing a decrease in the function and number of platelets and plasma factors involved in coagulation (42–44). This effect is directly proportional to the volume infused (42–44). Available evidence indicates that 0.9% saline at low doses does not affect coagulation (45) or has a minimal hypercoagulable effect, while at higher doses the effect is the opposite (hypocoagulable), associated with increased bleeding (42–44, 46–49). Infusions of more than 2,000 mL of fluids have been identified as an independent risk factor for the development of C-iTBI (51). Because the osmotically active solutions used for the control of intracranial hypertension such as hypertonic saline or mannitol are crystalloids, they retain their dose-dependent haemodilution capacity and cause hypocoagulable states (42–44). However, clinical studies have shown that neither 20% mannitol nor 3.5% saline affect coagulation at equimolar doses and limited infusion volumes during neurosurgery or in moderate TBI (50, 51). On the other hand, all colloids (dextran, gelatins, and hydroxyethyl starches) invariably affect coagulation, compromising platelet function and fibrin formation, decreasing the activity of coagulation factors, and increasing fibrinolysis (42–44).

3.6 Factor 6: low calcium levels was defined by participants as a "necessary and essential co-factor"

Calcium (Ca++) is an essential co-factor for the enzymatic activation of the coagulation cascade, specifically K- Vitamin dependent factors (II, VII, IX, and X) and factor XIII, which is involved in the structure and strength of the clot (52–54). Additionally, it plays a central role in platelet activation (52–54). Eleven percent of individuals suffering from spontaneous intracerebral haemorrhage in the acute phase present hypocalcaemia (serum Ca++ < 8.4 mg/dL), which is associated with the development of coagulopathy, higher haematoma volume, and increased risk of haematoma expansion (55). Shock associated with polytrauma (without TBI), causes a decrease in ionised Ca++ (<1.1 mmol/L) in 50% of individuals, a condition associated with the development of coagulopathy, increased transfusion requirements, and death (55), findings corroborated in a recent systematic review (56). In patients with moderate and severe TBI, decreases in ionic Ca++ (<1.1 mmol/L) on the third day postadmission were found to be predictive of mortality and poor functional outcomes (57). The main pathophysiological reasons for the decrease in calcium are chelation phenomena, primarily through the action of inflammatory mediators, lactate, and intracellular proteins released by astrocytes or damaged neurons (58). Additional mechanisms of hypocalcaemia include hypoalbuminemia secondary to increased capillary permeability post-shock resuscitation and citrate chelation after transfusion of storage blood (56, 57, 59).

3.7 Factor 7: anaemia-transfusion was defined by participants as "Sometimes beneficial, sometimes dangerous"

Traumatic brain injury itself rarely causes blood loss requiring transfusion (60, 61). Most TBI patients are not severely anaemic at the time they are admitted to the ICU but develop anaemia during their stay (60, 61). Post-ICU anaemia is a secondary complication but its effect on outcome remains controversial (60–62). Since the landmark work of Hébert et al. (63) more than 20 years ago, the trend has been towards restrictive transfusion therapy (64, 65). There is no cutoff point to consider a haemoglobin (Hgb) level as optimal (61, 66). Inasmuch as red blood transfusion has been associated with poor outcomes after TBI (65–70), transfusion should be guided by clinical goals rather than a "magic" number (61, 64, 65).

Indications for transfusion in the context of iTBI should therefore follow the hemodynamic status, cerebral tissue oxygenation, and premorbid state (cardiopathic patients are less tolerant to anaemia) (61, 64, 65). Hgb is responsible for nearly all the O_2 transported by the blood, provided it is of good quality and in sufficient quantity. When these conditions are compromised, cerebral tissue hypoxia (anaemic hypoxia) may develop (71, 72). Physiologically, below 7 g/dL, O_2 transport capacity drops by half, while above 12 g/dL, O_2 availability provides no beneficial changes; on the contrary, O_2 transport may decrease due to increased blood viscosity and decreased CBF (61, 71). Transfusion does not ensure correction of cerebral tissue hypoxia, which is multifactorial and requires exhaustive analysis (71, 73). Blood stored for long periods decreases its 2,3-diphosphoglycerate component, which increases the affinity of Hgb for O_2, restricting the

availability of O_2 to the cell (65–69, 74). Transfusions can generate acute lung injury (TRALI), multiorgan dysfunction, and C-iTBI depending mainly on the volume (> risk if massive) and storage time, the so-called "blood bank coagulopathy," which causes a series of physiological alterations such as tissue hypoxia, hypocalcaemia (citrate intoxication), hypothermia, acidosis, hyperglycaemia and hyperkalaemia, generating a vicious circle that perpetuates and exacerbates C-iTBI. In sum, balancing the risks and benefits of transfusion is paramount (65, 67–69, 74). Available evidence suggests that it is reasonable to maintain Hgb values between 7 and 9 g/dL (61, 65, 66).

3.8 Factor 8: temperature was defined by participants as a factor where "Too low or too high, both are detrimental"

Post-trauma and shock hypothermia affect the coagulation system, resulting in an independent risk factor for mortality and poor outcomes (75–77). Hypothermia increases the affinity of Hgb for O_2 (shift to the left), making it more difficult to transfer the necessary O_2 to the cell (high-affinity hypoxia) (72, 78). Hypothermia generates a hypocoagulable state, facilitating bleeding by blocking coagulation enzymatic cascades, prolonging the initiation phase, altering platelet aggregability, and decreasing fibrinogen synthesis (79–82). These mechanisms are mediated by thrombin (79, 80). Hyperthermia is prevalent after TBI (83–85). During the first hours of evolution, this constitutes a secondary insult, associated with the severity of the injury and with poor outcomes (83–85). Hyperthermia increases ICP, exacerbates oedema and inflammation, causes rupture of the blood–brain barrier (BBB), and can trigger cerebral hypoxia (83–85). Temperatures between 38 and 39 degrees Celsius generally either do not modify coagulation or cause a prothrombotic state without major consequences (86), but in cases of extreme elevation (heatstroke), the fibrinolytic mechanism initially ceases to function to then consume coagulation factors and platelets, leading to DIC and multiple organ dysfunction (87).

3.9 Factor 9: international normalised ratio-monitoring was defined by participants as a factor in which "Interpreting information is key"

For years and through today, in ICUs worldwide, coagulation monitoring has been based on the determination of conventional coagulation assays (CCA) such as International Normalised Ratio (INR), platelet count, activated partial thromboplastin time (aPPT), and D-dimer, which have also been used to define C-iTBI (Table 1) (6–10, 12, 88).

Two issues are important to highlight. On the one hand, the methods of analysis and cohort points from which to define alterations have been variable and heterogeneous. On the other, multiple limitations emerge in assessing the role of the components during the different phases of coagulation, among which we found (6–10, 12, 88):

- → Non-standardised parameters and cutoff points
- → Do not assess the onset of clot formation

TABLE 1 Conventional coagulation assays (CCA).

CCA	Evaluation	Cohort value to C-iTBI definition
Platelet count	Number only	< 100,000/mm (11)
INR	Prothrombin time	>1,2
PT	Time to clot formation in blood. Extrinsic pathway (old coagulation physiology)	>15 s
Thrombin time	Similar to PT	>20 s
aPPT	Contact activation in plasma	>35 s
	Heparin-sensitive	
	Intrinsic pathway	
Fibrinogen		<1.5 gr/L
D-dimer	Fibrinolysis. Unspecific	>0.5 µg/mL
Fibrinogen degradation products	Fibrinolysis	>11 mg/mL

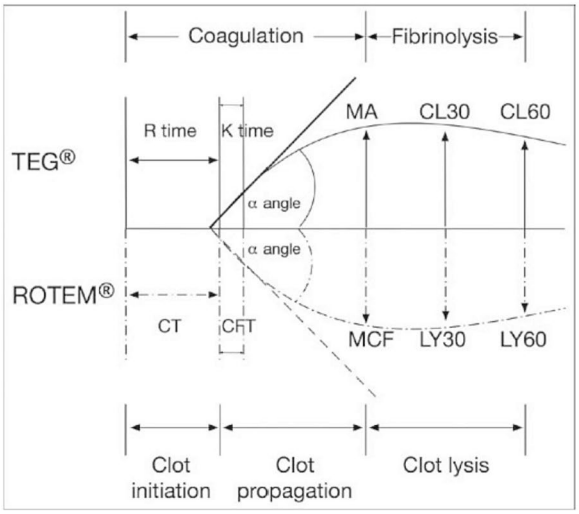
- → Do not assess clot strength and integrity
- → Low sensitivity especially in trauma
- → Do not assess platelet function
- → Non-specific and inaccurate assessment of the fibrinolytic system
- → Do not detect the broad spectrum of clotting alterations when antithrombotic drugs were used pre-injury

As a point of comparison, viscoelastic tests (VET) such as TEG thromboelastography or ROTEM thromboelastometry and their variations allow for a more detailed and rapid evaluation of the full spectrum of coagulation physiology at the bedside using whole blood (6–10, 12, 88). Clot formation kinetics, fibrin-platelet interactions, platelet functionality, and fibrinolysis can be graphically monitored (6–10, 12, 88). The parameters evaluated with VETs and their cohort points reported in the literature are outlined in Figure 2.

Viscoelastic tests are more sensitive than CCAs for the full evaluation of the coagulation system (6–10, 12, 87) and their use is recommended in current guidelines for the management of bleeding secondary to trauma (89); however, these modalities also have limitations, including the following:

- → Not widely available
- → Longer learning curve
- → They are *in vitro* tests (like CCA) that do not allow for assessment of endothelial contribution or von Willebrand factor deficiency.
- → Low sensitivity for detecting abnormalities when using low molecular weight heparins or the new oral anticoagulants
- → High negative predictive power, but low positive predictive power
- → Quality control and maintenance of difficult systems

Coagulation disorder therapy guided by VETs following craniotomy has shown promising results (90). A recent clinical



Parameter (Normal value)	Name	Evaluation
R / CT (4-8 minutes)	Reaction time	Activated factor VII / Tissue factor / Coagulation factors
K / CFT (1-4 minutes)	Clot kinetics/Amplification time	Thrombin / Platelet activation / Coagulation factors
A (47-74 grades)	Alpha angle	Fibrin formation and cross linking
MA / MCF (55-73 minutes)	Maximun amplitude	Fibrin / Overall platelet function
CL_{30} / LY_{30} (0-8%)	Lysis index. % decrease amplitude from time to MA to 30 minutes post MA	Fibrinolysis

FIGURE 2
Parameters evaluated with VET.

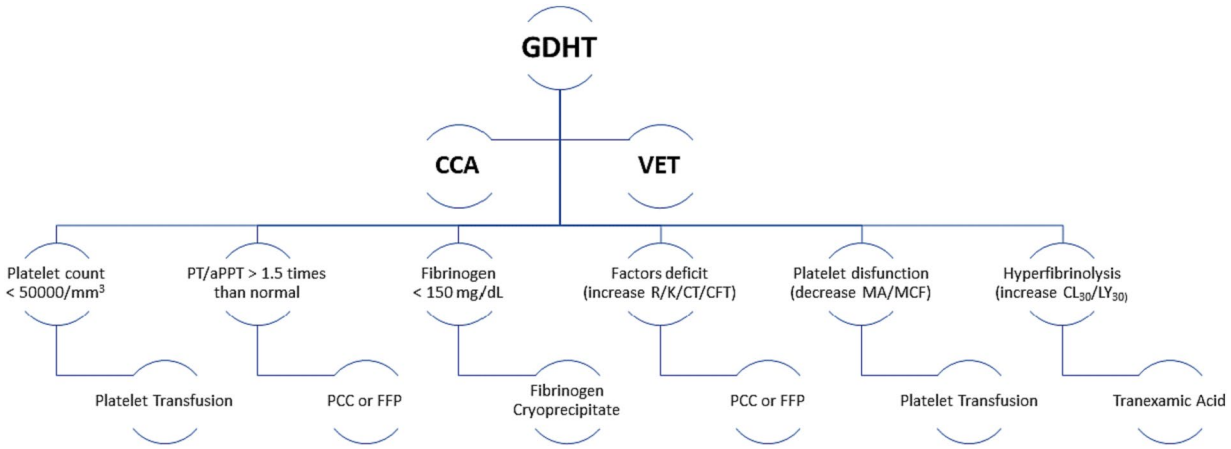


FIGURE 3
Monitoring methodology. PCC, Prothrombin complex concentrate; FFP, Fresh frozen plasma; GDHT, Goal-directed haemostatic therapy; CCA, Conventional coagulation assays; VET, Viscoelastic tests.

study comparing the management of coagulation disorders during trauma resuscitation demonstrated improved survival and less use of plasma and platelets in patients managed by GDHT directed by VETs vs. GHDT guided by CCA, although an analysis of the subgroup of individuals with TBI found no difference (91). Similar results were observed in the ITACTIC trial, specifically in terms of mortality (92). In the pre-defined TBI group, 64% of individuals managed and guided by VETs did not undergo massive transfusion protocols during the first day of trauma compared to 40% of patients in the group whose therapy was CCA-guided (OR 2.12, 95% CI 0.84–5.34) (91). Irrespective of the monitoring methodology available, we can direct therapy more precisely based on the results obtained. An example found in the evidence is outlined in Figure 3.

3.10 Factor 10: oral antithrombotic reversal was defined by participants as "Urgent, emergent, or both?"

We have previously explained why clinicians must be on "high alert" when a patient with TBI has a history of pre-injury antithrombotic drugs (12, 28–35). Due to the risk of lesion progression, and that of haemorrhagic lesions in particular, and given its close association with poor outcomes, this is a situation that requires prompt resolution (12, 28–35). Once the administration of the causative agent has been suspended, this next step must emphasise the assessment and clinical-tomographic categorisation of the TBI, establishing the presence of bleeding (volume, location, extension, anatomical distortions or displacements, and associated lesions); its

TABLE 2 Summary of agents employed for reversal of anti-thrombotic drugs.

Antithrombotic	Reversal agent	Evidence quality
Oral anticoagulants (K-vitamin dependents)	PCC + vitamin K	1A
	If PCC is not available, FFP is an alternative	
New oral anticoagulants factor Xa inhibitors (apixaban, endoxaban, rivaroxaban)* Thrombin inhibitor (dabigatran)**	PCC + Tranexamic Acid + Activated charcoal***	2C
	PCC + Idarucizumab + Activated charcoal***	2C + 1B
Antiplatelet agents (aspirin, dipyridamole, ticlopidine, cilostazol, clopidogrel)	Platelet transfusion****	2C
	Desmopressin*****	2C
Unfractionated heparin (UFH)	Protamine sulphate	Strong recommendation, moderate quality of evidence***
Low weight molecular heparin (LWMH)	Protamine sulphate	Strong recommendation, moderate quality of evidence***

PCC, Prothrombin complex concentrate; FFP, Fresh frozen plasma. *Guidelines suggest plasma dosing of the drugs in question, calibrating the anti-factor Xa activity for each agent. Consult with Haematologist (Level of evidence 2C) (89). **Guidelines suggest plasma dosing of dabigatran. If not available, follow up with thrombin time. Consult with Haematologist (Level of evidence 2C) (89). ***Taken from reference (94). ****Only for individuals with active bleeding and demonstrated evidence of platelet dysfunction (2C) or undergoing surgery (2B) (89). *****Suggested for individuals with pre-injury intake of antithrombotic agents or von Willebrand disease (89).

severity and therapeutic possibilities, including the need for surgical intervention (89, 93–95). Coagulation monitoring (CCA or VET) is essential to establish the starting point, follow-up, and above all to avoid the undesirable effects of "overtreatment" (89). Undoubtedly, severe life-threatening bleeding associated with pre-injury use of antithrombotic agents requires immediate reversal (89, 94). In other situations, the need for reversal must be balanced against the risks involved (thrombosis) (89, 94). A detailed analysis of how and with what agents this reversal should be implemented is beyond the scope of this manuscript; but generally the strategy depends fundamentally on the antithrombotic agent in question (89, 93–97). In Table 2, we summarise the agents used in accordance with current guidelines (89).

Although they are not oral agents, we have included unfractionated heparins (UFH) and low molecular weight heparins (LWMH) here due to their frequent pre-injury use, particularly in older adult with significant prothrombotic comorbidities or undergoing prolonged postoperative periods, especially orthopaedic surgery (94).

3.11 Factor 11: normal acid–base status was defined by participants as "Neither acid nor alkaline; neutral"

During acute TBI it is essential to achieve a balanced physiological microenvironment to prevent secondary insults (1, 4, 36, 71). Acidosis causes cerebral vasodilation, increased cerebral blood volume, and consequent increase in ICP; while alkalosis increases the affinity of Hgb for O_2 without the possibility of ceding it to the cells, causing cerebral tissue hypoxia of low extraction (1, 4, 36, 71, 72). Coagulation is not exempt from modifications if the acid–base state is modified (75, 76, 80). Although alkalosis does not modify coagulation parameters (86); acidosis compromises both platelet aggregation and clot formation, increases the prolongation phase due to alterations in thrombin, and accelerates the degradation of fibrinogen (75–77).

4 Summary of the design and implications of the mnemonic device "Coagulation"

In this study, we complemented our narrative analysis with evidence-based studies. The methodological premise was that analytic actions should coincide with both evidence-based studies and the characteristics of the factor identified by study participants.

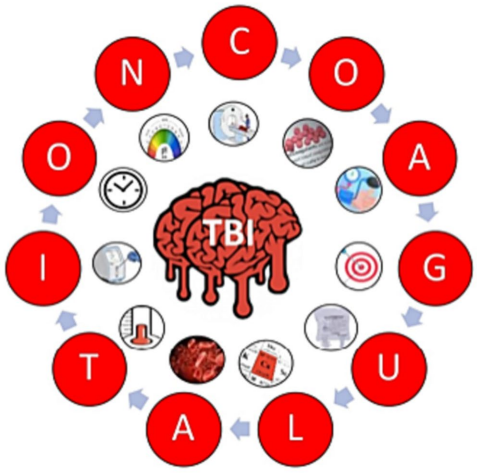
In the Figure 4, we summarise the algorithm based on the comprehensive analysis of the acronym "Coagulation," we designed as a mnemonic device encompassing the primary factors involved in the management of C-iTBI in the intensive care unit. In the process, we have suggested a comprehensive clinical approach to the variables and situations that may predispose patients to developing this condition, as well as the management of risk factors and complications requiring prompt resolution.

5 Conclusion

Narrative analysis is an interdisciplinary tool that may be applied as a qualitative approach to research using temporally situated data from a wide range of discourses. In this study, we conducted a narrative analysis based on the responses of intensive care physicians regarding the key factors associated with C-iTBI.

Knowledge and detection of the risk factors, pathophysiology, and kinetics of the coagulation system and its alterations allow for the prompt recognition and management of traumatic injury to the brain. The essential information obtained from the serial monitoring of coagulation in conjunction with clinical judgement allows for a personalised and goal-directed therapy that can help prevent or minimise the adverse effects of conventional therapies and reduce the negative consequences of coagulopathy.

Given the time-sensitive nature of such decisions, in this study, we aimed to design an acronym as a mnemonic device to provide clinicians with a simple auxiliary tool in the treatment of this critical



Cerebral Computed Tomography
Oral Preinjury Antithrombotics
Arterial Blood Pressure
Goal Directed Hemosthatic Therapy
Use Fluids Cautiously
Low Calcium Levels
Anemia-Transfusion
Temperature (Hypothermia)
INR-Coagulation Monitoring
Oral Antithrombotic Reversion
Normal Acid-Basic Status

FIGURE 4
The mnemonic device "Coagulation."

complication. We believe that the acronym "Coagulation" meets the stated objectives. It is a simple and easy to apply tool for anyone facing the challenge of treating victims of moderate or severe TBI on a daily basis.

Numerous challenges remain to prevent or minimise secondary damage and ensure improved patient outcomes in C-iTBI. Issues such as the harmonisation of definitions and validation of timely, appropriate, personalised, goal-based management require the prompt design and execution of large-scale clinical studies. The dissemination of knowledge as well as technological advances in the monitoring systems of the coagulation system offer a unique opportunity to unify criteria aimed at achieving increasingly precise medicine in this critical field.

6 Limitations and bias

This study is presented as a narrative method supported by scientific evidence and must take into account the limitations that such an approach entails. It is also specifically focused on TBI.

7 Clinical implications

In this article, we provide a mnemonic device for critical situations that will improve clinical care in the context of intensive care units.

Data availability statement

The raw data supporting the conclusions of this article will be made available upon request to the last author.

Author contributions

MQ-D: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing, Supervision, Validation. PA: Investigation, Methodology, Project administration, Writing – original draft. RJ-V: Methodology, Project administration, Resources, Writing – original draft. EE-S: Software, Validation, Writing – original draft. CT-G: Data curation, Formal Analysis, Investigation, Writing – review & editing. KN-N: Software, Validation, Writing – original draft. AS-L: Methodology, Project administration, Writing – original draft. PM-N: Validation, Visualization, Writing – original draft. MG-E: Resources, Validation, Visualization, Writing – review & editing. JG-C: Methodology, Project administration, Validation, Visualization, Writing – review & editing. DG: Supervision, Writing – original draft, Writing – review & editing. PS-C: Software, Writing – original draft.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This article has been funded by the scientific research grants of Mutua Madrileña.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Stocchetti N, Carbonara M, Citerio G, Ercole A, Skrifvars MB, Smielewski P, et al. Severe traumatic brain injury: targeted management in the intensive care unit. *Lancet Neurol.* (2017) 16:452–64. doi: 10.1016/S1474-4422(17)30118-7

2. Godoy DA, Murillo-Cabezas F. Evolutionary conceptualization of the injury mechanisms in traumatic brain injury. *Med Intens.* (2020) 46:90–3. doi: 10.1016/j.medin.2020.02.003

3. Gennarelli TA. Mechanisms of brain injury. *J Emerg Med.* (1993) 11:5–11.

4. Bouzat P, Sala N, Payen JF, Oddo M. Beyond intracranial pressure: optimization of cerebral blood flow, oxygen, and substrate delivery after traumatic brain injury. *Ann Intensive Care.* (2013) 3:23. doi: 10.1186/2110-5820-3-23

5. Yuan Q, Sun YR, Wu X, Yu J, Li ZQ, du ZY, et al. Coagulopathy in traumatic brain injury and its correlation with progressive hemorrhagic injury: a systematic review and meta-analysis. *J Neurotrauma.* (2016) 33:1279–91. doi: 10.1089/neu.2015.4205

6. Maegele M. Coagulopathy and progression of intracranial hemorrhage in traumatic brain injury: mechanisms, impact, and therapeutic considerations. *Neurosurgery.* (2021) 89:954–66. doi: 10.1093/neuros/nyab358

7. Laroche M, Kutcher ME, Huang MC, Cohen MJ, Manley GT. Coagulopathy after traumatic brain injury. *Neurosurgery.* (2012) 70:1334–45. doi: 10.1227/NEU.0b013e31824d179b

8. Zhang J, Zhang F, Dong JF. Coagulopathy induced by traumatic brain injury: the systemic manifestation of a localized injury. *Blood.* (2018) 131:2001–6. doi: 10.1182/blood-2017-11-784108

9. Mathur R, Suarez JI. Coagulopathy in isolated traumatic brain injury: myth or reality. *Neurocrit Care.* (2022) 38:429–38. doi: 10.1007/s12028-022-01647-4

10. Maegele M, Schöchl H, Menovsky T, Maréchal H, Marklund N, Buki A, et al. Coagulopathy and hemorrhagic progression in traumatic brain injury: advances in mechanisms, diagnosis, and management. *Lancet Neurol.* (2017) 16:630–47. doi: 10.1016/S1474-4422(17)30197-7

11. Roozenbeek B, Maas AI, Menon DK. Changing patterns in the epidemiology of traumatic brain injury. *Nat Rev Neurol.* (2013) 9:231–6. doi: 10.1038/nrneurol.2013.22

12. Böhm JK, Güting H, Thorn S, Schäfer N, Rambach V, Schöchl H, et al. CENTER-TBI participants and investigators. Global characterization of coagulopathy in isolated traumatic brain injury (iTBI): a CENTER-TBI analysis. *Neurocrit Care.* (2021) 35:184–96. doi: 10.1007/s12028-020-01151-7

13. Wafaisade A, Lefering R, Tjardes T, Wutzler S, Simanski C, Paffrath T, et al. Acute coagulopathy in isolated blunt traumatic brain injury. *Neurocrit Care.* (2010) 12:211–9. doi: 10.1007/s12028-009-9281-1

14. Solla DJF, de Amorim RLO, Kolias AG, Hutchinson PJ, de Andrade AF, Teixeira MJ, et al. Incremental prognostic value of coagulopathy in addition to the crash score in traumatic brain injury patients. *Neurocrit Care.* (2021) 34:130–8. doi: 10.1007/s12028-020-00991-7

15. Murray GD, Butcher I, McHugh GS, Lu J, Mushkudiani NA, Maas AI, et al. Multivariable prognostic analysis in traumatic brain injury: results from the IMPACT study. *J Neurotrauma.* (2007) 24:329–37. doi: 10.1089/neu.2006.0035

16. Greuters S, van den Berg A, Franschman G, Viersen VA, Beishuizen A, Peerdeman SM, et al. Acute and delayed mild coagulopathy are related to outcome in patients with isolated traumatic brain injury. *Crit Care.* (2011) 15:R2. doi: 10.1186/cc9399

17. Brain Trauma Foundation. American association of neurological surgeons, congress of neurological surgeons. Guidelines for the management of severe traumatic brain injury. *J Neurotrauma.* (2007) 24:i–vi. doi: 10.1089/neu.2007.9999

18. Lolli V, Pezzullo M, Delpierre I, Sadeghi N. MDCT imaging of traumatic brain injury. *Br J Radiol.* (2016) 89:20150849. doi: 10.1259/bjr.20150849

19. Mutch CA, Talbott JF, Gean A. Imaging evaluation of acute traumatic brain injury. *Neurosurg Clin N Am.* (2016) 27:409–39. doi: 10.1016/j.nec.2016.05.011

20. Marshall LF, Marshall SB, Klauber MR, Clark MB, Eisenberg HM, Jane JA, et al. A new classification of head injury based on computerized tomography. *J Neurosurg.* (1991) 75:S14–20. doi: 10.3171/SUP.1991.75.1S.0S14

21. Daiute C, Lightfoot C. Theory and craft in narrative inquiry In: C Daiute and C Lightfoot, editors. *Narrative Analysis: Studying the Development of Individuals in Society.* Thousand Oaks, CA: Sage (2004). vii–xviii.

22. Narayan RK, Greenberg RP, Miller JD, Enas GG, Choi SC, Kishore PRS, et al. Improved confidence of outcome prediction in severe head injury: a comparative analysis of the clinical examination, multimodality evoked potentials, CT scanning, and intracranial pressure. *J Neurosurg.* (1981) 54:751–62. doi: 10.3171/jns.1981.54.6.0751

23. Servadei FD, Murray GD, Key P, Teasdale GM, Dearden M, Iannotti F, et al. The value of the "worst" computed tomographic scan in clinical studies of moderate and severe head injury. *Neurosurgery.* (2000) 46:70–7. doi: 10.1097/00006123-200001000-00014

24. Maas AI, Hukkelhoven CW, Marshall LF, Steyerberg EW. Prediction of outcome in traumatic brain injury with computed tomographic characteristics: a comparison between the computed tomographic classification and combinations of computed tomographic predictors. *Neurosurgery.* (2005) 57:1173. doi: 10.1227/01.neu.0000186013.63046.6b

25. Mishra R, Vasquez Ucros HE, Florez-Perdomo WA, Suarez JR, Moscote-Salazar LR, Rahman MM, et al. Predictive value of Rotterdam score and Marshall score in traumatic brain injury: a contemporary review. *Ind J Neurotrauma.* (2021) 19:069–77. doi: 10.1055/s-0041-1727404

26. Raj R, Siironen J, Skrifvars MB, Hernesniemi J, Kivisaari R. Predicting outcome in traumatic brain injury: development of a novel computerized tomography classification system (Helsinki computerized tomography score). *Neurosurgery.* (2014) 75:632–47. doi: 10.1227/NEU.0000000000000533

27. Batchelor JS, Grayson A. A meta-analysis to determine the effect of anticoagulation on mortality in patients with blunt head trauma. *Br J Neurosurg.* (2012) 26:525–30. doi: 10.3109/02688697.2011.650736

28. Fabbri A, Servadei F, Marchesini G, Bronzoni C, Montesi D, Arietta L. et al., Antiplatelet therapy and the outcome of subjects with intracranial injury: the Italian SIMEU study. *Crit Care.* (2013) 17:R53. doi: 10.1186/cc12575

29. Uccella L, Zoia C, Bongetta D, Gaetani P, Martig F, Candrian C, et al. Are antiplatelet and anticoagulants drugs a risk factor for bleeding in mild traumatic brain injury? *World Neurosurg.* (2018) 110:e339–45. doi: 10.1016/j.wneu.2017.10.173

30. Kobayashi L, Barmparas G, Bosarge P, Brown CV, Bukur M, Carrick MM, et al. Novel oral anticoagulants and trauma: the results of a prospective American Association for the Surgery of Trauma multi-institutional trial. *J Trauma Acute Care Surg.* (2017) 82:827–35. doi: 10.1097/TA.0000000000001414

31. Zeeshan M, Jehan F, O'Keeffe T, Khan M, Zakaria ER, Hamidi M, et al. The novel oral anticoagulants (NOACs) have worse outcomes compared to warfarin in patients with intracranial hemorrhage after TBI. *J Trauma Acute Care Surg.* (2018) 85:915–20. doi: 10.1097/TA.0000000000001995

32. Markou M, Pleger B, Grötzlinger M, Pintea B, Hamsen U, Könen S, et al. Intake of NOAC is associated with hematoma expansion of intracerebral hematomas after traumatic brain injury. *Eur J Trauma Emerg Surg.* (2021) 47:565–71. doi: 10.1007/s00068-019-01228-9

33. Mathieu F, Güting H, Gravesteijn B, Monteiro M, Glocker B, Kornaropoulos EN, et al. Collaborative European NeuroTrauma effectiveness research in traumatic brain injury (CENTER-TBI) investigators and participants. Impact of antithrombotic agents on radiological lesion progression in acute traumatic brain injury: a CENTER-TBI propensity-matched cohort analysis. *J Neurotrauma.* (2020) 37:2069–80. doi: 10.1227/NEU.0000000000000575

34. Vedin T, Lundager Forberg J, Anefjäll E, Lehtinen R, Faisal M, Edelhamre M. Antiplatelet therapy contributes to a higher risk of traumatic intracranial hemorrhage compared to anticoagulation therapy in ground-level falls: a single-center retrospective study. *Eur J Trauma Emerg Surg.* (2022) 48:4909–17. doi: 10.1007/s00068-022-02016-8

35. Mathieu F, Malhotra AK, Ku JC, Zeiler FA, Wilson JR, Pirouzmand F, et al. Pre-injury antiplatelet therapy and risk of adverse outcomes after traumatic brain injury: a systematic review and meta-analysis. *Neurotrauma Rep.* (2022) 3:308–20. doi: 10.1089/neur.2022.0042

36. Godoy DA, Videtta W, Santa Cruz R, Silva X, Aguilera-Rodríguez S, Carreño-Rodríguez JN, et al. General care in the management of severe traumatic brain injury: Latin american consensus [published online ahead of print, 2020 may 3]. *Med Int.* (2020) 44:500–8. doi: 10.1016/j.medin.2020.01.014

37. Chesnut RM, Marshall LF, Klauber MR, Blunt BA, Baldwin N, Eisenberg HM, et al. The role of secondary brain injury in determining outcome from severe head injury. *J Trauma.* (1993) 34:216–22. doi: 10.1097/00005373-199302000-00006

38. Manley G, Knudson MM, Morabito D, Damron S, Erickson V, Pitts L. Hypotension, hypoxia, and head injury: frequency, duration, and consequences. *Arch Surg.* (2001) 136:1118. doi: 10.1001/archsurg.136.10.1118

39. Maegele M. Coagulopathy after traumatic brain injury: incidence, pathogenesis, and treatment options. *Transfusion.* (2013) 53:28S–37S. doi: 10.1111/trf.12033

40. Lustenberger T, Talvig P, Kobayashi L, Inaba K, Lam L, Plurad D, et al. The time course of coagulopathy is isolated severe traumatic brain injury. *Injury.* (2010) 41:924–8. doi: 10.1016/j.injury.2010.04.019

41. Oddo M, Poole D, Helbok R, Meyfroidt G, Stocchetti N, Bouzat P, et al. Fluid therapy in neurointensive care patients: ESICM consensus and clinical practice recommendations. *Intensive Care Med*. (2018) 44:449–63. doi: 10.1007/s00134-018-5086-z

42. Coats TJ, Brazil E, Heron M. The effects of commonly used resuscitation fluids on whole blood coagulation. *Emerg Med J*. (2006) 23:546–9. doi: 10.1136/emj.2005.032334

43. Kozek-Langenecker SA. Fluids and coagulation. *Curr Opin Crit Care*. (2015) 21:285–91. doi: 10.1097/MCC.0000000000000219

44. Kozek-Langenecker SA. Influence of fluid therapy on the hemostatic system of intensive care patients. *Best Pract Res Clin Anaesthesiol*. (2009) 23:225–36. doi: 10.1016/j.bpa.2008.11.002

45. Konrad C, Markl T, Schuepfer G, Schmeck J, Gerber HR. In vitro effects of different medium molecular hydroxyethyl starch solutions and lactated Ringer's solution on coagulation using SONOCLOT. *Anesth Analg*. (2000) 90:274–9. doi: 10.1097/00000539-200002000-00007

46. Tocantins LM. The clot accelerating effect of dilution on blood and plasma. Relation to the mechanism of coagulation of normal and haemophiliac blood. *Blood*. (1951) 6:720–39. doi: 10.1182/blood.V6.8.720.720

47. Ruttmann TG, James MF, Viljoen JF. Haemodilution induces a hypercoagulable state. *Br J Anaesth*. (1996) 76:412–4. doi: 10.1093/bja/76.3.412

48. Jamnicki M, Zollinger A, Seifert B, Popovic D, Pasch T, Spahn DR. The effect of potato starch derived and corn starch derived hydroxyethyl starch on in vitro blood coagulation. *Anaesthesia*. (1998) 53:638–44. doi: 10.1046/j.1365-2044.1998.489-az0565.x

49. Roche AM, James MFM, Grocott MPW, Mythen MG. Coagulation effects of in vitro haemodilution with a balanced electrolyte hetastarch solution and lactated Ringer's solution. *Anaesthesia*. (2002) 57:950–5. doi: 10.1046/j.1365-2044.2002.02707.x

50. Wang H, Cao H, Zhang X, Ge L, Bie L. The effect of hypertonic saline and mannitol on coagulation in moderate traumatic brain injury patients. *Am J Emerg Med*. (2017) 35:1404–7. doi: 10.1016/j.ajem.2017.04.020

51. Hernández-Palazón J, Fuentes-García D, Doménech-Asensi P, Piqueras-Pérez C, Falcón-Araña L, Burguillos-López S. Equiosmolar solutions of hypertonic saline and mannitol do not impair blood coagulation during elective intracranial surgery. *J Neurosurg Anesthesiol*. (2017) 29:8–13. doi: 10.1097/ANA.0000000000000255

52. Palta S, Saroa R, Palta A. Overview of the coagulation system. *Ind J Anaesth*. (2014) 58:515–23. doi: 10.4103/0019-5049.144643

53. O'Donnell JS, O'Sullivan JM, Preston RJS. Advances in understanding the molecular mechanisms that maintain normal haemostasis. *Br J Haematol*. (2019) 186:24–36. doi: 10.1111/bjh.15872

54. Singh S, Dodt J, Volkers P, Hethershaw E, Philippou H, Ivaskevicius V, et al. Structure functional insights into calcium binding during the activation of coagulation factor XIII a. *Sci Rep*. (2019) 9:11324. doi: 10.1038/s41598-019-47815-z

55. Morotti A, Charidimou A, Phuah CL, Jessel MJ, Schwab K, Ayres AM, et al. Calcium level and extent of bleeding in patients with intracerebral hemorrhage. *JAMA Neurol*. (2016) 73:1285–90. doi: 10.1001/jamaneurol.2016.2252

56. Vasudeva M, Mathew JK, Groombridge C, Tee JW, Johnny CS, Maini A, et al. Hypocalcemia in trauma patients: a systematic review. *J Trauma Acute Care Surg*. (2021) 90:396–402. doi: 10.1097/TA.0000000000003027

57. Manuel VR, Martin SA, Juan SR, Fernando MA, Frerk M, Thomas K, et al. Hypocalcemia as a prognostic factor in mortality and morbidity in moderate and severe traumatic brain injury. *Asian J Neurosurg*. (2015) 10:190–4. doi: 10.4103/1793-5482.161171

58. DeBot M, Sauaia A, Schaid T, Moore EE. Trauma-induced hypocalcemia. *Transfusion*. (2022) 62:S274–80. doi: 10.1111/trf.16959

59. Vasudeva M, Mathew JK, Fitzgerald MC, Cheung Z, Mitra B. Hypocalcaemia and traumatic coagulopathy: an observational analysis. *Vox Sang*. (2020) 115:189–95. doi: 10.1111/vox.12875

60. Vanhala H, Junttila E, Kataja A, Huhtala H, Luostarinen T, Luoto T. Incidence and associated factors of Anemia in patients with acute moderate and severe traumatic brain injury. *Neurocrit Care*. (2022) 37:629–37. doi: 10.1007/s12028-022-01561-9

61. Lelubre C, Bouzat P, Crippa IA, Taccone FS. Anemia management after acute brain injury. *Crit Care*. (2016) 20:152. doi: 10.1186/s13054-016-1321-6

62. Salim A, Hadjizacharia P, DuBose J, Brown C, Inaba K, Chan L, et al. Role of anemia in traumatic brain injury. *J Am Coll Surg*. (2008) 207:398–406. doi: 10.1016/j.jamcollsurg.2008.03.013

63. Hébert PC, Wells G, Blajchman MA, Marshall J, Martin C, Pagliarello G, et al. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. Transfusion requirements in critical care investigators, Canadian critical care trials group. *N Engl J Med*. (1999) 340:409–17. doi: 10.1056/NEJM199902113400601

64. Egea-Guerrero JJ, García-Sáez I, Quintana-Díaz M. Trigger transfusion in severe traumatic brain injury. *Med Intens*. (2021) S0210-5691:00071. doi: 10.1016/j.medin.2021.03.012

65. Montgomery EY, Barrie U, Kenfack YJ, Edukugho D, Caruso JP, Rail B, et al. Transfusion guidelines in traumatic brain injury: a systematic review and Meta-analysis of the currently available evidence. *Neurotrauma Rep*. (2022) 3:554–68. doi: 10.1089/neur.2022.0056

66. Florez-Perdomo WA, García-Ballestas E, Martinez-Perez R, Agrawal A, Deora H, Joaquim AF, et al. Hemoglobin levels as a transfusion criterion in moderate to severe traumatic brain injury: a systematic review and meta-analysis. *Br J Neurosurg*. (2021) 37:1473–9. doi: 10.1080/02688697.2021.1940850

67. Boutin A, Moore L, Lauzier F, Chassé M, English S, Zarychanski R, et al. Transfusion of red blood cells in patients with traumatic brain injuries admitted to Canadian trauma health centres: a multicentre cohort study. *BMJ Open*. (2017) 7:e014472. doi: 10.1136/bmjopen-2016-014472

68. Spahn DR, Rossaint R. Coagulopathy and blood component transfusion in trauma. *Br J Anaesth*. (2005) 95:130–9. doi: 10.1093/bja/aei169

69. Vernon T, Morgan M, Morrison C. Bad blood: a coagulopathy associated with trauma and massive transfusion review. *Acute Med Surg*. (2019) 6:215–22. doi: 10.1002/ams2.402

70. Zhang W, Du K, Chen X. Benefits of red blood cell transfusion in patients with traumatic brain injury. *Crit Care*. (2019) 23:218. doi: 10.1186/s13054-019-2498-2

71. Godoy DA, Lubillo S, Rabinstein AA. Pathophysiology and management of intracranial hypertension and tissular brain hypoxia after severe traumatic brain injury: an integrative approach. *Neurosurg Clin N Am*. (2018) 29:195–212. doi: 10.1016/j.nec.2017.12.001

72. Siggaard-Andersen O, Gothgen IH, Fogh-Andersen N, Larsen VH. Oxygen status of arterial and mixed venous blood. *Crit Care Med*. (1995) 23:1284–93. doi: 10.1097/00003246-199507000-00020

73. Oddo M, Levine JM, Mackenzie L, Frangos S, Feihl F, Kasner SE, et al. Brain hypoxia is associated with short-term outcome after severe traumatic brain injury independently of intracranial hypertension and low cerebral perfusion pressure. *Neurosurgery*. (2011) 69:1037–45. doi: 10.1227/NEU.0b013e3182287ca7

74. MacLeod JB. Trauma and coagulopathy: a new paradigm to consider. *Arch Surg*. (2008) 143:797–801. doi: 10.1001/archsurg.143.8.797

75. Eddy VA, Morris JA Jr, Cullinane DC. Hypothermia, coagulopathy, and acidosis. *Surg Clin North Am*. (2000) 80:845–54. doi: 10.1016/s0039-6109(05)70099-2

76. Gerecht R. The lethal triad. Hypothermia, acidosis & coagulopathy create a deadly cycle for trauma patients. *JEMS*. (2014) 39:56–61.

77. Vardon F, Mrozek S, Geeraerts T, Fourcade O. Accidental hypothermia in severe trauma. *Anaesth Crit Care Pain Med*. (2016) 35:355–61. doi: 10.1016/j.accpm.2016.05.001

78. Zauner A, Daugherty WP, Bullock MR, Warner DS. Brain oxygenation and energy metabolism: part I-biological function and pathophysiology. *Neurosurgery*. (2002) 51:289–301. doi: 10.1097/00006123-200208000-00003

79. Wallner B, Schenk B, Hermann M, Paal P, Falk M, Strapazzon G, et al. Hypothermia-associated coagulopathy: a comparison of viscoelastic monitoring, platelet function, and real time live confocal microscopy at low blood temperatures, an *in vitro* experimental study. *Front Physiol*. (2020) 11:843. doi: 10.3389/fphys.2020.00843

80. De Robertis E, Kozek-Langenecker SA, Tufano R, Romano GM, Piazza O, Zito Marinosci G. Coagulopathy induced by acidosis, hypothermia and hypocalcaemia in severe bleeding. *Minerva Anestesiol*. (2015) 81:65–75.

81. Mitrophanov AY, Szlam F, Sniecinski RM, Levy JH, Reifman J. Controlled multifactorial coagulopathy: effects of dilution, hypothermia, and acidosis on thrombin generation in vitro. *Anesth Analg*. (2020) 130:1063–76. doi: 10.1213/ANE.0000000000004479

82. Levi M. Hemostasis and thrombosis in extreme temperatures (hypo and hyperthermia). *Semin Thromb Hemost*. (2018) 44:651–5. doi: 10.1055/s-0038-1648231

83. Badjatia N. Hyperthermia and fever control in brain injury. *Crit Care Med*. (2009) 37:S250–7. doi: 10.1097/CCM.0b013e3181aa5e8d

84. Bao L, Chen D, Ding L, Ling W, Xu F. Fever burden is an independent predictor for prognosis of traumatic brain injury. *PLoS One*. (2014) 9:e90956. doi: 10.1371/journal.pone.0090956

85. Puccio AM, Fischer MR, Jankowitz BT, Yonas H, Darby JM, Okonkwo DO. Induced normothermia attenuates intracranial hypertension and reduces fever burden after severe traumatic brain injury. *Neurocrit Care*. (2009) 11:82–7. doi: 10.1007/s12028-009-9213-0

86. Ramaker AJ, Meyer P, van der Meer J, Struys MM, Lisman T, van Oeveren W, et al. Effects of acidosis, alkalosis, hyperthermia and hypothermia on haemostasis: results of point-of-care testing with the thromboelastography analyser. *Blood Coagul Fibrinolysis*. (2009) 20:436–9. doi: 10.1097/MBC.0b013e32832dc327

87. Iba T, Connors JM, Levi M, Levy JH. Heatstroke-induced coagulopathy: biomarkers, mechanistic insights, and patient management. *EClinicalMedicine*. (2022) 44:101276. doi: 10.1016/j.eclinm.2022.101276

88. Bradbury JL, Thomas SG, Sorg NR, Mjaess N, Berquist MR, Brenner TJ, et al. Viscoelastic testing and coagulopathy of traumatic brain injury. *J Clin Med*. (2021) 10:5039. doi: 10.3390/jcm10215039

89. Spahn DR, Bouillon B, Cerny V, Duranteau J, Filipescu D, Hunt BJ, et al. The European guideline on management of major bleeding and coagulopathy following trauma. *Crit Care*. (2019) 23:98. doi: 10.1186/s13054-019-2347-3

90. Rimaitis M, Bilskienė D, Tamošuitis T, Vilcinis R, Rimaitis K, Macas A. Implementation of thromboelastometry for coagulation management in isolated

traumatic brain injury patients undergoing craniotomy. *Med Sci Monit*. (2020) 26:e922879. doi: 10.12659/MSM.922879

91. Gonzalez E, Moore EE, Moore HB, Chapman MP, Chin TL, Ghasabyan A, et al. Goal-directed hemostatic resuscitation of trauma-induced coagulopathy: a pragmatic randomized clinical trial comparing a viscoelastic assay to conventional coagulation assays. *Ann Surg*. (2016) 263:1051–9. doi: 10.1097/SLA.0000000000001608

92. Baksaas-Aasen K, Gall LS, Stensballe J, Juffermans NP, Curry N, Maegele M, et al. Viscoelastic haemostatic assay augmented protocols for major trauma haemorrhage (ITACTIC): a randomized, controlled trial. *Intensive Care Med*. (2021) 47:49–59. doi: 10.1007/s00134-020-06266-1

93. Sperry JD, Rose AE, Williams E, Dierks MR, Medow JE. Emergent reversal of antithrombotics and treatment of life-threatening bleeding from coagulopathies: a clinical review. *J Emerg Med*. (2022) 63:17–48. doi: 10.1016/j.jemermed.2022.05.011

94. Frontera JA, Lewin III JJ, Rabinstein AA, Aisiku IP, Alexandrov AW, Cook AM, et al. Guideline for reversal of Antithrombotics in intracranial hemorrhage. A statement for healthcare professionals from the neurocritical care society and society of critical care medicine. *Neurocrit Care*. (2016) 24:6–46. doi: 10.1007/s12028-015-0222-x

95. Ammar AA, Ammar MA, Owusu KA, Brown SC, Kaddouh F, Elsamadicy AA, et al. Andexanet alfa versus 4-factor prothrombin complex concentrate for reversal of factor Xa inhibitors in intracranial hemorrhage. *Neurocrit Care*. (2021) 35:255–61. doi: 10.1007/s12028-020-01161-5

96. Badiwhala JH, Wilson JR, Fehlings MG. Global burden of traumatic brain and spinal cord injury. *Lancet Neurol*. (2019) 18:24–5. doi: 10.1016/S1474-4422(18)30444-7

97. Vinas-Rios JM, Sanchez-Rodriguez JJ, Kretschmer T. Hypocalcaemia and its role in traumatic brain injury. *J Neurol Disord*. (2017) 5:342. doi: 10.4172/2329-6895.1000342

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 13 December 2023
DOI 10.3389/fmed.2023.1294301

OPEN ACCESS

EDITED BY
Giancarlo Castaman,
University of Florence, Italy

REVIEWED BY
Akbar Dorgalaleh,
Iran University of Medical Sciences, Iran
Alessandro Casini,
University Hospitals of Geneva, Switzerland

*CORRESPONDENCE
Rosa Toenges
✉ Rosal_Toenges@DFCI.Harvard.edu

RECEIVED 14 September 2023
ACCEPTED 27 November 2023
PUBLISHED 13 December 2023

CITATION
Toenges R, Steiner M, Weber CF and Miesbach W (2023) Investigation of acquired dysfibrinogenaemia in adult patients with sepsis using fibrinogen function vs. concentration ratios: a cross-sectional study.
Front. Med. 10:1294301.
doi: 10.3389/fmed.2023.1294301

Investigation of acquired dysfibrinogenaemia in adult patients with sepsis using fibrinogen function vs. concentration ratios: a cross-sectional study

Rosa Toenges[1]*, Michael Steiner[2], Christian Friedrich Weber[3,4] and Wolfgang Miesbach[1]

[1]Department of Medicine, Hemostaseology, University Hospital, Goethe University Frankfurt, Frankfurt, Germany, [2]Medizinisches Labor Rostock, Rostock, Germany, [3]Department for Anesthesiology, Intensive Care Medicine and Pain Therapy, University Hospital, Goethe University Frankfurt, Frankfurt, Germany, [4]Department for Anesthesiology, Intensive Care Medicine and Emergency Medicine, Asklepios Clinics Hamburg, AK Wandsbek, Hamburg, Germany

Introduction: Inherited or acquired molecular abnormalities form a clinically heterogeneous group of fibrinogen disorders called dysfibrinogenaemia. Apart from a pediatric case report and in contrast to other clinical conditions, acquired dysfibrinogenaemia has not been previously reported in septic patients.

Methods: In an observational cohort study, 79 adult septic patients were investigated for the presence of acquired dysfibrinogenaemia at the time of their admission to the intensive care unit (ICU) of the University Hospital Frankfurt. Following established recommendations, fibrinogen clotting activity vs. antigen ratios were analyzed using Clauss fibrinogen, prothrombin-derived fibrinogen, and radial immunodiffusion (RID) fibrinogen concentration.

Results: Prothrombin-derived fibrinogen levels were highest (527±182mg/dL) followed by Clauss fibrinogen (492±209mg/dL) and radial immunodiffusion fibrinogen (426±159mg/dL). Very few cases demonstrated hypofibrinogenaemia making overt disseminated intravascular coagulation (DIC) unlikely in the cohort investigated. Clauss/RID fibrinogen ratios were lower (1.17±0.19) compared to prothrombin time-derived/RID ratios (1.35±0.33). Using the Clauss/RID dataset, 21% of patients (16/76 patients) demonstrated values below a threshold ratio for suspected acquired dysfibrinogenaemia arbitrarily set at 1.0. In contrast, prothrombin-derived ratios were below the threshold in only 7% (4/58 patients).

Discussion: The results point to the presence of acquired dysfibrinogenaemia in part of adult septic patients. If confirmed in further studies, this may form part of a specific laboratory signature of a sepsis-associated coagulation phenotype.

KEYWORDS

fibrinogen, dysfibrinogenaemia, sepsis, disseminated intravascular coagulation (DIC), clauss/RID fibrinogen ratios, prothrombin time-derived/RID ratios

1 Introduction

Fibrinogen is a critically important plasma protein expressed by hepatocytes as a hexameric glycoprotein. Its main functions in primary and secondary hemostasis include fibrin clot formation, fibrin crosslinking by factor XIIIa, platelet aggregation, and fibrinolysis. Being a positive acute phase reactant, fibrinogen levels may increase 2–4-fold during an inflammation response.

Inherited or acquired structural abnormalities of fibrinogen are collectively referred to as dysfibrinogenaemia and may interfere with any of its multiple functions (1). Congenital fibrinogen disorders are rare and include hypo- and afibrinogenaemia, dysfibrinogenaemia, and combinations thereof. Clinical presentations are dominated by bleeding events of varying severity. However, thrombotic events occur in up to 20% of patients. The most common acquired fibrinogen disorder is hypofibrinogenaemia typically resulting from consumptive coagulopathy or hemodilution. Acute or chronic liver disease with reduced liver synthesis may also cause and contribute to hypo(dys) fibrinogenaemia. Additional causes of acquired dysfibrinogenaemia include drug therapy, malignancy, and the presence of anti-fibrinogen antibodies in autoimmune disorders. Patients may be asymptomatic or present with either bleeding and/or thrombotic events (2, 3).

The laboratory approach to congenital or acquired dysfibrinogenaemia relies on the use of different fibrinogen assays with the aim to demonstrate a critical difference between functional and antigen concentration levels. Screening tests including thrombin time and reptilase time may hint at the presence of dysfibrinogenaemia but are not performed routinely in the clinical laboratory. The diagnosis of dysfibrinogenaemia can be established by obtaining a fibrinogen activity vs. antigen ratio (4). For this purpose, most commonly thrombin-clottable fibrinogen is determined by the Clauss method or the fibrinogen activity is derived from the prothrombin time polymerization curve. Immunoreactive fibrinogen can be determined by different immunologic methods including enzyme-linked immunosorbent assays, turbidimetry/nephelometry, or radial immunodiffusion (5). Method-specific reference intervals should be considered and have an impact on resulting activity-antigen ratios. The diagnosis of inherited dysfibrinogenaemia can be verified at the molecular level using genetic analysis. In contrast, acquired dysfibrinogenaemia is considered a transient phenomenon with no specific molecular abnormalities (6).

Sepsis is a complex syndrome resulting from a dysregulated host response to infection. Increasing incidence and a high number of fatal outcomes are hallmarks of this life-threatening condition. Coagulation abnormalities have long been recognized to be present in virtually all patients with sepsis. They range from minimal activation of coagulation to overt disseminated intravascular coagulation (DIC) and variably contribute to sepsis morbidity and mortality. Diagnostic criteria for DIC were first established in 2001 and supplemented in 2019 with sepsis-induced coagulopathy (*SIC*) scoring system to detect the compensated phase of DIC. Hypofibrinogenaemia (<100 mg/dL) is an established diagnostic criterion for the overt DIC scoring system whereas no *SIC* range has as yet been added (7). Dysfibrinogenaemia is not included in the currently available scoring systems. Apart from a pediatric case report (8), the issue of acquired dysfibrinogenaemia in sepsis has not been approached. The aim of the present study was to investigate a clinical cohort of adult patients fulfilling the diagnostic criteria of sepsis for the presence of acquired dysfibrinogenaemia at the time of their admission to the intensive care unit (ICU) using three different fibrinogen assays and establishing fibrinogen activity-antigen ratios.

2 Materials and methods

2.1 Patients and study design

The present observational study was approved by the local ethics committee (protocol number 368/2010), and informed consent was obtained from all patients or their legal representatives, respectively. We included 79 adult patients, fulfilling the diagnostic criteria of sepsis according to the Consensus Criteria Sepsis-3 (9, 10), consecutively admitted to the ICU of the University Hospital Frankfurt. A cohort of 60 males and 19 females, aged between 18 and 80 years, was assessed for acquired dysfibrinogenaemia. Except for thromboprophylaxis with fixed-dosed subcutaneous low molecular weight heparin to 58 patients (74%), no further coagulation-directed drug therapy was administered. Patient characteristics are summarized in Table 1.

2.2 Fibrinogen assays

Plasma samples were obtained at the time of admission to the ICU from venous citrate-anticoagulated blood and stored in

TABLE 1 Characteristics of a cohort of 79 septic patients, at the time of admission to the intensive care unit.

Patient characteristics	Total cohort (*n* = 79)
Age, median, years	62
Sex, male, %	67
Reason for admission	
Cardiac	16
Hepatic	5
Abdominal	18
Renal	2
Pulmonary	30
Hematologic	3
Oncologic	5
Comorbidities, median (range)	3 (1–5)
Comorbidity conditions, % (*n*)	
Diabetes mellitus	22 (17)
Chronic pulmonary disease	11 (9)
Chronic kidney disease	19 (15)
Coronary heart disease	43 (34)
Malignancy	13 (10)
Liver disease	15 (12)
Neurologic disease	11 (9)
Rheumatoid arthritis/autoimmune disorder	6 (5)
Thromboprophylaxis, % (*n*)	74 (58)

aliquots at −80°C. Hemolytic, lipaemic, icteric, and turbid plasma samples were excluded for their potential interference with the fibrinogen assays. Samples demonstrating hemolysis, Thrombin-clottable fibrinogen was assessed using Clauss method reagents (STA Fibrinogen). Prothrombin time (PT)-derived fibrinogen was obtained from PT curves using PT reagents (STA Neoplastin Plus). The coagulation analyzer STAR-R Evolution (Stago) was used in automated mode according to the manufacturer's instructions including internal and external quality control assessment. Immunoreactive fibrinogen was analyzed by radial immunodiffusion using NOR-Partigen fibrinogen plates (Siemens), and readings were obtained at 18 and at 48 h after incubation, respectively, to not miss late diffusion endpoints. Fibrinogen ratios were calculated using Clauss fibrinogen and PTder fibrinogen, respectively, divided by RID fibrinogen. The "relative fibrinogen deficit" was obtained by subtracting fibrinogen activity from fibrinogen concentration.

3 Results

Using three methods, fibrinogen data were obtained from 79 adult septic patients consecutively admitted to the ICU. Two assays, clottable fibrinogen using the Clauss assay and prothrombin time-derived (PTder) fibrinogen, reflect functional fibrinogen levels whereas fibrinogen antigen concentration was determined by radial immunodiffusion (RID). For the lack of sufficient sample volume, prothrombin time-derived fibrinogen could not be analyzed for 21 patients. Prothrombin time-derived fibrinogen levels were highest (527 ± 182 mg/dL) followed by Clauss fibrinogen (492 ± 209 mg/dL), and radial immunodiffusion (426 ± 159 mg/dL). Hypofibrinogenaemia defined as fibrinogen below 100 mg/dL was detected in two cases by Clauss and RID assays, respectively. PTder fibrinogen was not below 100 mg/mL. Conversely, the proportion of hyperfibrinogenaemia defined as fibrinogen above 450 mg/dL was highest using PTder fibrinogen (65.5%) followed by Clauss fibrinogen (54.5%) and RID fibrinogen (48.7%; Table 2).

We next calculated fibrinogen activity vs. antigen ratios using Clauss fibrinogen and PTder fibrinogen, respectively, divided by RID fibrinogen. Clauss/RID fibrinogen ratios were lower (1.17 ± 0.19, $n = 76$) compared to PTder/RID fibrinogen ratios (1.35 ± 0.33, $n = 58$). Giving the expectation that fibrinogen activity should align with its concentration, a threshold ratio for suspected dysfibrinogenaemia was arbitrarily set at 1.0 with lower ratios being a possible indicator for the presence of dysfibrinogenemia. Using the Clauss/RID fibrinogen dataset, 16 of 76 ratios (21%) were below the threshold ranging from 0.81 to 0.99. In contrast, the PTder/RID fibrinogen dataset yielded only 4 of 58 ratios (7%) below the threshold ranging from 0.69 to 0.99 (Figure 1). The corresponding "relative fibrinogen deficit" (fibrinogen concentration minus fibrinogen activity) was between 4 and 158 mg/dL.

4 Discussion

The present observational cohort study aimed to investigate the presence of acquired dysfibrinogenaemia in adult septic patients at the time of their admission to the ICU. Fibrinogen clotting activity vs. antigen ratios were calculated for 97 patients. Using the Clauss fibrinogen/radial immunodiffusion dataset, 21% of patients (16/76 patients) demonstrated values below a threshold ratio for suspected acquired dysfibrinogenaemia. Prothrombin-derived fibrinogen ratios were below the threshold in only 7% (4/58 patients). Collectively, these

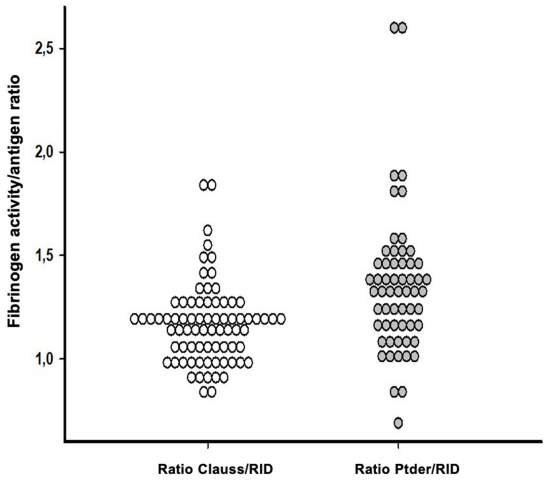


FIGURE 1
Fibrinogen activity vs. antigen ratios were calculated using Clauss fibrinogen (white) or PTder fibrinogen (gray), divided by RID in adult septic patients. Clauss/RID fibrinogen ratios were lower (1.17 ± 0.19, $n = 76$) compared to PTder/RID fibrinogen ratios (gray; 1.35 ± 0.33, $n = 58$). Ratios below the threshold of 1 were suspected as being a possible indicator for dysfibrinogenemia. PTder, Prothrombin time-derived fibrinogen; RID, Radial immunodiffusion (RID).

TABLE 2 Fibrinogen data obtained from adult septic patients ($n = 79$).

	Clauss	PTder	RID
n	77	58	78
Mean ± SD (mg/dL)	492 ± 209	527 ± 182	426 ± 159
Hypofibrinogenaemia (< 100 mg/dL), *n* (%)	2 (2.6)	n.d.	2 (2.6)
Range (mg/dL)	66–70		50–93
Hyperfibrinogenaemia (> 450 mg/dL), *n* (%)	42 (54.5)	38 (65.5)	38 (48.7)
Range (mg/dL)	457–1,026	453–940	452–736

Fibrinogen was analyzed as clottable fibrinogen (Clauss), as prothrombin time-derived fibrinogen (PTder), and as immunoreactive fibrinogen using radial immunodiffusion (RID). n.d., none detected.

results point to the occurrence of acquired dysfibrinogenaemia in part of adult septic patients.

Dysfibrinogenaemia comprise a group of inherited or acquired structurally abnormal fibrinogen molecules with variable pathophysiology and clinical features (1). Knowledge on inherited dysfibrinogenaemia has been steadily accumulating with most details gained from studies of rare bleeding disorders (3). In contrast, the issue of acquired dysfibrinogenaemia has not been comprehensively addressed in the past. Most studies were performed in patients with liver or biliary tract disease, with fewer studies and case reports on its presence in malignancy and other clinical conditions. The pathophysiology of acquired dysfibrinogenaemia in liver disease involves increased sialylation of carbohydrate side chains whereas cancer-associated dysfibrinogen is supposed to be synthesized and secreted by tumor cells (7). The pathogenesis of acquired dysfibrinogenaemia in sepsis has not been formally approached in studies. We propose that it may involve inflammation-induced abnormal glycosylation of plasma fibrinogen and/or yet unidentified additional posttranslational protein modifications. Clinically, it remains largely unknown if acquired dysfibrinogenaemia contributes to either bleeding or thrombotic events in addition to the disease-specific bleeding or thrombotic risk.

Being an example of significant cross talk between inflammation and coagulation, sepsis-induced coagulopathy is a continuum ranging from subclinical coagulopathy to overt, potentially fatal disseminated intravascular coagulation (DIC) (11) with a severely deranged hemostatic balance. Diagnostic criteria for overt, decompensated DIC were first established in 2001 and recently supplemented by the sepsis-induced coagulopathy (*SIC*) scoring system to allow for detecting earlier stages of compensated DIC in sepsis (7). With regard to fibrinogen, hypofibrinogenaemia defined as values below 100 mg/dL is only included in the overt DIC diagnostic scoring system. To the authors' best knowledge, the presence of acquired dysfibrinogenaemia in sepsis has not been systematically studied in the past. Only one study investigated a fibrinogen function vs. antigen ratio in sepsis and concluded that ratios were largely aberrant in septic patients compared to normal values with plasmin-mediated fibrin(ogen) breakdown products possibly contributing to over-proportionally decreased fibrinogen function versus antigen ratios (12).

We conducted an observational cohort study in adult septic patients at the time of their admission to the intensive care unit. Fibrinogen was investigated using three different methods. Hypofibrinogenaemia defined as values below 100 mg/dL were observed in only very few patients. As this finding would be consistent with overt DIC, this advanced sepsis-induced hemostatic derangement could be excluded for the majority of the patients investigated. However, it should be noted that fibrinogen was assessed only at a single time point instead of being followed sequentially (13).

Using a generally accepted diagnostic approach to suspected dysfibrinogenaemia employing a fibrinogen clotting activity to antigen ratio (4), we investigated its presence in 79 sepsis patients at the time of their admission to the intensive care unit. Fibrinogen assays were selected according to their availability in a routine coagulation laboratory. It should be emphasized that several methods have been developed to determine fibrinogen in plasma (5). Assays vary in technical principles, equipment and reagents requirements, degree of expertise, and turnaround time, to name but a few. Under routine conditions, most laboratories use either the Clauss fibrinogen assay or a prothrombin time-derived fibrinogen estimation. The Clauss assay's readout is clot formation (14) and it is generally accepted as a measure of clottable fibrinogen. Fibrinogen estimation derived from the prothrombin time is an attractive alternative as it provides a fibrinogen value at no extra time and cost (15). Depending on the method used and the cohort of patients under consideration, PT-derived fibrinogen concentrations have been reported increased compared to the Clauss method with varying discrepancies (6). Correlations between either fibrinogen value or *in vivo* hemostasis remain difficult to judge. True clottable fibrinogen assays are time consuming and technically difficult precluding their routine use. A number of immunological assays are available to measure fibrinogen concentration including ELISA, radial immunodiffusion, and nephelometry. The disadvantage for clinical interpretation is that a protein concentration is measured rather than functional activity. However, it is exactly this combination of measures that is required to investigate inherited or acquired dysfibrinogenaemia (4). Harmonization efforts notwithstanding (16), no consensus has been reached as to which methods should be preferentially employed for the investigation of dysfibrinogenaemia (17).

Confirming data from previous investigations, the present study yielded discrepant fibrinogen values being highest for PT-derived fibrinogen followed by Clauss fibrinogen and radial immunodiffusion. Accordingly, the percentage of hyperfibrinogenaemia ranked in the same order. Increased fibrinogen in a substantial number of septic patients is expected to result from its behavior as a positive acute phase reactant.

The focus of the present study was to investigate the presence of acquired dysfibrinogenaemia in adult septic patients using the concept of calculated fibrinogen activity versus antigen ratios. Clauss/RID ratios were lower compared to PT-derived/RID ratios. Applying a threshold ratio of 1.0 for suspected dysfibrinogenaemia, the Clauss/RID approach yielded 21% values below threshold compared to 7% using the PT-derived/RID ratio (Figure 1). It may be argued that the acute phase response would have a critical impact on the ratio concept. However, it has been previously demonstrated that the fibrinogen function vs. antigen concentration ratio can be applied regardless of the presence of an acute phase reaction (18). In contrast to previous studies using a fibrinogen activity vs. antigen ratio threshold of 0.7 to identify dysfibrinogenaemia, the present analysis considered a threshold ratio of 1.0 with the aim to demonstrate substantial ratio heterogeneity within the dataset (Figure 1), and identified a subset of patients with evidence of dysfibrinogenaemia. Receiver operating characteristic (ROC) analyses to compare different threshold ratios were beyond the scope of the present analysis.

If validated by independent studies, our results point to a significant proportion of adult septic patients displaying evidence of acquired dysfibrinogenaemia. It should be emphasized that this potential hemostatic derangement occurs before fibrinogen consumption reaches the limit of hypofibrinogenaemia as defined by values below 100 mg/dL and may therefore be part of sepsis-induced coagulopathy (*SIC*). Data from the present study apply only for the methods employed as variability among fibrinogen methods is well known (5) and have an impact on calculated ratios

between any two of them. This is further exemplified in the present study by the varying proportion of acquired dysfibrinogenaemia depending on the fibrinogen activity assay used for calculating ratios. Future studies may consider cross-calibrating various fibrinogen assays used. This would equally apply to both functional as well as immunological assays. However, this approach would be beyond the manufacturers' assay specification and require validation efforts. Nevertheless, improved comparability between fibrinogen assays may thus be obtained and equimolarity with a fibrinogen activity vs. antigen ratio of 1.0 may form the basis for future studies in both inherited and acquired dysfibrinogenaemia. A different approach has recently been selected with a fibrinogen ratio obtained from two functional assays to identify inherited dysfibrinogenaemia (19) However, this concept has not yet been employed for acquired dysfibrinogenaemia.

The functional implications of sepsis-induced acquired dysfibrinogenaemia remain unknown and call for confirmatory studies with a focus on fibrin structure and clot properties in septic patients. Viscoelastic tests including thromboelastography (TEG) and rotational thromboelastography (ROTEM) should be added in future *SIC* studies focusing on acquired dysfibrinogenaemia (20). We propose that acquired dysfibrinogenaemia in part of septic patients may belong to a laboratory signature of a sepsis-associated coagulation phenotype. It remains to be investigated if this will translate into a risk indicator for clinical outcome.

In contrast to inherited dysfibrinogenaemia, acquired dysfibrinogenaemia has been less extensively studied (1). Except for a pediatric case report (19), the lack of studies in sepsis appears surprising given the central role of fibrinogen in inflammation (21). Although not focusing on dysfibrinogenaemia, a recent study compared Clauss and PT-derived fibrinogen with specific categories of disease and concluded that both methods are not interchangeable for certain clinical conditions raising the possibility of dysfibrinogenaemia (22). Another study investigated hemostatic impairment in postpartum hemorrhage being a specific form of DIC and reported evidence of acquired (hypo)dysfibrogenaemia in acute obstetric coagulopathy (23). Collectively, these data call for further studies to more fully characterize the occurrence and clinical significance of acquired dysfibrinogenaemia in a wider variety of clinical settings.

Limitations of the present study include:

First, the study included only a single point of analysis at the time of admission of adult septic patients to the ICU. Dynamic studies with multiple analyses are required to follow the natural history of acquired dysfibrinogenaemia and specifically to answer the question if this condition will resolve with clinical improvement. Second, data reported in the present study apply only to the fibrinogen methods used and future studies should include additional fibrinogen methods for comparison. Specifically, more sophisticated immunological assays should be used to allow for high throughput and short turnaround times. Third, the possible importance of acquired dysfibrinogenaemia on *in vivo* hemostasis in septic patients is at present unknown and should be investigated in future studies by adding measures of fibrin and clot properties including thrombin and reptilase times, D-dimer, and/or other fibrin(ogen) degradation products. Similarly, clinical outcome measures should be determined in adult septic patients in the presence of acquired dysfibrinogenaemia.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Institutional Review Boards of the UCT and the Ethical Committee at the University Hospital Frankfurt. The studies were conducted in accordance with or their legal representatives local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MS: Data curation, Formal analysis, Software, Writing – original draft. CW: Investigation, Project administration, Supervision, Writing – review & editing. WM: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. RT: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We thank all medical technologists and support staff of Department of Medicine, Hemostaseology, University Hospital Frankfurt.

Conflict of interest

WM has received research support and paid consultancies from Bayer, Biomarin, Biotest, CSL Behring, Chugai, Freeline, LFB, Novo Nordisk, Octapharma, Pfizer, Regeneron, Roche, Sanofi, Takeda/Shire, and uniQure.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. May JE, Wolberg AS, Lim MY. Disorders of Fibrinogen and fibrinolysis. *Hematol Oncol Clin North Am*. (2021) 35:1197–217. doi: 10.1016/j.hoc.2021.07.011

2. Casini A, de Moerloose P, Neerman-Arbez M. Clinical features and Management of Congenital Fibrinogen Deficiencies. *Semin Thromb Hemost*. (2016) 42:366–74. doi: 10.1055/s-0036-1571339

3. Casini A, Blondon M, Lebreton A, Koegel J, Tintillier V, de Maistre E, et al. Natural history of patients with congenital dysfibrinogenemia. *Blood*. (2015) 125:553–61. doi: 10.1182/blood-2014-06-582866

4. Cunningham MT, Brandt JT, Laposata M, Olson JD. Laboratory diagnosis of dysfibrinogenemia. *Arch Pathol Lab Med*. (2002) 126:499–505. doi: 10.5858/2002-126-0499-LDOD

5. Mackie IJ, Kitchen S, Machin SJ, Lowe GDO. Hemostasis and thrombosis task force of the British Committee for Standards in Haematology. Guidelines on fibrinogen assays. *Br J Haematol*. (2003) 121:396–404. doi: 10.1046/j.1365-2141.2003.04256.x

6. Mackie J, Lawrie AS, Kitchen S, Gaffney PJ, Howarth D, Lowe GDO, et al. A performance evaluation of commercial fibrinogen reference preparations and assays for Clauss and PT-derived fibrinogen. *Thromb Haemost*. (2002) 87:997–1005. doi: 10.1055/s-0037-1613124

7. Iba T, Levi M, Thachil J, Helms J, Scarlatescu E, Levy JH. Communication from the scientific and standardization Committee of the International Society on thrombosis and Haemostasis on sepsis-induced coagulopathy in the management of sepsis. *J Thromb Haemost*. (2023) 21:145–53. doi: 10.1016/j.jtha.2022.10.022

8. Torres M, Lucia JF, Giralt M, Pérez Lungmus G, Ercoreca L, Raichs A. Acquired dysfibrinogenaemia in a newborn with bacterial sepsis (author's transl). *Sangre*. (1976) 21:399–406.

9. Singer M, Deutschman CS, Seymour C, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (sepsis-3). *J Am Med Assoc*. (2016) 315:801–10. doi: 10.1001/jama.2016.0287

10. Dellinger RP, Levy M, Rhodes A, Annane D, Gerlach H, Opal SM, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med*. (2013) 39:165–228. doi: 10.1007/s00134-012-2769-8

11. Giustozzi M, Ehrlinder H, Bongiovanni D, Borovac JA, Guerreiro RA, Gąsecka A, et al. Coagulopathy and sepsis: pathophysiology, clinical manifestations and treatment. *Blood Rev*. (2021) 50:100864. doi: 10.1016/j.blre.2021.100864

12. Stief TW, Ijagha O, Weiste B, Herzum I, Renz H, Max M. Analysis of hemostasis alterations in sepsis. *Blood Coagul Fibrinolysis*. (2007) 18:179–86. doi: 10.1097/MBC.0b013e328040bf9a

13. Levi M, Meijers JC. DIC: which laboratory tests are most useful. *Blood Rev*. (2011) 25:33–7. doi: 10.1016/j.blre.2010.09.002

14. Clauss A. Rapid physiological coagulation method in determination of fibrinogen. *Acta Haematol*. (1957) 17:237–46. doi: 10.1159/000205234

15. Rossi E, Mondonico P, Lombardi A, Preda L. Method for the determination of functional (clottable) fibrinogen by the new family of ACL coagulometers. *Thromb Res*. (1988) 52:453–68. doi: 10.1016/0049-3848(88)90029-1

16. Casini A, Undas A, Palla R, Thachil J, de Moerloose PSubcommittee on Factor XIII and Fibrinogen. Diagnosis and classification of congenital fibrinogen disorders: communication from the SSC of the ISTH. *J Thromb Haemost*. (2018) 16:1887–90. doi: 10.1111/jth.14216

17. Miesbach W, Schenk J, Alesci S, Lindhoff-Last E. Comparison of the fibrinogen Clauss assay and the fibrinogen PT derived method in patients with dysfibrinogenemia. *Thromb Res*. (2010) 126:e428–33. doi: 10.1016/j.thromres.2010.09.004

18. Krammer B, Anders O, Nagel HR, Burstein C, Steiner M. Screening of dysfibrinogenaemia using the fibrinogen function versus antigen concentration ratio. *Thromb Res*. (1994) 76:577–9. doi: 10.1016/0049-3848(94)90287-9

19. Xiang L, Luo M, Yan J, Liao L, Zhou W, Deng X, et al. Combined use of Clauss and prothrombin time-derived methods for determining fibrinogen concentrations: screening for congenital dysfibrinogenemia. *J Clin Lab Anal*. (2018) 32:e22322. doi: 10.1002/jcla.22322

20. Tsantes AG, Parastatidou S, Tsantes EA, Bonova E, Tsante KA, Mantzios PG, et al. Sepsis-induced coagulopathy: an update on pathophysiology, biomarkers, and current guidelines. *Lifestyles*. (2023) 13:350. doi: 10.3390/life13020350

21. Luyendyk JP, Schoenecker JG, Flick MJ. The multifaceted role of fibrinogen in tissue injury and inflammation. *Blood*. (2019) 133:511–20. doi: 10.1182/blood-2018-07-818211

22. Guven B, Can M, Tekin A. Comparison of Fibrinogen concentrations determined by the Clauss method with prothrombin-derived measurements on an automated Coagulometer. *J Appl Lab Med*. (2022) 7:1337–45. doi: 10.1093/jalm/jfac066

23. de Lloyd L, Jenkins PV, Bell SF, Mutch NJ, Martins Pereira JF, Badenes PM, et al. Acute obstetric coagulopathy during postpartum hemorrhage is caused by hyperfibrinolysis and dysfibrinogenemia: an observational cohort study. *J Thromb Haemost*. (2023) 21:862–79. doi: 10.1016/j.jtha.2022.11.036

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 11 January 2024
DOI 10.3389/fmed.2023.1344769

OPEN ACCESS

EDITED BY
Felix Carl Fabian Schmitt,
Heidelberg University Hospital, Germany

REVIEWED BY
Vivienne Theobald,
Heidelberg University Hospital, Germany
Chiara Middel,
Heidelberg University Hospital, Germany

*CORRESPONDENCE
Larissa Henze
larissa.henze@med.uni-rostock.de

RECEIVED 26 November 2023
ACCEPTED 15 December 2023
PUBLISHED 11 January 2024

CITATION
Henze L, Grunwald L, Felser S, Witte M, Grosse-Thie C, Roolf C, Murua Escobar H and Junghanss C (2024) Abdominal venous thromboses: detection of the *JAK2* p.V617F mutation by next-generation ultradeep sequencing—A prevalence study of patients in Mecklenburg-West Pomerania (2017–2021). *Front. Med.* 10:1344769. doi: 10.3389/fmed.2023.1344769

Abdominal venous thromboses: detection of the *JAK2* p.V617F mutation by next-generation ultradeep sequencing—A prevalence study of patients in Mecklenburg-West Pomerania (2017–2021)

Larissa Henze[1]*, Luise Grunwald[1], Sabine Felser[1], Maria Witte[2], Christina Grosse-Thie[1], Catrin Roolf[1], Hugo Murua Escobar[1] and Christian Junghanss[1]

[1]Department of Medicine, Clinic III—Hematology, Oncology, and Palliative Medicine, Rostock University Medical Center, Rostock, Germany, [2]Department of General, Visceral, Thoracic, Vascular, and Transplant Surgery, Rostock University Medical Center, Rostock, Germany

Background: Abdominal venous thromboses are rare thrombotic events with heterogeneous etiologies. They are related to myeloproliferative neoplasms (MPNs) in some patients and can occur as first signs of the disease. MPNs are characterized by mutations in the genes of Janus kinase 2 (JAK2), myeloproliferative leukemia virus oncogene (MPL), and calreticulin (CALR).

Methods: Within the prospective trial "Prevalence of JAK2 mutations in patients with abdominal venous thromboses" (JAK2 MV study; German Clinical Trials Register: DRKS00026943), the peripheral blood of patients with abdominal venous thromboses in Mecklenburg-West Pomerania, a federal state located in north-east Germany, was analyzed by next-generation ultradeep sequencing for MPN-associated mutations. Clinical characteristics and blood cell counts were also of interest. The primary endpoint was the detection of the mutation *JAK2* p.V617F. Secondary endpoints were the detection of other acquired variants of JAK2, as well as MPL and CALR.

Results: A total of 68 patients with abdominal venous thromboses were included from February 2017 to January 2021, with splanchnic veins affected in 65 patients. The mutation *JAK2* p.V617F was present in 13 patients (19%), with four patients showing low variant allele frequencies (VAF 0.1% to 1.9%). The time interval from the thrombotic event to analysis was longer for patients with the mutation. The mutation *MPL* p.W515R was detected in three cases, all of them with low VAF. One patient among them had a concurrent mutation of *JAK2* p.V617F. The mutations *CALR* type I or type II were not found.

Discussion: By analyzing peripheral blood for the mutation *JAK2* p.V617F, an important cause of these rare thrombotic events can be identified. The development of a diagnostic workup with next-generation ultradeep sequencing for the analysis of the *JAK2* p.V617F mutation and further mutations has the potential to better understand the etiology of abdominal venous thromboses in

individual patients in regional clinical care, as abdominal venous thromboses are diagnosed by various medical disciplines.

KEYWORDS

abdominal venous thromboses, splanchnic vein thrombosis, JAK2 mutation, ultradeep sequencing, anticoagulation

Introduction

Abdominal venous thromboses are rare events (1). In the literature, hepatic vein thromboses (also known as Budd-Chiari syndrome), portal vein thrombosis, splenic vein thrombosis, and mesenteric vein thromboses are grouped as splanchnic vein thromboses (SVT) (1–4). Incidences range from 0.7 per million people per year for hepatic vein thromboses to 70 per million people for portal vein thromboses (5–7). Thromboses in the abdominal region may also involve the vena cava inferior and other abdominal veins such as renal or ovarian veins. Multiple causes and triggers of abdominal venous thromboses have been described: cirrhosis, inflammatory disease, particularly pancreatitis and inflammatory bowel disease (8), abdominal surgery (9), malignancies (8), myeloproliferative neoplasms (MPN) (1, 10), and congenital or acquired coagulation disorders (1, 11). Abdominal venous thromboses can be complicated by acute sequelae such as liver failure, gastrointestinal bleeding (1), intestinal infarction (12), perforation, peritonitis, and sepsis (11) and can lead to chronic complications, mostly the formation of varices with bleeding risk or re-thrombosis (1, 13). Therefore, in addition to the diagnosis of abdominal venous thrombosis, clarification of causality is fundamental for further treatment decisions and clinical outcomes (2).

The study "Prevalence of JAK2 Mutations in Patients with Abdominal Venous Thrombosis" (JAK2 MV Study) prospectively investigated patients with abdominal venous thromboses in the federal state of Mecklenburg-West Pomerania (MV) in north-east Germany (see Supplementary Figure 1) for the presence of mutations in the gene of Janus kinase 2 (JAK2). The mutation *JAK2* p.V617F represents one of the major diagnostic criteria of MPN (14, 15) and is frequently found in patients with abdominal venous thromboses. JAK2 is a non-receptor tyrosine kinase (16). Activated JAK2 generates the transcription factors known as "Signal transducers and activators of transcription." This JAK-STAT pathway is involved in the proliferation, differentiation, and self-renewal of the hematopoietic system. The mutation *JAK2* p.V617F constitutively activates the tyrosine kinase (16), leading to increased erythropoiesis and thrombopoiesis and thus resulting in the phenotype MPN (17). Other known driver mutations of MPN, though less commonly detected, exist for myeloproliferative leukemia virus oncogene (*MPL* p.W515R) and calreticulin (*CALR* type I and *CALR* type II) (18). Both of these also result in the activation of the JAK-STAT pathway: mutations in the MPL gene that encodes the thrombopoietin receptor by affecting the intracellular domain of the protein followed by ligand-independent signaling through JAK2, mutations in the CALR gene that alter binding of CALR to MPL associated with JAK2, and thereby over-activating the JAK-STAT signaling pathway (19–22). Both mutations of MPL and CALR were included in the JAK2 MV study as secondary endpoints.

The implementation of the study in the federal state of Germany allows for the depiction of the reality of clinical care for patients with abdominal venous thromboses in a defined region with existing data about population and infrastructure. It also helps to better characterize one of the main causes of abdominal venous thromboses. Potentially, it can lay the groundwork for the development of a diagnostic workup in general clinical care in this area.

Methods

Study design

The investigator-initiated JAK2 MV trial was a prospective cohort study to analyze patients in MV with abdominal venous thromboses for the presence of acquired single nucleotide variants in the JAK2 gene. The primary endpoint was the detection of the *JAK2* p.V617F mutation. Secondary endpoints were the detection of other acquired variants of JAK2, as well as the mutations *MPL* p.W515R and *CALR* type I and type II, which are also part of the diagnostic criteria of MPN (14, 15).

Patients in MV with thromboses in hepatic veins, portal veins, splenic veins, mesenteric veins, and/or other abdominal veins were included in the study, regardless of the timepoint of the thrombotic event. For this study, all thrombotic events within 1 month before inclusion were classified as recent events, whereas the others were termed past events. Thrombosis had to be confirmed by imaging [ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI)] or surgically for inclusion in the study. Written informed consent by the patient was required. Patients < 18 years were excluded.

A total of 5 ml of peripheral blood (anticoagulant EDTA) was collected for next-generation sequencing (NGS) and a blood smear for cytology. A standardized recording of demographic, clinical, and laboratory parameters using a questionnaire was within the scope of the study.

The organization of the study, data collection, and analysis, as well as laboratory work (NGS and cytology) were performed at Clinic III—Hematology, Oncology, and Palliative Medicine, Rostock University Medical Center.

The Ethics Committee of Rostock University Medical Center has approved the trial (approval date: 22 November 2016, A 2016–0200), and the trial is registered in the German Clinical Trials Register (DRKS00026943).

Study implementation

At the beginning of the study, all departments of internal medicine ($n = 27$) and surgery ($n = 28$) at hospitals in MV with at least 80 beds, all specialists in hematology and oncology ($n = 17$), and ~7 months after the start of the study, all specialists in gastroenterology ($n = 20$) were informed about the study by post. In addition to a general brochure about the trial, a patient information sheet, an informed consent form, and a clinical questionnaire were included in the postal mailing. The trial was also promoted at regional educational and scientific meetings.

Patients were informed and included by their treating physicians, who also collected peripheral blood and answered the clinical questionnaire. Blood samples were sent by standard mail. Only upon receipt of the blood sample, together with a valid informed consent form and clinical questionnaire, the analysis of the blood sample was proceeded within the study. Sample processing and analysis were performed pseudonymously.

In cases where a mutation was identified, the respective physician was informed by phone and by written report.

Next-generation sequencing

A dedicated targeted sequencing gene panel for NGS was designed and validated for use in routine diagnostics. In addition to the total protein-coding sequences of JAK2, the panel also captures the total protein-coding sequences of MPL and CALR. Nucleic acid segments with known mutation hotspots were covered by at least two amplicons in the panel design. Nucleic acid isolation as well as the performance of NGS are outlined in the study by Grunwald et al. (23). Generally, in routine diagnostics for conventional applications, a sensitivity of NGS of approximately 2% is described (24). Here, validation of the above-described NGS panel detected variant allele frequencies (VAF) of 1% and below as the condition of high amplicon coverage (> 2,000) was fulfilled as ultradeep sequencing.

Statistical analysis

Descriptive participants' characteristics were calculated. Interval-scaled data were tested for normal distribution using the Shapiro-Wilk test. Mean differences were tested using Fisher's exact test or the Mann–Whitney U-test. The significance level was set at $p \leq 0.050$. SPSS 25.0 (SPSS Inc., Chicago, IL, United States) was used for the analysis.

Results

Study participants

Between 22 February 2017 and 31 January 2021, 73 blood samples were submitted, of which 68 could be included and analyzed in the study (Figure 1). From Rostock originated 84% of samples, and 16% were from different locations in MV ($n = 4$ from Ribnitz-Damgarten, $n = 2$ from Greifswald, $n = 2$ from Stralsund, $n = 1$ from Neubrandenburg, $n = 1$ from Waren, and $n = 1$ from Wismar) (see Supplementary Figure 1). Figure 2 lists the medical disciplines that submitted blood samples.

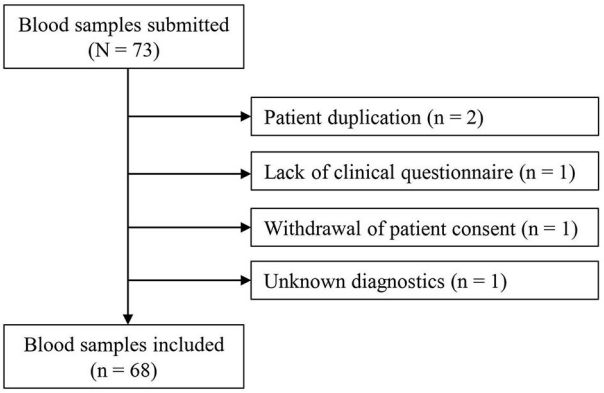


FIGURE 1
Flowchart of the study. Unknown diagnostics: the diagnostic method for the detection of the thrombosis was not known (Thrombosis had to be confirmed by imaging or surgically for inclusion in the study).

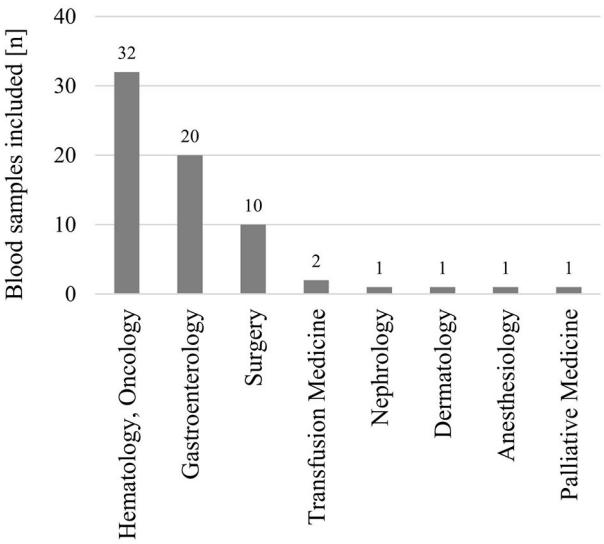


FIGURE 2
Medical disciplines that submitted blood samples to be included in the study ($n = 68$).

Patients' characteristics are summarized in Table 1. Overall, 40 (59%) patients were male, 28 (41%) were female. The median age was 62.0 years (range 28–84 years) for male patients and 53.5 years (range 29–80 years) for female patients. In 65 of the 68 patients with abdominal venous thromboses (96%), splanchnic veins were involved. Thrombosis was most commonly localized in the portal vein ($n = 52$), followed by the splenic vein ($n = 27$) and mesenteric veins ($n = 27$). In 33 patients, thromboses were located in two or more abdominal veins. In total, 37 patients had a recent thrombotic event. For the other patients, the thrombotic diagnosis lasted from 32 days to 20 years (in the mean 12 months).

10.3389/fmed.2023.1344769

TABLE 1 Characteristics of study participants ($n = 68$).

	Total	*JAK2* p.V617F negative	*JAK2* p.V617F positive		*p* (negative vs. positive)
	$n = 68$	$n = 55$	VAF 0.1–1.9 % $n = 4$	VAF $\geq$ 2 % $n = 9$	
Sex, *n* (%)					0.123
Female	28 (41)	20 (36)	3 (75)	5 (56)	
Male	40 (59)	35 (64)	1 (25)	4 (44)	
Age (years)					0.167
Median (min–max)	61 (28–84)	60 (28–84)	69 (60–77)	64 (44–73)	
Thrombotic site, *n*					
Portal vein	52	40	3	9	0.636
Hepatic vein	5	4	0	1	0.22
Splenic vein	27	22	1	3	0.502
Mesenteric vein	27	22	1	3	0.553
SVT	65	53	3	9	0.353
Other veins	9	6	2	1	0.327
Single vs. multiple sites, *n* (%)					0.107
Single site	26 (38)	23 (42)	2 (50)	1 (11)	
Multiple sites	33 (49)	27 (49)	2 (50)	4 (44)	
Unknown	9 (13)	5 (9)	0	4 (44)	
Date of thrombotic event, *n* (%)					**0.041***
</= 1 month	37 (54)	33 (60)	2 (50)	2 (22)	
>1 month	29 (43)	20 (36)	2 (50)	7 (78)	
Unknown	2 (3)	2 (4)	0	0	
Treatment, *n*					
Surgery	8	4	1	3	**0.041***
Thrombolysis°	3	3	0	0	
Anticoagulation	54	45	4	5	0.13
Not specified	7	6	0	1	
Hepatomegaly, *n* (%)					0.142
Yes	10 (15)	9 (16)	0	1 (11)	
No	49 (72)	40 (73)	4 (100)	5 (56)	
Unknown	6 (9)	3 (6)	0	3 (33)	
Not specified	3 (4)	3 (6)	0	0	
Splenomegaly, *n* (%)					0.373
Yes	19 (28)	15 (27)	0	4 (44)	
No	41 (60)	34 (62)	4 (100)	3 (33)	
Unknown	5 (7)	3 (6)	0	2 (22)	
Not specified	3 (4)	3 (6)	0	0	
Previous thrombosis, *n* (%)					0.239
Yes	7 (10)	4 (7)	0	3 (33)	
No	44 (65)	37 (67)	3 (75)	4 (44)	
Unknown	14 (21)	12 (22)	1 (25)	1 (11)	
Not specified	3 (4)	2 (4)	0	1 (11)	

(Continued)

TABLE 1 (Continued)

	Total	*JAK2* p.V617F negative	*JAK2* p.V617F positive		*p* (negative vs. positive)
	$n = 68$	$n = 55$	VAF 0.1–1.9 % $n = 4$	VAF $\geq$ 2 % $n = 9$	
Thrombophilia, *n* (%)					1
Yes	3 (4)	3 (6)	0	0	
No	37 (54)	30 (55)	2 (50)	5 (56)	
Unknown	27 (40)	22 (40)	2 (50)	3 (33)	
Not specified	1 (2)	0	0	1 (11)	
Malignancy, *n* (%)					0.291
Yes°°	21 (31)	19 (35)	1 (25)	1 (11)	
No	34 (50)	25 (46)	2 (50)	7 (78)	
Unknown	12 (18)	10 (18)	1 (25)	1 (11)	
Not specified	1 (2)	1 (2)	0	0	
Liver cirrhosis, *n* (%)					0.193
Yes	8 (12)	5 (9)	0	3 (33)	
No	49 (72)	42 (76)	2 (50)	5 (56)	
Unknown	11 (16)	8 (15)	2 (50)	1 (11)	

SVT, splanchnic vein thrombosis; VAF, variant allele frequency. °No systematic recording of lysis therapy in the JAK2-MV trial. °° Malignancies reported in patients without detection of the JAK2 p.V617F mutation: (suspected) pancreatic cancer (n = 6), lymphoma, carcinoma of unknown primary (suspected,) gastric cancer (suspected), lung cancer (suspected), cholangiocellular carcinoma, lymphoma (n = 2 each), rectal cancer, intraductal papillary mucinous neoplasia, liver tumor, chronic lymphocytic leukemia (n = 1 each). Malignancies reported in patients with the mutation JAK2 p.V617F are listed in Table 2. *Statistical significant.

Data on the clinical situation were presented by the referring physician and recorded as text on the questionnaire. The presenting symptoms were variable. Pain, gastrointestinal complaints, and liver-associated symptoms were most often mentioned. Of note, the diagnosis was incidental (no symptoms) in 13 patients.

CT was used most frequently ($n = 54$), followed by ultrasound ($n = 21$) and MRI ($n = 5$) as diagnostic procedures. Multiple methodologies were applied to 13 patients. In three patients, diagnosis was established during surgery.

As therapeutic procedure, surgery was performed on eight patients (12%) (Table 1). The use of anticoagulation was reported for 54 (79%) patients in total (Table 1) with the following distribution: low-molecular-weight heparins (LMWH) ($n = 24$), heparin ($n = 3$), phenprocoumon ($n = 7$), direct oral anticoagulants ($n = 10$), and unspecified ($n = 6$). Furthermore, enoxaparin in prophylactic dosage was mentioned for two patients, as was clopidogrel ± acetylsalicylic acid for two patients. For three patients, information about thrombolysis was listed. It has to be noted, though, that this treatment option was not systematically queried in the standardized reporting form (Table 1).

Prevalence of the mutation *JAK2* p.V617F

The mutation *JAK2* p.V617F was detected in 13 of 68 study participants (VAF 0.2–43%), corresponding to 19%. Four of the thirteen patients had VAF between 0.1% and 1.9%, which is below the conventional cut-off used for routine diagnostics by NGS. The characteristics of study participants with the mutation *JAK2* p.V617F are summarized in Table 2. Strikingly, all but one study participant with the mutation had portal vein thrombosis. Five patients also had thromboses in other abdominal veins, and two patients had experienced a previous thrombotic event. Tumor diseases were reported in these patients as "suspected leukemia" (MPN38; VAF 43.0%), possibly MPN, and carcinoma of the esophagogastric junction (MPN36; VAF 1.4%).

Table 1 presents the characteristics of the total study population for patients without mutation of *JAK2* p.V617F and for patients with *JAK2* p.V617F mutation (VAF 0.1–1.9% and VAF $\geq$ 2%, respectively). Patients with a mutation of *JAK2* p.V617F, regardless of VAF, were summarized for statistical analysis. Individuals with the mutation were more likely to be female and older. Patients with the mutation also tended more often to have suffered from a previous thrombotic event or to have liver cirrhosis, whereas malignancy was less commonly suspected. Hepatomegaly and splenomegaly were comparable between patients with and without the mutation of *JAK2* p.V617F. Figure 3 visualizes the findings of the hemoglobin and platelet counts of the three groups, showing a scattering of the values without classification of the groups. Patients with the mutation *JAK2* p.V617F had a significantly longer history of abdominal venous thromboses. They were significantly more often treated by surgery (4 of 13 patients). Nine of the thirteen patients with the mutation *JAK2* p.V617F received anticoagulants.

TABLE 2 Patients with the mutation *JAK2* p.V617F: site of thrombosis, previous thrombotic event, malignancy, and liver cirrhosis.

Code	*JAK2* p.617F VAF (%)	Portal vein	Hepatic vein	Splenic vein	Mesenteric vein	Other veins	Previous thrombosis	Suspected malignancy	Liver cirrhosis
MPN38	43.0	+	Unknown	Unknown	Unknown	Unknown	+	+ [4]	+
MPN18	31.4	+	Unknown	Unknown	Unknown	Unknown	Not specified	-	-
MPN43	28.4 [6]	+	Not specified	Not specified	Not specified	Not specified	-	-	+
MPN30	24.6	+	Unknown	Unknown	Unknown	Unknown	-	Unknown	Unknown
MPN42	17.3	+	+	+	Not specified	-	-	-	-
MPN11	13.2	+	Unknown	+	+	+ [1]	Unknown	-	-
MPN44	13.2	+	-	-	-	-	+	-	-
MPN67	12.3	+	-	-	+	-	+	-	+
MPN72	4.5	+	-	+	+	-	-	-	-
MPN36	1.4	-	-	-	-	+ [2]	-	+ [5]	-
MPN34	0.6	+	-	+	+	Not specified	Unknown	Unknown	Unknown
MPN45	0.3	+	-	-	-	+ [3]	-	-	-
MPN28	0.2	+	Not specified	Not specified	Not specified	Not specified	-	-	Unknown

VAF, variant allele frequency; +, yes; –, no. [1] venous confluence; [2] left renal veins; [3] plexus venosus uterinus. [4] suspected leukemia; [5] carcinoma of the esophagogastric junction. [6] in addition *MPL* p.W515R with VAF 0.2%.

10.3389/fmed.2023.1344769

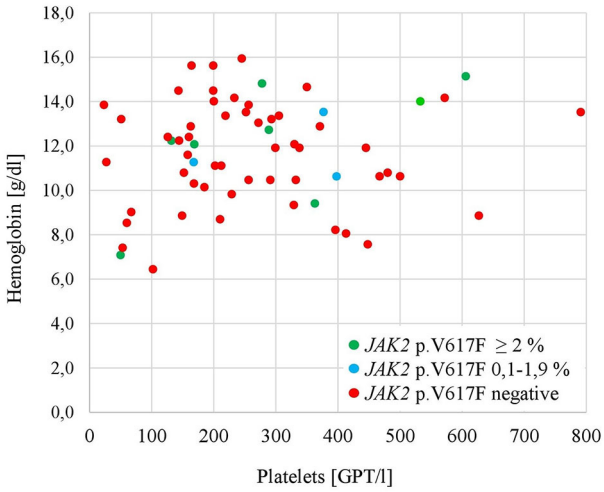


FIGURE 3
Hemoglobin and platelet count for blood samples included in the study ($n = 68$).

Prevalence of the mutations *MPL* p.W515R and *CALR* type I and type II

Mutations of *MPL* p.W515R were detected in three cases with VAFs of 1.2%, 0.4%, and 0.2%, thus showing low VAF. The latter patient had a concurrent mutation of *JAK2* p.V617F (MPN43; VAF 28.4%) (Table 2). CALR mutations were not found.

Discussion

In the literature, in 50%−75% of patients with abdominal venous thromboses, the etiology could be identified (8, 11, 22). In addition to thrombophilia, acquired local and systemic pathophysiological factors are responsible; among the latter, MPNs are of particular importance (3, 22). MPNs associated with abdominal venous thromboses predominantly show the mutation *JAK2* p.V617F (2). In the prospective study "Prevalence of JAK2 mutations in patients with abdominal venous thrombosis" (JAK2 MV study) described here, a prevalence of 19% was found for the mutation *JAK2* p.V617F, whereas the mutation *MPL* p.W515R was only detected in two additional patients (3%) with low VAF. This is in line with other reports about the distribution of the driver mutations in the genes of JAK2, MPL, and CALR in patients with SVT with and without MPN (25–27). While all driver mutations can cause hyperproliferation of myeloid progenitor cells, the mutation *JAK2* p.V617F has been described to contribute to a prothrombotic state by different mechanisms, like enhanced vascular and intercellular adhesion of neutrophils and increased formation of neutrophil extracellular traps (18, 28–30), all of which have not yet been fully elucidated (31). However, as shown in this study and others, the association between the mutation *JAK2* p.V617F and abdominal venous thromboses is clinically evident. Ageno et al. (1) reported a frequency of *JAK2* p.V617F of 20.1 % in a multicentric cohort of patients with SVT with thrombotic events ranging back < 6 months. In the prospective multicenter pilot study for rivaroxaban as a treatment for SVT, *JAK2* p.V617F was detected in 13 of 50 patients tested (26%); patients with Budd-Chiari syndrome or liver cirrhosis were excluded (32). In a monocentric study from 1994 to 2021, published by Colaizzo et al. (13), *JAK2* p.V617F was found in 29.8% by systematic screening of 152 patients at the time of diagnosis of SVT without concomitant cirrhosis or carcinoma. In this study, patients were followed up (median for 64 months, range 1–214 months) for both detection of *JAK2* p.V617F and diagnosis of MPN: 9 of 13 patients with the initial finding of *JAK2* p.V617F and 6 of 8 patients with detection of *JAK2* p.V617F during follow-up were diagnosed with MPN during the course of the study. Analysis of *JAK2* p.V617F was performed using real-time quantitative PCR; the detection limit was defined at 1.5%, but information on VAF was not given.

In the JAK2 MV study, ultradeep sequencing was used to detect and quantify the mutation *JAK2* p.V617F. Given the sensitivity of ultradeep sequencing, VAF from 0.1% to 1.9% could also be described. In the study cohort, four patients showed *JAK2* p.V617F with VAF in this range, and nine patients showed VAF >/= 2% (range 4.5%−43.0%). Patients with evidence of the mutation were diagnosed earlier with abdominal venous thromboses, in line with the observation of Colaizzo et al. (13). How et al. (2, 33) postulate that abdominal venous thromboses are early events, preceding the diagnosis of MPN in some patients. In a Danish population study with nearly 20,000 participants, Cordua et al. (17) showed an increased odds ratio for venous and arterial thromboses in case *JAK2* p.V617F was present with low VAF without a diagnosis of MPN. In light of this, the high sensitivity of the sequencing method is particularly important. In parallel, within the JAK2 MV study, the presence of *JAK2* p.V617F in patients was not associated with hepatomegaly, splenomegaly, or blood count abnormalities (clinical or laboratory signs of MPN). As depicted in Figure 1, hemoglobin levels and platelet counts varied. Particularly hemoglobin levels > 16.5 g/dl or > 16.0 g/dl (referring to men or women), or platelet counts > 450 GPT/l as major diagnostic criteria for MPN (polycythemia vera and essential thrombocythemia, respectively), according to the International Consensus Classification of 2022 (15), were only seen in a few cases with the presence of *JAK2* p.V617F. This implies that high blood cell counts are not the main trigger factors for thrombotic events. The abdominal venous thromboses may influence the development of blood count abnormalities through portal hypertension, inflammation, or bleeding episodes (27). More importantly, as described above, abdominal venous thromboses can be found early before evidence of MPN by diagnostic criteria (14, 15).

Consistent with the literature (2, 13, 33), individuals with abdominal venous thromboses and *JAK2* p.V617F were more likely to be female. Individuals with *JAK2* p.V617F tended to be older. In the literature, comparative data about age refer to individuals with SVT compared to patients with other thromboses (2) or patients with MPN (4, 33). In this regard, patients with SVT tend to be younger.

Population aspects

The JAK2 MV study had no defined exclusion criteria except for age < 18 years. The study was open to all patients with current or precedent evidence of abdominal venous thromboses in MV. As a consequence, the study cohort was heterogeneous, with competing causes of the thrombotic event, but it represented general clinical care. This highlights the study because, in contrast to other reports, it was not confined to patients of referral centers (1, 7, 12, 13, 34) or MPN or SVT registries (4). Compared to the literature (1, 13), the prevalence of *JAK2* p.V617F in total was lower, but the mutation was still found in about one in five patients. Patients with SVT were represented within the study cohort at 96%.

The study was restricted to MV. The federal state in northeast Germany is an area of low population density with 69 inhabitants per km^2, consisting of 1.6 million inhabitants (35). In contrast to urban areas, the distribution of medical specialists is limited, and specialized diagnostic centers are scarce. Patients with rare medical problems might be underdiagnosed. Initiating the prospective trial "Prevalence of JAK2 mutations in patients with abdominal venous thromboses" was a step toward distributing highly sophisticated diagnostic tools to potentially all patients in MV.

Diagnostic aspects

A unique feature of this study was the application of a highly sensitive and quantitative diagnostic method for the detection of the mutation *JAK2* p.V617F: ultradeep sequencing. This allowed the detection of VAF below the sensitivity limitation of NGS or conventional applications such as quantitative polymerase chain reaction (36, 37). Baumeister et al. (37) mention that VAFs of 1% lead to subclinical manifestations of MPN. The JAK2 MV study was not designed to confirm the diagnosis of MPN by further investigation or to follow-up patients. Nevertheless, this aspect is of particular interest for patients with VAF of *JAK2* p.V617F between 0.1% and 1.9%. It should be emphasized that with the detection of *JAK2* p.V617F, further hematological diagnostics regarding MPN are required. This was communicated to the submitting colleagues. Due to the study design, no information about the results was available.

Limitations

The following limitations of the study have to be acknowledged. First, the study is restricted to MV and might therefore be influenced by the geographic or genetic characteristics of its population. Second, due to the population of MV and the rarity of the thrombotic manifestations, the cohort is small. Still, the results are comparable to data from the literature, taking into account the differences in the inclusion criteria. Third, because the study period ranged from 22 February 2017 to 31 January 2021, the data might be influenced by the COVID-19 pandemic in the last 12 months. During this time, 12 patients were included. Finally, the study was designed as a prevalence study, and a follow-up was not within the scope of the study protocol. Nevertheless, receiving results of further hematological diagnostics would be of great interest for patients with mutations in *JAK2* V617 with VAF $\geq$ 2% to differentiate MPN from clonal hematopoiesis of indeterminate potential (CHIP) and in those with VAF between 1.0% and 1.9%.

Conclusion

The JAK2 MV study presents relevant aspects of general clinical care in patients with abdominal venous thromboses: Patients were diagnosed by various medical disciplines. Clinical criteria and blood counts were not conclusive for etiology. By ultradeep next-generation sequencing of peripheral blood samples, the mutation *JAK2* p.V617F was detected in 19% of patients included in the JAK2 MV study. Looking ahead, longitudinal examination of VAF is of interest, as is patients' follow-up with a focus on additional hematological results, especially in the light of evolving data about the pathophysiological influence of CHIP (38). The JAK2 MV study suggests, along with others (38), that "CHIP-associated SVT" (38) or "SVT-predominant MPN" may become a novel clinical entity consisting of patients with abdominal venous thrombosis with the mutation *JAK2* p.V617F. It has therapeutic and prognostic implications before fulfilling the diagnostic criteria of MPN, for example, continuation of anticoagulation, regular surveillance of blood cell counts, and clinical follow-up. Corresponding to the rapid development and distribution of highly sensitive diagnostic procedures like next-generation ultradeep sequencing patients with VAF < 2% will perhaps be included in the future (39). Meanwhile, a larger cohort of patients can contribute to better characterizing different patient groups with abdominal venous thromboses.

Implications

Patients with abdominal venous thromboses are seen by various medical disciplines.

In a prospective prevalence study in MV, a federal state in Germany, the mutation *JAK2* p.V617F was detected in 19% of patients with abdominal venous thromboses. Clinical or laboratory criteria did not provide evidence for the mutation *JAK2* p.V617F.

Establishing a diagnostic workout for peripheral blood samples in a region can help clarify the etiology of abdominal venous thrombotic events.

Peripheral blood samples are suitable for mutation analysis of *JAK2*. Ultradeep sequencing, as a highly sensitive and quantitative detection method, is advantageous. By using next-generation sequencing, other mutations of interest can be investigated at the same time.

If mutations are detected, further hematological diagnostics should be initiated, and regular follow-up is warranted.

Data availability statement

The data presented in this study are deposited in the sequence read archive (SRA) by the NIH, accession number SUB10682917.

Ethics statement

This study involving humans was approved by the Ethikkommission an der Medizinischen Fakultät der Universitaet Rostock, Rostock, Germany. The study was conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

LH: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Supervision, Validation, Writing – original draft. LG: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. SF: Formal analysis, Visualization, Writing – review & editing. MW: Investigation, Writing – review & editing. CGT: Conceptualization, Funding acquisition, Writing – review & editing. CR: Conceptualization, Funding acquisition, Writing – review & editing. HME: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing – original draft. CJ: Resources, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. We thank the society of internal medicine in Mecklenburg West-Pomerania (Gesellschaft der Internisten Mecklenburg-Vorpommern e.V.) for funding the JAK2-MV study by the research grant of the year 2016 (received by LH, CGT, CR, and HME).

Acknowledgments

We thank all the patients who participated in the study. We thank the colleagues in Mecklenburg-West Pomerania for their contribution to the JAK2 MV study by including their patients.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1344769/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Regional distribution of blood samples included in the study ($n = 68$).

References

1. Ageno W, Riva N, Schulman S, Beyer-Westendorf J, Bang SM, Senzolo M et al. Long-term clinical outcomes of splanchnic vein thrombosis: results of an international registry. *JAMA Intern Med.* (2015) 175:1474–80. doi: 10.1001/jamainternmed.2015.3184

2. How J, Zhou A, Oh T. Splanchnic vein thrombosis in myeloproliferative neoplasms: pathophysiology and molecular mechanisms of disease. *Ther Adv Hematol.* (2017) 8:107–18. doi: 10.1177/2040620716680333

3. Mathew C, Zumberg M. Clots in unusual places: lots of stress, limited data, critical decisions. *Hematology Am Soc Hematol Educ Program.* (2021) 2021:92–9. doi: 10.1182/hematology.2021000237

4. Alvarez-Larrán A, Pereira A, Magaz M, Hernández-Boluda JC, Garrote M, Cuevas B et al. Natural history of polycythemia vera and essential thrombocythemia presenting with splanchnic vein thrombosis. *Ann Hematol.* (2020) 99:791–8. doi: 10.1007/s00277-020-03965-z

5. Rajani R, Melin T, Björnsson E, Broomé U, Sangfelt P, Danielsson A et al. Budd-Chiari syndrome in Sweden: epidemiology, clinical characteristics and survival - an 18-year experience. *Liver Int.* (2009) 29:253–9. doi: 10.1111/j.1478-3231.2008.01838.x

6. Acosta S, Alhadad A, Svensson P, Ekberg O. Epidemiology, risk and prognostic factors in mesenteric venous thrombosis. *Br J Surg.* (2008) 95:1245–51. doi: 10.1002/bjs.6319

7. Stefano V de, Vannucchi AM, Ruggeri M, Cervantes F, Alvarez-Larrán A, Iurlo A et al. Splanchnic vein thrombosis in myeloproliferative neoplasms: risk factors for recurrences in a cohort of 181 patients. *Blood Cancer J.* (2016) 6:e493. doi: 10.1038/bcj.2016.103

8. Riva N, Beyer-Westendorf J, Contino L, Bucherini E, Sartori MT, Grandone E et al. Treatment of portal, mesenteric, and splenic vein thrombosis with rivaroxaban: a pilot, prospective cohort study. *Blood.* (2021) 138:671. doi: 10.1182/blood-2021-153813

9. Valeriani E, Di Nisio M, Riva N, Cohen O, Garcia-Pagan J-C, Magaz M et al. Anticoagulant therapy for splanchnic vein thrombosis: a systematic review and meta-analysis. *Blood.* (2021) 137:1233–40. doi: 10.1182/blood.2020006827

10. Felser S, Rogahn J, Le Coutre P, Al-Ali HK, Schulze S, Muegge L-O et al. Anxieties, age and motivation influence physical activity in patients with myeloproliferative neoplasms - a multicenter survey from the East German study group for hematology and oncology (OSHO #97). *Front Oncol.* (2022) 12:1056786. doi: 10.3389/fonc.2022.1056786

11. Praktiknjo M, Meyer C, Strassburg CP, Trebicka J. Frische venöse Thrombose splanchnischer Gefäße: Zwei Fallberichte zur kathetergestützten lokalen Thrombolyse und -aspiration. *Internist.* (2017) 58:82–9. doi: 10.1007/s00108-016-0120-0

12. Abbattista M, Capecchi M, Martinelli I. Treatment of unusual thrombotic manifestations. *Blood.* (2020) 135:326–34. doi: 10.1182/blood.2019000918

13. Colaizzo D, Amitrano L, Guardascione MA, Tiscia GL, D'Andrea G, Longo VAC et al. Outcome of patients with splanchnic venous thrombosis presenting without overt MPN: a role for the JAK2 V617F mutation re-evaluation. *Thromb Res.* (2013) 132:e99–e104. doi: 10.1016/j.thromres.2013.07.014

14. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood.* (2016) 127:2391–405. doi: 10.1182/blood-2016-03-643544

15. Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka H-M et al. International consensus classification of myeloid neoplasms and acute leukemias: integrating morphologic, clinical, and genomic data. *Blood.* (2022) 140:1200–28. doi: 10.1182/blood.2022015850

16. Demiriz IS, Kazanci MH, Menfaatli E, Jafari-Gharabaghlou D, Zarghami N. Allelic burden of Janus kinase 2 in a 6-month course of therapy for myeloproliferative neoplasms. *Mol Biol Rep.* (2023) 50:5687–95. doi: 10.1007/s11033-023-08511-4

17. Etheridge SL, Roh ME, Cosgrove ME, Sangkhae V, Fox NE, Chen J et al. JAK2V617F-positive endothelial cells contribute to clotting abnormalities in myeloproliferative neoplasms. *Proc Natl Acad Sci U S A.* (2014) 111:2295–300. doi: 10.1073/pnas.1312148111

18. Torres DG, Paes J, da Costa AG, Malheiro A, Silva GV, Mourão LPdS et al. JAK2 variant signaling: genetic, hematologic and immune implication in chronic myeloproliferative neoplasms. *Biomolecules.* (2022) 12:291. doi: 10.3390/biom12020291

19. Vainchenker W, Kralovics R. Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. *Blood.* (2017) 129:667–79. doi: 10.1182/blood-2016-10-695940

20. Falchi L, Kantarjian HM, Verstovsek S. Assessing the thrombotic risk of patients with essential thrombocythemia in the genomic era. *Leukemia.* (2017) 31:1845–54. doi: 10.1038/leu.2017.150

21. Cazzola M, Kralovics R. From Janus kinase 2 to calreticulin: the clinically relevant genomic landscape of myeloproliferative neoplasms. *Blood.* (2014) 123:3714–9. doi: 10.1182/blood-2014-03-530865

22. Colaizzo D, Amitrano L, Tiscia GL, Scenna G, Grandone E, Guardascione MA, et al. The JAK2 V617F mutation frequently occurs in patients with portal and mesenteric venous thrombosis. *J Thromb Haemost.* (2007) 5:55–61. doi: 10.1111/j.1538-7836.2006.02277.x

23. Grunwald L, Grosse-Thie C, Sender S, Knuebel G, Krohn S, Roolf C et al. Ultradeep targeted sequencing reveals low allele frequencies of somatic JAK2 and MPL variants in patients with abdominal vein thromboses: results of an ongoing prospective prevalence study in Mecklenburg-West Pomerania. *Biomark Res.* (2020) 8:73. doi: 10.1186/s40364-020-00254-9

24. Maslah N, Verger E, Schlageter M-H, Miclea J-M, Kiladjian J-J, Giraudier S et al. Next-generation sequencing for JAK2 mutation testing: advantages and pitfalls. *Ann Hematol.* (2019) 98:111–8. doi: 10.1007/s00277-018-3499-y

25. Cattaneo D, Bucelli C, Marchetti A, Lionetti M, Fermo E, Bellani V et al. Pathological and genomic features of myeloproliferative neoplasms associated with splanchnic vein thrombosis in a single-center cohort. *Ann Hematol.* (2023) 102:1409–20. doi: 10.1007/s00277-023-05217-2

26. Colaizzo D, Amitrano L, Guardascione MA, Favuzzi G, Tiscia GL, D'Andrea G et al. Clinical utility of screening for CALR gene exon 9 mutations in patients with splanchnic venous thrombosis. *Thromb Haemost.* (2015) 113:1381–2. doi: 10.1160/TH14-12-1055

27. Debureaux P-E, Cassinat B, Soret-Dulphy J, Mora B, Verger E, Maslah N et al. Molecular profiling and risk classification of patients with myeloproliferative neoplasms and splanchnic vein thromboses. *Blood Advances.* (2020) 4:3708–15. doi: 10.1182/bloodadvances.2020002414

28. Wolach O, Sellar RS, Martinod K, Cherpokova D, McConkey M, Chappell RJ et al. Increased neutrophil extracellular trap formation promotes thrombosis in myeloproliferative neoplasms. *Sci Transl Med.* (2018) 10:8292. doi: 10.1126/scitranslmed.aan8292

29. DeZern AE, Malcovati L, Ebert BL. CHIP, CCUS, and other acronyms: definition, implications, and impact on practice. *Am Soc Clin Oncol Educ Book.* (2019) 39:400–10. doi: 10.1200/EDBK_239083

30. Edelmann B, Gupta N, Schnoeder TM, Oelschlegel AM, Shahzad K, Goldschmidt J et al. JAK2-V617F promotes venous thrombosis through β1/β2 integrin activation. *Journal of Clin Inv.* (2018) 128:4359–71. doi: 10.1172/JCI 90312

31. Eaton N, Subramaniam S, Schulte ML, Drew C, Jakab D, Haberichter SL et al. Bleeding diathesis in mice lacking JAK2 in platelets. *Blood Advances.* (2021) 5:2969–81. doi: 10.1182/bloodadvances.2020003032

32. Ageno W, Beyer Westendorf J, Contino L, Bucherini E, Sartori MT, Senzolo M et al. Rivaroxaban for the treatment of noncirrhotic splanchnic vein thrombosis: an interventional prospective cohort study. *Blood Adv.* (2022) 6:3569–78. doi: 10.1182/bloodadvances.2022007397

33. How J, Trinkaus KM, Oh ST. Distinct clinical, laboratory and molecular features of myeloproliferative neoplasm patients with splanchnic vein thrombosis. *Br J Haematol.* (2018) 183:310–3. doi: 10.1111/bjh.14958

34. Klute K, DeFilippis EM, Shillingford K, Chapin J, DeSancho MT. Clinical presentations, risk factors, treatment and outcomes in patients with splanchnic vein thrombosis: a single-center experience. *J Thromb Thrombolysis.* (2016) 42:267–71. doi: 10.1007/s11239-016-1337-4

35. Statistiken zu Mecklenburg-Vorpommern. (2023) Available online at: https://de.statista.com/themen/2450/mecklenburg-vorpommern/#editorsPicks (accessed August 5, 2023).

36. Gondek LP. CHIP is clonal hematopoiesis a surrogate for aging and other disease? *Hematology Am Soc Hematol Educ Program.* (2021) 2021:384–9. doi: 10.1182/hematology.2021000270

37. Baumeister J, Chatain N, Sofias AM, Lammers T, Koschmieder S. Progression of myeloproliferative neoplasms (MPN): diagnostic and therapeutic perspectives. *Cells.* (2021) 10:551. doi: 10.3390/cells10123551

38. Camerlo S, Ligato J, Rosati G, Carrà G, Russo I, Gobbi M de et al. Shedding light on the pathogenesis of splanchnic vein thrombosis. *Int J Mol Sci.* (2023) 24:262. doi: 10.3390/ijms24032262

39. Luque Paz D, Kralovics R, Skoda RC. Genetic basis and molecular profiling in myeloproliferative neoplasms. *Blood.* (2023) 141:1909–21. doi: 10.1182/blood.2022017578

frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 24 January 2024
DOI 10.3389/fpubh.2023.1303787

OPEN ACCESS

EDITED BY
Felix Carl Fabian Schmitt,
Heidelberg University Hospital, Germany

REVIEWED BY
Rosa Sonja Alesci,
Hochtaunus Coagulation Center, Germany
Carina Schey,
Global Market Access Solutions, Switzerland

*CORRESPONDENCE
Xiaoyu Xi
✉ xixy@cpu.edu.cn

†These authors have contributed equally to this work and share first authorship

RECEIVED 28 September 2023
ACCEPTED 15 December 2023
PUBLISHED 24 January 2024

CITATION
Huang Y, Wan C, Guan T and Xi X (2024) Current status of treatment and disease burden of a cohort of hemophilia B in China. *Front. Public Health* 11:1303787. doi: 10.3389/fpubh.2023.1303787

Current status of treatment and disease burden of a cohort of hemophilia B in China

Yiwen Huang[1†], Chuchuan Wan[1†], Tao Guan[2] and Xiaoyu Xi[1*]

[1]The Research Center of National Drug Policy & Ecosystem, China Pharmaceutical University, Nanjing, China, [2]Beijing Hemophilia Home Care Center, Beijing, China

Objective: Hemophilia B is a rare X-chromosome linked hereditary bleeding disorder. Patients require lifelong treatment and it is costly, but there is a lack of research in China on the treatment and burden for this group. Our aim was to review the actual treatment pattern of hemophilia B patients in China, and describe the financial burden and other disease burden from the patient's perspective.

Methods: Using data collected by the Beijing Hemophilia Home Care Center, descriptive statistics were made on the sociodemographic characteristics and treatment of patients. The annual drug costs were calculated according to the actual factor dosage and price.

Results: During the study period, 29.9% of the patients only received on-demand treatment, while the rest of the patients received varying numbers of prophylaxis treatment. The total cost of clotting factors for 341 patients in one year was 16.0 million CNY ($2.5 million), with 46990.8 CNY ($7283.7) per patient. The drug cost of prophylaxis was significantly higher than that of on-demand treatment. The amount of prothrombin complex concentrates used by patients was the largest, more than 5 times of recombinant coagulation factor IX. Based on the average annual wage and average working time of Chinese employees in 2021, the average annual wage loss of HB patients reached 31544.2 CNY ($4889.4). The results of the questionnaire showed that 77.1% and 65.3% of patients had chronic pain and acute pain of different frequencies.

Conclusion: The level of prophylaxis for Chinese patients is low; safer and more effective recombinant drugs are not widely available. Patients also face a high burden of drug costs, as well as indirect costs that cannot be underestimated. Therefore, continued efforts are needed to improve the quality of life of patients by reducing their financial burden and promote standardized treatment.

KEYWORDS

China, hemophilia B, economic burden, cost, prophylaxis

1 Introduction

Hemophilia B (HB), a rare X-chromosome linked hereditary bleeding disorder (1), has been included in the Circular of Publishing the First List of Rare Diseases in China (2). HB usually occurs in males [global prevalence is 1 in 30,000 (3), and China's is about 1 in 100,000 (4)], while it is extremely rare in females. Patients with HB have a deficiency

of coagulation factor IX (FIX) activity due to mutations in the genes encoding FIX, which leads to coagulation dysfunction (5, 6). Bleeding is the most prominent clinical manifestation of HB, among which spontaneous bleeding, minor post-traumatic bleeding, and severe bleeding after wound or surgery are more common (7). As a lifelong disorder, hemophilia is associated with significant clinical burden driven by haemarthrosis, joint damage and pain, with subsequent negative impact on patients' mental health, daily functioning and overall quality of life (8).

Given the nature of HB, replacement therapy of high-quality factor concentrates is essential (9). Patients with HB mainly receive clotting factor replacement therapy and surgical treatment for bleeding and joint problems. In addition, gene therapy which has shown promising results in some clinical studies (10). Replacement therapy is currently the primary and most effective clinical treatment for HB (11). It is used to replenish the FIX deficiency in patients and includes on-demand treatment and prophylaxis. The former is to address timely hemostasis after bleeding episodes, and the latter is a regular continuous replacement therapy to prevent bleeding (12). The *Chinese guidelines on the treatment of hemophilia (version 2020)* (5) and The *WFH Guidelines for the Management of Hemophilia, 3rd edition* (13) state that recombinant FIX (rFIX) or viral-inactivated prothrombin complex concentrates (PCCs) is the preferred alternative treatment for HB. Fresh frozen plasma (FFP) can be used if these clotting factor concentrates (CFCs) are not available. PCCs are the main product of HB replacement therapy in China today. However, they are not the most ideal products. Because PCCs contain coagulation factors II, VII, and X in addition to FIX components, some of which may lead to the risk of venous and arterial thrombosis or disseminated intravascular coagulation (DIC) formation with long-term high dose use.

The implementation of replacement therapy means that patients will be on medication for the rest of their lives, resulting in ongoing treatment costs, most notably for clotting factor medications. During the course of replacement therapy, some patients may develop inhibitors to clotting factor, which can reduce the effectiveness of replacement therapy. Additional costs and medication burden will be incurred if patients are treated with bypass agents [e.g., genetically recombinant activated coagulation Factor VII (rFVIIa) and immune tolerance induction (ITI) therapy]. Furthermore, incomplete treatment of long-term recurrent joint bleeding can damage the joint structure, resulting in joint deformity and loss of function. Worse still, joint replacement may be required, and patients have to endure pain during the recovery process while bearing the high cost of surgery. Studies have been conducted in Europe and the United States to demonstrate the clinical, human, and economic burden of severe HB patients (14, 15). However, most available studies (over 70%) on the burden of hemophilia in China did not distinguish between HA and HB or only targeted HA patients, and were conducted from a third-party perspective, analyzing direct treatment costs through health insurance system data. There is a lack of studies specifically focusing on patients with HB, where the sample size of hemophilia B patients is small or does not indicate the type of hemophilia (16, 17).

In view of the above, this study aims to review the actual treatment pattern of HB in China using data from the Beijing Hemophilia Home Care Center patient database, thereby aiming to identify the shortcomings in treatment. At the same time, the study also describes the financial burden and other disease burden from the patient's perspective, so as to better understand the HB patients' burden. These will help the relevant parties to explore better treatment strategies and burden reduction measures.

2 Method

2.1 Study design

The study was conducted using data from current patients (with a confirmed diagnosis of HB, excluding carriers) in the Beijing Hemophilia Home Care Center database. Sociodemographic information and treatment information in the database were mainly accessed. For the reason of data availability, usability, completeness, and quality, only patient data within one consecutive year from September 1, 2020 to August 31, 2021 were extracted. We also added a patient questionnaire study to investigate other patient burden, which consisted of two parts. One part was about health service utilization (visits within two weeks, hospitalizations within one year) and patient labor loss (work time lost due to illness, actual work time, and impact on productivity). The questions in this section were generated from literature reviews and expert interviews. The other part assessed pain levels using a visual analogue scale (VAS).

2.2 Data collection

The Beijing Hemophilia Home Care Center is the largest organization for hemophiliacs in China, with over 14,000 registered patients. The organization collects data on hemophiliacs on Chinese mainland through an application called "Hemophilia Home." The App allows patients and their families to record information on bleeding, treatment, medical appointments, medication costs, etc.

Patient inclusion criteria: (1) be a citizen of the People's Republic of China, regardless of age or sex; (2) knowingly and voluntarily register for the Hemophilia Home APP and agree to the APP registration agreement; (3) be diagnosed with hemophilia B by a regular hospital, excluding carriers. Patients register in the APP on their own, and their caregivers can register on behalf of infants, the elder or other people with mobility difficulties who cannot complete the registration on their own. Patients will record their use of each treatment in the APP for each episode, along with the cause, site, and symptoms of each bleeding. All other information will be registered only once.

In this study, we mainly used sociodemographic information and treatment data of patients in the database. Specifically, the sociodemographic included gender, age, education and employment status, marital status and health insurance information; the disease treatment information included disease severity and treatment records (including type of treatment, type and dosage of medication for each treatment). Additionally, a convenience sample of the active patient users was conducted in the form of an online questionnaire that patients voluntarily completed via the Internet, ultimately collecting data on patient health service utilization and loss to the labor force.

2.3 Statistical analysis

The raw database data were cleaned before analysis. The sociodemographic information of individual patients was corrected by verifying with the staff of the Beijing Hemophilia Home Care Center. We merged two types of medical insurance (i.e., urban resident basic medical insurance and new rural cooperative medical scheme) used in the APP into urban and rural resident basic medical insurance to reflect the current status in China. Also, patients with obvious logical errors in information were excluded.

The study could be divided into three parts: basic patient characteristics, treatment status, and patient burden. Only patients with relevant data were considered in the treatment and burden analysis. Basic patient characteristics included gender, age, marital status, education, and employment status. The treatment status includes disease severity, treatment modality, type of drug used and factor dosage. The financial burden of patients was reflected by direct costs. Only factor treatment costs were considered due to data limitations. Furthermore, since the database only counted patients' drug use and did not record each drug cost in detail, it was necessary to use an appropriate formula to estimate the cost. The annual drug cost calculation formula is as follows. The conversion between the Chinese Yuan (CNY) and the US dollar was made using the average exchange rate of the CNY to the US dollar in 2021, 6.4515 *(Number of people completely unemployed due to illness * average weekly working hours + number of people whose working hours are partially affected by illness * average weekly working hours affected) * average hourly wage * 52#.*

#The average weekly working hours and average wages are obtained from the public data of the National Bureau of Statistics of China.

Data were presented as means with standard deviation (SD) or medians for continuous variables and as frequencies with percentages for categorical variables. Given that the distribution of drug cost data did not satisfy normality and chi-square, a nonparametric test, the Kruskal-Wallis H test, was used in this study to compare differences in factor doses and drug costs between patients with different treatment modalities or different levels of severity.

All statistical analyses were performed using SPSS 26, Microsoft® Excel 2016 and Stata 15.

3 Results

3.1 Sociodemographic characteristics

A total of 1,654 patients' sociodemographic information was collected, the majority of whom were male (99.8%, $n = 1{,}651$). The mean age of the patients was 22.4 (±15.1) years, with 53.7% ($n = 889$) and 46.3% ($n = 765$) of adult (≥18) and underage (<18) patients, respectively. In our sample, 54.2% ($n = 411$) of the patients were of marriage age (The legal marriage age in China is 22 for men and 20 for women.) were unmarried, 4.7% ($n = 36$) were divorced or widowed, and the rest were married. Considering the large age span of patients, the education and employment status of patients were combined and analyzed. Of the total patients, 163 (9.9%) patients were in the preschool stage; 769 (46.5%) patients were in the education stage, but 41.4% ($n = 318$) of them were out of school. Additionally, 519 (31.4%) patients were unemployed, and the rest worked normally or retired (12.2%, $n = 203$) (Table 1).

TABLE 1 Sociodemographic characteristics of HB patients.

Items ($N = 1{,}654$)	*n* (%)/mean (SD)
Gender	
Male	1,651 (99.8%)
Female	3 (0.2%)
Age	**22.4 (15.1)**
Minors (<18)	765 (46.3%)
Adults (≥18)	889 (53.7%)
Marital status	
Unmarried	411 (54.2%)
Married	312 (41.1%)
Others (divorced/widowed)	36 (4.7%)
Education and employment status	
Preschool	163 (9.9%)
Educated and in school	451 (27.3%)
Educated but out of school	318 (19.2%)
Employed	191 (11.5%)
Unemployed	519 (31.4%)
Retired	12 (0.7%)

SD, standard deviation.

3.2 Treatment

A total of 341 patients were included in the analysis of treatment. Patients were diagnosed as having mild (5 < IU/dl < 40), moderate (1 < IU/dl < 5), or severe (IU/dl < 1) hemophilia based on factor activity levels. The vast majority of the 341 patients had moderate and severe HB, 48.4% ($n = 165$) and 48.7% ($n = 166$), respectively, and only 10 (2.9%) had mild HB (Table 1).

Because the database recorded the type of each treatment during the study period, patient treatment types were classified into three categories: having had on-demand treatment only, prophylactic treatment only, or both. During the study period, 178 (52.2%) had received both on-demand and prophylactic treatment, 61 (17.9%) received only prophylaxis, and the remaining 102 (29.9%) received only on-demand therapy. In terms of different severity levels, the percentages of the three treatment modalities were basically the same. About half (52.7%) of moderate patients and half (50.0%) of severe patients administered a combination of on-demand therapy and prophylaxis. Relatively, the proportion varied more due to the small number of patients with mild disease. Over the study period, the total amount of FIX used was 7.7×10^6IU, with 22660.7 IU per patient. The mean factor dosage was higher in moderate and severe patients than in mild, but the Kruskal-Wallis H test did not show a significant difference ($p > 0.05$). The total amount of PCCs used was the highest, reaching 6.5×10^6IU, followed by rFIX (1.2×10^6 IU), while human coagulation factor IX (1.3×10^4IU) was the least (Table 2).

In view of the treatment level, the average annual factor dosage of 341 patients was 619.5 IU/Kg, of which the average annual factor

TABLE 2 Treatment status.

Items	*n* (%)			
Severity		Only on-demand	Only prophylaxis	A combination of both
Mild	10 (2.9%)	2 (20.0%)	0	8 (80.0%)
Moderate	165 (48.4%)	46 (27.9%)	32 (19.4%)	87 (52.7%)
Severe	166 (48.7%)	54 (32.5%)	29 (17.5%)	83 (50.0%)
Treatment modality				
Only on-demand	102 (29.9%)			
Only prophylaxis	61 (17.9%)			
A combination of both	178 (52.2%)			
clotting factor				
Total quantity (IU)	7,727,300			
Per patient (IU/person)	22660.7			
Mild	8390.0		chi2 (2)=1.711 Prob = 0.4251	
Moderate	21160.0			
Severe	25012.0			
PCCs (IU)	6,522,300			
rFIX (IU)	1,192,000			
human coagulation factor IX (IU)	13,000			
Average annual factor dosage (IU/Kg)	619.5			
For on-demand therapy (IU/Kg)	194.3			
For prophylaxis (IU/Kg)	656.2			
Minors (<18 years)	845.8			
Adults (≥18 years)	318.9			
Number of injections per year				
Mean (±SD)	29 (±31.8)			
Range	(1–176)			

IU, international unit; PCCs, prothrombin complex concentrates; rFIX, recombinant factor IX; SD, standard deviation.

dosage was 656.2 IU/Kg in 239 patients with prophylaxis and 194.3 IU/Kg in 280 patients with on-demand treatment. The mean factor dosage of patients with prophylaxis showed a fluctuating upward trend (37.0–74.3 IU/Kg), while there was no significant trend in the mean dosage of on-demand treatment patients (Figure 1).

3.3 Economic burden

The total cost of clotting factors for 341 patients during the study period (total of one year) was 16.0 million CNY ($2.5 million) calculated according to the aforementioned method, with a mean of 46,990.8 CNY ($7283.7) per patient and a median of 15,262.5 CNY ($2365.7). Of these, the cost for prophylaxis amounted to 11.7 million CNY ($ 1.8 million), with an average cost of 48,962.2 CNY ($7589.3) per patient with prophylaxis ($n = 239$), and the cost for on-demand therapy mounted to 4.3 million CNY ($0.7 million), with an average cost of 15434.9 CNY ($2392.5) per on-demand patient ($n = 280$) (Table 3). As each treatment was recorded as either "prophylaxis" or "on-demand," the number of people corresponding to the two treatments mentioned above ($n = 519$) was greater than the total number of patients ($n = 341$).

The results of the Kruskal-Wallis H test for drug costs between patients with different treatment modalities and between severity levels are shown in Table 3. There was a significant difference in drug cost between patients with the three treatment modalities ($p < 0.05$), while there was no significant difference among patients with different levels of severity.

3.4 Other disease burden

For the further questionnaire study, 153 valid questionnaires were obtained. In the two weeks prior to the questionnaire study, 52 (34.0%) had a visit (both outpatient and emergency) with an average of 1.9 visits in two weeks. This indicated that there was a 60% average visit rate of patient within a two-week period. Thirty-two patients (20.9%) had been hospitalized in the previous year, with an average of 1.9 hospitalizations per year (Table 4).

Thirty-five of 153 (22.9%) reported never having chronic pain, the majority of patients (64.7%, $n = 99$) had chronic pain occasionally or sometimes, 90.7% ($n = 107$) of those with chronic pain had mild or moderate pain, and 8.5% ($n = 10$) had severe chronic pain. Regarding the frequency of acute pain, 53 (34.6%)

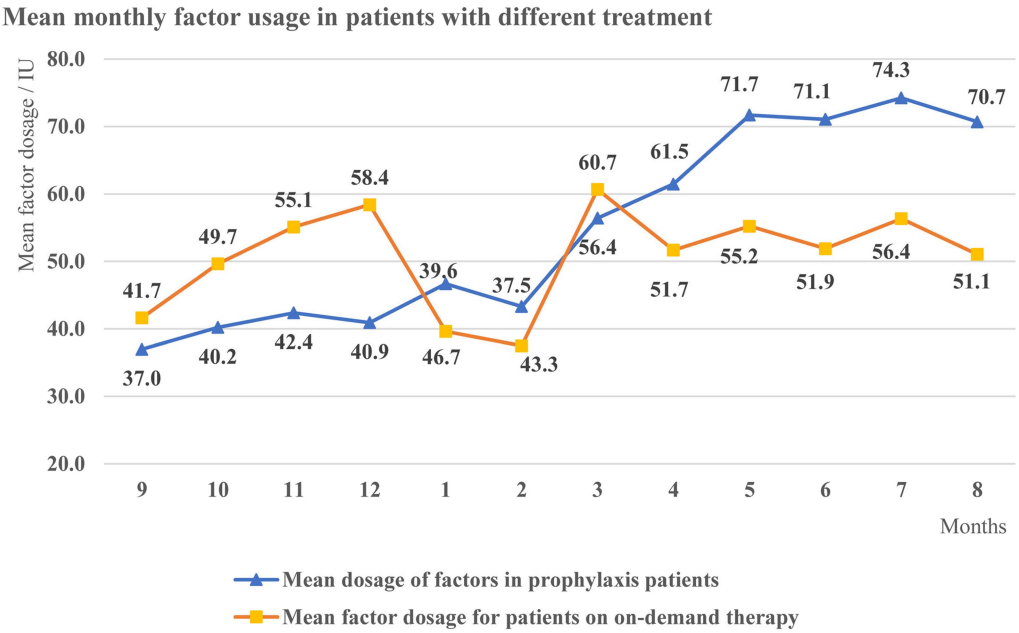


FIGURE 1
Mean monthly factor dosage in patients with different treatment.

TABLE 3 Result of Kruskal-Wallis H.

	Obs	Rank sum	chi2 (2)	Prob
Treatment			72.858	0.0001
On-demand (1)	102	10822.00		
prophylaxis (2)	61	10042.50		
combination (3)	178	37446.50		
Severity			4.325	0.1151
Mild	10	1172.50		
Moderate	165	27475.50		
Severe	166	29663.00		

Groups	Rank means difference	Critical value	Prob
(1) & (2)	58.52	38.20	0.000123 (S)
(1) & (3)	104.26	29.31	0.000000 (S)
(2) & (3)	45.75	35.01	0.000880 (S)

Obs, the number of observations in the current dataset.

patients had no acute pain and the majority of patients with occasional or sometimes acute pain (61.4%, $n = 94$). Again, mild to moderate pain was predominant in those with acute pain (74.0%, $n = 74$) (Table 4).

The results from the 153 responses to the questionnaire showed that the work time lost due to HB was 30.2 h per week. This numerical value was used to estimate the work time and wages lost due to illness for that patient overall ($n = 1{,}654$). The average annual wage in China in 2021 was 85,945 CNY ($13321.8), and the average weekly working time was 47.8 h, or an average wage of 34.6 CNY ($5.4) per hour, resulting in a lost wage of 52,170,000.4 CNY ($8,087,129.3) for 1,654 patients in one year, or 31,544.2 CNY ($4889.4) per patient.

Using the sum of patient's drug costs and lost wages to represent the total patient costs, the total annual cost was 68,197,963.3 CNY ($10,570,869.3), or an average of 78,530.0 CNY ($12,173.1) per patient.

4 Discussion

This study provides an overview of the treatment and disease burden of 1,654 hemophilia B patients from across China based on the Beijing Hemophilia Home Care Center's patient database. As few studies in China have focused on this special group of HB patients, this study analyzes their treatment and burden at the national level, which can fill the gap in knowledge on burden to a certain extent. Meanwhile, it objectively established that HB patients are mostly troubled by the disease in daily life; and they faced a high financial burden and need improved treatment level.

A total of 97.1% of patients in this study had moderate or severe disease, similar to existing studies [81.7% (18), 90.0% (19)]. Nevertheless, mild patients may have been overlooked because of the lack of obvious preexisting bleeding resulting in delayed diagnosis. Approximately 30% of patients received only on-demand treatment during the study, and not all of those receiving prophylaxis received standard prophylaxis on a regular basis, leaving a gap in treatment patterns. Sixty-four per cent of patients with prophylaxis were under 18 years of age. The average annual factor dosage among prophylaxis patients was 656.2 IU/Kg, including 845.8 IU/Kg in children (<18 years) and 318.9 IU/Kg in adults (≥18 years), which is lower than in a single-center study at the Tianjin Blood Center (1328.0 IU/kg for prophylaxis and 878.8 IU/Kg in adults from 2014 to 2018) (18), while our low-dose prophylaxis regimen (5) (hemophilia B FIX preparation 20 IU/Kg once a week) requires at least 1,000 IU/Kg per year, which also indicates that there are still a large number of patients with

TABLE 4 Other disease burden.

items	*n* (%)/mean (SD)
Visits within two weeks	
No	101 (66.0%)
Yes	52 (34.0%)
Average number of visits (per fortnight)	1.9
Hospitalization within one year	
No	121 (79.1%)
Yes	32 (20.9%)
Average number of hospitalizations (per year)	1.9
Chronic pain frequency	
Never	35 (22.9%)
Occasionally	64 (41.8%)
Sometimes	35 (22.9%)
Often	15 (9.8%)
Always	4 (2.6%)
Chronic pain frequency	
No pain	1 (0.8%)
Mild	70 (59.3%)
Moderate	37 (31.4%)
Severe	10 (8.5%)
Acute pain frequency	
Never	53 (34.6%)
Occasionally	71 (46.4%)
Sometimes	23 (15%)
Often	6 (3.9%)
Always	0
Acute pain level	
No pain	0
Mild	37 (37.0%)
Moderate	37 (37.0%)
Severe	26 (26.0%)
Work status ($n = 26$)	
Work normally	7 (26.9%)
Work affected by HB	19 (73.1%)
Average time of work affected (hours/week)	30.2 (40.4)

extremely low levels of prophylaxis nationwide. For presentation purposes, we calculated the overall average patient dose monthly. The upward trend of dosing during prophylaxis may be caused by implementation of more intense regimens, but due to limited data, it is uncertain to accurately infer whether patients' prophylaxis levels have improved over time. The treatment was the same among patients with moderate and severe disease, which suggests that those with moderate disease had similar phenotypes to those with severe disease, and were therefore included in the Registry, hence in our study. However, another reason may be that only those with severe disease felt the need to register on the APP.

The per patient factor cost calculated in this study was 46,990.8 CNY ($7283.7), and the median of 15,262.5 CNY ($2365.7) may be more representative due to the skewed distribution of the data. In a study of medical costs for urban hemophilia patients in China from 2010 to 2016 (16), the cost of coagulation factors for HB patients was 7,128.0 CNY ($1,104.9), and some difference between them may be due to the study being from a third-party payer perspective, including only inpatients, and having a small sample size ($n = 80$). The total cost of factor IX drugs for only 341 patients amounted to 16,024,000 CNY ($2,483,763.5) a year. The reported prevalence of hemophilia B in Chinese mainland was about 0.5/100,000 (4), which would result in over 300 million CNY ($46.5 million) in factor IX drug costs given the large population base in China. The ANOVA showed no significant differences in factor drug costs between patients with different severity levels. However, in the study by Buckner et al. (20), the total annual medical costs of hemophilia B patients increased with severity. This difference possibly resulted from inconsistencies in the calculation of costs, differences in patient baseline (only adult patients in their study), and differences in severity classification (mild, moderate, moderate–severe, and severe in their study). Again, another likely explanation is the difference in the diagnosis (and inclusion into the studies) of patients with mild bleeding phenotype (in mild and moderate patients).

In addition to economic losses, the physical and psychological harm to patients may be more serious. The average number of injections per year was 29, with a maximum of 176. Frequent injections brought physical injury to patients. Patients suffer from pain due to bleeding. In our study, about 77.1% of the patients experience chronic pain on a daily basis, which is generally consistent with existing studies [85% (14), 81% (15)], suggesting the importance of improving nursing care to alleviate the pain of patients. Better overall management including early diagnosis, early and adequate treatment as well as the availability of multidisciplinary comprehensive care etc. are of great benefit to patients.

It is necessary to note that this study has certain limitations. Firstly, only 341 patients had complete treatment records in the database, which may make the analysis of treatment modality and factor dosage different from the actual situation. Similarly, there is a bias in estimating the total indirect cost (wage loss) of patients based on the results of 153 supplementary questionnaires. Although some studies believed that the direct medical cost of hemophilia B was almost entirely caused by factor therapy (99%) (15), some studies showed that the treatment service accounted for 1/3 of the total medical cost (21), and other medical expenses of patients (such as other drug costs, joint surgery costs, etc.) were also worth considering. Furthermore, the analysis of patient bleeding pattern and comparison of pain in children and adults was hindered due to data limitations.

5 Conclusion

This study demonstrates the treatment profile and burden of hemophilia B patients in China. The level of prophylaxis for Chinese patients is low; safer and more effective recombinant drugs are not

widely available. Inadequate treatment causes overwhelming suffering. Patients also face a high burden of drug costs, as well as indirect costs that cannot be underestimated. Therefore, patients with HB need access to more preferential policies to improve their quality of life by reducing their financial burden and promoting better, standardized treatment.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

YH: Writing – original draft. CW: Writing – original draft. TG: Writing – review & editing. XX: Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We thank Beijing Hemophilia Home Care Center for the support of this research. And we would like to thank the patients and data manager staff who took part in the study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Thrombosis and Hemostasis Group HSoCMA, China HTCCNo. Consensus of Chinese expert on the diagnosis and treatment of hemophilia (version 2017). *Zhonghua Xue Ye Xue Za Zhi*. (2017) 38:364–70. doi: 10.3760/cma.j.issn.0253-2727.2017.05.002

2. Circular of Publishing the First List of Rare Diseases. *Gazette of the National Health Commission of People's Republic of China*. National Health Commission of the People's Republic of China (2018) 15–9.

3. Mannucci PM, Tuddenham EG. The hemophilias–from royal genes to gene therapy. *N Engl J Med*. (2001) 344:1773–9. doi: 10.1056/NEJM200106073442307

4. Qu Y, Nie X, Yang Z, Yin H, Pang Y, Dong P, et al. The prevalence of hemophilia in mainland China: a systematic review and meta-analysis. *Southeast Asian J Trop Med Public Health*. (2014) 45:455–66.

5. Thrombosis and Hemostasis Group CSoH, Chinese Medical Association., Hemophilia Treatment Center Collaborative Network of C. Chinese guidelines on the treatment of hemophilia(version 2020). *Chin J Hematol*. (2020) 265–71

6. Shih MY, Wang JD, Yin JD, Tsan YT, Chan WC. Differences in major bleeding events between patients with severe hemophilia a and hemophilia B: a Nationwide, population-based cohort study. *Clin Appl Thromb Hemost*. (2019) 25:107602961988802. doi: 10.1177/1076029619888023

7. Ding QL, Wang XF, Wang HL, Sun J, Hua BL, Wu JS. Consensus of Chinese expert on the diagnosis and treatment of hemophilia. *J Clin Hematol*. (2010) 23:49–53.

8. O'Hara J, Martin AP, Nugent D, Witkop M, Buckner TW, Skinner MW, et al. Evidence of a disability paradox in patient-reported outcomes in haemophilia. *Haemophilia*. (2021) 27:245–52. doi: 10.1111/hae.14278

9. Franchini M, Mannucci PM. Past, present and future of hemophilia: a narrative review. *Orphanet J Rare Dis*. (2012) 7:24. doi: 10.1186/1750-1172-7-24

10. Nathwani AC, Reiss UM, Tuddenham EGD, Rosales C, Chowdary P, McIntosh J, et al. Long-term safety and efficacy of factor IX gene therapy in hemophilia B. *N Engl J Med*. (2014) 371:1994–2004. doi: 10.1056/NEJMoa1407309

11. Srivastava A. Current issues in hemophilia: recognizing clinical heterogeneity, replacement therapy, and outcome assessment preface. *Semin Thromb Hemost*. (2015) 41:816–8. doi: 10.1055/s-0035-1564801

12. Blanchette VS, Key NS, Ljung LR, Manco-Johnson MJ, van den Berg H, Srivastava A, et al. Definitions in hemophilia: communication from the SSC of the ISTH. *J Thromb Haemost*. (2014) 12:1935–9. doi: 10.1111/jth.12672

13. Srivastava A, Santagostino E, Dougall A, Kitchen S, Sutherland M, Pipe SW, et al. WFH guidelines for the Management of Hemophilia, 3rd edition. *Haemophilia*. (2020) 26:1–158. doi: 10.1111/hae.14046

14. Burke T, Asghar S, O'Hara J, Sawyer EK, Li N. Clinical, humanistic, and economic burden of severe hemophilia B in the United States: results from the CHESS US and CHESS US+ population surveys. *Orphanet J Rare Dis*. (2021) 16:143. doi: 10.1186/s13023-021-01774-9

15. Burke T, Asghar S, O'Hara J, Chuang M, Sawyer EK, Li N. Clinical, humanistic, and economic burden of severe haemophilia B in adults receiving factor IX prophylaxis: findings from the CHESS II real-world burden of illness study in Europe. *Orphanet J Rare Dis*. (2021) 16:521. doi: 10.1186/s13023-021-02152-1

16. Huang ZW, Nicholas S, Yang Y, Chen X, Maitland E, Ma Y, et al. Medical costs and hospital utilization for hemophilia a and B urban inpatients in China: a national cross-sectional study. *BMC Health Serv Res*. (2022) 22:9. doi: 10.1186/s12913-022-07626-x

17. Gong G-w, Chen Y-c, Fang P-q, Min R. Medical expenditure for patients with hemophilia in urban China: data from medical insurance information system from 2013 to 2015. *Orphanet J Rare Dis*. (2020) 15:137. doi: 10.1186/s13023-020-01423-7

18. Song XW, Liu W, Xue F, Zhong J, Yang Y, Liu Y, et al. Real-world analysis of haemophilia patients in China: a single centre's experience. *Haemophilia*. (2020) 26:584–90. doi: 10.1111/hae.14029

19. Keshavarz K, Bordbar M, Hashemipoor Z, Jalali FS, Ravangard R. Economic burden of hemophilia a and B: a case in Iran. *Hematology*. (2020) 25:149–55. doi: 10.1080/16078454.2020.1741205

20. Buckner TW, Bocharova I, Hagan K, Bensimon AG, Yang H, Wu EQ, et al. Health care resource utilization and cost burden of hemophilia bin the United States. *Blood Adv*. (2021) 5:1954–62. doi: 10.1182/bloodadvances.2020003424

21. Gharibnaseri Z, Davari M, Cheraghali A, Eshghi P, Ravanbod R, Espandar R, et al. Health care resource utilization and cost of care for haemophilia a and B patients in Iran. *Transfus Apher Sci*. (2016) 54:122–6. doi: 10.1016/j.transci.2016.01.025

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 05 February 2024
DOI 10.3389/fmed.2024.1347024

OPEN ACCESS

EDITED BY
Felix Carl Fabian Schmitt,
Heidelberg University Hospital, Germany

REVIEWED BY
Felix Hezel,
Heidelberg University Hospital, Germany
Emilia Lutz,
Heidelberg University Hospital, Germany

*CORRESPONDENCE
Rosa Sonja Alesci
✉ s.alesci@gerinnungszentrum-hochtaunus.de

RECEIVED 30 November 2023
ACCEPTED 15 January 2024
PUBLISHED 05 February 2024

CITATION
Alesci RS, Goldmann G, Halimeh S, Holstein K, Königs C, Miesbach W, Pfrepper C and Olivieri M (2024) Patient perspective on living with mild hemophilia in Germany: results from a nationwide survey.
Front. Med. 11:1347024.
doi: 10.3389/fmed.2024.1347024

Patient perspective on living with mild hemophilia in Germany: results from a nationwide survey

Rosa Sonja Alesci[1]*, Georg Goldmann[2], Susan Halimeh[3], Katharina Holstein[4], Christoph Königs[5], Wolfgang Miesbach[6], Christian Pfrepper[7] and Martin Olivieri[8]

[1]IMD Blood Coagulation Center Hochtaunus, Bad Homburg, Germany, [2]Institute of Experimental Hematology and Transfusion Medicine, University of Bonn, Bonn, Germany, [3]Blood Coagulation Center Rhein-Ruhr, Duisburg-Altstadt, Germany, [4]II. Medical Department, Coagulation and Hemophilia Center, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [5]Department of Paediatrics and Adolescent Medicine, Clinical and Molecular Haemostasis, Goethe University, University Hospital Frankfurt, Frankfurt, Germany, [6]Department of Hemostaseology and Hemophilia Center, Medical Clinic 2, University Hospital Frankfurt, Frankfurt, Germany, [7]Division of Hemostaseology, Department of Hematology, Cellular Therapy, Hemostaseology and Infectiology, University of Leipzig Medical Center, Leipzig, Germany, [8]Pediatric Thrombosis and Hemostasis Unit, Pediatric Hemophilia Center, Dr. von Hauner Children's Hospital, LMU München, Munich, Germany

Introduction: The disease burden and bleeding risk of patients with mild hemophilia may be underestimated. Their health-related quality of life (QoL) may be negatively impacted by insufficient treatment and bleed-related joint damage connected to a potentially delayed diagnosis.

Aim: This study aims to gain information on the care reality and QoL of patients aged ≥12 years with mild hemophilia in Germany.

Methods: An anonymous cross-sectional patient survey using standardized questionnaires was conducted in a validated electronic patient-reported outcome system. Medical specialists, hemophilia centers, patient organizations, and support groups across Germany invited the patients.

Results: A total of 43 patients (35 patients with hemophilia A, 5 patients with hemophilia B, and 3 patients for whom the information was missing) with a median age of 33 years were analyzed. The median age at diagnosis was 6.0 years (interquartile range [IQR] 2.0–15.0), and the median factor activity was 14.0% (IQR 12.0–25.0). Nearly 85% of the patients received factor concentrates in the past, and the most common reasons for the treatment were surgery or joint bleeding (each 65.6%). Half of the patients who provided feedback experienced complications during bleeding episodes. Prophylactic treatment with factor concentrates was rare (10.3%). The patients had minor problems regarding their health status.

Conclusion: Bleeding complications and joint bleeding, in particular, may be highly underestimated in patients with mild hemophilia, highlighting a medical need in this population. Patients with a potential benefit from prophylaxis need to be identified. Mild hemophilia has a negative impact on patients' QoL. Hemophilia centers satisfied the patients' needs. Further research is needed to address the current lack of awareness and improve adequate treatment in the future.

KEYWORDS

mild hemophilia, hemophilia A, hemophilia B, care reality, joint bleeding, quality of life

1 Introduction

Hemophilia refers to a rare congenital bleeding disorder (1) mainly affecting men (2). It is characterized by a coagulation factor deficiency of factor VIII (FVIII; hemophilia A, HA) or factor IX (FIX; hemophilia B, HB) (1).

Normal factor (FVIII or FIX) activity ranges from 50 to 150% (3) Residual factor activity in a patient's plasma is used to differentiate three severity degrees of hemophilia: mild with a relatively broad range of >5 to <40%, moderate with 1 to 5%, and severe with <1% of normal factor activity. Those with the normal factor activity ranging from 40 to 50% are not yet classified (4) Mild hemophilia with its wide range shows a varying phenotype. Some patients may present with a bleeding phenotype overlapping with severe hemophilia, possibly requiring prophylactic treatment (5), highlighting the importance of an individualized treatment strategy (1, 6).

Unlike severe hemophilia, mild phenotypes are often diagnosed later in life (5, 7), usually as a result of extended bleeding episodes provoked by injury or medical interventions; however, recurrent spontaneous bleeding episodes are rare in mild hemophilia. Recently, patient age was reported to correlate with arthropathy, a most relevant factor negatively affecting the quality of life (QoL) (7).

In mild HA, the development of FVIII neutralizing antibodies (inhibitors) is usually due to intensive exposure to factor concentrates (7, 8); the risk of inhibitor development may be associated with certain F8 mutations (8). While occurring less frequently in patients with mild or moderate HA compared to the severe form, these conditions pose a lifelong risk (8). Inhibitors can increase the severity of bleeding episodes (7) and may even shift a mild phenotype to a severe one (9), thereby complicating the treatment (10). FIX inhibitors are rare (1.5 to 3% of patients) and are almost exclusively found in patients with severe HB (1, 11), for whom an HB-specific formal clinical guidance may be lacking due the rarity of their condition (12). FIX inhibitor formation is unrelated to the type of FIX clotting factor concentrate (CFC) and is considered a most serious complication as anaphylaxis and nephrotic syndrome may occur (11).

Exact numbers of patients with (mild) hemophilia are unknown (1) due to various reasons (2, 7), and the number of undiagnosed patients is likely to far exceed (1) than those reported (2): in 2020, 4,518 patients with HA (mild type: 738 patients) and 860 patients with HB (mild type: 152 patients) were newly registered in the German Hemophilia Registry (13).

In patients with mild HA without medical contraindications, desmopressin is recommended for minor bleeding episodes, surgeries, and other invasive procedures (1). Desmopressin, a synthetic vasopressin analog, induces von Willebrand factor release from endothelial organelles and simultaneously increases FVIII levels by two- to six-fold (7, 14, 15). Factor replacement therapy may be required upon serious trauma or surgical procedures. Anti-fibrinolytic therapy with or without desmopressin can be used for the treatment of mucosal bleeding or invasive dental procedures. The use of desmopressin is cheaper than CFCs, avoids exposure to FVIII concentrates, and reduces the risk of inhibitor development (1, 16). It is ineffective in patients with HB (1, 7).

Abbreviations: DHG, Deutsche Hämophiliegesellschaft e.V.; IGH, Interessengemeinschaft Hämophiler e.V.

Patients with mild hemophilia or their families may initially lack awareness of the disease (9), leading to a delay in diagnosis (7). Suspicion may only be raised upon severe symptom development or complications, such as prolonged bleeding episodes after medical interventions, resulting from inadequate management. The literature on mild hemophilia including diagnosis and management is limited (7), and mild hemophilia may be underdiagnosed and undertreated compared to severe hemophilia (7, 17).

The current treatment of severe hemophilia aims to change its phenotype to that of moderate-to-mild hemophilia (1, 18) by prophylaxis (1). However, for patients with mild-to-moderate hemophilia, unmet needs (18), such as recommendations for physical activity (19), remain.

This survey aimed to investigate the care reality and QoL of patients with mild hemophilia, as daily problems and restraints may remain largely neglected (7).

2 Materials and methods

This cross-sectional survey was conducted to collect information on the care reality and the QoL of patients aged ≥12 years in Germany, diagnosed with mild HA or HB. The main objectives of this study were to assess the patient satisfaction with the therapy and support provided by hemophilia centers, the impact of hemophilia on daily life, QoL [EQ-5D-5L questionnaire (20)], and medical treatments. The web-based survey collected anonymous data directly from patients in a single session using standardized electronic questionnaires filled out in the AMS-ePRO® tool, a validated electronic patient-reported outcome system. Consequently, the captured data were encrypted.

Various medical specialists, hemophilia centers, patient organizations, and support groups across Germany distributed invitations. Distribution and individualized one-time QR codes on invitations were not tracked, thereby ensuring anonymity. To minimize the selection bias, invitations were handed out to all eligible patients. Patients accessed the survey using their own smartphones/tablets by scanning the QR code. Internet connection via a standard web browser was secured by hypertext transfer protocol secure, and the database server was hosted under controlled conditions in an off-site facility.

Easy-to-complete lay language questions focused on demographics, diagnosis, and main objectives discussed earlier. It is important to note that drug-related adverse events were not collected, and there were no free-text fields. However, respondents were able to skip questions, and while logged in, questions could be answered in any order and ticked answers could be amended. Data capture could end prematurely, and re-entry was not possible. Logic and plausibility checks were implemented to ensure data quality and to minimize data inconsistencies.

Based on the number of patients and demographics in Germany, 150–200 patients were expected to participate. The sample size was based on the estimated number of suitable patients and was not formally calculated, as this was an exploratory survey without formal hypothesis testing. Statistical analyses were conducted using SAS® (v9.4 or later; SAS Institute Inc., Cary, NC, United States). The analysis set included all eligible patients who answered at least one question. All results were reported descriptively. Continuous variables were represented using mean, standard deviation, median, quartiles (Q1 and Q3), range (minimum/maximum), and the number of missing values. Categorical variables were represented as absolute and

percentage frequencies of answers. All available data were included in the analyses and summarized as far as possible. Unless otherwise specified, missing data were not replaced. The survey started in September 2021 and ended in July 2022.

3 Results

3.1 Patient characteristics

A total of 44 datasets were collected, and 43 were included in the final analysis set; one was excluded because it was a test input. The median age was 33.0 years (min–max 12–75; 41 answers; Table 1). The sex of the patients was recorded. The median age at the initial diagnosis of mild hemophilia was 6.0 years (min–max 0–37; 39 answers). Most patients had HA (87.5%, 40 answers).

The median factor activity at diagnosis was 14.0% (min–max 4–55), with a mean (SD) activity of 18.1% (11.8), and 10 patients (23.8%) did not know their factor activity at diagnosis (30 answers). One patient with HA had factor levels within the lower limit of normal (4), experiencing increased hematoma frequency/intensity and unusual blood test results, suggesting a possible coagulopathy. In another patient with HA, factor levels below the limit of "mild" (4) were detected by coincidence.

Patients could provide multiple answers regarding the reasons for which blood coagulation was checked at hemophilia diagnosis (41 answers). The three most common reasons for hemophilia diagnosis were familial predisposition (46.3%), bleeding episodes during/after surgery or dental treatment (36.6%), and increased hematoma frequency or intensity (24.4%, 41 answers). The time elapsed between first symptoms and hemophilia diagnosis was <3 months for one-third of the patients (33.3%). Another third of the patients (35.9%) did not know how much time passed between first symptoms and hemophilia diagnosis. Approximately one-quarter of patients received their diagnosis >1 year after their first symptoms (39 answers, Table 1).

TABLE 1 Self-reported patient characteristics (full analysis set: *N* = 43).

Patient characteristics	FAS *N* = 43
Age (years)[a]	
Median (IQR)	33.0 (23.0–48.0)
Mean (SD)	36.0 (16.8)
Min–Max	12–75
Age at diagnosis of mild hemophilia (years)[b]	
Median (IQR)	6.0 (2.0–15.0)
Mean (SD)	10.6 (10.6)
Min–Max	0–37
Unknown, *n* (%)[†]	1 (2.4)
Hemophilia type, n (%)[c]	
Mild hemophilia A	35 (87.5)
Mild hemophilia B	5 (12.5)
Factor activity at diagnosis[d]	
Median (IQR)	14.0 (12.0–25.0)
Min–Max	4–55
Unknown, *n* (%)[†]	10 (23.8)
Reason for which blood coagulation was checked at hemophilia diagnosis, n (%)[‡,e]	
Familial predisposition	19 (46.3)
Bleeding episode during/after surgery or dental treatment	15 (36.6)
Increased hematoma frequency/intensity	10 (24.4)
Problems with stopping the bleeding episode	7 (17.1)
Coincidence	4 (9.8)
Frequent and/or severe nosebleeds	4 (9.8)
Unusual blood test results, indicating a possible coagulopathy	3 (7.3)
Frequent and/or severe gum bleeding	2 (4.9)
Unusual bleeding episode, e.g., knee, gastrointestinal	1 (2.4)
Unknown[†]	0 (0)
Time elapsed between first symptoms and diagnosis of hemophilia, n (%)[f]	
<3 months	13 (33.3)
3 to 12 months	2 (5.1)
>1 year	10 (25.6)
Unknown[†]	14 (35.9)

Percentages are calculated for patients with non-missing data; number (missing) *n* = [a]41 (2), [b]39 (4), [c]40 (3), [d]30 (13), [e]41 (2), [f]39 (4). [†]Patients could answer "I do not know". [‡]Multiple answers were possible. FAS, full analysis set; IQR, interquartile range 25–75; n, number; SD, standard deviation.

3.2 Previous hemophilia treatment

The majority of the patients (84.2%) had received the factor concentrate for hemophilia treatment in the past (38 answers, Table 2). The median age of first factor administration was 10.0 years (min–max 0–56; 27 answers; Table 2).

Almost half of the patients with HA (43.6%) had not received desmopressin treatment in the past (39 answers). The median age at first desmopressin administration was 27.5 years (min–max 4–49); the mean age (SD) was 23.0 years (14.9, 8 answers, including three patients who could not recall).

Half of the patients (50.0%, 38 answers) experienced complications due to untreated bleeding episodes in the past.

3.3 Reasons for previous hemophilia treatment and for complications due to untreated bleeding episodes

Patients could provide multiple answers regarding the reasons for previous treatments (ranging from 11 to 32 answers). There were multiple reasons for the treatment with the factor concentrate (Table 3), with the most common being joint bleeding and/or a perioperative setting (21; 65.6% each). The most common reasons for using desmopressin were medical procedures (dental treatments and surgery) or accidents (11 answers). Spontaneous or joint bleeding was less frequently treated with desmopressin. The most common reasons for complications due to untreated bleeding episodes—19 patients (50%) who answered that they had experienced complications—were due to medical procedures, such as dental

TABLE 2 Previous hemophilia treatment—type of treatment and age of first treatment administration (full analysis set: *N* = 43).

Characteristics	FAS *N* = 43
Treatment with a factor concentrate in the past, n (%)[a]	
Yes	32 (84.2)
No	6 (15.8)
Age at the first factor concentrate administration (years)[b]	
Median (IQR)	10.0 (6.0–19.0)
Mean (SD)	16.0 (14.1)
Min–Max	0–56
Unknown, *n* (%)[†]	5 (15.6)
Treatment with desmopressin in the past, n (%)[c]	
Yes	11 (28.2)
No[‡]	20 (51.3)
Unknown[†]	8 (20.5)
Age at the first desmopressin administration (years)[d]	
Median (IQR)	27.5 (9.0–29.0)
Mean (SD)	23.0 (14.9)
Min–Max	4–49
Unknown, *n* (%)[†]	3 (27.3)
Complications due to untreated bleeding episodes, n (%)[e]	
Yes	19 (50.0)
No	18 (47.4)
Unknown[†]	1 (2.6)

Percentages are calculated for patients with non-missing data; number (missing) n = [a]38 (5); [b]27 (16); [c]39 (4), [d]8 (35), [e]38 (5). [†]Patients could answer "I do not know".
[‡]Includes 3 patients with hemophilia B; 17 patients (43.6%) with hemophilia A did not receive desmopressin. FAS, full analysis set; IQR, interquartile range 25–75; n, number; SD, standard deviation.

TABLE 3 Reasons for previous hemophilia treatments and for complications due to untreated bleeding episodes (full analysis set: *N* = 43).

Characteristics	FAS *N* = 43
Reason for treatment with factor concentrate, n (%)[†, ‡, a]	
Joint bleeding	21 (65.6)
Perioperative setting	21 (65.6)
Dental treatment/tooth replacement	19 (59.4)
Accident	15 (46.9)
Spontaneous bleeding	4 (12.5)
Unknown	0 (0)
Reason for treatment with desmopressin, n (%)[†, ‡, b]	
Dental treatment/tooth replacement	6 (54.5)
Perioperative setting	4 (36.4)
Accident	3 (27.3)
Joint bleeding	2 (18.2)
Spontaneous bleeding	2 (18.2)
Unknown	0 (0)
Reason for complications due to untreated bleeding episodes, n (%)[†, ‡, c]	
Bleeding after surgery	11 (57.9)
Dental treatment/tooth replacement	10 (52.6)
Joint bleeding	10 (52.6)
Accident	4 (21.1)
Spontaneous bleeding	2 (10.5)
Unknown	0 (0)

Percentages are calculated for patients with non-missing data; number (missing) n = [a]32 (11), [b]11 (32), [c]19 (24). [†]Patients could answer "I do not know".
[‡]Multiple answers were possible. FAS, full analysis set; n, number.

treatment (10/19 patients; 52.6%), surgery (11/19 patients; 57.9%), and joint bleeding (10/19 patients; 52/69%). Patients could provide multiple answers to this question.

3.4 Current hemophilia treatment and reasons for choosing prophylaxis with factor concentrate

Patients could provide multiple answers regarding their current treatment/the use of prophylaxis (39 answers). Most patients (69.2%) received the factor concentrate on-demand. One-quarter of the patients used medications other than factor concentrates, such as tranexamic acid or desmopressin. Furthermore, 59% of the patients received non-medicinal treatment or no medication for the treatment of bleeding episodes (Table 4) and used, e.g., plasters or dressings.

Additionally, four patients (10.3%), two with HA and two with HB, currently used prophylaxis with factor concentrate. They indicated multiple reasons for choosing prophylaxis (Table 4). The three most frequent reasons were a desire for better control of bleeding episodes even in the case of non-apparent bleeding (100%), a reduction of bleeding episode frequency (100%), or severity (75%). The desire for more safety in daily life or a more carefree life was not in focus (0%).

Concentrates with an extended half-life were rarely used in this patient population (27 answers; Table 4), and most patients (63%) were unaware of whether they were receiving them.

3.5 Visits at hemophilia centers

Patients visited hemophilia centers only when needed (55.3%) or on a regular basis, i.e., more often than every six months and every 6 or 12 months (44.7%; 38 answers). Half of those patients (nine, 52.9%) who needed regular visits (17 patients) attended the hemophilia center about every six months or only about once a year (seven, 41.2%). One patient required more frequent visits (Table 5).

3.6 Satisfaction with treatment and support

There were 37 patients who provided feedback on their satisfaction with the support and treatment received at the hemophilia center (Figure 1). Generally, patients were very satisfied (*n* = 16; 43.2%) or satisfied (n = 17; 45.9%) with the support from their hemophilia center. Then, two patients each (5.4%) were neutral or unsatisfied in this respect. The results were similar for the satisfaction with therapy

TABLE 4 Current hemophilia treatment of bleeding episodes and reasons for choosing prophylaxis with factor concentrate (full analysis set: *N* = 43).

Characteristics	FAS *N* = 43
Current treatment/use of prophylaxis, n (%)[†, a]	
Current treatment of bleeding episodes	
Factor concentrate if required	27 (69.2)
Non-medicinal treatment or no medication	23 (59.0)
Other medications except factor concentrate	10 (25.6)
Current use of prophylaxis	
Factor concentrate	4 (10.3)
Use of factor concentrate with extended half-life, n (%)[‡, b]	
No	8 (29.6)
Yes	2 (7.4)
Unknown	17 (63.0)
Reasons for choosing prophylaxis with factor concentrate, n (%)[†, c]	
Better bleeding episode control even in the case of non-apparent bleeding	4 (100.0)
Reduction of bleeding episode frequency	4 (100.0)
Reduction of bleeding episode severity	3 (75.0)
Desire for a more active life	2 (50.0)
Reduction of hospitalizations	2 (50.0)
Prevention of joint damage	1 (25.0)
Not specified	0 (0.0)

Percentages are calculated for patients with non-missing data; number (missing) n = [a]39 (4), [b]27 (16), [c]4 (39). [†]Multiple answers were possible.
[‡]Based on patients using "prophylaxis" and/or "factor concentrate if required". FAS, full analysis set; n, number.

for the treatment of bleeding episodes (very satisfied: $n = 18$; 48.6% and satisfied: $n = 17$; 45.9%). Two patients (5.4%) were neutral, and none was unsatisfied regarding the therapy for the treatment of bleeding episodes received at the hemophilia center. None of the patients was very unsatisfied with either the support or the treatment received at their hemophilia center (Figure 1).

The majority of the patients (73.7%) did not seek medical care from other physicians other than their hemophilia center, while approximately one-quarter of patients (26.3%) sought another medical opinion (38 answers).

Patients could provide multiple answers regarding the use of additional services for medical support (39 answers). Again, most patients (74.4%) did not utilize services in addition to their contact with the hemophilia centers (Table 5). Online services of patient societies or online communities/personal exchange with other patients were rarely used (39 answers). Nearly half of the patients (45.9%) used a diary to track bleeding episodes and treatments (37 answers; Table 5), while others did not. If patients used a diary, 64.7% of physicians incorporated this information into their decision-making (17 answers; Table 5).

3.7 Impact of hemophilia on daily life

Answers were provided by 37 patients; most patients could usually (67.6%) or always (13.5%) detect the bleeding episodes and assess when they needed treatment (Figure 2A). Hemophilia was sometimes (43.2%) a burden, for example, at work, school, or during leisure time, and seldom for one-third (35.1%) of the patients (Figure 2B). Patients needed support always (40.5%), usually (32.4%), or sometimes (18.9%) from the hemophilia center upon bleeding episodes (Figure 2C).

3.8 Health questionnaire (EQ-5D-5L)

EQ-5D-5L includes the dimensions "mobility", "self-care", "usual activities", "pain/discomfort", and "anxiety/depression", with the ratings ranging from "no problems" to "unable" and "no" to "extreme" (20). The number of answers varied in different dimensions:

The answers were provided by 37 patients for mobility, 35 for self-care, and 34 patients for each of the dimensions: usual activities, pain/discomfort, and anxiety/depression. Most patients had no problems in all five dimensions, except for pain/discomfort (Figure 3). Here, the results were roughly similar for no and slight impairment (41.2 and 47.1%, respectively). For mobility and usual activities, >10% of the patients experienced slight problems (21.6 and 14.7%, respectively), and for anxiety/depression, >20% of the patients had slight symptoms (26.5%; Figure 3). Information was missing for eight patients regarding self-care and nine patients each for usual activities, pain/discomfort, and anxiety/depression.

The EQ-VAS scale allows patients to self-report their health status on a scale from 0 ("worst imaginable health") to 100 ("best imaginable health"). The median EQ-VAS was 80.0 (range 30–100; based on 33 answers).

4 Discussion

This survey aimed to shed light on the reality of care for patients with mild hemophilia, as daily problems and restrains may remain largely neglected (7).

The proportion of hemophilia types in our patient population examined (HA: 87.5% and HB: 12.5%; 40 answers) was in line with that of the population affected (HA: 80 to 85% and HB: 15 to 20%) (1). The median age at diagnosis reported here (6.0 years) was similar to other publications (2.4 to 6.5 years), (9, 10, 21, 22), and the median factor activity at diagnosis (14%) was also in line with other findings (15%) (21). One patient indicated FVIII levels within the lower limit of normal (4), and medical history suggested a possible coagulopathy. Due to limited data, we can only speculate if this was due to temporarily elevated FVIII activity, or artifacts in applied assays (1), reporting an error or a misunderstanding by the patient. The two latter reasons or false low values (1) may be causal for the factor levels below the limit of "mild" (4), which were detected by coincidence in another patient with FVIII deficiency. Both patients were included in the analysis as local laboratory standards on categorization levels for hemophilia slightly differ.

The main reasons for diagnosis in this survey, such as familial predisposition, bleeding episodes during/after a surgery or dental treatment, and increased hematoma frequency or intensity, were also reported by other publications (7, 21). The diagnosis of mild hemophilia is often delayed compared to that of more severe phenotypes (7, 9, 18) and this delay is highly dependent on a country's

TABLE 5 Frequency of visits to the hemophilia center, use of additional services, and patient diaries (full analysis set *N* = 43).

Characteristics	FAS *N* = 43
Frequency of visits to the hemophilia center, n (%)[a]	
Only when needed	21 (55.3)
Regularly	17 (44.7)
Visit frequency by patients who regularly visit the hemophilia center, n (%)[b]	
About every 6 months	9 (52.9)
About once a year	7 (41.2)
More often than every 6 months	1 (5.9)
Use of additional services, n (%)[†,c]	
No additional services	29 (74.4)
Online services of the IGH	6 (15.4)
Online services of the DHG	5 (12.8)
Online community	2 (5.1)
Personal exchange with other patients	2 (5.1)
Tracking of bleeding episodes and treatments in a diary, n (%)[d]	
No	20 (54.1)
Yes	17 (45.9)
Physician takes a diary into account when deciding on treatment, n (%)[e]	
No	6 (35.3)
Yes	11 (64.7)

Percentages are calculated for patients with non-missing data; number (missing) *n* = [a] 38 (5), [b] 17 (26), [c]39 (4), [d]37 (6), [e]17 (26). [†]Multiple answers were possible. FAS, full analysis set; DHG: Deutsche Hämophiliegesellschaft e.V.; IGH: Interessengemeinschaft Hämophiler e.V.; n, number.

economic status (2, 9). Patients unaware of their condition may neglect symptoms until late (7, 9). The main reason for a late(r) diagnosis is the late(r) onset of bleeds as patients with a mild phenotype often do not have spontaneous bleeds and require "adequate" trauma (7).

In our survey, most patients (84.2%) had previously received the factor concentrate for the treatment of hemophilia. Other studies report lower proportions to treat bleedings (51 to 75%) (16, 21) or joint bleeds (40%) (21); an exact comparison of the results is limited as, due to limited data, we cannot report the exact specification of the CFC use. Generally, the use of CFCs bears the risk of inhibitor development, which may eventually complicate treatment (10, 23). The age at first FVIII treatment largely depends on the level of the baseline factor activity. In addition, patients with mild HA may receive their first CFC dose at 4.4 years. A known family history of HA may lead to an earlier treatment (3.9 years), while a negative family history can lead to a delay in therapy (6.4 years). Patients with mild HA and a negative familial history tended to be older if factor activities ranged between 10 and 15% (7.2 years) and 25 and 40% (12.1 years) (24). This is approximately in line with our cohort: The familial predisposition was known by less than half of the patients (46.3%), and they initiated the CFC treatment at a median age of 10.0 years, with a median factor activity at diagnosis of 14.0% (12.0–25.0%). The patients in our survey used factor concentrates mainly to treat joint bleeding and/or in a perioperative setting, as recommended (1).

Most patients (69.2%) received the factor concentrate on-demand as a current treatment option, which was lower than that reported in the DYNAMO study (98%), an international multicenter study including men aged 12 to 55 years with non-severe hemophilia

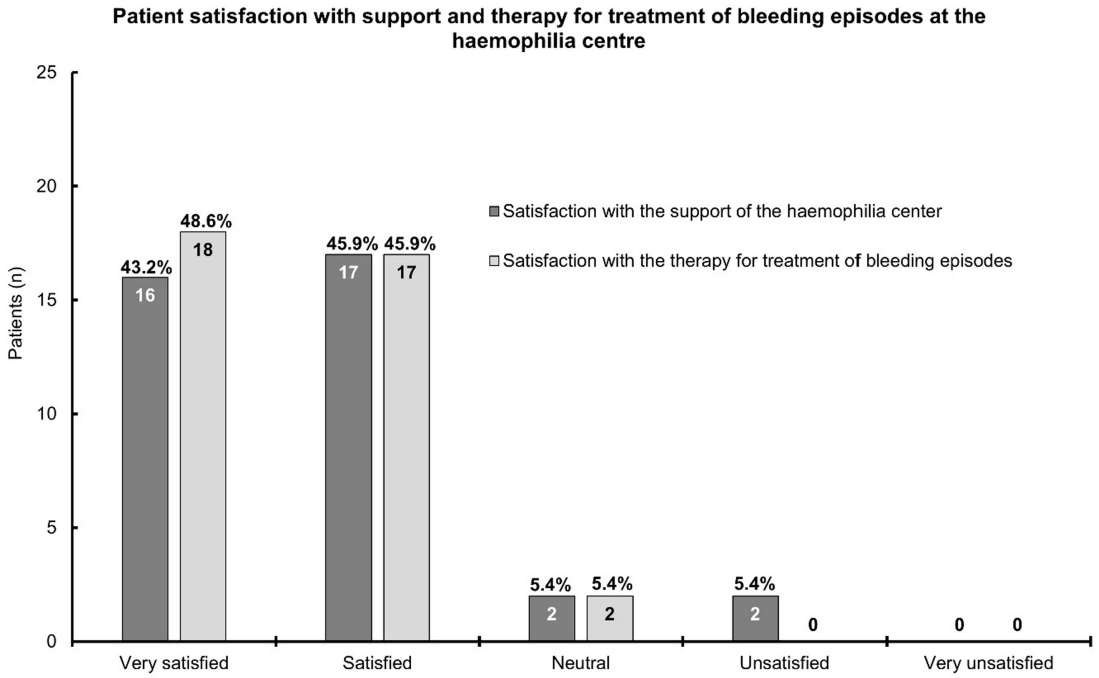


FIGURE 1
A bar chart depicting patient satisfaction with the support and therapy for the treatment of bleeding episodes at the hemophilia center. This chart is based on the information provided by 37 patients; the answers for six patients per topic were missing. The bars show the patient numbers at the inside end of the bars; the corresponding percentages are shown on top of the bars. Percentages relate to the number of patients who provided answers.

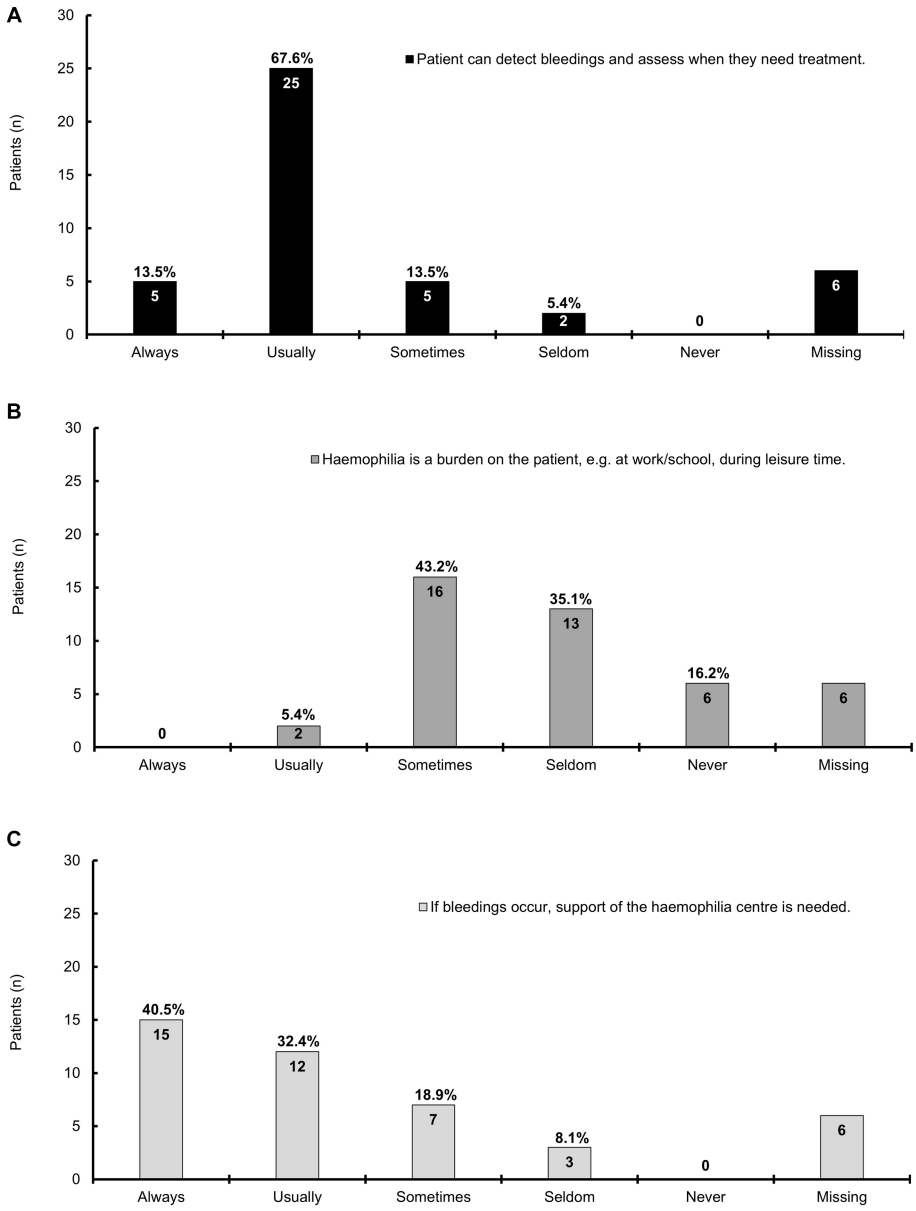


FIGURE 2
A bar chart representing the impact of mild hemophilia on daily life; this chart is based on the information provided by 37 patients; the answers for six patients were missing. The bars show the patient numbers at the inside end of the bars; the corresponding percentages are shown on top of the bars. Percentages relate to the number of patients who provided answers. **(A)** The majority of patients can always or usually detect bleeding episodes and assess when they need treatment; **(B)** Hemophilia is sometimes a burden on the patients, e.g., at work, school, or during leisure time; **(C)** The majority of patients always or usually needs support from the hemophilia center if bleeding occurs, which is sometimes the case for seven patients.

(residual FVIII/IX activity: 2 to 35%) (21). Other patients (59.0%) chose non-medicinal treatment or no medication for the treatment of bleeding episodes and used plasters or dressings or other medications except the factor concentrate (25.6%; see below).

We observed a higher prophylaxis rate (10.3%) than the DYNAMO study (1.7% prophylaxis/intermittent prophylaxis), which included patients with a lower factor activity range (2 to 35%) determined by the central laboratory upon inclusion (21), while we report a patient-reported factor activity at diagnosis. The prophylaxis rate in our study was similar to the PROBE study (11.8% of men with mild hemophilia with regular/intermittent prophylaxis [$n = 12$] and 2.63% of women [$n = 1$] with regular prophylaxis) (25); however, we did not overtly offer the prophylaxis subcategories "regular/intermittent" (4) and did not ask for the participants' sex. Knowledge on the benefits of prophylaxis is scarce (18). Patients with mild hemophilia may receive prophylaxis for the treatment of acute bleeding episodes or before invasive procedures (7, 25), potentially at a later stage than patients with severe hemophilia (5). There may be patients with mild hemophilia, who would benefit from prophylaxis, fostering "adequate" hemostasis and protection from the consequences of the diseases (5).

Factor concentrates with an extended half-life were rarely used by patients using "factor concentrate if required" and/or "on current prophylaxis", and most patients (63%) were unsure if they are treated

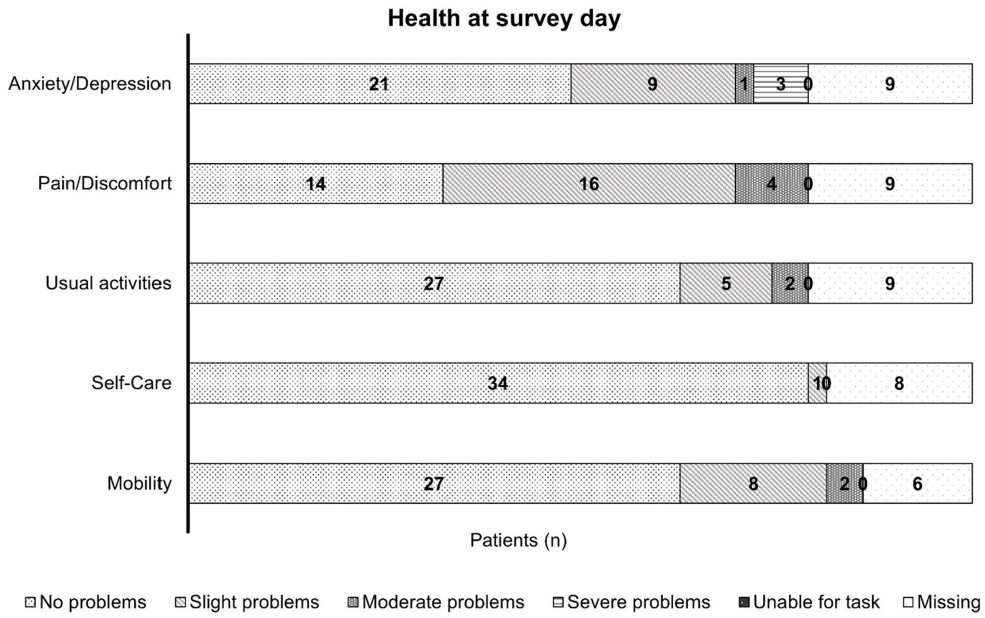


FIGURE 3
A bar chart representing the health of patients with mild hemophilia at the day of the survey; this chart is based on the information provided by 34, 35, and 37 patients, with missing information for six patients in mobility, eight patients in self-care, and nine patients each in their usual activities, pain/discomfort, and anxiety/depression. The bars show the patient numbers in the center of the respective bar section. Most patients experienced no limitations due to their condition in all five dimensions, except for the pain/discomfort dimension where nearly half of the patients experienced slight impairment.

with those concentrates. FVIII concentrates with extended half-life, non-replacement, or gene therapy may even raise target trough levels (26). Gene therapy, non-replacement, or novel concentrates might be a future option, at least for some carefully selected patients (1, 27).

Less than half of the patients (43.6%) had received desmopressin in the past, and one-quarter of the patients (25.6%) used medications other than factor concentrates such as tranexamic acid or desmopressin as a current treatment option. Desmopressin is recommended for most patients with mild hemophilia A (28). The majority of the patients will reach adequate peak FVIII levels (≥30%) post-desmopressin and almost all if treatment decisions are adapted to desmopressin response testing results (16). However, FVIII levels >50% are considered safe for major surgery may not be reached (29). Desmopressin, recommended for patients with mild (and moderate) HA (1), was used by the patients in our survey for medical procedures, surgery, or accidents. Spontaneous or joint bleeding was less frequently treated with desmopressin, which is similar to the DYNAMO study. In the DYNAMO study, desmopressin was most commonly used for the treatment of minor wounds, oral cavity bleeds, and soft-tissue/(sub)cutaneous bleeds (16).

Approximately 60% of patients chose non-medicinal treatment or no medication for the treatment of bleeding episodes as compressions, a procedure applied by others for small bleeds or cuts, that is following the standard RICE (rest, ice, compression, and elevate) principle (30).

Half of the patients (50.0%) experienced complications due to untreated bleeding episodes in the past, which was lower than the percentage reported in the DYNAMO study (75%) (21). The treatment of mild hemophilia may be suboptimal regarding joint outcomes (25, 31). Increasing factor levels do not automatically correspond to a less severe phenotype (32); for individuals with HA, it is necessary to maintain factor levels of ≥15% (33) or > 20% (34) to prevent all spontaneous joint hemorrhages. Regression models of a longitudinal study (34) predicted 1.4 and 0.6 bleeds/year for patients with hemophilia A and B and factor levels of 15%, which seemed unlikely to prevent all joint bleeds. This may highlight the importance of adequate prophylaxis to avoid loss of joint range-of-motion and final hemophilic arthropathy after infrequent but ongoing bleeding episodes over time (35). Patients with mild HA may experience destruction of cartilage or mild-to-moderate synovitis (17). Repeated (limited or subclinical) or even single joint bleeding may lead to joint arthropathy, resulting in pain and decreased mobility of affected joints (1, 17, 18, 26). A large proportion of patients with non-severe hemophilia may therefore be at risk of long-term sequelae if not receiving more intensive treatment (31). Therefore, early detection of signs of joint damage (26), and patient education on prevention and early recognition of joint bleeding (21) are crucial (36).

Approximately, half of our patients (55.3%) visited the hemophilia center only when needed. Those patients requiring regular visits (44.7%) had to visit either once in every six months (52.9%) or only about once a year (41.2%). Patients with mild or mild-to-moderate hemophilia should visit a hemophilia treatment−/−comprehensive care center at least every two years (37). In daily routine, visits to hemophilia centers may be much more frequent with every 6 to 12 months, while the mean frequency could be even higher (19).

The generally (very) high satisfaction of the patients in our survey with the support from their hemophilia center and the treatment of bleeding episodes was also reported in another, yet small, survey from

the United Kingdom, (30) and a larger study from the United States comprised patients with all severities (mild 32.8%), females patients with hemophilia, and patients with other bleeding disorders, such as von Willebrand disease (38).

In our survey, patients perceived hemophilia sometimes (43.2%) a burden during their daily life, and one-third (35.1%) of patients seldom had this feeling. This is slightly less than reported by other publications (26, 39). Mild hemophilia may negatively impact employment (19, 39) and have a moderate (59%) or large (53%) impact on education or work (39). Interestingly, the impact on education and work may be lower for patients with severe hemophilia, suggesting a better care reality for these patients (39). QoL data in mild hemophilia and comparisons to healthy controls are rare (26) indicating an unaddressed unmet need.

Most patients (73.0%) always/usually needed support from the hemophilia center, indicating the need for adequate treatment (10). However, patients may choose a wait-and-see approach before seeking healthcare services, depending on the severity of bleeding episodes and pain (30). Improved patient education may lead to earlier intervention and, thereby, timely treatment (7, 9).

Most patients had no problems in all five EQ-5D-5L dimensions ("mobility", "self-care", "usual activities", "pain/discomfort", and "anxiety/depression"), with the only exception of pain/discomfort. In this dimension, approximately similar proportions of patients experienced either no (41.2%) or a slight (47.1%) impairment. The patients experienced slight problems with mobility (21.6%) and usual activities (14.7%), and 26.5% of patients had slight symptoms of anxiety/depression. Our findings was similar to that of the B-Natural study HB cohort (31). The QoL was found to be affected in our study as well as in other studies (26). Patients with mild hemophilia may experience pain and long-term disabilities typically associated with severe hemophilia (40). Here, the percentage of slight/moderate pain/discomfort and no severe pain was lower than that in the B-HERO-S study, which included adult patients and caregivers of children with HB of all grades (19, 41). Patients with mild (and moderate) hemophilia reported higher acute and chronic pain levels than people without bleeding disorder, suggesting suboptimal treatment of joint disease (25), which again negatively impacts QoL (25, 31), strengthening the need for regular, close surveillance (17).

In this study, the median patient self-rated health, as reported by the EQ-VAS scale, was 80.0, ranging from 30 to 100, with a score of 100 equaling "best imaginable health". This score was higher than that in the B-HERO-S study (41) and was similar to the P-FiQ study, which included both patients with HA and HB (19).

4.1 Limitations

As with any observational study, there is a risk of bias such as the selection bias due to differing patient populations visiting different centers. The patients who came to the centers in a certain period of time were approached, i.e., potentially those with a lower factor activity. It cannot be excluded that patients with more problems were more motivated to answer the questionnaire. The participation was lower (21.5 to 28.7%) than expected, which was also frequently observed in previous electronic/web-based surveys (14–18%) (42, 43). Increasing these through supportive measures, e.g., reminders, was not possible in our setting. Results may not be representative of all patients with mild hemophilia, as only those with smartphone/tablet access and the ability to scan QR codes could participate. Motivation/approaches in filling out questionnaires may differ, but retrospective verification is impossible due to anonymized data capture. Captured data were patient-reported and were not validated by a physician. Due to the survey design, reviewing unclear/incorrect information was impossible. While automated measures reduced the possibility of implausible data, it is important to note that missing/implausible data may still exist. The generation of irrelevant/double/fake datasets cannot be completely ruled out. Additionally, some data may be missing due to accidental or purposeful premature survey termination.

5 Conclusion

The definition "mild" based on the residual factor activity may not result in an actual perception of a mild disease by the affected patients. Mild hemophilia can be easily overlooked, leading to inadequate patient management, especially regarding joint problems/hemophilic arthropathy and lowering the patients' QoL. Further research, such as longitudinal studies to track the progression of clinical outcomes or a study to determine whether individuals with higher factor activity have fewer emergency visits or scheduled appointments, might be useful in this patient population. Increased awareness of mild hemophilia is needed to improve diagnosis and treatment for these patients.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

RA: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing, Resources. GG: Resources, Writing – review & editing. SH: Resources, Writing – review & editing. KH: Resources, Writing – review & editing. CK: Investigation, Resources, Writing – review & editing. WM: Investigation, Resources, Writing – review & editing. CP: Resources, Supervision, Writing – review & editing. MO: Investigation, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors thank all the participants of this survey and the members of the DHG, IGH, and Deutsche Bluthilfe for their support. Medical writing support was provided by Ina Heppel and Hanna Neymeyer, AMS Advanced Medical Services GmbH, Mannheim, Germany, prior to the review, revision, and the decision to publish by the authors, and funded by CSL Behring, Germany.

Conflict of interest

RA has acted as a paid consultant to CSL Behring and has received funding for research carried out in this work. She has received funding from Takeda, Sobi, Octapharma, Grifols, and Novartis and meeting/congress support from Takeda, Sobi, and Bayer. Furthermore, RA acted as a paid consultant to Amgen, Bayer, BFSH, Grifols, LFB Pharma, Medlearning, Octapharma, onkowissen, STREAMED UP (Pfizer, Grifols), Sobi, and Takeda Pharma. GG reports receiving honoraria for lectures from Bayer, Biotest, Novo Nordisk, Octapharma, Roche, Sobi, and Takeda, and reimbursement for travel expenses from Biotest, Novo Nordisk, and Sobi. SH reports grants or contracts from Bayer Healthcare, Baxalta Innovations (now Takeda), Biotest, CSL Behring, Novo Nordisk Pharma, Octapharma, Pfizer Pharma. Further, SH has received honoraria from Bayer Healthcare, Baxalta Innovations (now Takeda), Biotest, CSL Behring, Novartis Pharma, Novo Nordisk Pharma, Octapharma, Pfizer Pharma, Roche Pharma; Swedish Orphan Biovitrum and consulting fees from Bayer Healthcare, Biotest, CSL Behring, Novo Nordisk Pharma, Octapharma, Chugai Pharma Germany, Swedish Orphan Biovitrum. KH reports grants for research or clinical studies (to institution): Bayer, CSL Behring, Novo Nordisk, Pfizer, Sobi and GWT/Roche, and honoraria for lectures or consultancy (to person): Bayer, BioMarin, Biotest, Chugai, CSL Behring, LFB, Novo Nordisk, Pfizer, Roche, Sobi and Takeda. KH has served as a consultant for Bayer, Biotest, CSL Behring, LFB, Novo Nordisk, Pfizer, Roche and Sobi, and in an advisory board for Octapharma. She has received meeting/congress support from Pfizer, Sobi, Takeda, Bayer, CSL Behring, Novo Nordisk and has served as a speaker for the Haemophilia Board of the GTH and is a member of the Ärztlicher Beirat of the Deutsche Hämophiliegesellschaft e.V. (DGH; Medical Advisory Board of the German Haemophilia Society e.V.). CK has received consulting fees or honoraria for lectures and meeting/congress support from BFSH, CSL Behring, MSD, Novo Nordisk, Roche/Chugai, Sobi/Sanofi, and Takeda. CK's institution has received grants from Bayer Vital GmbH, Biotest, CSL Behring, Intersero, Novo Nordisk, Pfizer, Roche/Chugai, Sanofi/Sobi, Takeda, and the European Union (H2020) and federal funding. He is a member of the German Paediatric AIDS Society and Head of the German Haemophilia PUP Registry. WM has acted as a paid consultant to Bayer, BioMarin, Biotest, CSL Behring, Chugai, Freeline, LFB, Novo Nordisk, Octapharma, Pfizer, Regeneron, Roche, Sanofi, Sigilon, Sobi, Takeda/Shire, uniQure and has received research funding form Bayer, Biotest, CSL Behring, LFB, Novo Nordisk, Octapharma, Pfizer, Sobi, Takeda/Shire. WM has received honoraria for lectures from Bayer, BioMarin, Biotest, CSL Behring, Chugai. LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sobi, and Takeda/Shire and has received meeting/congress support from Bayer, BioMarin, Biotest, CSL Behring, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sobi, Takeda/Shire, uniQure. CP reports grants for studies and research from Chugai, Roche, LeoPharma, Zacros, and Takeda; and personal fees for lectures or consultancy from Alexion, Bayer, BioMarin, Chugai Pharma, Roche, CSL Behring, Leo Pharma, Novo Nordisk, Pfizer, BMS, Sanofi, Sobi, Takeda, and Zarcos. MO has received honoraria or consulting fees from Bayer, BioMarin, Biotest, Chugai, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Roche, Sobi, Stago and Takeda. MO has participated in DSMB/advisory boards from Bayer, Biomarin, Biotest, Chugai, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Roche, Sobi and Takeda and has received meeting/travel support from Bayer, Biotest, CSL Behring, Novo Nordisk, Pfizer, Roche, Sobi, Takeda. This study received funding from CSL Behring, Germany. The funder had the following involvement with the study: study design, the writing of this article (funding of medical writing support), and the decision to submit it for publication.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Srivastava A, Santagostino E, Dougall A, Kitchen S, Sutherland M, Pipe SW, et al. The WFH guidelines for the Management of Hemophilia panelists and co-authors WFH guidelines for the management of hemophilia, 3rd edition. *Haemophilia*. (2020) 26:1–58. doi: 10.1111/hae.14046

2. WFH World Federation of Hemophilia. *Report on the annual global survey*. Geneva: WFH (2021).

3. Barthels M, Bergmann F, Czwalinna A. *Das Gerinnungskompendium*. 2nd ed. M. Barthels

Stuttgart: Thieme (2012). doi: 10.1055/b-002-13417

4. Blanchette VS, Key NS, Ljung LR, Manco-Johnson MJ, van den Berg HM, Srivastava A. Definitions in hemophilia: communication from the SSC of the ISTH. *J Thromb Haemost*. (2014) 12:1935–9. doi: 10.1111/jth.12672

5. Castaman G, Peyvandi F, De Cristofaro R, Pollio B, Di Minno DMN. Mild and moderate hemophilia a: neglected conditions, still with unmet needs. *J Clin Med*. (2023) 12:1368. doi: 10.3390/jcm12041368

6. Stephensen D, Bladen M, McLaughlin P. Recent advances in musculoskeletal physiotherapy for haemophilia. *Ther Adv Hematol*. (2018) 9:227–37. doi: 10.1177/2040620718784834

7. Benson G, Auerswald G, Dolan G, Duffy A, Hermans C, Ljung R, et al. Diagnosis and care of patients with mild haemophilia: practical recommendations for clinical management. *Blood Transfus*. (2018) 16:535–44. doi: 10.2450/2017.0150-17

8. Eckhardt CL, van Velzen AS, Peters M, Astermark J, Brons PP, Castaman G, et al. Factor VIII gene (F8) mutation and risk of inhibitor development in nonsevere hemophilia a. *Blood*. (2013) 122:1954–62. doi: 10.1182/blood-2013-02-483263

9. Boeriu E, Arghirescu TS, Serban M, Patrascu JM, Boia E, Jinca C, et al. Challenges in the diagnosis and management of non-severe hemophilia. *J Clin Med*. (2022) 11:3322. doi: 10.3390/jcm11123322

10. Franchini M, Favaloro EJ, Lippi G. Mild hemophilia a. *J Thromb Haemost*. (2010) 8:421–32. doi: 10.1111/j.1538-7836.2009.03717.x

11. Santoro C, Quintavalle G, Castaman G, Baldacci E, Ferretti A, Riccardi F, et al. Inhibitors in hemophilia B. *Semin Thromb Hemost*. (2018) 44:578–89. doi: 10.1055/s-0038-1660817

12. Hart DP, Matino D, Astermark J, Dolan G, d'Oiron R, Hermans C, et al. International consensus recommendations on the management of people with haemophilia B. *Ther Adv Hematol*. (2022) 13:10852. doi: 10.1177/20406207221085202

13. Deutsches Hämophilieregister (DHR). Jahresbericht (2020). 2020:14.

14. Mannucci PM, Ruggeri ZM, Pareti FI, Capitanio A. 1-Deamino-8-d-arginine vasopressin: a new pharmacological approach to the management of haemophilia and von Willebrands' diseases. *Lancet*. (1977) 1:869–72. doi: 10.1016/S0140-6736(77)91197-7

15. Kaufmann JE, Vischer UM. Cellular mechanisms of the hemostatic effects of desmopressin (DDAVP). *J Thromb Haemost*. (2003) 1:682–9. doi: 10.1046/j.1538-7836.2003.00190.x

16. Zwagemaker AF, Kloosterman FR, Coppens M, Gouw SC, Boyce S, Bagot CN, et al. Desmopressin for bleeding in non-severe hemophilia a: suboptimal use in a real-world setting. *Res Pract Thromb Haemost*. (2022) 6:e12777. doi: 10.1002/rth2.12777

17. Álvarez Román MT, de la Corte RH, Bonanad Boix S, Mingot-Castellano ME, Fernández MN. The factor VIII treatment history of non-severe hemophilia a: COMMENT. Joint damage in adult patients with mild or moderate hemophilia a evaluated with the HEAD-US system. *J Thromb Haemost*. (2021) 19:2638–41. doi: 10.1111/jth.15475

18. Rajpurkar M, Forsyth A, Manco-Johnson M. Current challenges for men and women with mild-to-moderate haemophilia. *Haemophilia*. (2021) 27:5–7. doi: 10.1111/hae.14193

19. Witkop M, Wang M, Hernandez G, Recht M, Baumann K, Cooper DL. Impact of haemophilia on patients with mild-to-moderate disease: results from the P-FiQ and B-HERO-S studies. *Haemophilia*. (2021) 27:8–16. doi: 10.1111/hae.14251

20. EuroQol group. EuroQol--a new facility for the measurement of health-related quality of life. *Health Policy*. (1990) 16:199–208. doi: 10.1016/0168-8510(90)90421-9

21. Kloosterman FR, Zwagemaker AF, Bagot CN, Beckers EAM, Castaman G, Cnossen MH, et al. The bleeding phenotype in people with nonsevere hemophilia. *Blood Adv*. (2022) 6:4256–65. doi: 10.1182/bloodadvances.2022007620

22. Chambost H, Gaboulaud V, Coatmélec B, Rafowicz A, Schneider P, Calvez T. What factors influence the age at diagnosis of hemophilia? Results of the French hemophilia cohort. *J Pediatr*. (2002) 141:548–52. doi: 10.1067/mpd.2002.128115

23. Peerlinck K, Jacquemin M. Mild haemophilia: a disease with many faces and many unexpected pitfalls. *Haemophilia*. (2010) 16:100–6. doi: 10.1111/j.1365-2516.2010.02306.x

24. Abdi A, Kloosterman FR, Eckhardt CL, Male C, Castaman G, Fischer K, et al. The factor VIII treatment history of non-severe hemophilia a. *J Thromb Haemost*. (2020) 18:3203–10. doi: 10.1111/jth.15076

25. Chai-Adisaksopha C, Noone D, Curtis R, Frick N, Nichol MB, Germini F, et al. Non-severe haemophilia: is it benign? - insights from the PROBE study. *Haemophilia*. (2021) 27:17–24. doi: 10.1111/hae.14105

26. Peyvandi F, Tavakkoli F, Frame D, Quinn J, Kim B, Lawal A, et al. Burden of mild haemophilia a: systematic literature review. *Haemophilia*. (2019) 25:755–63. doi: 10.1111/hae.13777

27. Miesbach W, Oldenburg J, Klamroth R, Eichler H, Koscielny J, Holzhauer S, et al. *Gentherapie der Hämophilie: Empfehlung der Gesellschaft für Thrombose- und Hämostaseforschung (GTH)*. Hamostaseologie. (2023) 43:196–207. doi: 10.1055/a-1957-4477

28. Bundesärztekammer. *Querschnitts-Leitlinien zur Therapie mit Blutkomponenten und Plasmaderivaten*, Gesamtnovelle 2020. Deutsches Ärzteblatt, 117:1–287. (2020).

29. Castaman G, Eckhardt C, van Velzen A, Linari S, Fijnvandraat K. Emerging issues in diagnosis, biology, and inhibitor risk in mild hemophilia a. *Semin Thromb Hemost*. (2016) 42:507–12. doi: 10.1055/s-0036-1571309

30. Othman S, Alavian S, Norton C. Living with mild haemophilia and care after a bleed – a qualitative study. *J Haemophilia Pract*. (2016) 3:14–23. doi: 10.17225/jhp00063

31. Berntorp E, LeBeau P, Ragni MV, Borhany M, Abajas YL, Tarantino MD, et al. Quality of life in a large multinational haemophilia B cohort (the B-natural study) - unmet needs remain. *Haemophilia*. (2022) 28:453–61. doi: 10.1111/hae.14525

32. Walsh C, Boggio L, Brown-Jones L, Miller R, Hawk S, Savage B, et al. Identified unmet needs and proposed solutions in mild-to-moderate haemophilia: a summary of opinions from a roundtable of haemophilia experts. *Haemophilia*. (2021) 27:25–32. doi: 10.1111/hae.14168

33. Den Uul IEM, Fischer K, van der Bom JG, Grobbee DE, Rosendaal FR, Plug I. Analysis of low frequency bleeding data: the association of joint bleeds according to baseline FVIII activity levels. *Haemophilia*. (2011) 17:41–4. doi: 10.1111/j.1365-2516.2010.02383.x

34. Soucie JM, Monahan PE, Kulkarni R, Konkle BA, Mazepa MA. The frequency of joint hemorrhages and procedures in nonsevere hemophilia a vs B. *Blood Adv*. (2018) 2:2136–44. doi: 10.1182/bloodadvances.2018020552

35. Wang M, Recht M, Iyer NN, Cooper DL, Soucie JM. Hemophilia without prophylaxis: assessment of joint range of motion and factor activity. *Res Pract Thromb Haemost*. (2020) 4:1035–45. doi: 10.1002/rth2.12347

36. Daffunchio C, Landro ME, Galatro G, Neme D, Cambiaggi G, Moretti N, et al. How mild is mild haemophilia? *Haemophilia*. (2023) 29:530–7. doi: 10.1111/hae.14750

37. Eichler H, Pedroni MA, Halimeh S, Königs C, Langer F, Miesbach W, et al. Leitlinie der Gesellschaft für Thrombose- und Hämostaseforschung (GTH) zur Struktur- und Prozessqualität von Hämophilie-Zentren. *Hamostaseologie*. (2019) 39:311–21. doi: 10.1055/s-0039-1688450

38. Riske B, Shearer R, Baker JR. Patient satisfaction with US hemophilia treatment center care, teams and services: the first National Survey. *Haemophilia*. (2020) 26:991–8. doi: 10.1111/hae.14176

39. Cutter S, Molter D, Dunn S, Hunter S, Peltier S, Haugstad K, et al. Impact of mild to severe hemophilia on education and work by US men, women, and caregivers of children with hemophilia B: the bridging hemophilia B experiences, results and opportunities into solutions (B-HERO-S) study. *Eur J Haematol*. (2017) 98:18–24. doi: 10.1111/ejh.12851

40. den uijl IE, Fischer K, Van Der Bom JG, Grobbee DE, Rosendaal FR, Plug I. Clinical outcome of moderate haemophilia compared with severe and mild haemophilia. *Haemophilia*. (2009) 15:83–90. doi: 10.1111/j.1365-2516.2008.01837.x

41. Buckner TW, Witkop M, Guelcher C, Sidonio R, Kessler CM, Clark DB, et al. Impact of hemophilia B on quality of life in affected men, women, and caregivers-assessment of patient-reported outcomes in the B-HERO-S study. *Eur J Haematol*. (2018) 100:592–02. doi: 10.1111/ejh.13055

42. Pedersen MJ, Nielsen CV. Improving survey response rates in online panels:effects of low-cost incentives and cost-free text appeal interventions. *Soc Sci Comput Rev*. (2016) 34:229–43. doi: 10.1177/0894439314563916

43. Iversen HH, Holmboe O, Bjertnaes O. Patient-reported experiences with general practitioners: a randomised study of mail and web-based approaches following a national survey. *BMJ Open*. (2020) 10:e036533. doi: 10.1136/bmjopen-2019-036533

frontiers | Frontiers in Medicine

TYPE Case Report
PUBLISHED 30 May 2024
DOI 10.3389/fmed.2024.1391422

OPEN ACCESS

EDITED BY
Herbert Schöchl,
Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Austria

REVIEWED BY
Predrag S. Miljic,
University of Belgrade, Serbia
Oliver Grottke,
RWTH Aachen University, Germany

*CORRESPONDENCE
Nicola Curry
✉ nicola.curry@ouh.nhs.uk

RECEIVED 25 February 2024
ACCEPTED 15 May 2024
PUBLISHED 30 May 2024

CITATION
Goodarzi S, Abu-Hanna J, Harper S, Khan D, Morrow G and Curry N (2024) Are all fibrinogen concentrates the same? The effects of two fibrinogen therapies in an afibrinogenemic patient and in a fibrinogen deficient plasma model. A clinical and laboratory case report.
Front. Med. 11:1391422.
doi: 10.3389/fmed.2024.1391422

Are all fibrinogen concentrates the same? The effects of two fibrinogen therapies in an afibrinogenemic patient and in a fibrinogen deficient plasma model. A clinical and laboratory case report

Soutiam Goodarzi[1,2], Jeries Abu-Hanna[2,3], Sarah Harper[3], Dalia Khan[3], Gael Morrow[2,4] and Nicola Curry[2,3]*

[1]Oxford University Medical School, Medical Sciences Division, John Radcliffe Hospital, Oxford, United Kingdom, [2]Radcliffe Department of Medicine, Oxford University, Oxford, United Kingdom, [3]Oxford Haemophilia and Thrombosis Centre, Oxford University Hospitals National Health Service (NHS) Foundation Trust, Oxford, United Kingdom, [4]School of Pharmacy and Life Sciences, Robert Gordon University, Aberdeen, United Kingdom

The choice of treatments for inherited, or acquired, fibrinogen deficient states is expanding and there are now several fibrinogen concentrate therapies commercially available. Patients with the rare inherited bleeding disorder, afibrinogenemia, commonly require life-long replacement therapy with fibrinogen concentrate to prevent hemorrhagic complications. Recent reports in the setting of acquired bleeding, namely trauma hemorrhage, have highlighted the potential importance of the different compositions of fibrinogen supplements, including cryoprecipitate and the various plasma- derived concentrates. Clot strength and the subsequent susceptibility of a clot to lysis is highly dependent on the amount of fibrinogen as well as its structural composition, the concentration of pro- and anti-coagulant factors, as well as fibrinolytic regulators, such as factor XIII (FXIII). This report details the effects of two commercially available fibrinogen concentrates (Riastap®, CSL Behring and Fibryga®, Octapharma) on important functional measures of clot formation and lysis in a patient with afibrinogenemia. Our report offers insights into the differential effects of these concentrates, at the clot level, according to the variable constituents of each product, thereby emphasizing that the choice of fibrinogen concentrate can influence the stability of a clot *in vivo*. Whether this alters clinical efficacy is yet to be understood.

KEYWORDS

afibrinogenemia, fibrinogen concentrate, hemostasis, inherited bleeding disorder, fibrinolysis

Introduction

Fibrinogen (Fg) is the main architectural component of a blood clot (1). Following its cleavage by thrombin, soluble Fg is converted into insoluble fibrin monomers which can self-polymerize. The resultant protofibrils form a fibrin mesh at sites of injury (2). Clot formation, clot structure and clot stability are influenced by the interplay of many

factors during the process of fibrin formation. These influences can broadly be thought of as: (1) the effects of pro-/anti-coagulant and fibrinolytic regulatory proteins directly affecting either thrombin generation or fibrinolysis; (2) the natural variety in Fg structure (e.g., relative amounts of gamma-prime; post-translational modifications); (3) the effects of blood flow and the cell surface (e.g., endothelial, platelet) (3). The complex interplay of these interactions results in variations in the diameter, and density, of the fibrin strands that form, which affects clot strength and subsequently the susceptibility of the clot to lysis (2).

Fibrinogen deficiency is most often encountered clinically in an acquired setting, for example, during major bleeding after traumatic injury or childbirth (4). Much less commonly, it may be the result of an inherited rare bleeding disorder, namely hypo- or afibrinogenemia, which affect 1–2 individuals per million (5). These are hereditary conditions characterized by an abnormally low production of Fg, with Clauss Fg levels 1.0 g/L or less for hypofibrinogenemia, and undetectable levels for afibrinogenemia (5). Afibrinogenemia carries a lifelong high risk of bleeding and is often managed with regular infusions of fibrinogen concentrate on a prophylactic basis.

There are several Fg concentrates available commercially. Data from the trauma setting have shown that these concentrates are compositionally different and *in vitro* and *ex vivo* work has reported variation in both clot lysis and clot structure, dependent on the Fg supplementation used (6–8). One important difference noted between the Fg concentrates has been the concentration of factor XIII (FXIII), which is much higher in Fibryga® (Octapharma, Switzerland) concentrate when compared to RiaSTAP® (CSL Behring, Germany). FXIII plays a vital role in cross-linking fibrin gamma chains, thus stabilizing the fibrin mesh and reducing lytic susceptibility. This may alter a patient's hemostatic response to Fg therapy.

Here we present data from a young patient with congenital afibrinogenemia who was switched from RiaSTAP® to Fibryga® concentrate due to an operational change in hospital prescribing. The aim of this study was to explore, in detail, the differential effects of two Fg concentrates on clot formation, stability and lysis. Further experiments, to investigate the hemostatic differences in more detail, were conducted using fibrinogen-deficient plasma spiked with each concentrate, *in vitro*.

Case report

Our patient is a 26-year-old male of Pakistani descent, born to distantly related parents, who was diagnosed with congenital afibrinogenemia a few weeks after birth. He has given informed consent for his details to be presented. He was originally admitted to the Special Care Baby Unit at 4-days-old with jaundice and vomiting. There was no history of trauma or of a bleeding disorder in the family (Figure 1). He was born at 39 weeks via elective C-section. There were no known complications during pregnancy. The patient received his first dose of vitamin K immediately after birth.

He developed tonic-clonic seizures 6 h into his admission, treated with phenobarbitone, phenytoin and paraldehyde. He was diagnosed with a small germinal matrix hemorrhage on cranial ultrasound scan with no ventricular dilatation. He stabilized within a few days and was sent home. Three weeks later, at a routine check, another cranial ultrasound scan was taken due to an abnormal increase in his head circumference, upon which ventricular dilatation and parieto-occipital ischemia were noted, caused by the previous hemorrhage. Review of his blood tests showed abnormal coagulation: PT 31 s (normal range, NR: 9–12 s); APTT 41 s (NR: 20–30 s). Clauss Fg was undetectable at <0.3 g/L, as was Fg antigen.

Congenital afibrinogenemia was suspected and considering his clinical presentation of prior intracranial bleeding, cryoprecipitate was given immediately. His trough Fg level was then maintained above 0.8 g/L, necessitating cryoprecipitate infusion every 3 days. Genetic testing confirmed the homozygous variant c.78+5G>A (Supplementary Figure S1) (9). His parents were confirmed as heterozygote for the variant, both with a low Clauss Fg level (mother-−1.3 g/L; father-−1.2 g/L), and neither had a bleeding history.

Following a good recovery at 3 months, the cryoprecipitate was discontinued. Unfortunately, he developed a second intracranial hemorrhage at 8 months of age, and since then, has received Fg concentrate prophylaxis, maintaining a trough ≥1 g/L. He has had no further bleeding episodes and was taught to self-infuse at 18-years. He has not been exposed to any significant hemostatic challenges, such as surgery, since commencing prophylaxis. In 2023, the hospital routinely switched Fg concentrate therapy due to operational changes in hospital prescribing and we undertook extended laboratory testing to compare the two licensed products. At the time of testing, the patient weighed 82.7 kg, and had a body mass index of 25.6 kg/m^2. We report the results below.

Methods

Venous blood samples were taken from the patient at various time points, drawn into 3.2% citrate (Becton Dickinson®, New Jersey, USA), after written informed consent. Trough (taken at 72 h) and peak (10 min post-dose) samples were taken for RiaSTAP®. A second trough sample was taken for RiaSTAP® immediately prior to administration of the first Fibryga® dose to delineate how much RiaSTAP® had remained from the previous dose. A combined RiaSTAP® + Fibryga® sample was taken immediately after the first Fibryga® injection. Trough (taken at 72 h) and peak (10 min post-dose) samples were taken for Fibryga® several months after the switch to ensure complete clearance of any RiaSTAP®.

At each sample draw, the following tests were performed: Clauss Fg, Fg antigen; FXIII, alpha-2 anti-plasmin (A2AP), ROTEM EXTEM and FIBTEM (10); fibrin polymerization; plasmin generation; confocal microscopy. In some instances, these tests were conducted on increasing concentrations of RiaSTAP® or Fibryga® spiked into fibrinogen-deficient plasma (F1DP) (Affinity Biologicals, Canada). Briefly, Fg concentrate was reconstituted to the standard 20 g/L concentration, and in each assay, increasing volumes of Fg concentrate was added replacing the equivalent volume of buffer (assay dependent).

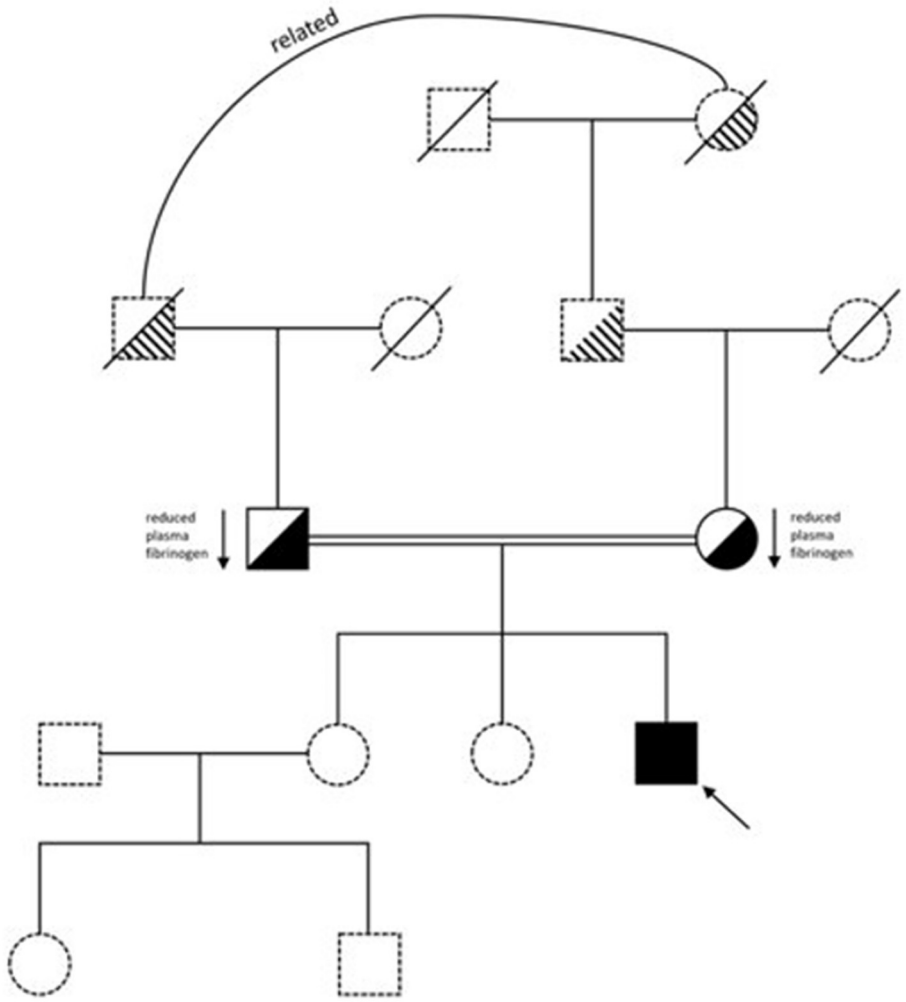


FIGURE 1
Family pedigree.

Standard and extended clotting factor tests

Clauss Fg (Dade Thrombin Reagent, Siemens, Germany) (NR: 1.5–4.5 g/L), Fg antigen (LIAPHEN, Hyphen BioMed, France) (NR: 1.94–4.17 g/L), FXIII (FXIIIA, Siemens) and alpha2 antiplasmin (α2AP, Siemens) were measured with chromogenic assays: NR: 50–150% and 80–130%, respectively. All assays were analyzed using a Sysmex CS-5100 analyzer in the specialist hemostasis laboratory.

Clot lysis

Patient or F1DP (30%), 16 μM phospholipids (Rossix, Molndal, Sweden), 45 pM tPA (NIBSC, Potters Bar, UK) in 10 mM TRIS pH 7.4 0.01% Tween20 was added to 96 well flat-bottom assay plates. In F1DP experiments an increasing dose of Fg (range: 0.5–6 g/L) was added, using RiaSTAP® or Fibryga®. Clotting was initiated with 0.01 U/mL thrombin (Sigma Aldrich, USA), 10.6 mM $CaCl_2$. Absorbance at 405 nm was recorded every 60 s for 4 h using Ascent software (version 2.6). Data were analyzed by calculating time to 50% lysis using Shiny App software.[1]

Fibrin polymerization

Several concentrations—0.5, 1.0, 2.0, 4.0, and 6.0 g/L of RiaSTAP® and Fibryga® were chosen to compare polymerization rates, under standard conditions. Briefly, polymerization was activated using 0.1 U/ml thrombin and 5 mM calcium chloride, in 10 mM TRIS buffer. Turbidity was measured every 10 s for 265 min.

Plasmin generation

10% plasma was mixed with 0.5 mM S-2251 chromogenic substrate (Chromogenix, Ohio, USA), and added to 10 nM tPA

1 Shiny Apps for clot lysis analysis. Available online at: https://drclongstaff.shinyapps.io/clotlysisCL_2019/ (accessed January 15, 2024).

(Actilyse, Boehringer Ingelheim, Germany). Absorbance readings at 405 nm were taken every 30 s for 8 h at 37 °C. The rate of plasmin generation was determined using the Shiny App for zymogen activation.[2]

Confocal microscopy

Clots were formed using 30% plasma (patient samples or F1DP with 3 or 6 g/L RiaSTAP® or Fibryga®), 0.25 μM Alexa Fluor 488 (AF488) fibrinogen (ThermoFisher Scientific, USA) and 16 μM phospholipids. Clotting was initiated with 0.1 U/ml thrombin and 10.6 mM CaCl2 before adding to Ibidi μ-slide VI0.4 chambers (Ibidi GmbH, Germany). Representative images are shown in the manuscript. Images were recorded on Zeiss LSM 880. Images were analyzed using FIJI v2.15.0 and the Diameter J plug-in.

SDS-PAGE

Proteins were boiled in NuPAGE LDS Sample Buffer (Invitrogen) and NuPAGE Sample Reducing Agent (Invitrogen) at 70°C for 10 min. Equal amounts of protein along with SeeBlue Plus 2 Pre-stained Protein Standards were loaded into wells of NuPAGE 4–12% Bis-Tris Protein Gels (Invitrogen) and run in NuPAGE MOPS SDS Running Buffer (Invitrogen) at 200 V for 50 min. Gels were stained with 0.1% (w/v) Coomassie Brilliant Blue in 10% (v/v) acetic acid, 50% (v/v) methanol, and 40% (v/v) distilled water for 3 h with shaking. Gels were washed with 10% acetic acid, 50% methanol, 40% distilled water 3 times for 2 h.

Statistical analysis

Data are presented descriptively using mean and standard deviation. Categorical data are presented as frequencies and percentages. Clinical and laboratory measures were compared using student's *t*-test (two-group comparisons) and one-way ANOVA (three-group comparisons). A *P*-value of <0.05 was chosen to represent statistical significance throughout. Data analysis was performed using GraphPad Prism 10, GraphPad Software LLC.

Results

Patient samples

The patient had no change in their clinical picture throughout this study and they did not experience any abnormal bleeding whilst receiving treatment with either Fg concentrate.

Prophylactic treatment with either concentrate, at a dose of 3 g, led to a similar incremental recovery: Clauss Fg rose from 0.9 to 2.0 g/L, Riastap® and 0.9 to 2.1 g/L, Fibryga®. Trough Clauss Fg was higher following Fibryga® infusion (1.4 g/L), compared to RiaSTAP® (0.9 g/L). FXIII levels were higher following Fibryga® infusion (1.59 IU/mL), vs. 0.96 IU/mL, RiaSTAP®, reflecting previously reported higher FXIII levels in Fibryga® (11, 12). EXTEM and FIBTEM ROTEM showed an expected increment in the maximum amplitudes, with no differences between the two products. FIBTEM maximum amplitude (MA) rose from 8 to 14 mm (after 3 g RiaSTAP®) and from 10 to 15 mm (after 3 g Fibryga®). At the same timepoints, EXTEM MA rose 57 to 63 mm (Riastap®) and 59 to 66 mm (Fibryga®). 6% lysis was detected in the Pre-Fibryga® FIBTEM sample, otherwise all samples showed 0 or 1% lysis.

At the trough sample timepoints, lysis was quicker in the Fibryga® sample (79.6 min) when compared to Riastap® (90.3 min) $p < 0.0001$ (Supplementary Figure S2A). 50% clot lysis times prolonged after both treatments. However, the increase with Fibryga® (~50% longer, to 119 min) was significantly greater than after Riastap® (~7% longer, to 97 min) (Supplementary Figure S2B). Clot turbidity rose after both treatments, as expected, but the change was less with RiaSTAP®; 0.095 (trough) to 0.098 (peak) compared with 0.0133 Fibryga® (trough) to 0.125 (peak). The first trough Riastap® sample was hemolyzed, which may have affected these results.

To evaluate these fibrinolytic differences further and explore fibrin clot structure, we performed confocal microscopy (Figure 2). Compared to PNP, the fibrin fibers in most patient samples were shorter and thinner. Treatment with Riastap®, or when the Fibryga® dose was given for the first time (e.g., with Riastap® still present), led to no change in diameter or length of fibrin fibers. Fibryga® treatment led to an increase in fiber length, with fibers showing a greater diameter, when compared to pre-treatment. There was a reduction in numbers of pores after treatment with both concentrates.

In vitro results, reconstituted fibrinogen concentrates

Factor levels for RiaSTAP® and Fibryga® concentrates are shown in Supplementary Table S1. Clauss Fg and antigen levels were comparable. A2AP levels were low in both products, Fibryga® (4%), RiaSTAP® (1%). Plasminogen levels were comparably low. FXIII levels were higher in Fibryga® (1.24 IU/mL) vs. negligible, RiaSTAP® (0.01 IU/mL), as previously reported (6).

Fibrin polymerization rates with RiaSTAP® were slower, across all Fg concentrations (Supplementary Figure S3). Fibrin polymerization was fastest at 2 g/L for both concentrates (772.51 Abs/s RiaSTAP® and 1,879.88 Abs/s Fibryga®). Maximum turbidity was lower with RiaSTAP® across all concentrations (0.12 RiaSTAP® vs. 0.46 Fibryga® at 0.5 g/L; 1.52 RiaSTAP® vs. 1.71 Fibryga® at 6 g/L), though the difference between the two products was much less at the higher concentrations.

SDS-Page analysis (Figure 3) of the reconstituted concentrates revealed differences. RiaSTAP® had uneven distribution of chains with a strong signal for the β chain and a lower signal for the α chain, unlike the Fibryga® product which produced an equal signal for the α, β, and γ chains. Addition of thrombin resulted in relatively small amounts of γ-γ crosslinks for RiaSTAP®. The γ

2 Shiny Apps for plasmin generation analysis. Available online at: https://drclongstaff.shinyapps.io/zymogenactnCL/ (accessed January 15, 2024).

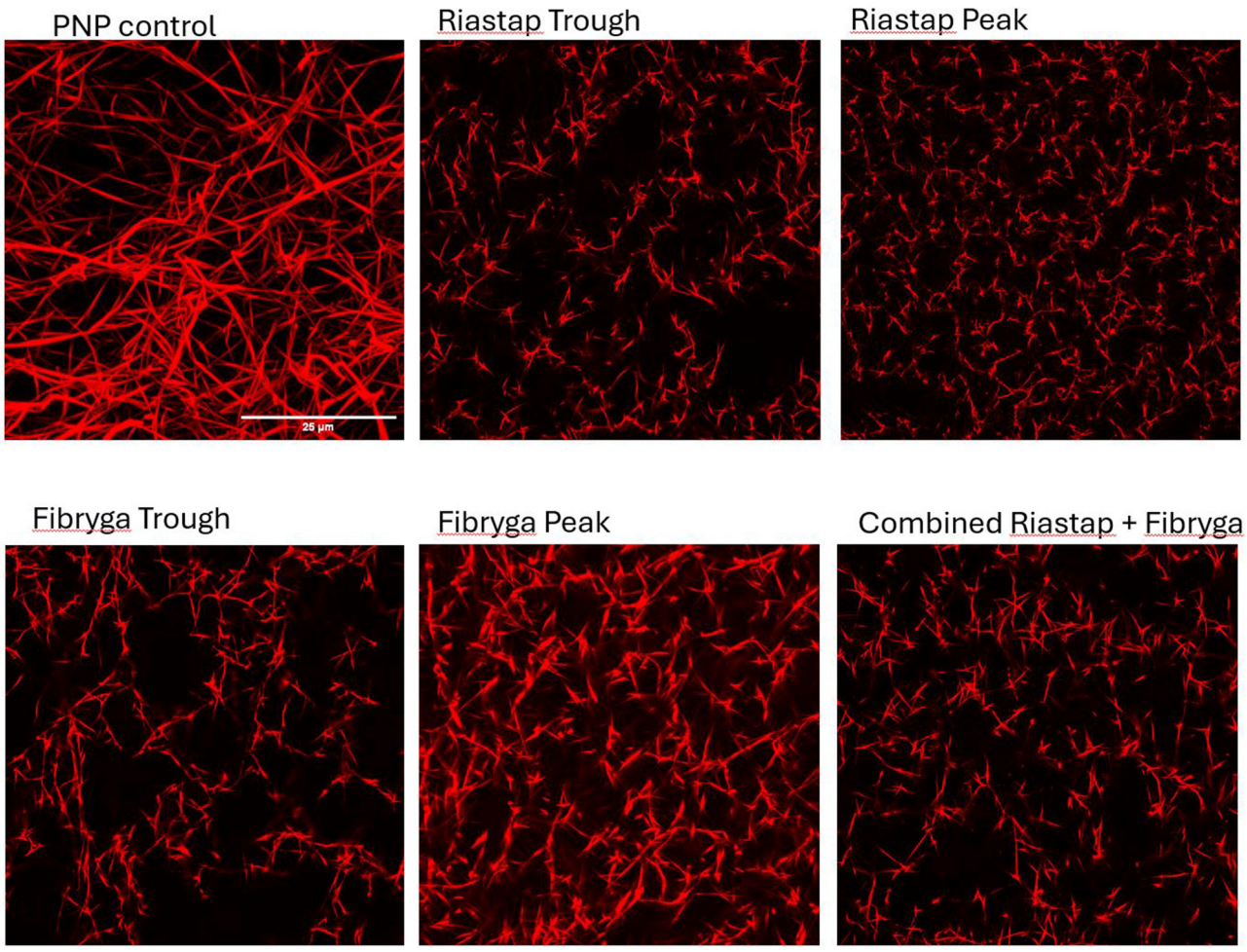


FIGURE 2
Confocal imaging of patient samples. Confocal microscopy, Alexa Fluor 488 (AF488) fibrinogen, delineating fibrin strands in: pooled normal plasma (PNP) as a control; Riastap® trough and peak samples; Fibryga® trough and peak samples and the "combined sample"—a mix of Riastap® -Fibryga®.

chain signal was reduced for Fibryga® on exposure to thrombin, with marked increases in γ-γ cross-linking, likely because of the higher FXIII levels. There was incomplete fibrin breakdown with Riastap® as the signal at the β position remained after plasmin treatment.

In vitro results, fibrinogen-deficient plasma

Like the fibrin polymerisation experiments, reduced turbidity was seen in F1DP clot lysis assays when spiked with Riastap®, compared to Fibryga®, at the same Fg concentration. Faster lysis was seen when Fibryga® was added to F1DP compared to RiaSTAP® at the lower concentrations (e.g., 0.5–4 g/L) but at 6 g/L, clot lysis was faster with Fibryga®. Despite slower lysis, there was faster plasmin generation in RiaSTAP® supplemented F1DP compared to Fibryga® and notably the rate of plasmin generation plateaued at 2 g/L with Riastap®, but not until 4 g/L with Fibryga® (Figure 4). Confocal imaging for spiked-F1DP at two concentrations (3 and 6 g/L) revealed differences (data not shown). Fibryga® fibers were longer with a greater diameter at the same Fg concentration, in line with the turbidity assays.

Discussion

We present detailed *in vivo* and *in vitro* data which explore the impact of two commercially available fibrinogen concentrates on standard laboratory coagulation tests, dynamic measures of fibrinolysis, and confocal microscopy. Our results show that in our patient with afibrinogenemia there were comparable effects of the two concentrates on measures of Fg activity and clot strength (Clauss Fg and ROTEM measures), in line with other data (13). However, there were differences seen, both in the patient samples and the *in vitro* experiments, on measures of FXIII (14) and fibrinolysis (clot lysis and plasmin generation). Confocal images confirm structural differences between the two concentrates, both in the *in vivo* samples and *in vitro* experimental F1DP samples.

In the patient, after treatment with either concentrate, clot lysis times prolonged when compared with pre-treatment, confirming both treatments increase resistance of clots to fibrinolysis. This

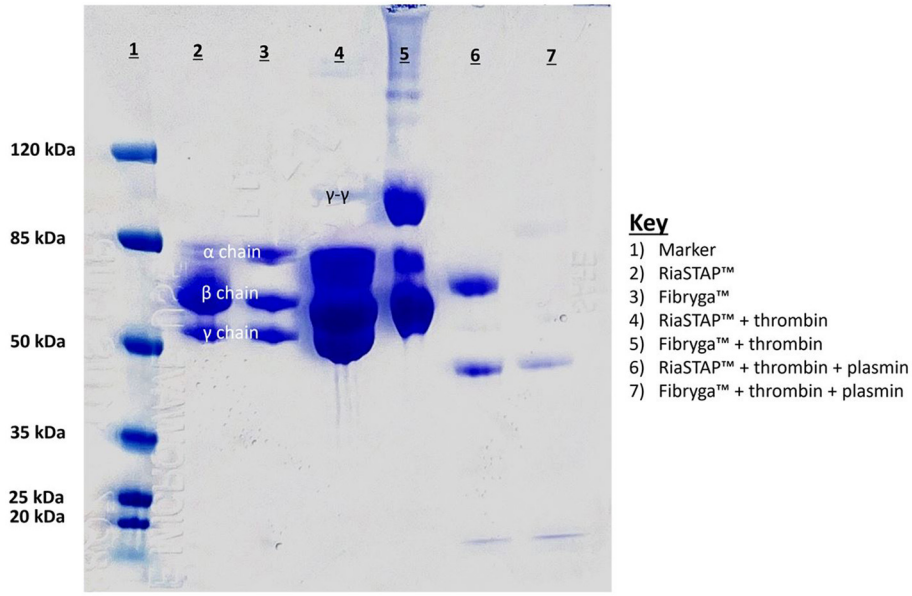


FIGURE 3
SDS Page analysis of reconstituted fibrinogen concentrates. Lanes of gel: 1. Ladder; 2. Riastap®; 3. Fibryga®; 4. Thrombin + Riastap®; 5. Thrombin + Fibryga®; 6. Thrombin + plasmin + Riastap®; 7. Thrombin + plasmin + Fibryga®.

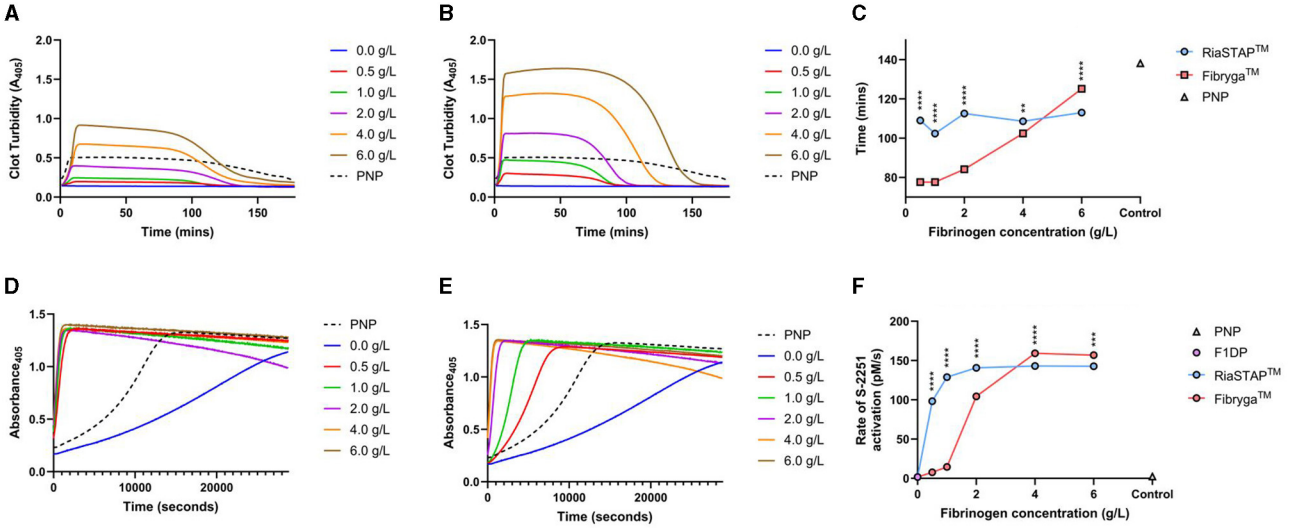


FIGURE 4
F1DP and increasing concentrations of Riastap® or Fibryga®: clot lysis and plasmin generation, dose response curves. **(A)** RiaSTAP® clot lysis curves. **(B)** Fibryga® clot lysis curves. **(C)** 50% clot lysis over the five fibrinogen concentrations. **(D)** Plasmin generation curves for RiaSTAP®. **(E)** Plasmin generation curves for Fibryga®. **(F)** Plasmin generation rates over varying concentrations for RiaSTAP® and Fibryga®. $^{**}P < 0.05$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

is as expected, and in line with many other published reports (1, 11, 12, 15). However, despite similar trough Clauss Fg levels and similar recovery after the two therapies, lysis was consistently slower in the samples with Fibryga® present.

Fibrin polymerization, fibrin structure and subsequent fibrinolysis are highly dependent on several variables. One important reason is the rate at which a clot is formed, which is influenced by the concentrations of thrombin and fibrinogen. High thrombin concentrations produce thinner fibrin fibers within dense networks that are less permeable and more resistant to fibrinolysis (1, 16). One of the other important factors in fibrin clot stability, and its subsequent resistance to breakdown, is the cross-linking of fibers resulting from the action of activated FXIII (17, 18).

Faster fibrin polymerization, and greater turbidity, was seen with Fibryga®, compared to Riastap®, across all Fg concentrations. These differences are important, particularly as the

thrombin concentration was controlled across experiments and suggests that the structure of the fibrinogen is different between concentrates (19). Certainly, there were differences seen in the Western blot comparative analysis, with less alpha chain in the Riastap®. The confocal images also provide some explanation for the changes in susceptibility to lysis. After both treatments, the fibrin structure became denser and pores less numerous. The fibers after Fibryga® treatment were visibly longer and the structure of the clot more readily resembled PNP. Denser fibrin structures, with fewer pores, are known to be more resistant to lysis (16, 17).

Rising concentrations of Fibryga®, both *in vivo* and *in vitro*, were strongly associated with longer 50% lysis times. In the patient samples, prolongation of lysis occurred in a dose dependent manner with Fibryga® e.g., the Riastap®-Fibryga® sample lysed more slowly than Riastap® alone, and the longest lysis time was seen in the sample containing only Fibryga® concentrate. This effect was mirrored in the F1DP-spiked plasma. Across the same concentration range of Riastap®, there was no important change in lysis. These data suggest that constituents within Fibryga®, that are absent in Riastap®, contribute strongly to lysis susceptibility.

Our subsequent experiments aimed to determine whether plasmin generation capacity explained the differences. Both products showed a sigmoidal dose response, with increasing plasmin generation with higher Fg concentrations. Contrary to the clot lysis experiments, the EC50 was markedly lower for Riastap® compared to Fibryga® (0.35 vs. 1.77 g/L). The possible reasons for more rapid plasmin generation in this experiment might be a lower A2AP or greater plasminogen concentration in Riastap®, although we were not able to show differences (Supplementary Table S1). Our findings require further exploration.

A notable difference between the Fg concentrates, however, is the FXIII concentration. Fibryga® contains substantially more FXIII (1.24 vs. 0.01 IU/mL). Our clot lysis data both *in vivo*, and *in vitro,* show that the lysis time is affected by the type of concentrate. The Western blot data lend further strength to the notion that one of the important effectors of the difference in lytic resistance is the FXIII concentration. Our data show that RiaSTAP®, when exposed to thrombin, does not form γ-γ crosslinks, whereas crosslinks are evident in the Fibryga® experiments; data which align with a prior publication (18). Lower quantities of FXIII crosslinks increases the susceptibility of a fibrin clot to breakdown (19, 20).

Notably, our patient has normal FXIII levels. The sample taken when he was in receipt of Riastap® (e.g., no supplementary FXIII) was 0.96 IU/mL. It might be hypothesized that additional FXIII in a Fg concentrate would be of no consequence, however, our data suggest that resistance to lysis is further enhanced by Fibryga®. This may have important clinical consequences, particularly if a Fg concentrate were to be used for a patient with dysfibrinogenemia (where some variants confer a pro-thrombotic phenotype) or conversely, for those patients where supplementing FXIII might also be beneficial; e.g., in trauma-hemorrhage (6, 13).

Both concentrates are derived from human plasma, and some of the manufacturing steps taken may have caused structural post-translational alterations in the fibrinogen chains. The viral inactivation steps particularly are different, involving a 20-h heating process for RiaSTAP® at 60°C.[3] This compares to Fibryga® which is treated with solvent-detergent for virus inactivation, and nanofiltration for virus removal (18).[4] Denaturation of Fg chains can occur above 65°C (21).

Our data suggest that more attention should be paid to how fibrinogen concentrates are used clinically (22) and which laboratory tests are conducted to monitor therapy, e.g., should measures of fibrinolysis be considered? Notably, there are no rapid and reliable tests available to clinicians which measure mild to moderate fibrinolysis (standard ROTEM and TEG assays detect more significant lysis only) and this is an area of active laboratory research. It may be that the differences shown between these two products are not sufficiently marked to manifest as differences in clinical outcome, but our data suggest there may be room to tailor the prescription of Fg concentrate more individually. For example, for those patients in receipt of fibrinogen replacement for vascular malformations (where FXIII may also be reduced) or for those patients where fibrinolysis is an important part of the acquired coagulopathy, for example after trauma, a product with greater FXIII or greater resistance to fibrinolysis, may be preferable. Conversely, for patients with a dysfibrinogenemia genotype that confers a thrombotic risk, fibrinolytic resistance may be preferred to be avoided.

The data we present highlight that fibrinogen concentrates should not be considered entirely interchangeable, and they have differences with regards to clot stability against lysis. Further evaluation in a larger group of patients is required to answer whether these fibrinolytic changes seen in laboratory assays can be translated into important clinical outcome differences.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

SG: Data curation, Formal analysis, Investigation, Writing—original draft. JA-H: Formal analysis, Investigation,

3 Available at: https://riastap-prescribing-information.pdf (https://cslbehring.com) (accessed February 23, 2024).

4 Pathogen Safety - Fibryga® Fibrinogen (Human) (https://fibrygausa.com) (accessed February 23, 2024).

Methodology, Writing—review & editing. SH: Investigation, Validation, Writing—review & editing. DK: Conceptualization, Writing—review & editing. GM: Formal analysis, Investigation, Methodology, Supervision, Validation, Writing—review & editing. NC: Conceptualization, Methodology, Supervision, Writing—original draft, Writing—review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We thank Prof. R. Ariens, Dr. C. Duval, and Prof. M. Laffan for their expert guidance during the development of this project. We also thank Prof. S. Srinivas and Dr. Shifaan Thowfeequ for their help and support with the microscopy.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1391422/full#supplementary-material

References

1. Wolberg AS. Fibrinogen and fibrin: synthesis, structure, and function in health and disease. *J Thromb Haemost.* (2023) 21:3005–15. doi: 10.1016/j.jtha.2023.08.014

2. Pieters M, Wolberg AS. Fibrinogen and fibrin: an illustrated review. *Res Pract Thromb Haemost.* (2019) 3:161–72. doi: 10.1002/rth2.12191

3. Kattula S, Byrnes JR, Wolberg AS. Fibrinogen and fibrin in hemostasis and thrombosis. *Arterioscler Thromb Vasc Biol.* (2017) 37:e13–21. doi: 10.1161/ATVBAHA.117.308564

4. Curry N. Fibrinogen replacement in haemostatic resuscitation: dose, laboratory targets and product choice. *Transfus Med Rev.* (2021) 35:104–7. doi: 10.1016/j.tmrv.2021.06.005

5. Mumford AD, Ackroyd S, Alikhan R, Bowles L, Chowdary P, Grainger J, 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) 167:304–26. doi: 10.1111/bjh.13058

6. Morrow GB, Feller T, McQuilten Z, Wake E, Ariëns RAS, Winearls J, et al. Cryoprecipitate transfusion in trauma patients attenuates hyperfibrinolysis and restores normal clot structure and stability: results from a laboratory sub-study of the FEISTY trial. *Crit Care.* (2022) 26:290. doi: 10.1186/s13054-022-04167-x

7. Morrow GB, Carlier MSA, Dasgupta S, Craigen FB, Mutch NJ, Curry N. Fibrinogen replacement therapy for traumatic coagulopathy: does the fibrinogen source matter? *Int J Mol Sci.* (2021) 22:2185. doi: 10.3390/ijms22042185

8. Whyte CS, Rastogi A, Ferguson E, Donnarumma M, Mutch NJ. The efficacy of fibrinogen concentrates in relation to cryoprecipitate in restoring clot integrity and stability against lysis. *Int J Mol Sci.* (2022) 23:2944. doi: 10.3390/ijms23062944

9. Asselta R, Duga S, Simonic T, Malcovati M, Santagostino E, Giangrande PL, et al. Afibrinogenemia: first identification of a splicing mutation in the fibrinogen gamma chain gene leading to a major gamma chain truncation. *Blood.* (2000) 96:2496–500. doi: 10.1182/blood.V96.7.2496

10. Ganter MT, Hofer CK. Coagulation monitoring: current techniques and clinical use of viscoelastic point-of-care coagulation devices. *Anesth Analg.* (2008) 106:1366–75. doi: 10.1213/ane.0b013e318168b367

11. Li W, Sigley J, Pieters M, Helms CC, Nagaswami C, Weisel JW, et al. Fibrin fiber stiffness is strongly affected by fiber diameter, but not by fibrinogen glycation. *Biophys J.* (2016) 110:1400–10. doi: 10.1016/j.bpj.2016.02.021

12. Gabriel DA, Muga K, Boothroyd EM. The effect of fibrin structure on fibrinolysis. *J Biol Chem.* (1992) 267:24259–63. doi: 10.1016/S0021-9258(18)35759-4

13. Haas T, Cushing MM, Asmis LA. Comparison of the efficacy of two human fibrinogen concentrates to treat dilutional coagulopathy *in vitro*. *Scand J Clin Lab Investig.* (2018) 78:230–5. doi: 10.1080/00365513.2018.1437645

14. Neisser-Svae A, Hegener O, Görlinger K. Differences in the biochemical composition of three plasma derived human fibrinogen concentrates. *Thromb Res.* (2021) 205:44–6. doi: 10.1016/j.thromres.2021.06.020

15. Wolberg AS, Gabriel DA, Hoffman M. Analyzing fibrin clot structure using a microplate reader. *Blood Coagul Fibrin.* (2002) 13:533–9. doi: 10.1097/00001721-200209000-00008

16. Wolberg AS. Thrombin generation and fibrin clot structure. *Blood Rev.* (2007) 21:131. doi: 10.1016/j.blre.2006.11.001

17. Bucay I, O'Brien ET III, Wulfe SD, Superfine R, Wolberg AS, Falvo MR, et al. Physical determinants of fibrinolysis in single fibrin fibers. *PLoS ONE.* (2015) 10:e0116350. doi: 10.1371/journal.pone.0116350

18. Schulz PM, Gehringer W, Nöhring S, Müller S, Schmidt T, Kekeiss-Schertler S, et al. Biochemical characterization, stability, and pathogen safety of a new fibrinogen concentrate (fibryga®). *Biologicals.* (2018) 52:72–7. doi: 10.1016/j.biologicals.2017.12.003

19. Byrnes JR, Duval C, Wang Y, Hansen CE, Ahn B, Mooberry MJ, et al. Factor XIIIa-dependent retention of red blood cells in clots is mediated by fibrin α-chain crosslinking. *Blood.* (2015) 126:1940–8. doi: 10.1182/blood-2015-06-652263

20. Duval C, Ali M, Chaudhry WW, Ridger VC, Ariëns RA, Philippou H. Factor XIII A-subunit V34L variant affects thrombus cross-linking in a murine model of thrombosis. *Arterioscler Thromb Vasc Biol.* (2016) 36:308–16. doi: 10.1161/ATVBAHA.115.306695

21. Marx G, Mou X, Hotovely-Salomon A, Levdansky L, Gaberman E, Belenky D, et al. Heat denaturation of fibrinogen to develop a biomedical matrix. *J Biomed Mater Res.* (2008) 84B:49–57. doi: 10.1002/jbm.b.30842

22. Weisel JW, Litvinov RI. Mechanisms of fibrin polymerization and clinical implications. *Blood.* (2013) 121:1712–9. doi: 10.1182/blood-2012-09-306639

frontiers | Frontiers in Medicine

TYPE Brief Research Report
PUBLISHED 10 June 2024
DOI 10.3389/fmed.2024.1399335

OPEN ACCESS

EDITED BY
Felix Carl Fabian Schmitt,
Heidelberg University Hospital, Germany

REVIEWED BY
Philipp Kaimann,
Heidelberg University Hospital, Germany
Felix Hezel,
Heidelberg University Hospital, Germany
Matthias Unterberg,
University Hospital Bochum GmbH, Germany

*CORRESPONDENCE
Claude Capron
✉ claude.capron@aphp.fr
Paul Coppo
✉ Paul.coppo@aphp.fr

†These authors have contributed equally to this work

RECEIVED 11 March 2024
ACCEPTED 03 May 2024
PUBLISHED 10 June 2024

CITATION
Zhu J, Bouzid R, Travert B, Géri G, Cohen Y, Picod A, Heming N, Rottman M, Joly-Laffargue B, Veyradier A, Capron C and Coppo P (2024) Combined coagulation and inflammation markers as predictors of venous thrombo-embolism and death in COVID-19.
Front. Med. 11:1399335.
doi: 10.3389/fmed.2024.1399335

Combined coagulation and inflammation markers as predictors of venous thrombo-embolism and death in COVID-19

Jaja Zhu[1,2†], Raïda Bouzid[3†], Benoît Travert[4], Guillaume Géri[5], Yves Cohen[6], Adrien Picod[6], Nicholas Heming[7,8,9,10], Martin Rottman[11,12], Bérangère Joly-Laffargue[3,13,14], Agnès Veyradier[3,13,14], Claude Capron[1,15*] and Paul Coppo[3,16,17*]

[1]Service d'Hématologie-Immunologie-Transfusion, AP-HP Paris Saclay, CHU Ambroise Paré, Université de Versailles Saint Quentin-Université Paris Saclay, Montigny-le-Bretonneux, France, [2]Laboratoire Cellules Souches et Applications Thérapeutiques, UMR INSERM 1184, Commissariat à l'Energie Atomique et aux Energies Alternatives, Fontenay-aux-Roses, France, [3]Centre de Référence des Microangiopathies Thrombotiques (CNR-MAT), AP-HP, Paris, France, [4]Service de Médecine Interne, Hôpital Ambroise-Paré, AP-HP, Boulogne-Billancourt, France, [5]Service de Médecine Intensive et Réanimation, Hôpital Ambroise-Paré, AP-HP, Boulogne-Billancourt, France, [6]Service de Médecine Intensive et Réanimation, Hôpital Avicenne, AP-HP, Paris, France, [7]Department of Intensive Care, Raymond Poincaré Hospital, APHP University Versailles Saint Quentin-University Paris Saclay, Garches, France, [8]Institut Hospitalo Universitaire PROMETHEUS, Garches, France, [9]Innovative Biomarkers Plateform, Laboratory of Infection & Inflammation-U1173, School of Medicine, INSERM, University Versailles Saint Quentin-University Paris Saclay, Garches, France, [10]FHU SEPSIS, Garches, France, [11]Innovative Biomarkers Plateform, Laboratory of Infection & Inflammation-U1173, School of Medicine, INSERM, University Versailles Saint Quentin-University Paris Saclay, Garches, France; FHU SEPSIS, Garches, France, [12]General Intensive Care Unit, Raymond Poincaré Hospital (AP-HP), FHU SEPSIS, Laboratory of Infection and Inflammation-U1173, School of Medicine Simone Veil, Université Versailles Saint Quentin, University Paris Saclay, INSERM, Garches, France, [13]EA3518, Institut de Recherche Saint Louis, Université de Paris, Paris, France,, [14]Service D'hématologie Biologique, Laboratoire ADAMTS13, Hôpital Lariboisière, AP-HP Nord, Université de Paris, Paris, France, [15]Université Paris-Saclay, Université de Versailles Saint Quentin en Yvelines (UVSQ), Biomarqueurs en cancérologie et onco-hématologie (BECCOH), Boulogne-Billancourt, France, [16]Service d'Hématologie, Hôpital Saint-Antoine, AP-HP-Sorbonne Université, Paris, France, [17]NSERM UMRS 1138, Centre de Recherche des Cordeliers, Paris, France

Background: The COVID-19 pandemic related to SARS-CoV-2 virus was responsible for global pandemic. The severe form of the disease was linked to excessive activation of immune pathways together with a systemic cytokine storm response and thrombotic venous or arterial complications. Factors predicting severe outcomes including venous and/or pulmonary thrombosis (VT) and death were identified, but the prognostic role of their combination was not addressed extensively.

Objectives: We investigated the role of prognostic factors from the coagulation or inflammatory pathways to better understand the outcome of the disease.

Methods: For this, we prospectively studied 167 SARS-CoV-2-positive patients from admission in intensive care units (ICU) or emergency departments from four academic hospitals over a 14-month period. Besides standard biology, we assessed serum concentrations of inflammatory markers, coagulation factors and peripheral blood cells immunophenotyping.

Results: Thirty-nine patients (23.3%) developed VT and 30 patients (18%) died. By univariate analysis, C-reactive protein (CRP) level $>$ 150 mg/L, interleukin-6 (IL-6) $\geq$ 20 pg/mL, D-dimers $>$ 1,500 μg/L, ADAMTS13 activity $\leq$ 50%, Von

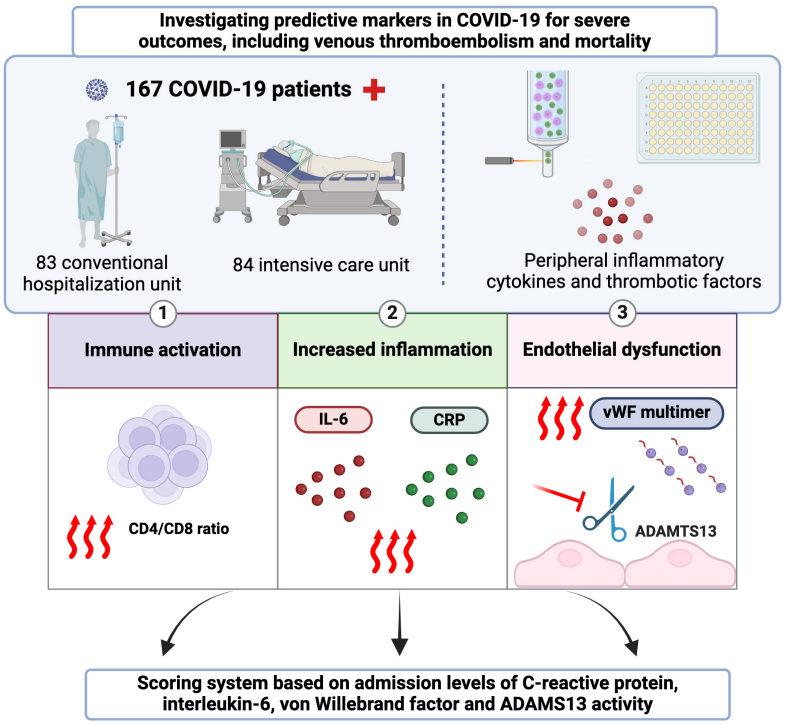
10.3389/fmed.2024.1399335

Willebrand factor Antigen (VWF:Ag) ≥ 400 IU/dL, VWF cofactor binding activity (VWF:CB) ≥ 350 IU/dL and VWF:Ag/ADAMTS13 activity ratio ≥ 10 were associated with VT, ICU admission or death. Using these thresholds, a scoring system was built by logistic regression based on the odds ratios derived from CRP, VWF, IL-6 and ADAMTS13 activity. This score was discriminant to predict ICU requirement, death and VT.

Conclusion: A combination of coagulation and inflammatory markers can refine the prognostication of severe outcome in COVID-19, and could be useful for the initial evaluation of other types of viral infection.

KEYWORDS

thrombosis, COVID-19, ADAMTS13, von Willebrand factor, interleukin-6, C-reactive protein, prognosis



GRAPHICAL ABSTRACT

Introduction

The severe acute respiratory syndrome (SARS) disease caused by the human SARS coronavirus 2 (SARS-CoV-2) has been responsible for the COVID-19 pandemic (1). The major advances in the understanding of the natural history and pathophysiology of the disease helped better clarifying its prognosis, now tampered by vaccination. This may apply in the future to other viral infections and could prove useful to explore and better manage infections by new SARS variants.

COVID-19 patients were mostly asymptomatic or mildly symptomatic. However, 5% of them developed a critical form of the disease with severe pulmonary damages with hypoxemia (2) The profound inflammatory response linked to an excessive activation of immune pathways, together with the related systemic cytokine storm response, led for these patients to an acute respiratory distress syndrome that induced thrombosis in pulmonary vessels (3), increasing mortality (4).

The coagulation system is activated in response to infection by a variety of different pathogens, including bacteria and viruses. This response appears to have developed as a host defense system to limit the spread of the pathogen. During infections, there is an interplay between blood coagulation, immune cells, and platelets to restrict dissemination of pathogens within the body. The activation of coagulation is beneficial for infections with bacteria and viruses by limiting pathogen dissemination and supports pathogen killing and tissue repair. On the other hand, over-activation can lead to thrombosis with subsequent depletion of hemostatic factors and secondary bleeding (5). This scenario applies for COVID-19 (6); in that regard, venous and/or

pulmonary thrombosis (VT) in patients with COVID-19 has been shown to involve multiple mechanisms, including activation of coagulation pathways, endothelial cells dysfunction, release of neutrophil extracellular traps (NETs), systemic inflammation and activation of the complement system (7). Consistent with endothelial cell activation, it has been proposed that abnormal, Von Willebrand factor (VWF)-mediated, interactions between platelets and the endothelium might also contribute to thrombosis in severe forms of COVID-19 (8–11). In this regard, it has been suggested that the VWF-cleaving protease ADAMTS13/VWF antigen ratio could be related to disease severity and predict poor outcome when elevated (12–14). Reflecting the coagulopathy of severe COVID-19, abnormal coagulation parameters including increased D-dimers and fibrinogen levels, mildly prolonged prothrombin time (PT) and mild thrombocytopenia have also been associated with disease severity and poor prognosis (15–18). A role for tissue factor (TF) (factor III, tissue thromboplastin or CD142) was also suggested in COVID-19-related thrombosis (7, 19). Lastly, the acute inflammatory response to SARS-CoV-2 has been shown to induce multiorgan failure, through the production of pro-inflammatory cytokines, notably interleukin (IL)-1β and IL-6 (20). In patients with severe COVID-19, a relationship has been reported between the extent of endothelial dysfunction and the magnitude of the immune inflammatory response (20). Especially, a unified pathophysiological hypothesis suggests that the imbalance between angiotensin-II and angiopoietin$_{1,7}$, caused by the interaction between SARS-CoV-2 and the angiotensin converting enzyme 2 (ACE2), results in an angiotensin-II "intoxication" with an abnormal activation of the angiotensin-II/angiotensin-II type 1 receptor, producing end-organ damage through the production of inflammatory cytokines and activation of the coagulation and complement cascades (21). In this way, several biomarkers of a pathway involving angiotensin-II, cytokines, C-reactive protein (CRP), coagulation (including ADAMTS13 and its substrate VWF) and finally peripheral blood immune cells have been individually described to predict the outcome of COVID-19 (22). Moreover, differences in ACE2 expression are linked to the severity and outcome of COVID-19 patients (23). Most of these markers were explored for their prognostic value; however, the role of their combination in the prognostication of severe outcomes including death, VT and ICU admission in COVID-19 patients was not fully addressed. We attempted here to assess simultaneously the role of coagulation and inflammation markers in order to better identify patients with COVID-19 at risk of severe outcomes.

Materials and methods

Patients

SARS-CoV-2-positive patients were prospectively and consecutively enrolled in four centers of the Assistance Publique-Hopitaux de Paris (AP-HP) (Avicenne, Ambroise Paré, Raymond-Poincaré and Lariboisière) from March 2020 to April 2021. Inclusion criteria allowed to select severe COVID-19 forms as enrolled patients had to be treated in intensive care unit (ICU) or hospitalized in emergency departments. All cases were confirmed as being positive by reverse transcription polymerase chain reaction (RT-PCR) of nasal swabs or tracheal aspirates. Clinical characteristics were collected from patient charts. VT was suspected clinically during hospitalization and systematically confirmed by Doppler-ultrasonography or computerized tomography.

Blood cell count, standard coagulation tests [prothrombin time (PT), activated partial thromboplastin time (ATT), fibrinogen and D-dimer] and serum CRP were assessed on admission.

Written informed consent was obtained from all participants or representatives. The study protocol at all sites was approved by the Ethics committee: CE SRLF 20-29 and CER-Paris-Saclay-2020-050. For all patients, the following blood sample analyses were issued on first admission as part of the routine care; therefore, no additional sampling was performed for the study.

Flow cytometry

Peripheral blood (PB) was processed for immunophenotyping as reported (24), Briefly, PB was stained with fluorochrome-conjugated antibodies to CD45, CD3, CD4, CD8, CD14, CD16, CD38 and HLA-DR to evaluate lymphocyte subsets, monocytes and activated cells. The expression of TF on monocytes was investigated with antibodies to CD142. All antibodies were purchased from BD Biosciences (**Supplementary Table 1**). Samples were acquired on a BD FACS Lyric instrument (BD Biosciences) and data were further analyzed with the Kaluza software (Beckman Coulter).

Peripheral inflammatory cytokines and thrombotic factors

IL-1β, IL-6, angiotensin-II and TF activity levels were measured using standard commercially available enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer instructions (Duoset reagents from Bio-Techne; LSBIO and Abcam, respectively). ADAMTS13 activity, VWF antigen (VWF:ag) and VWF cofactor binding activity (VWF:CB) were assessed in plasma as previously described (10).

Statistical analyses

Qualitative variables are reported as numbers and percentages. Quantitative discrete and continuous variables are reported as medians and interquartile ranges (IQR). Pearson's Chi Square was used for comparison of qualitative variables and Mann-Whitney test to compare quantitative variables between subgroups. In order to identify factors independently associated with VT and mortality, a Cox proportional hazards regression was performed. The Youden Index was calculated to identify optimal cut-off values for the parameters found to be significant in univariate analysis (sensitivity + specificity–1). The sensitivity, specificity, positive / negative predictive values and their respective confidence intervals (CIs) were determined. The discriminative performance of a scoring system in predicting death and the likelihood of being in ICU were assessed using the area under the receiver operating characteristic

TABLE 1 Patient characteristics at diagnosis.

	Total *n* = 167	No VT *n* = 128	VT *n* = 39	*p*-value	Survivors *n* = 137	Non-survivors *n* = 30	*p*-value	No ICU *n* = 83	ICU *n* = 84	*p*-value
Age (years)	72 [60–85]	73 [61–87]	71 [58–82]	0.38	71 [58–84]	74.5 [67–88]	0.11	83 [64–91]	66.5 [56–75]	< **0.001**
Gender										
Female, *n* (%)	64 (38.3)	49 (38.3)	15 (38.5)	0.98	58 (42.3)	6 (20)	< **0.001**	45 (54.2)	19 (22.6)	**0.023**
Male, *n* (%)	103 (61.7)	79 (61.7)	24 (61.5)		79 (57.7)	24 (80)		38 (45.8)	65 (77.4)	
Comorbidity										
Obesity, *n* (%)	43 (25.8)	37 (28.9)	6 (15.4)	0.09	34 (24.8)	9 (30)	0.56	12 (14.5)	31 (36.9)	**0.001**
Smoking, *n* (%)	37 (22.2)	26 (20.3)	11 (28.2)	0.30	28 (20.4)	9 (30.00)	0.25	16 (19.3)	21 (25.0)	0.37
Prior history of cancer, *n* (%)	34 (20.4)	29 (22.7)	5 (12.8)	0.18	26 (19.0)	8 (26.7)	0.34	20 (24.1)	14 (16.7)	0.23
Hypertension, *n* (%)	66 (39.5)	52 (40.6)	14 (35.9)	0.60	54 (39.4)	12 (40.0)	0.95	32 (38.6)	34 (40.5)	0.80
Body mass index (kg/m^2)	25.7 [22–29.1]	26.2 [22.6–29.4]	24.3 [21.7–27.6]	0.19	25.8 [22.5–29.4]	25.1 [22–27.6]	0.33	23.7 [20.3–27.2]	27.3 [23.3–30.5]	**0.001**
Intensive care unit, *n* (%)	84 (50.3)	56 (43.8)	28 (71.8)	**0.002**	66 (48.2)	18 (60.0)	0.24	0 (0.0)	84 (100.0)	
ARDS, *n* (%)	134 (80.7)	27 (21.26)	100 (78.7)	0.24	105 (77.2)	29 (96.7)	**0.01**	54 (65.9)	80 (95.2)	< **0.001**
SaO2 (%)	92 [87–96]	93 [88–96]	93 [84–94]	**0.04**	92 [88–96]	92 [84.5–94]	0.25	94 [92–97]	90 [84–93.5]	< **0.001**
pO2 (mmHg)	64 [55–75]	67 [58–76]	56.5 [50–72.5]	**0.02**	66 [56–76]	61 [49–72]	0.19	68.5 [54–87]	62 [56–74]	0.41
Non-invasive ventilation, *n* (%)	81 (49.7)	56 (45.2)	25 (64.1)	**0.04**	60 (45.1)	21 (70.0)	**0.01**	15 (18.8)	66 (79.5)	< **0.001**
Duration of non-invasive ventilation (days)	4 [2–7]	4 [2.5–7]	5 [2–8]	0.78	4 [2.5–7]	4 [2–8]	0.48	6 [3–10]	4 [2–7]	0.14
Mechanical ventilation, *n* (%)	40 (24.5)	27 (21.8)	13 (33.3)	0.14	25 (18.8)	15 (50)	< **0.001**	1 (1.3)	39 (47)	**NA**
Duration of mechanical ventilation (days)	12 [5–19]	11 [6–18]	15 [4–20]	0.83	13 [4.5–18.5]	12 [7–20]	0.75	16	11.5 [5–19]	NA
Venous thrombosis, *n* (%)	39 (23.4)	0 (0.0)	39 (100.0)	**NA**	32 (23.4)	7 (23.3)	0.99	11 (13.3)	28 (33.3)	**0.002**
Pulmonary embolism/thrombosis, *n* (%)	29 (17.4)	0 (0.0)	29 (74.4)	**NA**	23 (16.8)	6 (20)	0.67	3 (3.6)	26 (31.0)	**NA**
Treatments										
Corticosteroids, *n* (%)	63 (17.4)	46 (35.9)	17 (43.6)	0.39	50 (36.5)	13 (43.3)	0.48	12 (14.5)	51 (60.7)	< **0.001**
Antithrombotic therapy, *n* (%)	130 (77.8)	91 (71.1)	39 (100)	< **0.001**	107 (78.1)	23 (76.7)	0.86	51 (61.5)	79 (94.1)	< **0.001**

(Continued)

10.3389/fmed.2024.1399335

TABLE 1 (Continued)

	Total *n* = 167	No VT *n* = 128	VT *n* = 39	*p*-value	Survivors *n* = 137	Non-survivors *n* = 30	*p*-value	No ICU *n* = 83	ICU *n* = 84	*p*-value
Tocilizumab (anti-IL6), *n* (%)	22 (13.2)	16 (12.5)	6 (15.5)	0.64	18 (13.1)	4 (13.3)	NA	2 (2.4)	20 (23.8)	NA
Death, *n* (%)	30 (18.0)	23 (18.0)	7 (18.0)	0.99	0 (0.0)	30 (100.0)	NA	12 (14.5)	18 (21.4)	0.24
Laboratory findings										
C reactive protein (mg/L)	159.5 [70–236]	149.5 [52–219.5]	218 [142–296]	< **0.001**	149.5 [61–237]	177.5 [143–230]	0.22	97 [39–77]	208 [142–284]	< **0.001**
Hemoglobin (g/dL)	11.6 [9.6–13.3]	11.7 [10.1–13.3]	10.6 [9.1–12.4]	**0.02**	11.65 [9.95–13.3]	10.7 [9.2–12.4]	0.14	11.7 [10.4–13.3]	11.5 [9.5–13.25]	0.26
Platelets (x10^9/L)	272.5 [193–350]	261 [192–335]	311 [194–404]	0.15	269.5 [194.5–339.5]	290 [185–433.5]	0.32	256 [191–335]	286 [196–379]	0.34
MPV (fL)	10.6 [9.8–11.3]	10.65 [9.85–11.3]	10.5 [9.6–11.2]	0.32	10.6 [9.8–11.3]	10.9 [9.75–11.25]	0.96	10.45 [9.8–11.2]	10.8 [9.85–11.4]	0.17
Leucocytes (x10^9/L)	8.15 [5.4–10.75]	7.7 [5.3–10.59]	8.5 [6.5–12.3]	0.09	8.0 [5.3–10.4]	9.0 [6.6–12.3]	0.16	7.0 [4.5–8.6]	9.8 [6.8–13.4]	< **0.001**
Lymphocytes (x10^9/L)	0.95 [0.59–1.51]	0.98 [0.64–1.54]	0.86 [0.43–1.37]	0.38	0.99 [6.25–1.61]	0.75 [0.25–0.99]	**0.02**	1.05 [0.76–1.67]	0.82 [0.53–1.35]	**0.02**
Neutrophils (x10^9/L)	6.22 [4.07–8.79]	5.6 [3.75–8.68]	7.23 [8.84–8.86]	0.10	5.81 [3.81–8.69]	7.81 [4.42–10.1]	0.12	4.77 [2.58–6.45]	8.3 [5.6–10.5]	< **0.001**
Monocytes (x10^9/L)	0.5 [0.33–0.74]	0.51 [0.34–1.54]	0.48 [0.28–0.71]	0.22	0.52 [0.33–0.75]	0.48 [0.36–0.72]	0.93	0.55 [0.37–0.75]	0.48 [0.32–0.73]	0.30
Neutrophil-lymphocyte ratio	6.39 [3.01–12.96]	5.32 [3.01–12.09]	9.34 [3.11–19.95]	0.06	5.22 [2.82–12.49]	10.39 [6.43–12.96]	**0.023**	3.71 [2.52–6.39]	5.32 [10.89–21.94]	< **0.001**
Lymphocyte-monocyte ratio	1.93 [1.11–1.89]	1.93 [1.21–2.94]	2.15 [1.04–3.01]	0.85	1.99 [1.24–3.22]	1.5 [0.82–2.14]	**0.013**	1.94 [1.33–3.01]	1.88 [0.92–2.82]	**0.25**
Platelet-lymphocyte count ratio	264.8 [160–414]	248.2 [138.3–385.2]	348.0 [191.3–521.9]	**0.04**	251.5 [160–388.7]	336.8 [189.7–683.9]	0.12	220.5 [135.3–352]	301 [187.6–587.5]	**0.02**
Prothrombin ratio (%)	72 [60–81]	74 [60–82]	68.5 [61–80]	0.55	72 [60–82]	74.5 [59.5–80]	1.00	79.5 [64.5–91.5]	69 [57–80]	**0.02**
aPTT ratio	1.29 [1.11–1.53]	1.29 [1.08–1.5]	1.24 [1.12–1.63]	0.36	1.28 [1.1–1.57]	1.33 [1.17–1.48]	0.53	1.21 [1.0–1.33]	1.36 [1.14–1.65]	**0.007**
D-dimers (μg/L)	1,200 [750–3,214]	1,055 [596–1,962]	1,856 [1,150–8,994]	**0.005**	1,124 [669–3,214]	1,397 [1,127–4,680]	0.15	1,012 [669–1,207]	1,762 [952–6,093]	**0.008**
Fibrinogen (g/L)	6.65 [5.25–7.85]	1.29 [1.08–1.5]	1.24 [1.12–1.63]	0.48	6.6 [5.2–7.9]	6.7 [5.4–7.6]	0.95	5.5 [4.4–6.6]	7.3 [5.9–8.5]	< **0.001**
IL-6 (pg/mL)	17.7 [6.7–47.9]	14.8 [6.2–45.9]	26.5 [11.8–65.9]	0.14	14.6 [6.0–44.7]	45.2 [16.1–169]	**0.005**	9.4 [3.9–21.4]	33.0 [13.5–104.5]	< **0.001**
IL-1 (pg/mL)	39.3 [36.1–57.1]	40.5 [36.9–51.6]	37.4 [25.4–61.4]	0.20	39.8 [35.5–60.3]	38.2 [36.5–50.5]	0.88	40.5 [35.7–59.8]	38.2 [36.3–48.9]	0.55

(Continued)

10.3389/fmed.2024.1399335

TABLE 1 (Continued)

	Total *n* = 167	No VT *n* = 128	VT *n* = 39	*p*-value	Survivors *n* = 137	Non-survivors *n* = 30	*p*-value	No ICU *n* = 83	ICU *n* = 84	*p*-value
Angiotensin-II (pg/mL)	159 [2.0–782.3]	427 [4.5–797.6]	26.0 [0.5–631.3]	0.21	31.8 [0.9–774.2]	565 [44.5–791.2]	0.21	10.2 [0.5–785.9]	492.9 [15.7–782.3]	0.07
Tissue factor level (pg/mL)	8.0 [4.9–27.0]	8.0 [4.7–24.4]	8.2 [6.0–40.6]	0.47	8.7 [5.5–7.1]	7.1 [3.4–13.7]	0.11	7.2 [5.2–15.0]	11.9 [3.9–38.4]	0.13
Activated monocytes (MFI)	18,592 [12,017–31,408]	19,279 [11,932–31,804]	16,940 [13,286–8,994]	1.00	20,522 [13,189–33,467]	12,561 [8,154–24,221]	0.06	24,707 [15,138–31,804]	16,940 [9,534–36,545]	0.30
Classical monocytes (%)	92.0 [86.1–96.1]	91.8 [84.5–96.0]	94.3 [87.9–97.3]	0.39	92.3 [86.3–96.8]	89.4 [82.1–94.5]	0.10	92.0 [86.2–95.7]	92.0 [85.2–96.2]	0.09
Tissue factor$^+$ monocytes (%)	1.74 [0.77–3.84]	1.75 [0.79–4.42]	1.70 [0.66–2.7]	0.37	1.63 [0.75–4.14]	1.76 [0.81–2.57]	0.92	1.37 [0.72–3.27]	2.34 [0.81–3.99]	0.21
Tissue factor HLA-DR$^+$ monocytes (MFI)	1,449 [1,041–2,287]	1,461 [1,085–2,393]	1,372 [883–2,105]	0.38	1,504 [1,041–2,450]	1,283 [1,022–1,774]	0.30	1,452 [1,065–3,000]	1,405 [1,016–2,019]	0.30
CD3$^+$ T-cells (%)	74.5 [66.9–80.3]	75.1 [68.2–80.3]	71.1 [64.5–81.6]	0.30	74.5 [68.3–80.3]	74.5 [64.3–80.8]	0.57	74.8 [69.3–82.1]	74.5 [66.3–78.4]	0.54
CD4$^+$ T-cells (%)	67.7 [58.2–78.0]	66.8 [56.6–76.9]	75.3 [63.3–83.8]	**0.008**	67.4 [58.7–78.0]	69.5 [52.3–80.6]	1.00	61.8 [54.9–73.4]	72.6 [61.4–81.4]	**0.007**
CD8$^+$ T-cells (%)	27.5 [17.1–37.4]	28.8 [18.5–39.6]	21.8 [12.2–27.8]	**0.008**	28.3 [17.1–36.9]	24.2 [17.6–41.1]	0.75	31.9 [22.7–40.6]	22.6 [14.8–32.6]	**0.008**
CD4$^+$/CD8$^+$ T-cell ratio	2.46 [1.59–4.49]	2.31 [1.45–4.06]	3.57 [2.33–6.98]	**0.007**	2.31 [1.45–4.06]	3.57 [2.33–6.98]	0.87	2.37 [1.64–4.49]	2.81 [1.28–4.68]	**0.007**
HLA-DR$^+$/CD38$^+$/CD4$^+$ T-cells (%)	10.7 [7.0–17.8]	11.6 [7–18.0]	6.7 [8.5–16.9]	0.32	10.2 [6.3–16.7]	15.3 [9.9–24.5]	**0.019**	12.5 [8.1–20.8]	9.8 [7.0–16]	0.12
HLA-DR$^+$/CD38$^+$/CD8$^+$ T-cells (%)	34.3 [21.3–50.9]	35.8 [18.3–51.6]	31 [22.1–41.9]	0.45	34.7 [19.9–50.9]	34.0 [28.3–41.7]	0.98	34.4 [25.3–51.6]	34.3 [19.7–47.1]	0.21
ADAMTS13 activity (%)	55 [38.5–77.5]	62 [46–80]	39 [31–68]	**0.002**	66 [41–81]	38.5 [33–50]	**0.004**	73 [47–85]	49 [33–71]	**0.008**
vWF:Ag (IU/dL)	400.5 [312.9–521.5]	383.2 [264.3–522.5]	453.4 [362.4–510]	0.17	385.6 [293.6–509]	463.7 [375.3–529.6]	0.20	293.6 [197.9–410.6]	423.6 [355.7–555.5]	**0.002**
vWF:CB (IU/dL)	351 [282.5–477.5]	346 [236–442]	368 [293–489]	0.17	330 [237–438]	431 [316–546]	**0.03**	285 [181–340]	370 [308–483]	**0.005**
vWF:Ag/ADAMTS13 activity ratio	6.96 [4.22–12.72]	5.56 [3.77–10.76]	5.62 [11.83–19.53]	**0.005**	5.64 [4.10–11.69]	12.26 [7.82–15.45]	**0.02**	4.10 [1.76–5.70]	10.09 [5.32–15.01]	< **0.001**

VT, venous and pulmonary thrombosis; ICU, intensive care unit; BMI, body mass index; ARDS, acute respiratory distress syndrome; SaO2, oxygen saturation; pO2, oxygen pressure; MPV, mean platelet volume; aPTT, activated prothrombin time; IL, interleukin; TF, tissue factor; MFI, mean fluorescence intensity; VWF, von Willebrand factor. Qualitative variables are reported as numbers and percentages. Quantitative discrete and continuous variables are reported as medians and interquartile ranges (IQR). Pearson's Chi Square was used for comparison of qualitative variables and Mann-Whitney test to compare quantitative variables between subgroups. Bold values reflect statistically significant values.

10.3389/fmed.2024.1399335

(ROC) curve (AUC). Statistical analyses were performed with R version 3.6.1 (2019-07-05) (The R Project).[1]

Results

During the inclusion period, 167 COVID-19 patients were enrolled. About half of these patients (50.3%) were in ICU at the time of the study. Of the 167 patients, 39 (23.3%) developed VT and 30 (20%) died (Table 1 and Figures 1A, B).

Clinical features associated with VT

Patients with VT were more often admitted in ICU ($p = 0.002$), and had consistently lower O2 saturation ($p = 0.04$), lower pO2 ($p = 0.02$) and more non-invasive ventilation ($p = 0.04$). All these patients received anti-thrombotic therapy. They had more D-dimers ($p = 0.005$), a lower ADAMTS13 activity ($p = 0.002$) with higher vWF:Ag/ADAMTS13 activity ratios ($p = 0.005$), higher CRP levels ($p < 0.001$) and more anemia ($p = 0.02$). CD8+ T-cells ($p = 0.008$) were lower and CD4+ T-cells were higher ($p = 0.008$) in this population (Table 1 and Figure 2).

Clinical features associated with ICU hospitalization

Patients in ICU differed from those recruited through emergency rooms by several factors. They were younger ($p < 0.001$), more often males ($p < 0.001$) and mostly obese ($p = 0.001$). They presented more often with acute respiratory distress syndrome (ARDS) ($p < 0.001$) and received more non-invasive ventilation ($p < 0.001$). These patients had more VT ($p = 0.002$); they received more corticosteroids ($p < 0.001$) and more anti-thrombotic therapy ($p < 0.001$) (Table 1). They had more inflammation with higher CRP levels ($p < 0.001$), PB polymorphonuclears ($p < 0.001$) and IL-6 ($p < 0.001$), and a higher CD4/CD8 T-cell ratio ($p = 0.007$). Lastly, levels of D-dimers ($p = 0.008$), fibrinogen ($p < 0.001$) and VWF/ADAMS13 ratio ($p < 0.001$) were higher in this population (Figure 2).

Clinical features associated with survival

Non-survivors were predominantly males ($p = 0.023$) with more ARDS ($p = 0.01$) and requiring more mechanical ventilation ($p < 0.001$); they had more activated CD4+ T-cells ($p = 0.019$), higher IL-6 levels ($p = 0.005$) and a lower lymphocyte count with lower neutrophil-lymphocyte and lymphocyte-monocyte ratios (Table 1). Considering coagulation pathways, patients who deceased had a lower ADAMTS13 activity ($p = 0.004$), higher VWF:CB ($p = 0.03$) and a higher VWF:Ag/ ADAMS13 ratio ($p = 0.02$) (Table 1 and Figure 2).

1 https://www.r-project.org/

Predictive features of VT, ICU hospitalization or death at admission

By univariate analysis, CRP level > 150 mg/L, IL-6 ≥ 20 pg/mL, D-dimers > 1,500 μg/L, ADAMTS13 activity ≤ 50%, VWF:Ag ≥ 400 IU/dL, VWF:CB ≥ 350 IU/dL and VWF:Ag/ADAMTS13 activity ratio ≥ 10 were associated with VT, ICU admission or death (Table 2).

Using these thresholds, a scoring system was built based on the odds ratios derived from significant parameters identified by logistic regression: (i) CRP and VWF (3 points each) and (ii) IL-6 and ADAMS13 activity (4 points each).

The probability to require ICU hospitalization yielded an AUC of 0.74 with a sensitivity and a specificity of, respectively, 67.8 and 63.9% for a score ≥ 4; for a score of ≥ 7 sensitivity was 60.7% and specificity 74.7%. With an AUC of 0.73, death could be predicted with a sensitivity of 76.7% and a specificity of 53.3% for a score of ≥ 4; for a score of ≥ 7 sensitivity was 63.3% and specificity 61.3%. Death was significantly associated with a score of ≥ 4 ($p = 0.004$).

Discussion

By assessing simultaneously various inflammation and coagulation markers in a large cohort of COVID-19 patients, this study provides a comprehensive description of risk factors for VT, death or hospitalization in ICU in this viral infection. Conducted over 14 months, this work likely involved mostly alpha and beta SARS-CoV-2 variants (25) and provides risk factors associated with these viruses. Here, 23.3% of patients experienced VT. This rate is comparable to this of other non-COVID-19 cohorts with severe infection (26), although the pathophysiological mechanism may differ.

An activated angiotensin-II/IL-6/CRP pathway and a high pro-aggregant VWF/ADAMTS13 ratio, reflecting strong inflammation and endothelial damage axis, appeared as independent risk factors for VT, death and/or ICU hospitalization. This study confirms and extends the view that ADAMTS13 activity is a reliable marker to assess the severity of SARS-CoV-2 infection (10). In this regard, normal levels of ADAMTS13 activity were identified as a strong predictor of survival. By contrast, platelet parameters, previously reported as early predictive markers of severity (27, 28), had no prognostic value in this series (29). Although most prognostic factors identified here confirm previous findings (25), the strength of this work is that multiple markers were addressed simultaneously, allowing to provide a more comprehensive prognostication of patients with COVID-19. These results may therefore help identifying patients for whom a so far undiagnosed thrombosis (especially pulmonary embolism/thrombosis) should be investigated.

The strong association of the IL-6/CRP inflammation pathway with both severe thrombotic events, survival and ICU hospitalization supports therapeutic approaches based on monoclonal antibodies directed against IL-6 alone or with corticosteroids in patients with intermediate severity (30, 31). Interestingly, IL-6 but not CRP levels were significantly discriminative between survivors and non-survivors (27), confirming that IL-6 assessment should be preferred over CRP

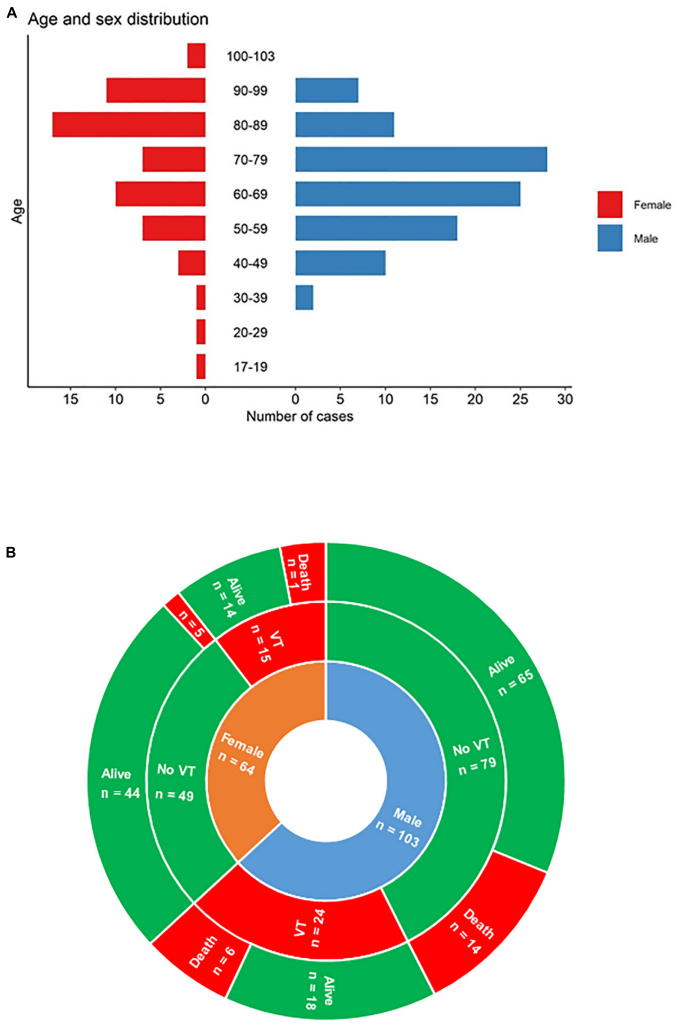


FIGURE 1
(A) Demographic data; **(B)** main severe outcomes (thrombo-embolic events and survival) according to sex. VT, venous or pulmonary thrombosis.

to evaluate critically ill patients (28). Similarly, the identification of a high pro-aggregant VWF/ADAMTS13 ratio as a strong prognostic factor, opens perspectives for VWF-targeted therapies (13). No influence of TF was observed in this series, confirming previous findings (32), although TF has been reported by others as a potential mediator of pathogenesis in COVID-19 by driving endothelial dysfunction and coagulopathy (29). Moreover, TF-expressing monocytes, that were found to be mildly increased in symptomatic vs. asymptomatic patients (30), were not different here between patient groups; hence, the role of serum or cell surface-expressed TF as a predictive marker of thrombo-embolism in patients with COVID-19 remains debatable.

T cells play are crucial in maintaining immune function and viral clearance. The impact of immune modulation as reflected here by an increased peripheral CD4/CD8 T-cell ratio, was confirmed to segregate patients with VT and those requiring ICU hospitalization. Yet, we did not confirm that a higher peripheral CD4/CD8 T-cell ratio was associated with a better outcome (31, 33), and whether peripheral CD4/CD8 T-cell ratio reflects prognosis in COVID-19 deserves further studies.

Taken together, these observations support a tentative unifying model where SARS-CoV-2 primarily induces a deregulation of the renin-angiotensin-aldosterone system axis. In this model, SARS-CoV-2 S protein binds to and internalizes its receptor ACE2, leading to a lack of conversion of angiotensin-II into the protective peptide angiopoietin$_{1,7}$ and thereby to the accumulation of angiotensin-II (21). In turn, angiotensin-II through the activation of its receptor AT1R induces the production of IL-6 and subsequently CRP. Lastly, the release of IL-6 may at least in part account for the high levels of circulating VWF, but also in the decrease of ADAMTS13 activity, leading to a pro-aggregant phenotype and thrombotic microangiopathy-like features (10, 13).

Altogether, this study suggests that the simultaneous analysis of a combination of multiple key coagulation and inflammatory markers in COVID-19 pathophysiology could refine the prognostication of severe outcome in COVID-19, and allow identifying patients at risk of VT, ICU hospitalization and death, and those in whom a VT (especially a pulmonary thrombosis) should be investigated. The score proposed here, relying on 4 key assessments could be refined/validated by retrospective analyses of COVID-19 patient cohorts where these markers were possibly

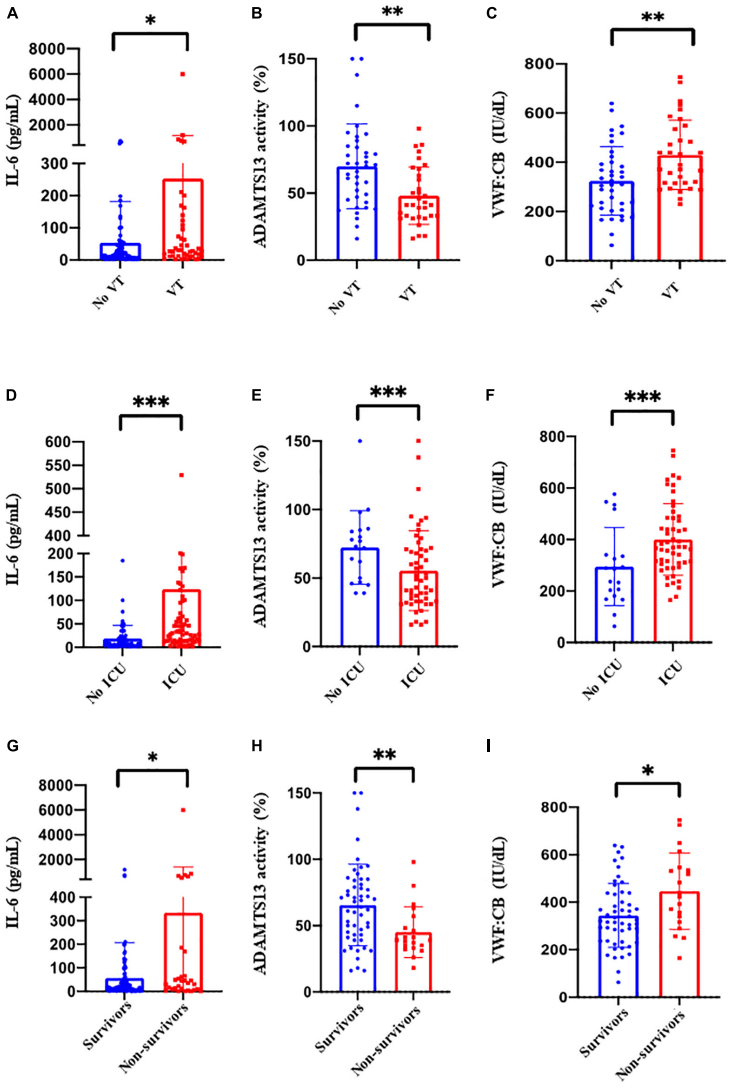


FIGURE 2
(A) C-reactive protein, **(A)** interleukin-6 (IL-6), **(B)** ADAMTS13 activity, **(C)** VWF:CB at the baseline between patients with (red bars) or without (blue bars) venous and pulmonary thrombosis (VT); **(D)** interleukin-6 (IL-6), **(E)** ADAMTS13 activity, **(F)** VWF:Ag, at the baseline between ICU patients (red bars) vs. non ICU patients (blue bars); **(G)** Interleukin-6 (IL-6), **(H)** ADAMTS13 activity, **(I)** VWF:CB at baseline between survivors (blue bars) and non-survivors (red bars). *$p < 0.05$; **$p <$ <0.01; ***$p < 0.001$.

TABLE 2 Clinical findings on admission associated with VT, death or ICU hospitalization by univariate analysis.

	VT		ICU		Death	
	OR (CI 2.5–97.5%)	*p*	OR (CI 2.5–97.5%)	*p*	OR (CI 2.5–97.5%)	*p*
CRP > 150 mg/L	1.16 (0.51–2.63)	0.73	3.02 (1.01–9.35)	**0.05**	3.19 (1.53- 6.62)	**0.002**
IL-6 ≥ 20 pg/mL	1.51 (0.70–3.32)	0.30	3.96 (1.54–10.15)	**0.004**	3.88 (1.93–7.81)	< **0.001**
D-dimers > 1,500 µg/L	2.72 (1.00–7.39)	**0.05**	1.28 (0.41–3.99)	0.68	12.6 (2.67–59.59)	**0.001**
ADAMTS13 activity ≤ 50%	3.26 (1.29–8.2)	**0.01**	4.32 (1.49–12.53)	**0.004**	2.09 (0.89–4.91)	0.09
VWF:Ag ≥ 400 IU/dL	1.73 (0.70–4.25)	0.23	2.23 (0.80–6.20)	0.13	1.37 (0.60–3.10)	0.75
VWF:CB ≥ 350 IU/dL	1.25 (0.46–3.42)	0.67	3.39 (1.06–10.88)	**0.04**	1.37 (2.10–21.09)	**0.001**
VWF:Ag/ADAMTS13 activity ≥ 10	2.92 (1.05–8.14)	**0.04**	5.2 (1.60–16.86)	**0.006**	3.89 (1.14–13.28)	**0.03**

VT, venous and/or pulmonary thrombosis; ICU, intensive care unit; OR, odds ratio; CI, confidence interval; CRP, C-reactive protein; TF, tissue factor; MFI, mean fluorescence intensity; VWF, von Willebrand factor. Bold values reflect statistically significant values.

independently measured. They should also be generalizable to other types of severe viral infections with evidence of prevalent venous and pulmonary thrombosis (34) where their prognostic value could be of help in patient management.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by the Ethics committee: CE SRLF 20-29 and CER-Paris-Saclay-2020-050. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JZ: Writing – original draft. RB: Writing – original draft. BT: Writing – original draft. GG: Writing – original draft. YC: Writing – original draft. AP: Writing – original draft. NH: Writing – original draft. MR: Writing – original draft. BJ-L: Writing – original draft. AV: Writing – original draft. CC: Writing – original draft. PC: Writing – original draft.

Funding

The authors declare that financial support was received for the research, authorship, and/or publication of this article. This work was partly funded by the Association de recherche sur les microangiopathies thrombotiques et autres maladies rares en Hématologie (ARMAT).

Acknowledgments

Medical writing for this manuscript was assisted by MPIYP (MC Béné), Paris, France.

Conflict of interest

PC was member of the Clinical Advisory Board for Alexion, Sanofi, Takeda, and Janssen. AV had participated to Advisory boards for Sanofi.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1399335/full#supplementary-material

References

1. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature.* (2020) 579:270–3.

2. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: Summary of a report of 72?314 cases from the Chinese center for disease control and prevention. *JAMA.* (2020) 323:1239–42. doi: 10.1001/jama.2020.2648

3. Klok FA, Kruip M, van der Meer NJM, Arbous MS, Gommers DAMPJ, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res.* (2020) 191:145–7.

4. Vabret N, Britton GJ, Gruber C, Hegde S, Kim J, Kuksin M, et al. Immunology of COVID-19: Current state of the science. *Immunity.* (2020) 52:910–41.

5. Antoniak S. The coagulation system in host defense. *Res Pract Thromb Haemost.* (2018) 2:549–57.

6. Lazzaroni MG, Piantoni S, Masneri S, Garrafa E, Martini G, Tincani A, et al. Coagulation dysfunction in COVID-19: The interplay between inflammation, viral infection and the coagulation system. *Blood Rev.* (2021) 46:100745.

7. Mackman N, Antoniak S, Wolberg AS, Kasthuri R, Key NS. Coagulation abnormalities and thrombosis in patients infected with SARS-CoV-2 and other pandemic viruses. *Arterioscler Thromb Vasc Biol.* (2020) 40:2033–44.

8. Turecek PL, Peck RC, Rangarajan S, Reilly-Stitt C, Laffan MA, Kazmi R, et al. Recombinant ADAMTS13 reduces abnormally up-regulated von Willebrand factor in plasma from patients with severe COVID-19. *Thromb Res.* (2021) 201:100–12. doi: 10.1016/j.thromres.2021.02.012

9. Ward SE, Fogarty H, Karampini E, Lavin M, Schneppenheim S, Dittmer R, et al. ADAMTS13 regulation of VWF multimer distribution in severe COVID-19. *J Thromb Haemost.* (2021) 19:1914–21. doi: 10.1111/jth.15409

10. Joly BS, Darmon M, Dekimpe C, Dupont T, Dumas G, Yvin E, et al. Imbalance of von Willebrand factor and ADAMTS13 axis is rather a biomarker of strong inflammation and endothelial damage than a cause of thrombotic process in critically ill COVID-19 patients. *J Thromb Haemost.* (2021) 19:2193–8. doi: 10.1111/jth.15445

11. Mancini I, Baronciani L, Artoni A, Colpani P, Biganzoli M, Cozzi G, et al. The ADAMTS13-von Willebrand factor axis in COVID-19 patients. *J Thromb Haemost.* (2021) 19(2):513–21.

12. Doevelaar AAN, Bachmann M, Holzer B, Seibert FS, Rohn BJ, Bauer F, et al. von Willebrand Factor multimer formation contributes to immunothrombosis in coronavirus disease 2019. *Crit Care Med.* (2021) 49:e512–20.

13. Philippe A, Gendron N, Bory O, Beauvais A, Mirault T, Planquette B, et al. Von Willebrand factor collagen-binding capacity predicts in-hospital mortality in COVID-19 patients: Insight from VWF/ADAMTS13 ratio imbalance. *Angiogenesis.* (2021) 24:407–11. doi: 10.1007/s10456-021-09789-3

14. von Meijenfeldt FA, Havervall S, Adelmeijer J, Lundström A, Rudberg AS, Magnusson M, et al. Prothrombotic changes in patients with COVID-19 are associated with disease severity and mortality. *Res Pract Thromb Haemost.* (2021) 5:132–41.

15. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost.* (2020) 18:844–7.

16. Levi M, Thachil J, Iba T, Levy JH. Coagulation abnormalities and thrombosis in patients with COVID-19. *Lancet Haematol.* (2020) 7:e438–40.

17. Berger JS, Kunichoff D, Adhikari S, Ahuja T, Amoroso N, Aphinyanaphongs Y, et al. Prevalence and outcomes of D-dimer elevation in hospitalized patients with COVID-19. *Arterioscler Thromb Vasc Biol.* (2020) 40:2539–47. doi: 10.1161/ATVBAHA.120.314872

18. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. *Lancet.* (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3

19. Bautista-Vargas M, Bonilla-Abadia F, Canas CA. Potential role for tissue factor in the pathogenesis of hypercoagulability associated with in COVID-19. *J Thromb Thrombol.* (2020) 50:479–83.

20. Dupont A, Rauch A, Staessens S, Moussa M, Rosa M, Corseaux D, et al. Vascular endothelial damage in the pathogenesis of organ injury in severe COVID-19. *Arterioscler Thromb Vasc Biol.* (2021) 41:1760–73.

21. Angeli F, Zappa M, Reboldi G, Trapasso M, Cavallini C, Spanevello A, et al. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection: One year later. *Eur J Intern Med.* (2021) 93:28–34. doi: 10.1016/j.ejim.2021.09.007

22. Hariyanto TI, Japar KV, Kwenandar F, Damay V, Siregar JI, Lugito NPH, et al. Inflammatory and hematologic markers as predictors of severe outcomes in COVID-19 infection: A systematic review and meta-analysis. *Am J Emerg Med.* (2021) 41:110–9. doi: 10.1016/j.ajem.2020.12.076

23. Alabsi S, Dhole A, Hozayen S, Chapman SA. Angiotensin-converting enzyme 2 expression and severity of SARS-CoV-2 infection. *Microorganisms.* (2023) 11:612.

24. Funderburg NT, Mayne E, Sieg SF, Asaad R, Jiang W, Kalinowska M, et al. Increased tissue factor expression on circulating monocytes in chronic HIV infection: Relationship to in vivo coagulation and immune activation. *Blood.* (2010) 115:161–7. doi: 10.1182/blood-2009-03-210179

25. Gorog DA, Storey RF, Gurbel PA, Tantry US, Berger JS, Chan MY, et al. Current and novel biomarkers of thrombotic risk in COVID-19: A consensus statement from the international COVID-19 thrombosis biomarkers colloquium. *Nat Rev Cardiol.* (2022) 19:475–95. doi: 10.1038/s41569-021-00665-7

26. Wang L, Ma X, Chen Y, Gao S, Pan W, Chen J, et al. Factors influencing DVT formation in sepsis. *Thromb J.* (2024) 22:11.

27. Lavillegrand JR, Garnier M, Spaeth A, Mario N, Hariri G, Pilon A, et al. Elevated plasma IL-6 and CRP levels are associated with adverse clinical outcomes and death in critically ill SARS-CoV-2 patients: Inflammatory response of SARS-CoV-2 patients. *Ann Intensive Care.* (2021) 11:9. doi: 10.1186/s13613-020-00798-x

28. Picod A, Morisson L, de Roquetaillade C, Sadoune M, Mebazaa A, Gayat E, et al. Systemic inflammation evaluated by interleukin-6 or C-reactive protein in critically ill patients: Results from the FROG-ICU study. *Front Immunol.* (2022) 13:868348. doi: 10.3389/fimmu.2022.868348

29. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in covid-19. *N Engl J Med.* (2020) 383:120–8.

30. Hottz, Azevedo-Quintanilha IG, Palhinha L, Teixeira L, Barreto EA, Pão CRR, et al. Platelet activation and platelet-monocyte aggregate formation trigger tissue factor expression in patients with severe COVID-19. *Blood.* (2020) 136:1330–41. doi: 10.1182/blood.2020007252

31. Pascual-Dapena A, Chillaron JJ, Llaurado G, Arnau-Barres I, Flores J, Lopez-Montesinos I, et al. Individuals with higher CD4/cd8 ratio exhibit increased risk of acute respiratory distress syndrome and in-hospital mortality during acute SARS-CoV-2 infection. *Front Med.* (2022) 9:924267. doi: 10.3389/fmed.2022.924267

32. Skendros P, Mitsios A, Chrysanthopoulou A, Mastellos DC, Metallidis S, Rafailidis P, et al. Complement and tissue factor-enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis. *J Clin Invest.* (2020) 130:6151–7. doi: 10.1172/JCI141374

33. De Zuani M, Laznickova P, Tomaskova V, Dvončová M, Forte G, Stokin GB, et al. High CD4-to-CD8 ratio identifies an at-risk population susceptible to lethal COVID-19. *Scand J Immunol.* (2022) 95:e13125. doi: 10.1111/sji.13125

34. Dolby HW, Potey P, Wilder-Smith AB, Clohisey S, Millar JE, Baillie JK, et al. Histological evidence of pulmonary microthrombosis and vasculitis in life-threatening respiratory virus diseases. *Open Forum Infect Dis.* (2021) 8:ofaa640. doi: 10.1093/ofid/ofaa640

Frontiers in Medicine

Translating medical research and innovation into improved patient care

A multidisciplinary journal which advances our medical knowledge. It supports the translation of scientific advances into new therapies and diagnostic tools that will improve patient care.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact