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Vascular malformations and thrombosis

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Vascular malformations and thrombosis

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Editorial: Vascular malformations and thrombosis

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Editorial on the Research Topic
Vascular malformations and thrombosis

Thrombotic disorders appear as acute diseases of arterial or venous vessels and represent the leading cause of morbidity and mortality in western countries. Risk factors differ among arterial or venous thrombosis, including molecular risk factors and clinical predisposing diseases. Together with predisposing diseases, congenital or acquired vascular malformations may also be associated with the occurrence of thrombosis or bleeding. Usually, thrombotic events of vascular malformations may appear as symptomatic disease, while bleedings are frequent in the case of vascular malformations such as aneurysms or arterio-venous fistulae. Furthermore, vascular malformations may be found in small or large vessels. From a clinical point of view, the most common difficulty for the management of vascular malformation is the absence of guidelines or best clinical practice. For this reason, and because patients with rare vascular malformations are not included in general guidelines, clinical evidence and experience in treating thrombotic or hemorrhagic complications in these patients are scarce, and further data regarding pathophysiological mechanisms and/or clinical outcomes are encouraged.

In this Frontiers Research Topic, several case reports described the occurrence of thrombotic events or bleeding in carriers of rare vascular malformations (Oualiken et al.; Li et al.; Zhou et al.). Furthermore, when a vascular malformation is identified before complication, a preventive strategy should be evaluated (Puhr-Westerheide et al.), as reported in the case of associated pregnancy (Di Micco et al.) or in the case of malformation of the face (Schmidt et al.). In other situations, such as acquired thrombophilia due to the presence of antiphospholipid syndrome, the clinical presentation may differ because antiphospholipid syndrome is a prothrombotic molecular abnormality that may simultaneously affect arterial and venous vessels in the case of vascular malformation (Jacintho et al.). On the other hand, a definite prothrombotic abnormality that can state the risk of thrombosis or bleeding of vascular malformations has not identified. Prothrombotic endothelial abnormalities may characterize clotting abnormalities, with a trend toward thrombosis in other prothrombotic diseases such as COVID-19 or Beçhet disease

(Ma et al.; Thangaraju et al.). Individual lifestyle is also important, and the protective role of environmental food should be investigated in-depth in the next number of years, in particular, in selected cohorts of patients that seem to have a favorable genetic predisposition (Jia et al.).

Little is known concerning prophylaxis or therapeutic therapies regarding vascular malformations. Di Micco et al. reported successful prophylaxis using enoxaparin in a pregnant woman with jugular agenesis, while RIETE investigators described the clinical characteristics of patients treated with DOACs after a venous thromboembolism (VTE) event (Lorenzo et al.). Molecular abnormalities that may explain drug resistance in prothrombotic diseases as essential thrombocytopenia have been reported by Yang et al.. Furthermore, the role of prophylaxis in patients with vascular malformation has been tested, with positive results obtained, in particular, the quality of life in patients treated with sirolimus (Harbers et al.).

All the articles included in this Research Topic will help clinicians dealing with patients with rare vascular malformations, improving the management of these complex situations.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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

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The Impact of Age and BMI on the VWF/ADAMTS13 Axis and Simultaneous Thrombin and Plasmin Generation in Hospitalized COVID-19 Patients

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Aging and obesity independently contribute toward an endothelial dysfunction that results in an imbalanced VWF to ADAMTS13 ratio. In addition, plasma thrombin and plasmin generation are elevated and reduced, respectively, with increasing age and also with increasing body mass index (BMI). The severity risk of Corona Virus Disease 2019 (COVID-19) increases in adults older than 65 and in individuals with certain pre-existing health conditions, including obesity (>30 kg/m^2). The present cross-sectional study focused on an analysis of the VWF/ADAMTS13 axis, including measurements of von Willebrand factor (VWF) antigen (VWF:AG), VWF collagen binding activity (VWF:CBA), Factor VIII antigen, ADAMTS13 antigen, and ADAMTS13 activity, in addition to thrombin and plasmin generation potential, in a demographically diverse population of COVID-19 negative (−) (n = 288) and COVID-19 positive (+) (n = 543) patient plasmas collected at the time of hospital presentation. Data were analyzed as a whole, and then after dividing patients by age (<65 and ≥65) and independently by BMI [<18.5, 18.5–24.9, 25–29.9, >30 (kg/m^2)]. These analyses suggest that VWF parameters (i.e., the VWF/ADAMTS13 activity ratio) and thrombin and plasmin generation differed in COVID-19 (+), as compared to COVID-19 (−) patient plasma. Further, age (≥65) more than BMI contributed to aberrant plasma indicators of endothelial coagulopathy. Based on these findings, evaluating both the VWF/ADAMTS13 axis, along with thrombin and plasmin generation, could provide insight into the extent of endothelial dysfunction as well as the plasmatic imbalance in coagulation and fibrinolysis potential, particularly for at-risk patient populations.

Keywords: COVID-19, plasmin, thrombin, von Willebrand factor, ADAMTS13

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INTRODUCTION

Coagulopathy is a sequela of COVID-19 that associates with the severity of disease progression (1–4). Venous thrombosis and thromboembolism, as well as arterial thrombosis were reported at a relatively higher frequency in COVID-19 patients (3, 5). Microvascular coagulation and endotheliopathy are critical pathophysiological consequences of COVID-19 immune activation that contribute to death (6, 7). Microthrombi are most often observed in lung vessels at autopsy, particularly in peripheral lung venules, arterioles, and alveolar capillaries (6, 8). In survivors of severe disease, long term exertional impairments may persist due to microvascular thrombosis and consequent lung injury (8).

A focus on endothelial dysregulation has emerged based on evidence of increased von Willebrand factor (VWF) antigen (AG) levels (i.e., Ultra-large Von Willebrand Factor (ULVWF) multimers), increased VWF collagen type I and III binding activity (9), along with mild to moderately decreased ADAMTS13 AG and ADAMTS13 activity in severely ill patients (10–13). Physiologically, VWF and ADAMTS13 play important roles in the maintenance of hemostasis in the microvasculature (14). VWF is a large multimeric glycoprotein secreted as ultra-large pro-thrombotic forms into the vascular lumen, primarily from endothelial cells and platelets. Although endothelial cells show both basal and stimulated secretion, platelets release VWF only upon activation (15). Factor VIII circulates in plasma as a complex with VWF and facilitates site-specific cleavage of ULVWF multimers under shear stress (16). The release of Factor VIII from VWF occurs in the presence of thrombin leading to a 4-fold increase in its plasma clearance. ADAMTS13 is the enzyme that regulates VWF activity by digesting shear stress elongated pro-thrombotic ULVWF multimers (17). Under pathophysiological states, such as thrombotic thrombocytopenic purpura (TTP), a severe deficiency in availability or activity of ADAMTS13 (<10%) results in accumulation of pro-thrombotic VWF multimer forms leading to the formation of microvascular platelet-rich thrombi, thrombocytopenia, secondary micro-hemorrhages, and peripheral blood schistocytes (14, 18). In addition, thrombotic microangiopathies (TMA) are caused by many different pathologies, with endothelial injury being a common denominator. Interestingly, elevated VWF levels, accompanied by increased Factor VIII levels (18) as well as mildly decreased ADAMTS13 activity (∼ 50%) and normal antigen levels (∼ 1 U/ml), are observed in severe COVID-19 infection (19). However, a complete loss of ADAMTS13 activity (i.e., <10%), thrombocytopenia, schistocytes are not common in COVID-19 infection. Nonetheless, COVID-19 disease progression is consistent with endothelial dysfunction and increased plasma VWF levels and VWF:AG/ADAMTS13 activity ratios are associated with COVID-19 disease severity and reported to be a predictor of morbidity and mortality (10–13).

In addition to VWF/ADAMTS13 axis dysregulation, plasma predictors of thrombosis and fibrinolysis potential, such as thrombin and plasmin generation, respectively, have not been well-defined in COVID-19 patients. Similarly to VWF/ADAMTS13 axis parameter evaluation, assays that assess thrombin and plasmin could add relevant information on coagulation risk. Thrombin is the primary mediator of fibrinogen cleavage to fibrin, and thrombin generation is a useful measure of both increased and reduced coagulation potential when measured in plasma. Conversely, fibrinolysis is mediated by the proteolytic action of plasmin, which accumulates as a result of enzymatic cleavage of plasminogen by tissue plasminogen activator. Plasmin generation offers insight into the amount of available plasmin that could participate in fibrin clot lysis. Both measurements, when evaluated simultaneously, provide information on the potential for clot formation and the impairment of clot lysis, respectively. These assessments can be made prior to the onset, or during the processes of, coagulopathy, and offer relevant insight into thrombin and plasmin function in disease diagnosis, disease severity, and drug therapy assessments.

Independent of COVID-19, VWF:AG and the VWF:AG/ADAMTS13 activity ratio increase with aging (≥65 years of age) and body mass index (BMI; >25 kg/m^2) (20, 21). Additionally, an underlying endotheliopathy is observed with aging and increasing BMI, potentially due to accumulating co-morbidities and declining organ function (22, 23). Understanding the impact of COVID-19 on endothelial markers of coagulation and more broadly, on plasma thrombin and plasmin generation, at early disease presentation may offer better insights into anticoagulation needs and monitoring as well as assessing early disease severity.

The current observational study is unique in that we evaluated the VWF/ADAMTS13 axis, as well as a simultaneous thrombin and plasmin generation assay that informs on amounts of functional thrombin and plasmin in plasma. This study evaluated individual plasmas of two large groups of demographically diverse hospitalized patients in a large urban medical center, to overcome the limitations of previous studies of endothelial dysregulation in COVID-19, which included small numbers of patients, which were then compared to healthy individuals. In contrast, we grouped hospitalized patients based on COVID-19 (−) or COVID-19 (+) status and these groups were comprised of 288 and 543 patients, respectively. Data was further evaluated based on age (i.e., <65 or ≥ 65 years), BMI (i.e., <18.5, 18.5–24.9, 25–29.9, >30 (kg/m^2). Finally, these parameters analyzed in the present study were evaluated in surviving and non-surviving patients within the COVID-19 (−) and COVID-19 (+) groupings. The data generated were used in correlation and association analysis with age and metabolic parameters (24).

PATIENTS, MATERIALS, AND METHODS

Patients and Sample Collection

Patients

This study was approved by the Institutional Review Board of Columbia University Irving Medical Center (CUIMC) (Protocol Number AAAT0680). Data were obtained from patients who were either admitted to the hospital or seen in the Emergency Department from April 14, 2020 through May 31, 2020 (i.e., before the identification of and routine testing for novel variants in the USA), and were evaluated for SARS-CoV-2 by RT-PCR and/or serology. COVID-19 (−) patients were identified and

selected based on a negative SARS-CoV-2 RT-PCR test and/or serology testing in the ED or within the initial 72 h after admission. To our knowledge, patients included in the COVID-19 (−) had no reported history of COVID-19 infection.

Patient Comparisons

First, patients were divided into COVID-19 (−) ($n = 288$) and COVID-19 (+) ($n = 543$) groups based on a positive SARS-CoV-2 RT-PCR test or positive serology. VWF, ADAMTS13, Factor VIII, and thrombin and plasmin generation parameters were compared between the groups.

Second, within the COVID-19 (−) and COVID-19 (+) groups, patients were split based on age <65 or age ≥ 65. Within the COVID-19 (−) group, age-dependent splitting resulted in $n = 156$ (<65 years of age) and $n = 132$ (≥65 years of age); within the COVID-19 (+) group, there were $n = 278$ patients <65 years of age and $n = 265$ patients ≥65 years of age. Comparisons for VWF, ADAMTS13, Factor VIII, and thrombin and plasmin generation parameters were made between COVID-19 (+) and (−) patients within the <65 and in the ≥65 years of age groupings. Further, parameters were compared within the COVID-19 (−) patient group based on age <65 and ≥ 65; similar comparisons were made for COVID-19 (+) patients.

Third, within COVID-19 (−) and COVID-19 (+) groups, patients were split based on CDC guidelines into four BMI categories: <18.5, 18.5–24.9, 25–29.9, >30 (kg/m^2). Within the COVID-19 (−) group this led to the following BMI category distribution: <18.5 ($n = 14$), 18.5–24.9 ($n = 53$), 25–29.9 ($n = 47$), >30 ($n = 92$) (kg/m^2). The COVID-19 (+) group had the following BMI category distribution: <18.5 ($n = 15$), 18.5–24.9 ($n = 112$), 25–29.9 ($n = 98$), >30 ($n = 253$) (kg/m^2). Further, within the COVID-19 (−) and COVID-19 (+) groups, parameters were compared across BMI categorizations. All statistical analyses and graphing of data were performed using Graphpad Prism software (version 9.2.0). Data are presented as Group median values and interquartile range [25–75 percentile]. Data between COVID-19 (−) and COVID-19 (+) groups were compared using a non-parametric Mann-Whitney U test. Comparisons across several groups within BMI categorizations were analyzed with a non-parametric One-way-ANOVA with multiple comparisons using a Kruskal-Wallis test.

Sample Collection and Handling

All initial blood samples were collected within 72 h of admission in sodium citrate and analyzed for routine clinical laboratory values at CUIMC and processed to platelet poor plasma for research based assays (24). To maintain continuity and quality of specimens, samples arrived at the University of Maryland Baltimore under dry ice as a single shipment. Samples were analyzed in blocks ($n = 50$) to allow for a single freeze thaw followed by evaluation of enzymatic and activity assays. Plasma samples were then aliquoted into multiple tubes containing 100–200 ul and refrozen for antigen-based assays.

VWF, ADAMTS13 and FVIII Measurements

The antigen and activity measurement of VWF and ADAMTS13 was performed by using commercial ELISA kits. VWF:AG and collagen type III binding activity (VWF:CBA) levels were measured by using Human von Willebrand Factor ELISA Kit (ab168548, Abcam, Cambridge, UK) and TECHNOZYM® vWF:CBA ELISA Kit (5450301, Technoclone, Vienna, Austria) to measure the quantity of VWF and its binding to collagen type III (therefore, an increase in VWF binding indicates more circulating ultra-large molecular weight multimers), respectively. ADAMTS13 antigen and activity levels were measured by using Human ADAMTS13 ELISA Kit (ab234559, Abcam) and TECHNOZYM® ADAMTS13 Activity ELISA (5450701, Technoclone), respectively. FVIII antigen levels were measured by using Human Factor VIII total antigen assay ELISA kit (HFVIIIKT-TOT, Molecular Innovations, Novi, MI, USA). All assays were performed following manufacturer's recommendations with additional dilution of plasma samples as required.

Simultaneous Thrombin and Plasmin Generation Assay (STPGA)

Simultaneous measurement of thrombin and plasmin generation potential of plasma samples were performed with modifications to previous methods (25, 26). Briefly, plasma samples were mixed with 512 μM of either thrombin specific substrate, Z-Gly-Gly-Arg-AMC (Bachem, Bubendorf, Switzerland) or plasmin specific substrate, Boc-Glu-Lys-Lys-AMC (Bachem) and 16 nM of thrombomodulin (PeproTech, Rocky Hill, NJ, USA) similar to a previous method designed to measure thrombin and plasmin in parallel (26).

The reaction was initiated by adding an activator solution that yielded a final concentration of 1 pM tissue factor (Diagnostica Stago, Parsippany, NJ, USA), 0.7 μg/mL of tissue plasminogen activator (Sigma-Aldrich, St. Louis, MO, USA) and 16 mM $CaCl_2$. Sample wells were supplemented with buffer (150 mM NaCl, 20 mM HEPES and pH 7.5) and AMC fluorophore instead of activator solution for background and calibrator measurements respectively. Calculation of thrombin and plasmin concentration was performed as described previously (25).

Clinical Laboratory Data

Laboratory tests were performed based on clinical necessity and not as directed by this study; the resulting values were obtained by request from the patients' charts. Therefore, not all patients had all of the tests ordered. As part of routine care, hemostasis was evaluated on STAR Evolution and STAR Max analyzers (Diagnostica Stago, Parsippany, NJ), hematology testing by Sysmex XN900 (Lincolnshire, IL), and chemistry testing by Roche Cobas c502 (Indianapolis, IN). Laboratory values, including antithrombin (AT), prothrombin time (PT)/international normalized ratio (INR), activated partial thromboplastin time (aPTT), fibrinogen, D-dimer, white blood cell count (WBC), absolute neutrophil count (ANC), absolute lymphocyte count (ALC), absolute monocyte count (AMC), hemoglobin, red blood cell count (RBC), RBC distribution width (RDW), reticulocyte count, platelet count, IL-6, lactate dehydrogenase (LDH), lactic acid, procalcitonin, troponin, blood urea nitrogen (BUN), creatinine, glucose, bilirubin (total, direct, and indirect), aspartate amino transferase (27),

alanine amino transferase (ALT), albumin, total protein, ferritin, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), creatine kinase (CK), triglycerides, and blood type, were collected. Laboratory data were obtained from the Clinical Data Warehouse at CUIMC after approval from the Tripartite Request Assessment Committee. Samples were obtained in the Emergency Department, at admission, and throughout the hospital stay, and were analyzed by the CUIMC Clinical Laboratories; residual samples that were no longer required for clinical purposes, were retrieved from the CUIMC Clinical Laboratories and banked for research studies. Clinical and demographic data, including name, medical record number (MRN), sex, date of birth, age, race, ethnicity, weight, body mass index, comorbidities (hypertension, diabetes mellitus, coronary artery disease, renal disease, hyperlipidemia, liver disease, lung disease), intubation/ventilator requirement, continuous veno-venous hemofiltration (CVVH) requirement, radiographically-confirmed thrombotic complications (deep vein thrombosis, pulmonary embolism, stroke), clotting of CVVH, hospitalization course (admission date, date of Emergency Department presentation, discharge date), mortality, and date of death were collected manually by reviewing the electronic medical record.

TABLE 1 | Patient demographics and clinical characteristics.

Patient characteristics	COVID-19 (−) ($n = 288$)	COVID-19 (+) ($n = 543$)
Age, median (range)	62 (1.0–101)	63 (3.0–99)
Sex		
Female	$n = 119$ (43 %)	$n = 203$ (44 %)
Male	$n = 159$ (57 %)	$n = 264$ (56 %)
Race/Ethnicity		
Asian	$n = 4$	$n = 3$
African	$n = 56$	$n = 83$
American/Black	$n = 56$	$n = 49$
Caucasian/White	$n = 13$	$n = 30$
Other	$n = 3$	$n = 1$
Multi–racial	$n = 104$ (Black:7,	$n = 211$ (Black:10,
Hispanic/Latino	White:21, other:18,	White: 35, other:32, Asian: 1, American Ind/Alaskan:1, Multiracial:1)
Declined	$n = 52$	$n = 166$
Body mass index (kg/m^2), median (range)	25.5 (13.7–53.2)	28.0 (14.1–63)
Ventilator	$n = 31$ (11 %)	$n = 67$ (12 %)
New thrombosis		
New–DVT/PE	$n = 22$ (8.0 %)	$n = 28$ (5.0 %)
New– Stroke	$n = 7$ (2.4 %)	$n = 21$ (4.0 %)
Chronic conditions*		
HTN	$n = 134$ (47 %)	$n = 262$ (48%)
DM	$n = 81$ (28 %)	$n = 184$ (34 %)
CAD	$n = 48$ (17 %)	$n = 54$ (10 %)
ESRD/CKD	$n = 35$ (13 %)	$n = 69$ (13 %)
Cancer	$n = 22$ (8.0 %)	$n = 38$ (7.0%)
Stroke	$n = 21$ (7.0 %)	$n = 32$ (6.0 %)
Hyperlipidemia	$n = 34$ (12 %)	$n = 94$ (17 %)
Heart Failure	$n = 39$ (14 %)	$n = 23$ (4.0 %)
Liver Disease	$n = 13$ (5.0 %)	$n = 11$ (2.0 %)
Lung Disease	$n = 50$ (17 %)	$n = 41$ (8.0 %)
Survivors	$n = 255$ (88.5 %)	$n = 433$ (80 %)
Non–survivors	$n = 33$ (11.5 %)	$n = 110$ (20 %)

**History of chronic conditions: HTN, Hypertension, DM, Diabetes Mellitus; CAD, Coronary heart disease; ESRD/CKD, End-stage renal failure/chronic kidney disease. The percentage of patients per group for binary variables are indicated.*

RESULTS

General and Clinical Characteristics of Study Subjects

Patient demographic data are shown in **Table 1**. Briefly, COVID-19 (−) and COVID-19 (+) groups were similarly split across age, sex and racial/ethnic background. COVID-19 (−) and COVID-19 (+) patients presented with a similar prevalence of chronic conditions (hypertension, diabetes mellitus, chronic kidney disease) and both COVID-19 (−) and COVID-19 (+) patients demonstrated high median BMIs. The COVID-19 (+) patient median values for pro-inflammatory markers (C-reactive protein, ferritin, fibrinogen, and IL-6) were all increased by 1.5–2.0-fold greater than that observed in COVID-19 (−) patients. Inflammatory markers tracked with increased D-dimer levels. All clinical laboratory data that were obtained by request from patient charts are shown in **Supplementary Table 1**. An illness severity scoring system was not applied to patients included in this study. Nonetheless, comparisons between COVID-19 (−) and COVID (+) patients suggest a greater state of inflammation in COVID-19 (+) patients based on increased CRP (570% increase, $p < 0.00010$), IL-6 (179% increase, $p < 0.014$), ferritin (287% increase, $p < 0.00010$), fibrinogen (130%, $p < 0.00010$), and erythrocyte sedimentation rate (157%, $p < 0.00010$).

VWF/ADAMTS13 Axis Changes in Acutely Ill COVID-19 (−) and COVID-19 (+) Patients

Increased VWF:AG and activity were observed in both COVID-19 (+) and COVID-19 (−) patients (**Figures 1A,B**, VWF:AG reference range: ∼ 0.5–2.0 U/mL). However, COVID-19 (+) patients demonstrated significantly higher VWF:AG and CBA levels compared to COVID-19 (−) patients ($p < 0.0001$). Respective median antigen and activity levels of VWF in the COVID-19 (+) group were 2.736 (IQR:1.822–4.060) and 3.745 (IQR:2.506–5.262) U/mL compared to 1.868 (IQR:1.257–2.770) and 2.989 (IQR:1.958–4.252) U/mL in the COVID-19 (−) group. A similar elevation of FVIII was observed in both COVID-19 (+) (Median:1.769 and IQR:1.031–3.366 U/mL) and COVID-19 (−) (Median:1.79 and IQR:0.898–3.283 U/mL) patients (**Figure 1C**, FVIII reference range: ∼0.5–1.5 U/mL) with no significant differences between the groups. ADAMTS13 activity levels on the other hand were found to be lower in both COVID-19 (+) and COVID (−) patient groups when compared to the normal reference range (**Figures 1D,E**, normal ADAMTS13 activity levels: ≥ 0.5 U/mL). Specifically,

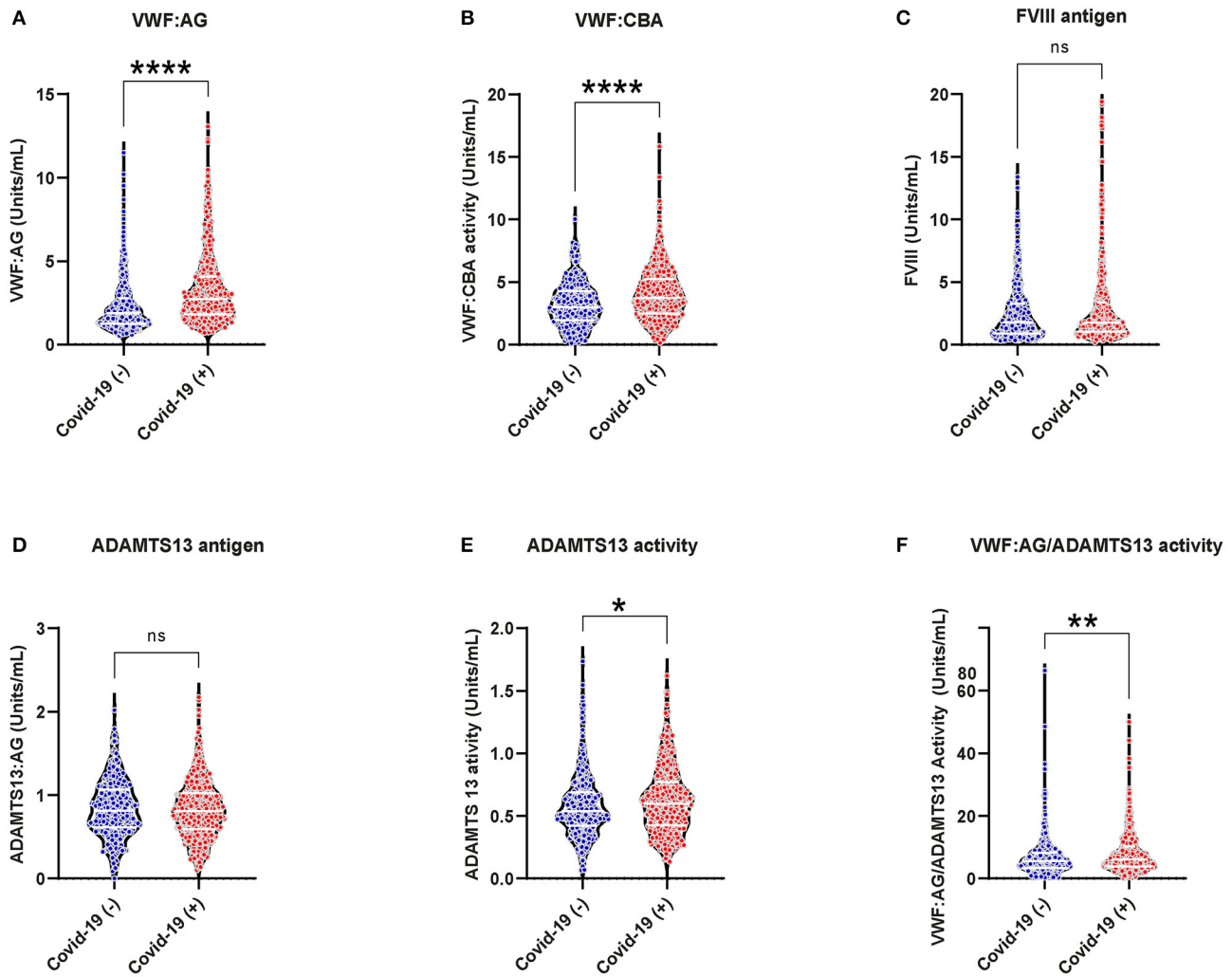

FIGURE 1 | VWF/ADAMTS13 axis changes and coagulation in acutely ill COVID-19 (–) and (+) patients: **(A)** VWF antigen, 1.868 (IQR, 1.257–2.770) (–), 2.736 (IQR, 1.822–4.060) (+), $p < 0.0001$; **(B)** VWF collagen binding activity (9) 2.989 (IQR, 1.958–4.252) (–), 3.745 (IQR, 2.506–5.262) (+), $p < 0.0001$; **(C)** FVIII antigen 1.79 (IQR, 0.898–3.283) (–), 1.769 (IQR, 1.031–3.366) (+), $p = 0.4154$; **(D)** ADAMTS13 antigen 0.806 (IQR, 0.592–1.023) (–), 0.813 (IQR, 0.614–1.069) (+), $p = 0.539$; **(E)** ADAMTS13 activity 0.540 (IQR, 0.420–0.689) (–), 0.597 (IQR, 0.427–0.767) (+), $p = 0.027$; **(F)** VWF:AG/ADAMTS13 activity 5.567 (IQR, 3.352–8.245) (–), 6.051 (IQR, 3.824–10.17) (+), $p < 0.0044$. Datapoints indicate individual measurements, and p-values are from the Mann-Whitney analysis for comparison within groups. Values are presented as median and interquartile range (IQR, 25th–75th percentile) for continuous variables. ns, $P > 0.05$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$.

ADAMTS13 activities in both groups were minimally decreased, but not lower than normal reference activity (50–160%). Respective median ADAMTS13 antigen and activity levels were 0.806 (IQR:0.592–1.023) and 0.597 (IQR:0.427–0.767) U/mL in COVID-19 (+) and 0.813 (IQR:0.614–1.069) and 0.54 (IQR:0.420–0.689) U/mL in COVID-19 (−) patients. The difference in ADAMTS13 activity levels between COVID-19 (+) and COVID-19 (−) patients was minimal, but statistically significant ($p = 0.027$). Subsequently, the VWF:AG/ADAMTS13 activity ratios in COVID-19 (+) patients (Median:6.051 and IQR:3.824–10.17) were significantly higher ($p < 0.0001$) than COVID-19 (−) patients (Median:5.567 and IQR:3.352–8.245) (**Figure 1F**). The data suggests that increased VWF:AG levels and VWF:CBA in plasmas of COVID-19 (+) patients occurred despite normal ADAMTS13 function. However, unlike in TTP the present data did not reveal thrombocytopenia in conjunction with increased VWF:AG levels and CBA in COVID-19 (+) patients (**Supplementary Figure 1**).

Plasma Coagulation in Acutely Ill COVID-19 (–) and COVID-19 (+) Patients

Thrombin generation increased, while plasmin generation decreased in the plasmas of COVID-19 (+) compared to COVID-19 (−) patients. An increased thrombin peak height and generation rate was observed with a simultaneously decreased

plasmin peak height and generation rate in COVID-19 (+) patients (**Figure 2**). The median peak heights and thrombin generation rates in COVID-19 (+) patients were significantly increased by 25% [230.0 (IQR:123.0–326.2) nM] and 21% [40.38 (IQR:20.39–67.33) nM/min], respectively, compared to COVID-19 (−) patients ($p < 0.01$), (**Figures 2A,B**). The area under curve (AUC) values, however, were similar between COVID-19 (+) (2,957 nM/min) and COVID-19 (−) (2,902 nM/min) patients (**Figure 2C**). Representative thrombin generation curves from COVID-19 (+) and COVID-19 (−) patient plasmas are shown in **Figure 2D**. Plasmin peak height and generation rate were decreased by 9 and 18%, respectively, in COVID-19 (+) compared to COVID-19 (−) patients ($p < 0.0001$, **Figures 2E,F**). The median peak height and plasmin generation rate in COVID-19 (+) patients were 535.2 (IQR: 458.5–624.3) nM and 20.97 (IQR: 15.31–28.57) nM/min compared to 585.5 (IQR: 497.5–665.5) nM and 25.2 (IQR: 19.26–33.69) nM/min in COVID-19 (−) patients. Relative to healthy donor PPP, run under the same conditions (25), the median plasmin generation rates in COVID-19 (+) patients were ∼40% lower. The AUC values were also significantly lower ($p = 0.0002$) in COVID-19 (+) patients (11,783 nM/min) compared to COVID-19 (−) patients (12,239 nM/min) (**Figure 2G**). Representative plasmin generation curves from COVID-19 (+) and COVID-19 (−) patient plasmas are shown in **Figure 2H**. These data demonstrate an increase in thrombin generation, suggesting a higher risk for thrombosis in COVID-19 (+) patients. Further, the observation of lower plasmin generation rates suggests an impaired fibrinolytic system in COVID-19 (+) patients. A similar distribution of platelet counts was observed in both COVID-19 (+) and COVID-19 (−) patients (**Supplemental Figure 1**).

Age Dependent Differences in VWF/ADAMTS13 Axis and Plasma Coagulation Parameters

Increasing age is a contributing factor to illness severity and death from COVID-19 infection. The differences in VWF, ADAMTS13, thrombin generation, and plasmin generation parameters were evaluated in plasmas from COVID-19 (+) and COVID-19 (−) patients that were <65 and ≥65 years of age (**Table 2**).

In patients <65 years of age, significant increases in median VWF:AG and VWF:CBA levels in COVID-19 (+) patients were observed. Specifically, the median VWF:AG, and VWF:CBA levels in the COVID-19 (+) group were increased by 28.8% and 17%, respectively, compared to the COVID-19 (−) group ($p < 0.0001$; $p = 0.002$) (**Table 2**). The increase in VWF levels and binding activity are consistent with endothelial dysfunction in patients <65 years of age. Despite the changes in VWF, no changes in VWF/ADAMTS13 activity were observed. Among patients <65 years of age, median plasmin generation rates reached statistical significance ($p < 0.05$). The median plasmin generation rates in COVID-19 (+) patients decreased by 9% compared to COVID-19 (−) patients ($p = 0.041$). Despite the changes in plasmin generation, no differences in thrombin generation were observed.

Among patients ≥65 years of age, no significant differences in ADAMTS13, ADAMTS13 activity or FVIII levels were observed between COVID-19 (+) or COVID-19 (−) groupings. On the other hand, significantly elevated VWF:AG, VWF:CBA and VWF:AG/ADAMTS13 activity ratios were observed in COVID-19 (+) patients (**Table 2**). Specifically, in the COVID-19 (+) group, median VWF:AG, VWF: CBA and VWF:AG/ADAMTS13 activity ratios increased (by 43, 23, and 21.5%, respectively) compared to the COVID-19 (−) group (**Table 2**). Among thrombin and plasmin parameters, elevated coagulation and decreased fibrinolysis was observed in COVID-19 (+) patients. Within this group, median thrombin peak heights and generation rates increased by 32.6% ($p = 0.0007$) and 21% ($p = 0.02$), respectively. Conversely, median plasmin peak heights and generation rates decreased by 11% ($p = 0.0006$) and 26% ($p < 0.0001$), respectively, compared to COVID-19 (−) patients (**Table 2**). Comparisons between the two age groups within the COVID-19 (+) patients (**Table 2**) indicates that patients ≥65 years of age have a reduced plasma ADAMTS13 activity (remaining in the reference range), as well as increased VWF:AG, VWF:CBA, and VWF/ADAMTS13 activity ratio. Further, thrombin and plasmin generation parameters were increased and decreased, respectively. Comparisons between < 65 and ≥ 65-year-old individuals are also provided for the COVID-19 (−) patient group (**Table 2**). A similar distribution of platelet count was observed in both COVID-19 (+) and COVID-19 (−) across patients grouped as <65 and ≥65 years of age (**Supplementary Figure 1**).

This data indicates that a main difference between younger and older COVID-19 (+) patients evaluated in the present study was increased VWF:AG levels and activities in older patients. More importantly the age of COVID-19 (+) patients defined a risk factor for promoting hemostasis and impairing fibrinolysis based on enhanced thrombin generation and impaired plasmin generation, respectively.

BMI Dependent Differences in VWF/ADAMTS13 Axis and Plasma Coagulation Parameters

A BMI greater than normal (> 25 kg/m^2) represents an important risk for COVID-19 illness severity. To assess the effect of BMI on VWF/ADAMTS13 axis changes, we grouped patients based on CDC guidelines into four BMI categories: <18.5, 18.5–24.9, 25–29.9, >30 (kg/m^2). Within underweight and normal healthy BMI grouping, VWF:AG, VWF: CBA, ADAMTS13 antigen, and ADAMTS13 activity levels did not differ based on COVID-19 (−) or COVID-19 (+) status. VWF:AG, VWF:CBA and VWF:AG/ADAMTS13 activity were significantly increased in COVID-19 (+) patients within the overweight (25–29.9 kg/m^2) and obese (>30 kg/m^2) BMI groupings (**Table 3**). However, ADAMTS13 levels and activities were unchanged within the overweight (25–29.9 kg/m^2) and obese (>30 kg/m^2) BMI groupings regardless of COVID-19 status (**Table 3**). Plasma coagulation and fibrinolysis parameters measured by simultaneous thrombin and plasmin generation showed a significant inhibition of fibrinolysis in the plasmas

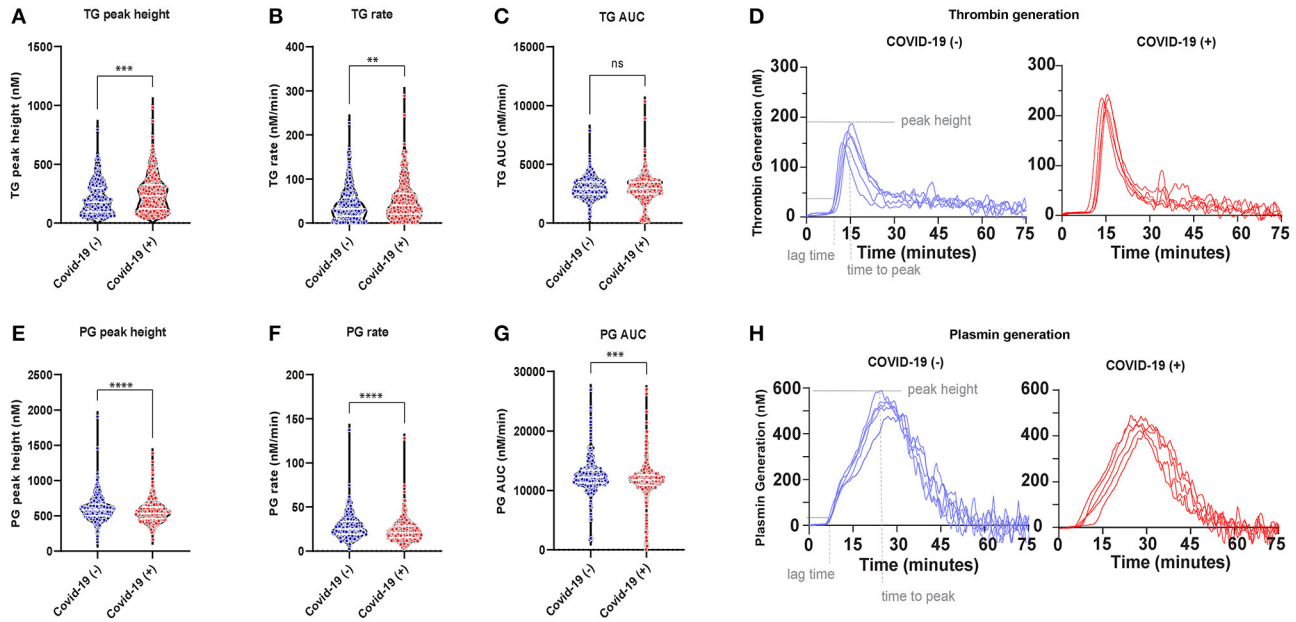

FIGURE 2 | Plasma coagulation in acutely ill COVID-19 (−) and (+) patients: **(A)** Thrombin generation (TG) peak height 177.5 (IQR, 94.24–297.6) (−), 230.0 (IQR, 23.0–326.2) (+), $p < 0.0005$; **(B)** TG rate 32.66 (IQR, 15.78–57.69) (−), 40.38 (IQR, 20.39–67.33) (+), $p = 0.0063$; **(C)** TG AUC 2902 (IQR, 2442–3503) (−), 2,957 (IQR, 2,486–3,502) (+), $p = 0.9187$; **(D)** Representative TG curves from patient plasma; **(E)** Plasmin generation (PG) peak height 585.5 (IQR, 497.5–665.5) (−), 535.2 (IQR, 458.5–624.3) (+), $p < 0.0001$; **(F)** PG rate 25.20 (IQR, 19.26–33.69) (−), 20.97 (IQR, 15.31–28.57) (+), $p < 0.0001$; **(G)** PG AUC 12,239 (IQR, 11,277–13,369), 11,783 (IQR, 10,884–26,994), $p = 0.0002$; **(H)** Representative PG curves from patient plasma. Data points indicate individual measurements, and p-value comes from the Mann-Whitney analysis for comparison within groups. Values are presented as median and interquartile range (IQR, 25th–75th percentile) for continuous variables. TG, thrombin generation; PG, Plasmin generation. ns, $P > 0.05$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$.

of obese (>30 kg/m^2, BMI) COVID-19 (+) patients. Median plasmin generation rates decreased by ∼25% in the plasma of COVID-19 (+) obese patients. Comparisons between BMI categorization in the COVID-19 (+) group demonstrated no significant differences in assayed parameters from plasmas collected at hospital presentation or admission. A similar distribution of platelet counts was observed in both COVID-19 (+) and COVID-19 (−) across patient BMI groupings (**Supplementary Figure 1**).

VWF/ADAMTS13 Axis Changes and Plasma Coagulation Parameters in Survivors and Non-survivors

The VWF/ADAMTS13 axis as well as plasma hemostasis and fibrinolysis were compared within the COVID-19 (−) and COVID-19 (+) groups to understand the differences in VWF/ADAMTS13 axis and plasma coagulopathy between surviving and non-surviving patients (**Table 4**). At the time of the initial blood draw, hospitalized COVID-19 (−) patients who ultimately did not survive their illness demonstrated significantly ($p < 0.05$) higher VWF levels and collagen binding activity as well as higher FVIII levels compared to COVID-19 (−) patients who survived their illness. The same parameters were also significantly ($p < 0.05$) increased in non-surviving COVID-19 (+) patients; however, survival was increased by 3.5-fold ($p < 0.0001$) in the COVID-19 (+) group compared to the COVID-19 (−) group.

DISCUSSION

COVID-19 infected patients are at greater risk for venous and arterial thrombosis, particularly once the severity of disease requires intensive care (5, 28–30). Several studies identify important links between metabolic and protein changes that indicate up-regulated coagulation linked to inflammation and complement and offer unique insight into the relevant changes in COVID-19 coagulation omics (31–33). However, to our knowledge, no study has specifically focused on VWF/ADAMTS13 axis changes of coagulation combined with thrombin and plasmin generation in COVID-19 (−) or COVID-19 (+) patient cohorts at the time of hospital presentation and admission. Further, the present analysis focuses on plasma coagulation parameters in these two cohorts and then, more specifically, based on aging or BMI categorization and finally on changes in the VWF/ADAMTS13 axis and plasma coagulation in survival. Here we evaluate VWF/ADAMTS13 axis changes that suggest an early endothelial-based coagulopathy along with imbalanced plasma thrombin and plasmin generation.

Microvascular thrombosis caused by endothelial dysregulation is tied to immune activation and is an important pathophysiological response to COVID-19 infection (34). A review of autopsy findings identified that ∼60% of deceased COVID-19 patients evaluated demonstrate microvascular thrombosis (35). Microthrombi are primarily observed in the lungs (∼75% of cases), but also in the kidneys, liver,

TABLE 2 | VWF, ADAMTS13, thrombin generation, and plasmin generation characteristics by age grouping.

Parameters	< 65 years			≥ 65 years			< 65 vs. ≥ 65 years	< 65 vs. ≥ 65 years
	COVID-19 (–)	COVID-19 (+)	*P*-value	COVID-19 (–)	COVID-19 (+)	*P*-value	COVID-19 (–) *P*-value	COVID-19 (+) *P*-value
ADAMTS13 Antigen (U/mL)	0.8805 (IQR, 0.6203–1.121)	0.8340 (IQR, 0.5950–1.084)	0.44	0.78 (IQR, 0.60–1.01)	0.7760 (IQR, 0.6033–1.013)	0.87	0.064	0.21
ADAMTS13 Activity (U/mL)	0.56 (IQR, 0.4483–0.7310)	0.6405 (IQR, 0.4743–0.8293)	0.0080	0.5085 (IQR, 0.3835–0.6673)	0.5460 (IQR, 0.4008–0.7203)	0.31	0.048	<0.00010
VWF: AG (U/mL)	1.690 (IQR, 1.236–2.395)	2.259 (IQR, 1.621–3.296)	<0.00010	2.019 (IQR, 1.303–3.119)	3.128 (IQR, 2.118–4.630)	<0.00010	0.050	<0.00010
VWF: CBA (U/mL)	2.875 (IQR, 1.591–4.115)	3.433 (IQR, 2.235–4.576)	0.0020	3.281 (IQR, 2.028–4.631)	4.147 (IQR, 2.742–5.597)	0.00010	0.021	<0.00010
VWF:AG/ADAMTS13 activity	4.827 (IQR, 2.857–7.709)	4.984 (IQR, 3.316–7.988)	0.27	5.923 (IQR, 4.108–8.843)	7.349 (IQR, 4.509–12.12)	0.0075	0.0065	<0.00010
FVIII (U/mL)	1.48 (0.77–3.32)	1.61 (0.84–2.88)	0.716	2.34 (1.30–3.87)	1.87 (1.17–3.61)	0.275	0.0021	0.0016
TG Peak Height (nM)	167.1 (IQR, 87.56–292.0)	211.4 (IQR, 101.1–306.0)	0.055	178.0 (IQR, 103.1–292.0)	247.4 IQR, (144.7–340.1)	0.00070	0.34	0.016
TG Rate (nM/min)	29.06 (IQR, 14.37–55.25)	35.63 (IQR, 17.15–63.08)	0.070	36.22 (IQR, 16.60–56.54)	44.76 (IQR, 21.42–69.19)	0.020	0.24	0.087
PG Peak Height (nM)	570.8 (IQR, 492.6–679.2)	562.6 (IQR, 464.8–643.6)	0.18	581.0 (IQR, 487.9–638.5)	519.8 (IQR, 446.6–602.2)	0.00060	0.75	0.0067
PG Rate (nM/min)	24.59 (IQR, 18.84–33.37)	22.29 (IQR, 16.18–29.65)	0.041	25.11 (IQR, 18.67– 32.48)	19.25 (IQR, 14.78– 25.99)	<0.00010	0.81	0.015

AG, antigen; CBA, Collagen binding activity.

and heart (35). Within lung tissue, histopathology and immunohistochemistry analyses provide evidence of widespread primary pathology across alveolar sites and the peripheral lung vasculature, including pre- and post-capillary pulmonary vessels (34, 36). The microthrombi described in small pulmonary arteries and veins demonstrate immunoreactivity for platelets and megakaryocytes (i.e., CD61), fibrin, VWF, and lymphocytes (i.e., CD4, CD8) (36). Interestingly, the localized pulmonary coagulopathy in COVID-19 pneumonia is more pronounced than that in influenza or bacterial pneumonia, and demonstrates an upregulated gene signature consistent with hypoxia-induced intussusceptive "splitting" angiogenesis (34). Platelet- and VWF-rich thrombi demonstrate greater resistance to thrombolytic therapies (37, 38) suggesting that treatment options are limited after established microvascular thrombosis in severe COVID-19 infection.

These observations of increased microvascular thrombosis caused by endothelial dysregulation influenced studies on the contributions of VWF and ADAMTS13 across a range of pro-thrombotic processes and COVID-19 disease severities (9, 39, 40). VWF is an acute-phase reactant and its secretion from endothelial cells increases in response to various stimuli, including shear stress and inflammation (41). During the inflammatory activation associated with COVID-19, the vascular imbalance of VWF and ADAMTS13 favors an elevated VWF:AG/ADAMTS13 activity ratio; this shift is implicated in localized endothelial dysfunction of COVID-19 infection (10–13). A close relationship with the VWF/ADAMTS13 axis and hospitalized COVID-19 (+) patients disease severity (low, intermediate, and high) is identified (11). This study also reports on VWF multimer accumulation in the plasmas of COVID-19 (+) patients suggesting a relationship between endothelial coagulation and COVID-19 disease severity. Two additional studies specifically identify the upper limits of VWF:AG levels (4.23-fold greater than normal) (12) and collagen binding activity (4.46-fold greater than normal) as predictors of mortality (13).

Our observations suggest that VWF:AG is increased in the plasma of both COVID-19 (−) and COVID-19 (+) patients at hospital presentation and admission. However, VWF:AG levels exceed the reference range (0.5–2 U/mL) and VWF collagen binding activity is significantly increased in COVID-19 (+) patients. Factor VIII levels were not found to be changed at the time of hospital presentation in the COVID-19 (+) patient plasmas analyzed in this study. Despite elevated VWF function in COVID-19 (+) patients' plasma, only mild changes in ADAMTS13 levels or activity are observed. Our rationale to measure ADAMTS13 levels was based on reports of ADAMTS13 antigen and activity decreases in other infections, including bacterial sepsis (42), and in viral infection-induced secondary TTP due to ADAMTS13 specific IgG inhibitor production (43). Nonetheless, the ratio of VWF:AG to ADAMTS13 activity does increase because of the higher VWF:AG levels. These observations differ from those observed in diseases of endothelial micro-thrombotic origin. For example, TTP is characterized by loss of ADAMTS13 function, thrombocytopenia, and schistocytosis (44). In the present study, COVID-19 (+) patient

TABLE 3 | VWF, ADAMTS13, thrombin generation, and plasmin generation characteristics by BMI groupings.

Parameters	BMI <18.5			BMI 18.5–24.9			BMI 25–29.9			BMI >30		
	COVID-19 (–)	COVID-19 (+)	*P*-value	COVID-19 (–)	COVID-19 (+)	*P*-value	COVID-19 (–)	COVID-19 (+)	*P*-value	COVID-19 (–)	COVID-19 (+)	*P*-value
ADAMTS13 Antigen (U/mL)	0.6540 (IQR, 0.5328–0.9453)	0.8530 (IQR, 0.4920–1.479)	0.43	0.7660 (IQR, 0.5750–0.9800)	0.7350 (IQR, 0.5030–0.9335)	0.30	0.9330 (IQR, 0.6360–1.148)	0.7950 (IQR,0.5883–1.002)	0.11	0.8510 (IQR, 0.6425–1.125)	0.8210 (IQR, 0.5660–1.027)	0.16
ADAMTS13 Activity (U/mL)	0.4785 (IQR, 0.4195–0.5963)	0.7580 (IQR, 0.5818–1.006)	0.054	0.5030 (IQR, 0.3950–0.6000)	0.6000 (IQR, 0.4298–0.7278)	0.032	0.6100 (IQR, 0.5090–0.7940)	0.6070 (IQR, 0.4190–0.7860)	0.27	0.5600 (IQR, 0.4250–0.7955)	0.5645 (IQR, 0.4030–0.7683)	0.40
VWF: AG (U/mL)	2.073 (IQR, 2.587–1.319)	3.443 (IQR, 4.877–1.819)	0.60	2.069 (IQR, 3.066–1.389)	2.725 (IQR, 4.414–1.843)	0.0029	1.661 (IQR, 2.944–1.252)	3.012 (IQR, 3.888–2.157)	<0.00010	1.855 (IQR, 2.943–1.268)	3.023 (IQR, 4.337–2.186)	<0.00010
VWF: CBA (U/mL)	2.821 (IQR, 2.501–4.313)	3.445 (IQR, 1.541–5.838)	0.60	3.355 (IQR, 4.378–2.092)	3.519 (IQR, 5.074–2.423)	0.17	2.945 (IQR, 4.161–2.097)	4.182 (IQR, 5.151–3.277)	0.00020	3.044 (IQR, 4.228–2.137)	4.292 (IQR, 5.688–2.884)	<0.00010
VWF:AG/ADAMTS13 activity	6.120 (IQR, 7.799–4.586)	3.315 (IQR−8.694–2.592)	0.18	6.138 (IQR, 9.228–3.680)	5.815 (IQR, 9.805–4.258)	0.79	4.752 (IQR, 6.759–3.386)	7.328 (IQR, 10.06–4.954)	<0.00010	5.131 (IQR, 7.025–3.172)	7.157 (IQR, 12.83–4.223)	0.0015
FVIII (U/mL)	3.444 (IQR, 2.025–5.537)	1.192 (IQR, 0.7350–4.455)	0.0674	2.172 (IQR, 1.262–4.063)	1.862 (IQR, 1.192–3.599	0.7619	1.927 (IQR, 0.7975–3.333	1.754 (IQR, 1.087–3.203	0.9008	1.829 (IQR, 0.7015–3.739	1.717 (IQR, 0.9930–3.231	0.7590
TG Peak Height (nM)	232.2 (IQR, 68.22–339.2	213.7 (IQR, 63.73–470.4	0.76	193.4 (IQR, 88.40–287.9)	226.9 (IQR, 127.3) 297.8	0.14	177.9 (IQR, 122.0–339.6	246.9 (IQR, 101.1–337.4	0.52	202.6 (IQR, 111.0–323.5	255.4 (IQR, 153.4–354.8	0.071
TG Rate (nM/min)	36.73 (IQR, 14.66–66.79)	46.58 (IQR, 12.75–107.5)	0.56	33.15 (IQR, 14.11–54.96)	37.48 (IQR, 18.50–59.06)	0.36	35.22 (IQR, 18.19–66.09)	46.16 (IQR, 17.30–71.33)	0.61	38.11 (IQR, 18.23–66.03	45.99 (IQR, 25.04–78.96	0.097
PG Peak Height (nM)	612.2 (IQR, 479.1–877.0)	505.0 (IQR, 459.8–624.6)	0.21	578.7(IQR, 498.6–636.6)	520.8 (IQR, 426.2–604.6)	0.013	593.2 (IQR, 504.8–641.9)	545.0 (IQR, 487.7–647.7)	0.40	585.0 (IQR, 527.7–682.0)	537.0 (IQR, 458.7–638.1)	0.026
PG Rate (nM/min)	32.48 (IQR, 19.32–42.19)	24.24 (IQR, 15.06–30.71)	0.12	22.75 (IQR, 16.68–28.41)	19.68 (IQR, 14.27–27.63)	0.11	23.87 (IQR, 18.15–30.68)	22.65 (IQR, 17.64–28.63)	0.31	26.36 (IQR, 18.49–32.43)	20.77 (IQR, 14.88–26.62)	0.0058

AG, antigen; CBA, Collagen binding activity.

TABLE 4 | VWF, ADAMTS13, thrombin generation, and plasmin generation characteristics by survivors and non–survivors in COVID-19 positive and negative groups.

Parameters	COVID-19 (–)			COVID-19 (+)		
	Survivors	Non–survivors	*P*-value	Survivors	Non–survivors	*P*-value
ADAMTS13 Antigen (U/mL)	0.8320 (IQR, 0.6285–1.081)	0.6670 (IQR, 0.4230–1.019)	0.067	0.8140 (IQR, 0.5688–1.014)	0.6990 (IQR, 0.5310–0.8870)	0.013
ADAMTS13 Activity (U/mL)	0.5560 (IQR, 0.4445–0.7070)	0.4000 (IQR, 0.3010–0.5720)	0.00020	0.5930 (IQR, 0.4150–0.7620)	0.4870 (IQR, 0.3563–0.6778)	0.0095
VWF: AG (U/mL)	2.937 (IQR, 1.920–4.191)	3.972 (IQR, 2.409–5.012)	0.0010	2.561 (IQR, 1.782–3.877)	3.327 (IQR, 2.366–5.838)	<0.00010
VWF: CBA (U/mL)	1.681 (IQR, 1.241–2.554)	2.268 (IQR, 1.799–4.649)	0.026	3.640 (IQR, 2.523–5.108)	4.506 (IQR, 3.286–5.900)	0.00040
VWF:AG/ADAMTS13 activity	5.185 (IQR, 3.212–7.886)	7.558 (IQR, 5.027–13.73)	0.00050	6.275 (IQR, 3.817–10.08)	8.105 (IQR, 5.043–13.44)	0.00010
FVIII (U/mL)	1.711 (IQR, 0.8755–3.392	2.740 (IQR, 2.028–4.888)	0.0101	1.678 (IQR, 0.9620–2.949)	2.706 (IQR, 1.561–5.594)	<0.0001
TG Peak Height (nM)	174.8 (IQR, 96.79–295.3)	176.9 (IQR, 83.24–252.7)	0.52	227.2 (IQR, 118.5–315.2)	227.7 (IQR, 119.5–334.8)	0.81
TG Rate (nM/min)	31.14 (IQR, 16.01–57.06)	39.31 (IQR, 10.26–56.15)	0.97	40.26 (IQR, 20.24–69.04)	39.55 (IQR, 19.63–64.72)	0.72
PG Peak Height (nM)	575.7 (IQR, 495.2–656.1)	568.0 (IQR, 443.3–546.4)	0.26	537.9 (IQR, 458.1–624.3)	506.1 (IQR, 406.5–576.9)	0.0064
PG Rate (nM/min)	24.78 (IQR, 19.30–33.50)	23.92 (IQR, 13.07–31.78)	0.12	21.53 (IQR, 14.78–28.85)	17.99 (IQR, 13.34–24.15)	0.0040

AG, antigen; CBA, Collagen binding activity.

plasma showed normal ADAMTS13 functional activity (≥50%) and normal platelet levels (∼250 × 10^9/L), consistent with prior studies of COVID-19 disease progression and severity (10, 13). Although not widespread across the spectrum of COVID-19-induced coagulopathy, some reports include case descriptions of TTP during ongoing infection; that is, microangiopathic hemolytic anemia with schistocytes and thrombocytopenia (19, 45).

COVID-19 disease outcome is widely reported to be affected by age and underlying comorbidities. For example, patients of increasing age and, independently, of increasing BMI are reported to be at greater risk for thrombosis based on underlying systemic organ functional decline and the likelihood of comorbidities (46, 47). In addition, comorbid states consistent with increasing age and increased BMI track with COVID-19 disease progression (48–50). Specifically, the median age in the present study was 62 and 63 years of age in the COVID-19 (−) and COVID-19 (+) cohorts, respectively, and, within the two groups, the patients were almost equally split between individuals younger and older than 65. Based on our current data with COVID-19 (−) and COVID-19 (+) patients, there was a clear age-dependent effect (i.e., ≥65) on VFW:AG, VWF collagen binding activity, and the VWF:AG/ADAMTS13 activity ratio, suggesting an enhanced potential for endothelial coagulopathy. A shift toward increased thrombin generation and decreased plasmin generation was observed in COVID-19 (+) patients >65 years of age in the present study, suggesting an increased risk for hemostasis and impaired fibrinolysis.

Assessment of coagulation in COVID-19 using viscoelastic coagulation tests (e.g., TEG and ROTEM) offers an important insight into the potential for hemostasis and the likelihood for effective fibrin clot lysis in whole blood and platelet rich plasma (51). These assays can be performed at bedside, and are potentially useful in the diagnosis and treatment of COVID-19-induced coagulopathy (27, 52). Several studies that utilize viscoelastic coagulation tests demonstrate elevated clot strength in COVID-19 infection (34, 53, 54). However, viscoelastic tests do not specifically determine the amount of thrombin or plasmin produced in the patient's sample, and the sensitivity of viscoelastic tests to detect fibrinolysis remains controversial (55). For example, in several cases of COVID-19 coagulopathy, analysis by ROTEM suggested that fibrinolysis is completely inhibited (56). However, we do not observe complete inhibition of plasmin generation in the plasma samples evaluated in the study described here. Our study employed a research-based simultaneous thrombin and plasmin generation enzymatic assay to assess the potential for hemostasis and fibrinolysis in PPP (26, 57–59). An important feature of this approach allows for an improved understanding of the rate of thrombin generation, but also an accurate assessment of plasmin generation rates and functional fibrinolysis within a sample. Analysis of 288 COVID-19 (−) and 543 COVID-19 (+) plasma samples obtained at the time of hospital presentation and admission suggested increased thrombogenic potential/ dysregulated hemostasis based on significantly greater thrombin peak heights and generation rates in COVID-19 (+) patients. In addition, impaired fibrinolysis was suggested by identifying significantly lower plasmin peak heights and generation rates in COVID-19 (+) patient samples (60). Interestingly, patients ≥65 years of age, which comprised ∼50% of the patient population studied, accounted for the

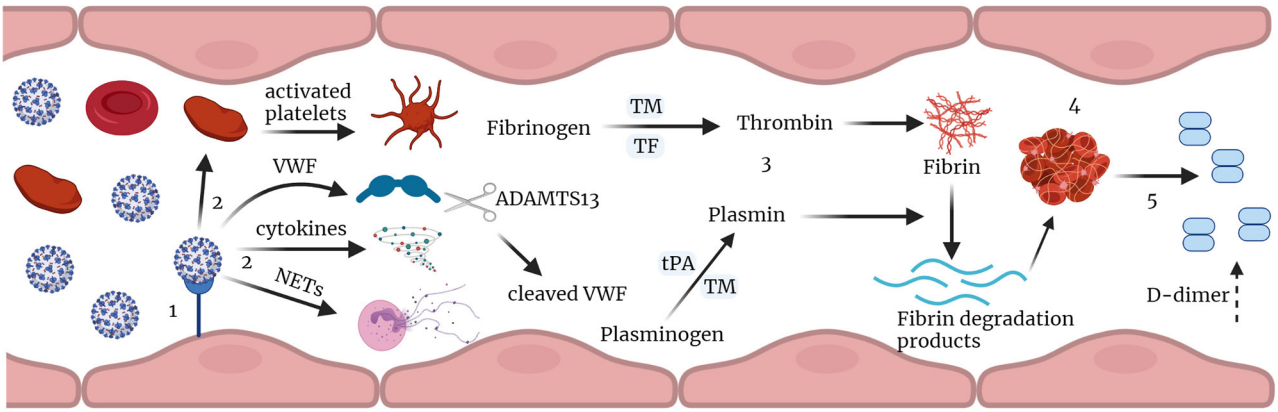

FIGURE 3 | Immune-thrombosis in COVID-19: endothelial and plasma coagulation: (1) Viral infection leading to endothelial damage and inflammation. (2) this leads to increased expression of cytokines, and procoagulant factors like VWF, activation of platelets and neutrophil extracellular traps (NETs) release; VWF is cleaved by ADAMTS13. (3) increased thrombin and plasmin generation potential in presence of thrombomodulin leading to increase in fibrin degradation products and D-dimer. (4) thrombus formation and (5) subsequent fibrin degradation that results in increased D-dimer in Covid-19. TM, thrombomodulin; tPA, tissue plasminogen activator; TF, tissue factor; VWF, von Willebrand factor; NETs, neutrophil extracellular traps (Figure created with Biorender.com).

highest thrombin generation rates and the lowest plasmin generation rates. Unexpectedly, neither overweight nor obese patients demonstrated increased thrombin generation, and only obese patients (i.e., $\geq$30 kg/m^2) demonstrated significantly lower plasmin generation. Collectively, this may indicate that age is one of the most important additive risk factors for dysregulated hemostasis in COVID-19 infection. This is not to say that all patients of increasing age develop thrombosis during COVID-19 infection, and these observations are likely due to existing comorbidities; for example, an aging endothelium and lower organ function naturally occurs over time. Finally, median D-dimer levels were increased in both COVID-19 (−) and (+) patients, but to a greater extent in the latter. However, active thrombosis was not ubiquitous in the patient cohorts described in our study, suggesting that ongoing fibrinolysis, unrelated to clot degradation, is relevant in COVID-19 (61).

The VWF/ADAMTS13 axis is significantly imbalanced in favor of higher VWF levels and activity and lower ADAMTS13 levels and activity in both acutely ill COVID-19 (−) and COVID-19 (+) non-survivors at the time of hospital admission. The VWF:AG/ADAMTS13 activity ratio was increased by 32 vs. 23% in COVID-19 (−) and COVID-19 (+) non-surviving patients, respectively. The samples in this study were analyzed in plasma from blood drawn at the time of hospital presentation or early after hospitalization and did not focus on temporal changes involved in disease progression. The most distinct difference between COVID-19 (+) and COVID-19 (−) non-survivors was a decrease in plasmin generation in COVID-19 (+) patients. This observation may suggest a COVID-19-induced impairment in fibrinolysis mediated by plasminogen activator inhibitor 1 (PAI-1) (62, 63), consistent with greater expression of the inhibitor in adipose tissue (64) and endothelium (65).

The present study defines VWF/ADAMTS13 axis parameters as markers of endothelial dysfunction, along with thrombin and plasmin generation as predictors of thrombosis and fibrinolysis, based on two important risk factors known to predict poor outcome in COVID-19 infection: increased age (66) and obesity (48). However, this study does have several acknowledged limitations. First, although most patients were admitted to inpatient care in both the COVID-19 (−) and COVID-19 (+) groups, some patients had blood draws in the Emergency Department and were discharged to home; therefore, only the sickest COVID-19 (−) patients are represented in this study. Second, hospitalized COVID-19 (−) and COVID-19 (+) patients demonstrate considerable differences in pathophysiology and not all co-morbidities could be captured based on the number of patients in need of care. Notably, COVID-19 (+) patients evaluated in this study demonstrated increased markers of inflammation as compared to COVID-19 (−) patients. Third, BMI values were not available for all patients. In the COVID-19 (−) group, 206 of 288 (72%) patient BMIs were available; in the COVID-19 (+) group 478 of 543 (88%) of patient BMIs were available. Fourth, the simultaneous measurement of thrombin and plasmin is a research-based methodological approach to assess thrombin and plasmin function and standardized reference values across laboratories are not available. Therefore, data can only be compared when evaluated across study groups. Nonetheless, this does not diminish the potential relevance of VWF/ADAMTS13 axis parameters, and of plasma thrombin and plasmin generation parameters, regarding the COVID-19 (+) patients evaluated in this study.

In conclusion, these data are consistent with early signs of endothelial damage that may reflect the pulmonary immune-thrombosis seen with COVID-19 (Schematic **Figure 3**). The median VWF:AG level, VWF: CBA, and VWF:AG/ADAMTS13 activity ratio were all increased in COVID-19 (+) patients, as compared to the acutely ill COVID-19 (−) cohort. However, changes in median ADAMTS13 levels and activity were not observed. Similarly, median platelet levels were unchanged, and thrombocytopenia was not a consistently seen clinical finding,

ruling out typical, and likely atypical, TMA. Furthermore, increased plasma coagulation, as determined by thrombin and plasmin generation, suggests the potential for dysregulated hemostasis in COVID-19 infection. This latter observation was almost exclusively weighted toward patients ≥65 years of age and surprisingly less relevant in overweight and obese COVID-19 (+) patients. Surprisingly, no differences in VWF/ADAMTS13 axis parameters were observed in critically ill COVID-19 (−) versus COVID-19 (+) non-survivors, while a significant imbalance, favoring endothelial coagulopathy was observed between surviving and non-surviving patients in each cohort. This retrospective analysis of acutely ill COVID-19 (−) and COVID-19 (+) patients suggests VWF/ADAMTS13 axis parameters, along with thrombin and plasmin generation, are relevant coagulation parameters to measure in early COVID-19 infection. The assessment of thrombin generation, but more specifically plasmin generation offers critical insight into impaired fibrinolysis not easily obtained by viscoelastic tests.

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 authors.

ETHICS STATEMENT

This study was approved by the Institutional Review Board of Columbia University Irving Medical Center (CUIMC) (Protocol Number AAAT0680). This study was conducted under a waiver of informed consent.

AUTHOR CONTRIBUTIONS

KTh, UK, IA, TT, SK, AW, JV, AD'A, SLS, ROF, and PB contributed to planning and design of the study and to the analysis and interpretation of data. KTh, UK, ROF, and PB drafted the manuscript. All authors critically revised the manuscript for content and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.817305/full#supplementary-material

Supplementary Figure 1 | Platelet levels in COVID-19 (–) and COVID-19 (+) patients: **(A)** Total platelets 222(IQR, 168–293.8) (–), 220(IQR, 152–287) (+), $p = 0.3977$; **(B)** Platelets levels by age groups: <65: 233(IQR, 177–313) (–); 232(IQR, 173–299.5) (+), $p = 0.6889$; >65: 214(IQR, 162–279) (–), 227.5(IQR, 164.8–287) (+), $p = 0.3809$; Platelet levels by BMI **(C)** BMI <18.5: 164.5(IQR, 105.5–232) (–), 188(IQR, 124–325.5) (+), $p = 0.5079$; **(D)** BMI 18.5–24.9: 220(IQR, 156.8–297) (–), 211.5(IQR, 148.8–271.3) (+), $p = 0.3904$; **(E)** BMI 25–29.9: 216(IQR, 184.8–299.3) (–), 252(IQR, 177.5–303) (+), $p = 0.6124$; **(F)** BMI >30: 241.5(170–341.5) (–), 238(179.8–300) (+), $p = 0.9451$. Datapoints indicate individual measurements, and *p*-values were obtained from the Mann-Whitney analysis for comparison within groups. Values are presented as median and interquartile range (IQR, 25th–75th percentile) for continuous variables.

REFERENCES

1. Sarkar M, Madabhavi IV, Quy PN, Govindagoudar MB. COVID-19 and coagulopathy. *Clin Respir J.* (2021) 15:1259–74. doi: 10.1111/crj.13438
2. Blot M, de Maistre E, Bourredjem A, Quenot JP, Nguyen M, Bouhemad B, et al. Specific features of the coagulopathy signature in severe COVID-19 pneumonia. *Front Med.* (2021) 8:675191. doi: 10.3389/fmed.2021.675191
3. Cheruiyot I, Kipkorir V, Ngure B, Misiani M, Munguti J, Ogeng'o J. Arterial thrombosis in coronavirus disease 2019. patients: a rapid systematic review. *Ann Vasc Surg.* (2021) 70:273–81. doi: 10.1016/j.avsg.2020.08.087
4. Neerukonda SN, Katneni U. A review on SARS-CoV-2 virology, pathophysiology, animal models, and anti-viral interventions. *Pathogens.* (2020) 9:426. doi: 10.3390/pathogens9060426
5. Boonyawat K, Chantrathammachart P, Numthavaj P, Nanthatanti N, Phusanti S, Phuphuakrat A, et al. Incidence of thromboembolism in patients with COVID-19: a systematic review and meta-analysis. *Thromb J.* (2020) 18:34. doi: 10.1186/s12959-020-00254-7
6. Bonaventura A, Vecchie A, Dagna L, Martinod K, Dixon DL, Van Tassell BW, et al. Endothelial dysfunction and immunothrombosis as key pathogenic mechanisms in COVID-19. *Nat Rev Immunol.* (2021) 21:319–29. doi: 10.1038/s41577-021-00536-9
7. Chen W, Pan JY. Anatomical and pathological observation and analysis of SARS and COVID-19: microthrombosis is the main cause of death. *Biol Proced Online.* (2021) 23:4. doi: 10.1186/s12575-021-00142-y
8. Fogarty H, Townsend L, Morrin H, Ahmad A, Comerford C, Karampini E, et al. Persistent endotheliopathy in the pathogenesis of long COVID syndrome. *J Thromb Haemost.* (2021) 2546–53. doi: 10.1111/jth.15578
9. Wysokinski WE, Melduni RM, Ammash NM, Vlazny DT, Konik E, Saadiq RA, et al. Von willebrand factor and ADAMTS13 as predictors of adverse outcomes in patients with nonvalvular atrial fibrillation. *CJC Open.* (2021) 3:318–26. doi: 10.1016/j.cjco.2020.10.018
10. 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
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) 19:513–21. doi: 10.1111/jth.15191
12. Philippe A, Chocron R, Gendron N, Bory O, Beauvais A, Peron N, et al. Circulating Von Willebrand factor and high molecular weight multimers as markers of endothelial injury predict COVID-19 in-hospital mortality. *Angiogenesis.* (2021) 24:505–17. doi: 10.1007/s10456-020-09762-6
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. Katneni UK, Alexaki A, Hunt RC, Schiller T, DiCuccio M, Buehler PW, et al. Coagulopathy and thrombosis as a result of severe COVID-19 infection: a microvascular focus. *Thromb Haemost.* (2020) 120:1668–79. doi: 10.1055/s-0040-1715841

15. Bryckaert M, Rosa JP, Denis CV, Lenting PJ. Of von Willebrand factor and platelets. *Cell Mol Life Sci.* (2015) 72:307–26. doi: 10.1007/s00018-014-1743-8
16. Cao W, Krishnaswamy S, Camire RM, Lenting PJ, Zheng XL. Factor VIII accelerates proteolytic cleavage of von Willebrand factor by ADAMTS13. *Proc Natl Acad Sci USA.* (2008) 105:7416–21. doi: 10.1073/pnas.0801735105
17. Dong JF, Moake JL, Nolasco L, Bernardo A, Arceneaux W, Shrimpton CN, et al. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. *Blood.* (2002) 100:4033–9. doi: 10.1182/blood-2002-05-1401
18. Escher R, Breakey N, Lammle B. Severe COVID-19 infection associated with endothelial activation. *Thromb Res.* (2020) 190:62. doi: 10.1016/j.thromres.2020.04.014
19. 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
20. Konkle BA. Von Willebrand factor and aging. *Semin Thromb Hemost.* (2014) 40:640–4. doi: 10.1055/s-0034-1389079
21. Kokame K, Sakata T, Kokubo Y, Miyata T. von Willebrand factor-to-ADAMTS13 ratio increases with age in a Japanese population. *J Thromb Haemost.* (2011) 9:1426–8. doi: 10.1111/j.1538-7836.2011.04333.x
22. Haidl H, Cimenti C, Leschnik B, Zach D, Muntean W. Age-dependency of thrombin generation measured by means of calibrated automated thrombography (CAT). *Thromb Haemost.* (2006) 95:772–5. doi: 10.1160/TH05-10-0685
23. Campello E, Zabeo E, Radu CM, Spiezia L, Gavasso S, Fadin M, et al. Hypercoagulability in overweight and obese subjects who are asymptomatic for thrombotic events. *Thromb Haemost.* (2015) 113:85–96. doi: 10.1160/TH14-02-0156
24. D'Alessandro A, Thomas T, Akpan IJ, Reisz JA, Cendali FI, Gamboni F, et al. Biological and clinical factors contributing to the metabolic heterogeneity of hospitalized patients with and without COVID-19. *Cells.* (2021) 10:2293. doi: 10.3390/cells10092293
25. Tarandovskiy ID, Shin HKH, Baek JH, Karnaukhova E, Buehler PW. Interspecies comparison of simultaneous thrombin and plasmin generation. *Sci Rep.* (2020) 10:3885. doi: 10.1038/s41598-020-60436-1
26. Tarandovskiy ID, Rajabi AA, Karnaukhova E, Buehler PW. Contradictory to its effects on thrombin, C1-inhibitor reduces plasmin generation in the presence of thrombomodulin. *J Thromb Thrombolysis.* (2019) 48:81–7. doi: 10.1007/s11239-019-01869-y
27. Investigators R-C, Investigators AC-a, Investigators A, Goligher EC, Bradbury CA, McVerry BJ, et al. Therapeutic anticoagulation with heparin in critically Ill patients with Covid-19. *N Engl J Med.* (2021) 385:777–89. doi: 10.1056/NEJMoa2103417
28. Mansory EM, Srigunapalan S, Lazo-Langner A. Venous thromboembolism in hospitalized critical and noncritical COVID-19 patients: a systematic review and meta-analysis. *TH Open.* (2021) 5:e286–94. doi: 10.1055/s-0041-1730967
29. Gonzalez-Fajardo JA, Ansuategui M, Romero C, Comanges A, Gomez-Arbelaez D, Ibarra G, et al. [Mortality of covid-19 patients with vascular thrombotic complications]. *Med Clin.* (2021) 156:112–7. doi: 10.1016/j.medcle.2020.10.008
30. Bilaloglu S, Aphinyanaphongs Y, Jones S, Iturrate E, Hochman J, Berger JS. Thrombosis in hospitalized patients with COVID-19 in a New York City health system. *JAMA.* (2020) 324:799–801. doi: 10.1001/jama.2020.13372
31. Marzelle J, Le Chevilliers B, Fourmestraux J, Dehni N, Dimaria G. [Current role of partial interruption of the inferior vena cava. Apropos of 100 cases]. *Ann Cardiol Angeiol (Paris).* (1988) 37:39–44.
32. Sullivan KD, Galbraith MD, Kinning KT, Bartsch KW, Levinsky NC, Araya P, et al. The COVIDome explorer researcher portal. *Cell Rep.* (2021) 36:109527. doi: 10.1016/j.celrep.2021.109527
33. Galbraith MD, Kinning KT, Sullivan KD, Baxter R, Araya P, Jordan KR, et al. Seroconversion stages COVID19 into distinct pathophysiological states. *Elife.* (2021) 10:e65508. doi: 10.7554/eLife.65508
34. 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. doi: 10.1056/NEJMoa2015432
35. Parra-Medina R, Herrera S, Mejia J. Systematic review of microthrombi in COVID-19 autopsies. *Acta Haematol.* (2021) 144:476–83. doi: 10.1159/000515104
36. Fox SE, Akmatbekov A, Harbert JL, Li G, Quincy Brown J, Vander Heide RS. Pulmonary and cardiac pathology in African American patients with COVID-19: an autopsy series from New Orleans. *Lancet Respir Med.* (2020) 8:681–6. doi: 10.1016/S2213-2600(20)30243-5
37. Jang IK, Gold HK, Ziskind AA, Fallon JT, Holt RE, Leinbach RC, et al. Differential sensitivity of erythrocyte-rich and platelet-rich arterial thrombi to lysis with recombinant tissue-type plasminogen activator. a possible explanation for resistance to coronary thrombolysis. *Circulation.* (1989) 79:920–8. doi: 10.1161/01.CIR.79.4.920
38. Denorme F, Langhauser F, Desender L, Vandenbulcke A, Rottensteiner H, Plaimauer B, et al. ADAMTS13-mediated thrombolysis of t-PA-resistant occlusions in ischemic stroke in mice. *Blood.* (2016) 127:2337–45. doi: 10.1182/blood-2015-08-662650
39. Taylor A, Vendramin C, Singh D, Brown MM, Scully M. von Willebrand factor/ADAMTS13 ratio at presentation of acute ischemic brain injury is predictive of outcome. *Blood Adv.* (2020) 4:398–407. doi: 10.1182/bloodadvances.2019000979
40. Cuker A, Cataland SR, Coppo P, de la Rubia J, Friedman KD, George JN, et al. Redefining outcomes in immune TTP: an international working group consensus report. *Blood.* (2021) 137:1855–61. doi: 10.1182/blood.2020009150
41. Gragnano F, Sperlongano S, Golia E, Natale F, Bianchi R, Crisci M, et al. The role of von willebrand factor in vascular inflammation: from pathogenesis to targeted therapy. *Mediators Inflamm.* (2017) 2017:5620314. doi: 10.1155/2017/5620314
42. Martin K, Borgel D, Lerolle N, Feys HB, Trinquart L, Vanhoorelbeke K, et al. Decreased ADAMTS-13 (A disintegrin-like and metalloprotease with thrombospondin type 1 repeats) is associated with a poor prognosis in sepsis-induced organ failure. *Crit Care Med.* (2007) 35:2375–82. doi: 10.1097/01.CCM.0000284508.05247.B3
43. Kosugi N, Tsurutani Y, Isonishi A, Hori Y, Matsumoto M, Fujimura Y. Influenza A infection triggers thrombotic thrombocytopenic purpura by producing the anti-ADAMTS13 IgG inhibitor. *Intern Med.* (2010) 49:689–93. doi: 10.2169/internalmedicine.49.2957
44. Chiasakul T, Cuker A. Clinical and laboratory diagnosis of TTP: an integrated approach. *Hematology Am Soc Hematol Educ Program.* (2018) 2018:530–8. doi: 10.1182/asheducation-2018.1.530
45. Kaufeld JK, Reinhardt M, Schroder C, Brasen JH, Wiech T, Brylka P, et al. Atypical HUS triggered by infection with SARS-CoV2. *Kidney Int Rep*. (2021) 6:2709–12. doi: 10.1016/j.ekir.2021.07.004
46. Wilkerson WR, Sane DC. Aging and thrombosis. *Semin Thromb Hemost.* (2002) 28:555–68. doi: 10.1055/s-2002-36700
47. Blokhin IO, Lentz SR. Mechanisms of thrombosis in obesity. *Curr Opin Hematol.* (2013) 20:437–44. doi: 10.1097/MOH.0b013e3283634443
48. Kompaniyets L, Goodman AB, Belay B, Freedman DS, Sucosky MS, Lange SJ, et al. Body mass index and risk for COVID-19-related hospitalization, intensive care unit admission, invasive mechanical ventilation, and death - United States, march-december 2020. *MMWR Morb Mortal Wkly Rep.* (2021) 70:355–61. doi: 10.15585/mmwr.mm7010e4
49. Kim SA, Park JB, O'Rourke MF. Vasculopathy of aging and the revised cardiovascular continuum. *Pulse.* (2015) 3:141–7. doi: 10.1159/000435901
50. Nogueira E. Rat renal carcinogenesis after chronic simultaneous exposure to lead acetate and N-nitrosodiethylamine. *Virchows Arch B Cell Pathol Incl Mol Pathol.* (1987) 53:365–74. doi: 10.1007/BF02890265
51. Sadd C, Rowe T, Nazeef M, Kory P, Sultan S, Faust H. Thromboelastography to detect hypercoagulability and reduced fibrinolysis in coronavirus disease 2019. *Acute Respiratory Distress Syndrome Patients. Crit Care Explor.* (2020) 2:e0192. doi: 10.1097/CCE.0000000000000192
52. Yuriditsky E, Horowitz JM, Merchan C, Ahuja T, Brosnahan SB, McVoy L, et al. Thromboelastography profiles of critically ill patients with coronavirus disease 2019. *Crit Care Med.* (2020) 48:1319–26. doi: 10.1097/CCM.0000000000004471
53. Spiezia L, Boscolo A, Poletto F, Cerruti L, Tiberio I, Campello E, et al. COVID-19-related severe hypercoagulability in patients admitted to intensive care

unit for acute respiratory failure. *Thromb Haemost.* (2020) 120:998–1000. doi: 10.1055/s-0040-1710018
54. Pavoni V, Gianesello L, Pazzi M, Stera C, Meconi T, Frigieri FC. Evaluation of coagulation function by rotation thromboelastometry in critically ill patients with severe COVID-19 pneumonia. *J Thromb Thrombolysis.* (2020) 50:281–6. doi: 10.1007/s11239-020-02130-7
55. Lisman T. Fibrinolytic shutdown in COVID-19 is likely a misnomer. *Shock.* (2021) 55:844–5. doi: 10.1097/SHK.0000000000001665
56. Creel-Bulos C, Auld SC, Caridi-Scheible M, Barker NA, Friend S, Gaddh M, et al. Fibrinolysis shutdown and thrombosis in a COVID-19 ICU. *Shock.* (2021) 55:316–20. doi: 10.1097/SHK.0000000000001635
57. Gailloud C, Raimondi S. [Diagnosis of intraocular tumors: phosphorus 32 test]. *Ophthalmologica.* (1978) 177:304–6. doi: 10.1159/000308785
58. Kessels H, Willems G, Hemker HC. Analysis of thrombin generation in plasma. *Comput Biol Med.* (1994) 24:277–88. doi: 10.1016/0010-4825(94)90024-8
59. van Geffen M, Loof A, Lap P, Boezeman J, Laros-van Gorkom BA, Brons P, et al. A novel hemostasis assay for the simultaneous measurement of coagulation and fibrinolysis. *Hematology.* (2011) 16:327–36. doi: 10.1179/102453311X13085644680348
60. Wright FL, Vogler TO, Moore EE, Moore HB, Wohlauer MV, Urban S, et al. Fibrinolysis shutdown correlation with thromboembolic events in severe COVID-19 infection. *J Am Coll Surg.* (2020) 231:193–203 e1. doi: 10.1016/j.jamcollsurg.2020.05.007
61. 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
62. Kellici TF, Pilka ES, Bodkin MJ. Therapeutic potential of targeting plasminogen activator inhibitor-1 in COVID-19. *Trends Pharmacol Sci.* (2021) 42:431–3. doi: 10.1016/j.tips.2021.03.006
63. D'Agnillo F, Walters KA, Xiao Y, Sheng ZM, Scherler K, Park J, et al. Lung epithelial and endothelial damage, loss of tissue repair, inhibition of fibrinolysis, and cellular senescence in fatal COVID-19. *Sci Transl Med.* (2021) 13:eabj7790. doi: 10.1126/scitranslmed.abj7790
64. Skurk T, Hauner H. Obesity and impaired fibrinolysis: role of adipose production of plasminogen activator inhibitor-1. *Int J Obes Relat Metab Disord.* (2004) 28:1357–64. doi: 10.1038/sj.ijo.0802778
65. Han M, Pandey D. ZMPSTE24 regulates SARS-CoV-2 spike protein-enhanced expression of endothelial PAI-1. *Am J Respir Cell Mol Biol.* (2021) 65:300–8. doi: 10.1165/rcmb.2020-0544OC
66. Pennington AF, Kompaniyets L, Summers AD, Danielson ML, Goodman AB, Chevinsky JR, et al. Risk of clinical severity by age and race/ethnicity among adults hospitalized for COVID-19-United States, March-September (2020). *Open Forum Infect Dis.* (2021) 8:ofaa638. doi: 10.1093/ofid/ofaa638

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CASE REPORT
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Case Report and Literature Review: Behçet's Disease With a Novel TFPI Gene Mutation

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We report a case of Behçet's disease (BD) with a newly identified tissue factor pathway inhibitor (TFPI) gene mutation. The patient suffered from recurrent deep vein thrombosis and dural sinus thrombosis which could not be relieved by constant anticoagulation therapy. Slight relapsing oral lesion was the initial manifestation of BD but was neglected. Genital ulcers and ocular symptoms were manifest 8-month later than vascular involvement. The patient was diagnosed with BD at last and a novel mutation in TFPI was identified simultaneously. After administration with azathioprine and dexamethasone, the clinical symptoms were quickly gone and no relapse was found during 7-month follow-up.

Keywords: thrombosis, Behçet's disease, TFPI, mutation, case report

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INTRODUCTION

Behçet's disease (BD) is a chronic multisystemic vasculitis which affects both arteries and veins with non-specific inflammation. The clinical symptoms of BD are protean and commonly characterized with oral aphthae, genital ulcerations and ocular involvement. Though the etiology of BD remains uncertain, many factors have been thought contributory, including geographic region, genetic variants, viruses' infection and so on (1, 2). Its prevalence and clinical manifestations also show regional differences (3). BD is most frequent from the Mediterranean region to the Far East, and gastrointestinal involvement is more commonly described in these areas (4–6). The onset age of BD is usually in the third decade, with significant morbidity and mortality reported to be associated with precocious onset, particularly in male patients.

Vascular involvement affects approximately 15 – 40% BD patients, and 27.5% of these patients may exhibit vascular lesion as their initial manifestation (7). Vascular complications before classic symptoms of BD or meeting the International Criteria for Behçet's disease (ICBD) greatly increase the difficulty in diagnosis. Deep vein thrombosis (DVT) is most common and mainly found in lower extremities. Venous thrombosis in other sites includes vena cava and dural sinus thrombosis. It was also reported that dural sinus thrombosis was significantly associated with systemic major vessel disease (8).

Tissue factor pathway inhibitor (TFPI) is a vital modulator in coagulation cascade. On one hand, it forms a quaternary complex with tissue factor, activated factor VII and X. On the other hand, it

binds with activated factor V, inhibiting the conversion from prothrombin to thrombin (9). The majority of TAPI is bound to vessel endothelial cells, and only 20 – 50% circulates in blood (10). Low levels of plasma TFPI are reported to increase the risk of thrombosis in both veins and arteries (11). Genetic variants may contribute to the heritable variation of TAFI level in plasma or affects its interaction with other cofactors (12–14).

Here we report a case in which a young man suffered from recurrent thrombosis and eventually identified with BD and a novel mutation in TFPI. The double hit of widespread vasculitis and disturbed modulating of coagulation may explain the refractory thrombophilia. To our knowledge, the mutation carried by this patient is newly identified and this is the first reported case presenting recurrent thrombosis caused by BD with thrombophilic genetic mutation. The patient and his family were fully informed and written consents were signed. This work is approved by the ethics committee of Union Hospital at Huazhong University of Science and Technology.

CASE PRESENTATION

A 21-year-old Chinese male patient who had suffered from recurrent thromboembolism events for nearly 1 year was admitted to our hospital. With no history of major health issues or family history regarding similar conditions reported, the patient exhibited swelling and pain in lower left limb 1 year ago. A proximal leg vein ultrasound scan indicated thrombosis and anticoagulation treatment was carried out with 2-week low molecular weight heparin (LMWH) and 8-month rivaroxaban. About 1 month after finished anticoagulation, he caught a cold and exhibited headache, vomiting and double vision in the left eye afterward. Cerebral angiography in local hospital indicated dural sinus thrombosis (**Figure 1**) and the patient was given anticoagulation with LMWH, dehydration therapy, neurotrophic treatment and so on. In the meantime, coagulation tests showed basically normal results. Nevertheless, after administration with LMWH for 50 days, the patient was diagnosed with DVT in lower right extremity again. Then the patient was transferred to our department.

In physical examination, limited abduction of his left eye, erythema at injection sites (**Figure 2A**) and raised temperature of skin on the right inner thigh were manifest, together with swelling of bilateral lower limbs, particularly in the right. Negative results were reported in investigations including extractable nuclear antigen, lupus anticoagulant and anti-cardiolipin antibodies. C-reactive protein (CRP) was 55 mg/L (normal: $<$ 5 mg/L). Considering the refractory thrombi in deep veins of lower limbs and dural sinus, genetic screening for thrombophilia which used panel-based next generation sequencing (NGS) targeting genes involved in hemostasis, anticoagulation and fibrinolysis system was also applied. While waiting for the results, treatment with argatroban, dehydration, anti-infection and neurotrophic therapy were used. Two days after transferred to our department, laboratory data involved with coagulation

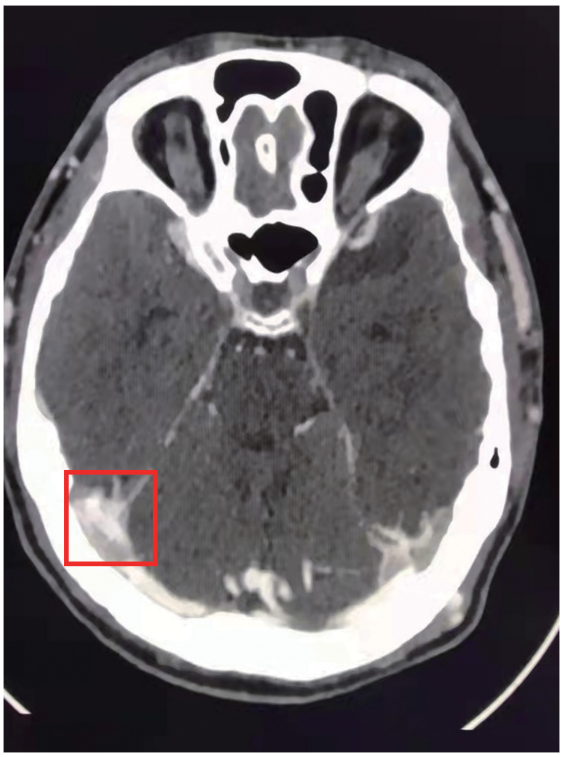

FIGURE 1 | MRI scan indicated dural sinus thrombosis.

testing, activities of coagulation factors showed prolonged APTT (64.5 s, normal: 20 – 47.1 s), TT (43.4 s, normal: 12.9 – 22.9 s) and reduction in activities of coagulation factors VIII (43%, normal: 70 – 150%), IX (51%, normal: 60 – 150%), XI (46%, normal: 70 – 120%) were observed. And treatment as described above for 2 weeks did not see release in right-leg swelling and the regional pain was even more severe. CRP consistently fluctuated between 50 and 60 mg/L.

Then the result of genetic screening came out, reporting a heterozygous mutation of TFPI (NM_006287; c.C403T: p.R135X; **Figure 3**). The variant was discovered by NGS and confirmed by Sanger sequencing. The variant of TFPI was also identified in his father but not his mother through further work-up. Meanwhile, the patient began to exhibit two ulcers on the scrotum (**Figure 2B**). But no oral aphthae were found with the patient. After repeated questioning, the patient reported recurrent genital ulcerations for half a year, and resolution always occurred spontaneously. He had gone to dermatologists and rheumatologists many times but no diagnosis had been made. There were also oral ulcerations three to four times a year for almost 2 years, along with occasional bloodshot eyes. Because of the mildness of these manifestations, he failed to put these signs together and did not mention them when admitted. According to the ICBD, the diagnosis of BD was made based on the oral and genital lesions, ocular involvement and vascular symptoms. The coexisting mutation in TFPI may also contribute to his refractory thrombophilia and resistance to anticoagulants.

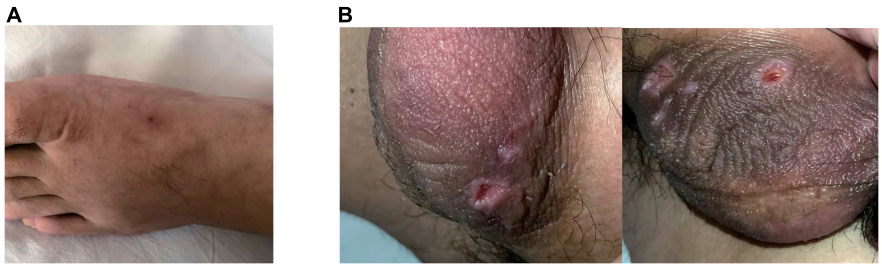

FIGURE 2 | Erythema at injection sites when the patient was admitted **(A)**. Ulcerations on the scrotum were manifest 2-weeks later **(B)**.

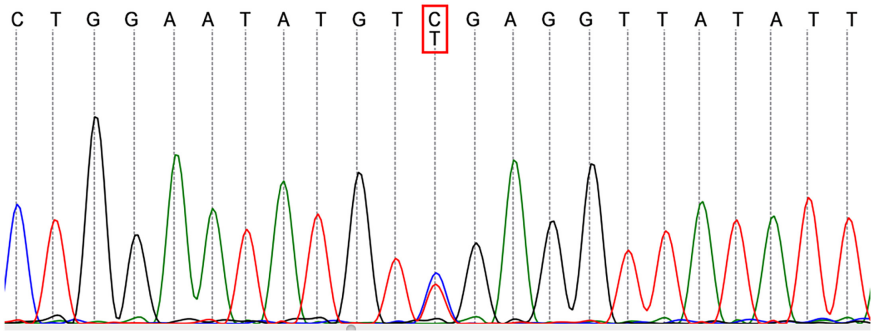

FIGURE 3 | Sequencing analysis of the patient revealed a heterozygous mutation in TFPI.

TREATMENT

After diagnosis of BD, azathioprine (100 mg qd), prednisone (4 mg qd) and continuous anticoagulation was administered to the patient.

OUTCOME AND FOLLOW-UP

The patient promptly recovered and the swelling and localized pain in his right leg disappeared quickly. So did the ulcerations in scrotum. Reexamination showed a decrease to normal range in CRP. During our 7-month follow-up, the patient exhibited no relapse in thrombosis or ulcerations.

DISCUSSION

In this study, we reported a case characterized with recurrent thrombi in lower extremities and dural sinus. Vascular involvement was 8-month earlier than ocular symptoms and genital aphthae. Slight relapsing oral lesion was the initial manifestation. However, it failed to draw attention from the patient himself or the clinicians. Diverse symptom combinations and timeframe make the diagnosis of BD sometimes quite challenging. The patient was also identified with a novel mutation in TFPI, which may contribute to the refractory thrombophilia. The effect of continuous anticoagulation treatment was proved to be unsatisfactory in this case. For the most part, laboratory data of coagulation studies was basically normal, while the prolonged APTT, TT, and reduction in activities of coagulation factors VIII,IX,XI were probably caused by the administration of argatroban at that time. On the contrary, immunosuppressive agents and glucocorticoids were significantly effective. This case highlights the importance of a broad differential diagnosis when the patient suffers from recurrent multiple thrombi under unknown cause.

Behçet's disease is classified as an inflammatory vascular disease affecting blood vessels of all sizes and kinds, which involves almost every organ and system. It is distributed globally, in spite of the regional differences of prevalence and manifestations. The most frequently described clinical symptom is mucocutaneous lesion. Oral aphthae is described in 98% of cases and genital aphthae in about 65% cases (15). While the spontaneously recovering ulcerations are quite negligible, diagnosis of BD is challenging -and relies on thorough medical history, which is indicated by our case.

The mechanism underlying vascular involvement in BD is not clear. Despite the fact that vascular syndrome is manifest in only approximately 15% patients, it contributes greatly to the morbidity and mortality of BD (16). Most BD patients with vascular involvement experience the first vascular event within 5 years since onset of the disease, which is earlier than fulfilling the diagnostic criteria in 10.8% of these patients (8). The primary objectives in treating BD patients with vascular involvement are suppressing vasculitis and preventing further complications (17). Standard guidelines regarding the management of thrombosis in BD patients are currently lacking. The first choice is immunosuppressive agents, while the usage of anticoagulants is controversial considering the risk of fatal bleeding when

pulmonary arterial aneurysm is coexisting. A retrospective analysis of 807 BD patients showed 4-fold decreased thrombosis relapse by immunosuppressive agents alone (18). The relapse rates were similar when comparing patients using anticoagulants together with immunosuppressant or only immunosuppressant (19, 20). The usage of azathioprine and dexamethasone was effective in this case and follow-up of longer duration is needed to testify its effect on relapse. To reach reliable consensus and tailor for specific phenotypes of patients, more randomize controlled studies regarding management of vascular BD are needed.

Tissue factor pathway inhibitor is a vital anticoagulant which inhibits the earliest steps in extrinsic coagulation pathway. Threshold effect of low plasma TFPI levels on thrombotic risk has already been clarified. About two-fold increased risk of incident VTE and myocardial infraction was found in subjects with baseline TFPI levels in the lowest 5 – 10% of the distribution (21, 22). Dennis et al. (11) systematically summarized the genetic factors associated with levels of plasma TFPI. They found that the minor allele of rs5940 was related with decreased TFPI level, and mutations causing deficiencies in protein S, factor V, apolipoprotein E and galactosidase α also had influences on plasma TFPI level. Studies have reported controversial conclusions regarding the relation between some variants of TFPI and the caused risk for thrombosis (14, 23). The mutation C-403T identified in this patient is located in exons of TFPI, and we presume that this mutation has deleterious effects on anti-coagulation which needs further study to verify.

There are also obvious limitations in our study. How the TFPI variant affects the plasma level and modulatory function of TFPI is unclear. Further investigation is needed to clarify its importance. Follow-up study of the patient will help monitor recurrence or aggravation. This case elucidates the importance of early diagnosis and identifying the causes in choosing appropriate treatment. A comprehensive medical history and broad differential diagnosis are vital when the patient exhibits recurrent multiple thrombi under unknown cause.

LEARNING POINTS

- The diagnosis of BD can be very challenging when vascular involvement is manifest earlier than the classical combinations of oral aphthae, genital ulcerations and ocular symptoms.
- A broad differential diagnosis and genetic screening for thrombophilia are vital when the patient exhibits recurrent multiple thrombi under unknown cause.
- A novel mutation in TFPI was identified in this case.

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.

ETHICS STATEMENT

Ethical Approval was provided by the Ethics Committee of Union Hospital at Huazhong University of Science and Technology. Written informed consent to participate was provided by the patient/next of kin. 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

LT and DY designed the study. DY and WS investigated and provided the clinical data. DY and JM wrote the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Hou S, Xiao X, Zhou Y, Zhu X, Li F, Kijlstra A, et al. Genetic variant on PDGFRL associated with Behçet disease in Chinese Han populations. *Hum Mutat.* (2013) 34:74–8. doi: 10.1002/humu.22208
2. Krause I, Leibovici L, Guedj D, Molad Y, Uziel Y, Weinberger A. Disease patterns of patients with Behçet's disease demonstrated by factor analysis. *Clin Exp Rheumatol.* (1999) 17:347–50.
3. Maldini C, Druce K, Basu N, LaValley MP, Mahr A. Exploring the variability in Behçet's disease prevalence: a meta-analytical approach. *Rheumatology (Oxford).* (2018) 57:185–95. doi: 10.1093/rheumatology/kew486
4. Kötter I, Vonthein R, Müller CA, Günaydin I, Zierhut M, Stübiger N. Behçet's disease in patients of German and Turkish origin living in Germany: a comparative analysis. *J Rheumatol.* (2004) 31:133–9.
5. Krause I, Mader R, Sulkes J, Paul M, Uziel Y, Adawi M, et al. Behçet's disease in Israel: the influence of ethnic origin on disease expression and severity. *J Rheumatol.* (2001) 28:1033–6.
6. Krause I, Yankevich A, Fraser A, Rosner I, Mader R, Zisman D, et al. Prevalence and clinical aspects of Behcet's disease in the north of Israel. *Clin Rheumatol.* (2007) 26:555–60. doi: 10.1007/s10067-006-0349-4
7. Fei Y, Li X, Lin S, Song X, Wu Q, Zhu Y, et al. Major vascular involvement in Behçet's disease: a retrospective study of 796 patients. *Clin Rheumatol.* (2013) 32:845–52. doi: 10.1007/s10067-013-2205-7
8. Tascilar K, Melikoglu M, Ugurlu S, Sut N, Caglar E, Yazici H. Vascular involvement in Behçet's syndrome: a retrospective analysis of associations and the time course. *Rheumatology (Oxford).* (2014) 53:2018–22. doi: 10.1093/rheumatology/keu233
9. Wood JP, Ellery PE, Maroney SA, Mast AE. Biology of tissue factor pathway inhibitor. *Blood.* (2014) 123:2934–43.
10. Broze GJ, Girard TJ. Tissue factor pathway inhibitor: structure-function. *Front Biosci (Landmark Ed).* (2012) 17:262–80. doi: 10.2741/3926
11. Dennis J, Kassam I, Morange PE, Trégouët DA, Gagnon F. Genetic determinants of tissue factor pathway inhibitor plasma levels. *Thromb Haemost.* (2015) 114:245–57. doi: 10.1160/TH14-12-1043
12. Ahnström J, Andersson HM, Hockey V, Meng Y, McKinnon TA, Hamuro T, et al. Identification of functionally important residues in TFPI Kunitz domain 3 required for the enhancement of its activity by protein S. *Blood.* (2012) 120:5059–62. doi: 10.1182/blood-2012-05-432005
13. Dahlbäck B. Novel insights into the regulation of coagulation by factor V isoforms, tissue factor pathway inhibitorα, and protein S. *J Thromb Haemost.* (2017) 15:1241–50. doi: 10.1111/jth.13665

14. Dennis J, Truong V, Aïssi D, Medina-Rivera A, Blankenberg S, Germain M, et al. Single nucleotide polymorphisms in an intergenic chromosome 2q region associated with tissue factor pathway inhibitor plasma levels and venous thromboembolism. *J Thromb Haemost.* (2016) 14:1960–70. doi: 10.1111/jth.13431
15. Cho SB, Cho S, Bang D. New insights in the clinical understanding of Behçet's disease. *Yonsei Med J.* (2012) 53:35–42.
16. Alakkas Z, Kazi W, Mattar M, Salem E, Seleem NF. Pulmonary artery thrombosis as the first presentation of Behçet's syndrome: a case report and review of the literature. *J Med Case Rep.* (2021) 15:322. doi: 10.1186/s13256-021-02931-1
17. Hatemi G, Christensen R, Bang D, Bodaghi B, Celik AF, Fortune F, et al. 2018 update of the EULAR recommendations for the management of Behçet's syndrome. *Ann Rheum Dis.* (2018) 77:808–18.
18. Desbois AC, Wechsler B, Resche-Rigon M, Piette JC, Huong DT, Amoura Z, et al. Immunosuppressants reduce venous thrombosis relapse in Behçet's disease. *Arthritis Rheum.* (2012) 64:2753–60. doi: 10.1002/art.34450
19. Ahn JK, Lee YS, Jeon CH, Koh EM, Cha HS. Treatment of venous thrombosis associated with Behcet's disease: immunosuppressive therapy alone versus immunosuppressive therapy plus anticoagulation. *Clin Rheumatol.* (2008) 27:201–5. doi: 10.1007/s10067-007-0685-z
20. Alibaz-Oner F, Direskeneli H. Management of vascular Behçet's disease. *Int J Rheum Dis.* (2019) 22 (Suppl. 1):105–8.
21. Zakai NA, Lutsey PL, Folsom AR, Heckbert SR, Cushman M. Total tissue factor pathway inhibitor and venous thrombosis. the longitudinal investigation of thromboembolism etiology. *Thromb Haemost.* (2010) 104:207–12. doi: 10.1160/TH09-10-0693
22. Morange PE, Simon C, Alessi MC, Luc G, Arveiler D, Ferrieres J, et al. Endothelial cell markers and the risk of coronary heart disease: the prospective epidemiological study of myocardial infarction (PRIME) study. *Circulation.* (2004) 109:1343–8. doi: 10.1161/01.CIR.0000120705.55512.EC
23. Zhang Y, Pang A, Zhao L, Guo Q, Zhang Z, Zhu X, et al. Association of TFPI polymorphisms rs8176592, rs10931292, and rs10153820 with venous thrombosis: a meta-analysis. *Medicine (Baltimore).* (2019) 98:e14978. doi: 10.1097/MD.0000000000014978

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A case report of cerebral venous sinus thrombosis presenting with rapidly progressive dementia

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Background: Cerebral venous sinus thrombosis (CVST) is a rare but serious and treatable cause of neurologic symptoms. Due to the variable clinical presentation, CVST was often misdiagnosed. According to published case reports, common clinical manifestations of CVST include headache, focal neurological deficit, epilepsy, papilledema, etc. It is rare, nevertheless, to mention cases of rapidly progressive dementia (RPD).

Case presentation: We reported a case of a 62-year-old retired male accountant, a Han Chinese from eastern China, who initially presented with slow response and memory decline. Until 2 months later, his memory declined and slow response deteriorated significantly, and he could not even complete simple tasks like brushing his teeth, washing his face, washing his feet, and dressing himself, and sometimes developed fecal incontinence. His neuropsychological test demonstrated severe cognitive decline. The cerebrospinal fluid (CSF) studies revealed markedly high opening pressure (260 mm of water), and coagulation tests indicated a mild elevation of D-Dimer of 1.19 mg/L. The magnetic resonance venography (MRV) showed thrombosis of the left transverse sinus, sigmoid sinus, and jugular venous bulb and was diagnosed as CVST. He switched from subcutaneous low molecular weight heparin (LMWH) and transitioned to oral anticoagulants at the time of discharge. The repeated CSF studies revealed normal opening pressure. After 5 days of anticoagulant treatment, his symptoms considerably improved, and a 1-month follow-up revealed that he had fully healed with no signs of recurrence.

Conclusion: This case demonstrated the clinical heterogeneity of CVST, which should be taken into account for differential diagnosis of RPD. This case study also offered fresh data for the categorization of the clinical traits and the diagnosis of CVST.

KEYWORDS

cerebral venous sinus thrombosis (CVST), dementia, rapidly progressive dementia, idiopathic intracranial hypertension (IIH), anticoagulant

Introduction

Cerebral venous sinus thrombosis (CVST) is a rare but serious and treatable cause of neurologic symptoms. Whereas CVST accounts for fewer than 1% of all strokes, which primarily affects younger adults and children, and is characterized by clinical signs and symptoms such as headache, nausea and vomiting, optic papilledema, limb paralysis, and epilepsy (1–4). The incidence of diagnosis and misdiagnosis is significant due to the complicated and diverse clinical presentations of CVST, and it has been reported in the literature that the misdiagnosed rate can be as high as 50% (5). The relative rarity of this disease, combined with the variable and subacute presentation of symptoms, is thought to contribute to the delay in diagnosis (6). The clinical presentation of CVST is highly heterogeneous, with headache symptoms being the most prevalent (7). However, rapidly progressive dementia (RPD) is rare as the first symptom of CVST (8). Here, we described a patient presenting with slow response and memory decline as the first symptom and had been misdiagnosed. He was finally diagnosed as CVST based on cranial magnetic resonance imaging (MRI) combined with MR venography (MRV). This case report describes a rare clinical manifestation of CVST, which will contribute to a better understanding of the clinical characteristics of CVST for better diagnosis in the future.

Case presenting

A 62-year-old retired male accountant, a Han Chinese from eastern China with a past medical history of significant hypertension and coronary artery disease, was admitted to a local hospital in September 2020 for "nephrotic syndrome" and was reported by his family to be cured and discharged on 50 mg of oral prednisone daily. The patient was admitted on December 21, 2020, for approximately 2 months for slow response and memory loss (**Figure 1**). He began to experience slow response, memory loss, personality change, irritability, and self-talk at times around October 2020, and the symptoms progressively worsened until 1 week ago, when he was unable to perform simple tasks like brushing teeth, washing face, washing feet, and dressing himself and sometimes had fecal incontinence. He denied complaints of fever, headache, slurred speech, numbness and weakness of the limbs, and convulsions. He visited a local hospital on December 12, 2020, and a head computed tomography (CT) indicated lacunar cerebral infarction with ischemic changes in the white matter of the brain. By using the Chinese medical treatment blood stasis-removing therapy using salvia miltiorrhiza, however, his symptoms deteriorated significantly.

On admission, he denied any history of smoking, alcohol abuse, or recreational drug use. He also had no known history of coronavirus disease-2019 (COVID-19) infection or vaccination with unremarkable family history. His body mass index at the time of admission was 25 kg/m^2, with no change in weight over 1 year, vital signs were within normal limits, and his room air was well saturated. Neurological examination revealed slow response, memory loss, poor computation power (100–7 = 9, 9–7 = 0), and could only answer simple questions. His neuropsychological test revealed severe cognitive decline with a Mini-Mental State Examination (MMSE) score of 14 and a Montreal Cognitive Assessment (MoCA) score of 10. The muscle strength of his limbs was grade 5/5, the muscle tone, and tendon reflexes were normal, and the remaining physical and neurological examination results were not significant.

On day 2 after admission, relevant examinations were carried out. No abnormality was revealed by blood routine, routine urine and stool testing, thyroid function, glycosylated hemoglobin, and tumor markers, homocysteine. Some biochemical indications revealed mild abnormalities, such as total bilirubin of 34.00 μmol/L (normal range, 3.4–20.5 μmol/L), direct bilirubin of 11.00 μmol/L (normal range, 0–6.8 μmol/L), total protein of 52.3 g/L (normal range, 60–80 g/L), Albumin of 33 g/L (normal range, 34–48 g/L), Alanine aminotransferase of 56 U/L (normal range, 8–40 U/L), and lactate dehydrogenase of 340 U/L (normal range, 81–234 U/L). Coagulation tests also revealed mild elevation of D-Dimer was 1.19 mg/L (normal range, 0–0.55 mg/L). In addition, the hepatitis B surface antigen, syphilis antibody, hepatitis C virus antibody, and anti-human immunodeficiency virus antibody were negative. Lung CT plain scan showed bronchitis, coronary lesions, a small amount of pleural effusion on both sides, and

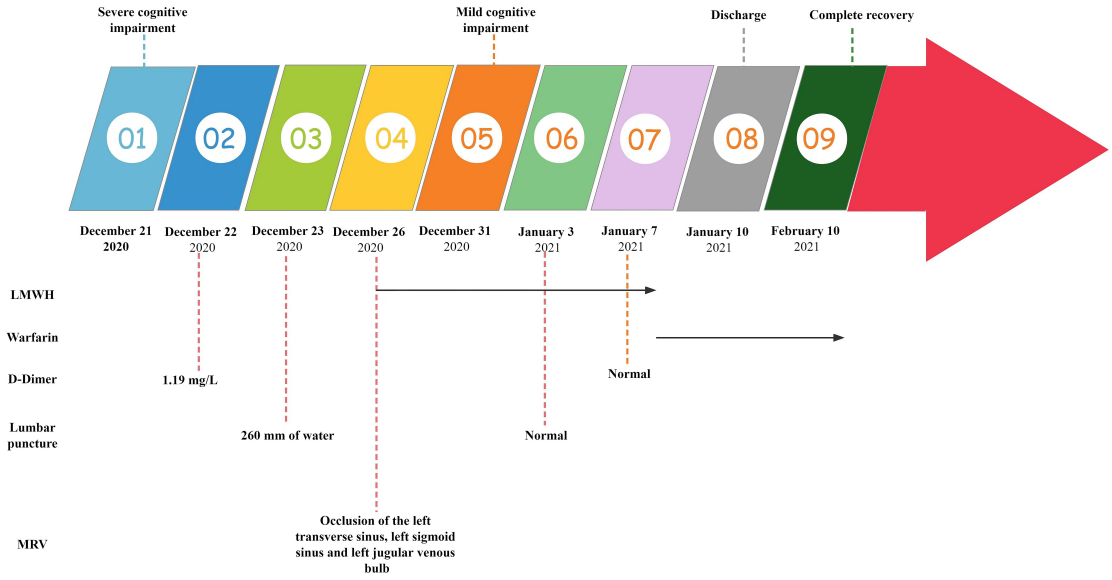

FIGURE 1
Summary of the clinical features, therapy, laboratory tests, and imaging tests. LMWH, low molecular weight heparin; MRV, magnetic resonance venography.

thickened adhesions on the left pleura. Upper abdomen CT scan showed limited hepatic contour dysplasia and cholecystitis.

On the third day of admission, the lumbar puncture was performed, showing high opening pressure (260 mm of water), normal cerebrospinal fluid (CSF) protein level (276.5 mg/L), normal cell count (3 × 10^6/L), normal CSF glucose (4.2 mmol/L), and normal CSF chloride (125 mmol/L). Considering the probability of autoimmune encephalitis (AE), an antibody testing of CSF was also performed on December 25 using a cell-based assay (EUROIMMUN Medical Diagnostics). However, the anti-NMDAR, anti-AMPAR1, anti-AMPAR2, anti-mGluR5, anti-GAD65, anti-lgLON5, and anti-DPPX antibodies were detected as negative. Electroencephalogram (EEG) revealed extensive mild abnormal EEG (alpha generalization). Brain MRI exhibited multiple lesions in the center of the centrum semiovale, with hypointensity on T1-weighted image (**Figure 2A**), hyperintensity on T2-weighted image (**Figure 2B**), on FLAIR (**Figure 2C**), and isointensity on DWI (**Figure 2D**). No intracranial aortic stenosis was evident on cranial magnetic resonance angiography (MRA) (**Figures 3A,B**). On December 26, MRV demonstrated thrombosis of the left transverse sinus, sigmoid sinus, and jugular venous bulb (**Figures 3C,D**), which was considered CVST. Subsequently, the patients were screened for predisposing factors for thrombosis. Factor V Leiden and prothrombin gene mutations were negative. Furthermore, his protein S and protein C were all within normal limits. He was administered low molecular weight heparin (LMWH) 100 unit/kg subcutaneously twice daily to maintain APTT between 1.5 and 2.5 times of control value for 14 days. His symptoms improved significantly after 5 days of anticoagulation therapy, in which computing power was fully restored. The MMSE scores and MoCA scores were 22 and 18, respectively. A repeat lumbar puncture on January 3 showed normal opening pressures (160 mm water) and the rest of the CSF test results were within normal limits. The repeated coagulation tests also revealed a normal D-dimer on January 7. On day 3 prior to discharge, he transitioned to oral anticoagulants (warfarin) with anticipated treatment lasting 6 months. He was discharged on January 10 with complete resolution of his symptoms at the follow-up 1 month later. The MMSE scores and MoCA scores were 28 and 26, respectively with no sign of recurrence at the follow-up after 6 months. As for his cognition, the patient himself stated that there was no change in his memory and his family did not notice any change in his personality or behavior.

Discussion

Goyal and colleagues summarized the clinical characteristics of 181 cases of CVST, most of which presented with the first and main clinical manifestations of headache. Other clinical symptoms of CVST included seizures, altered sensorium, focal neurological deficit, and vertigo (9). In the aforementioned study, no cases of RPD were mentioned. Even if the first symptom of the patient are slow response and memory decline, its progression was rapid. Here, we reported an RPD case with slow response and memory decline as the first symptom.

RPD is a group of RPD syndromes in which the decline in cognitive impairment to dementia is less than 2 years, or even months or weeks, and is partially reversible and treatable

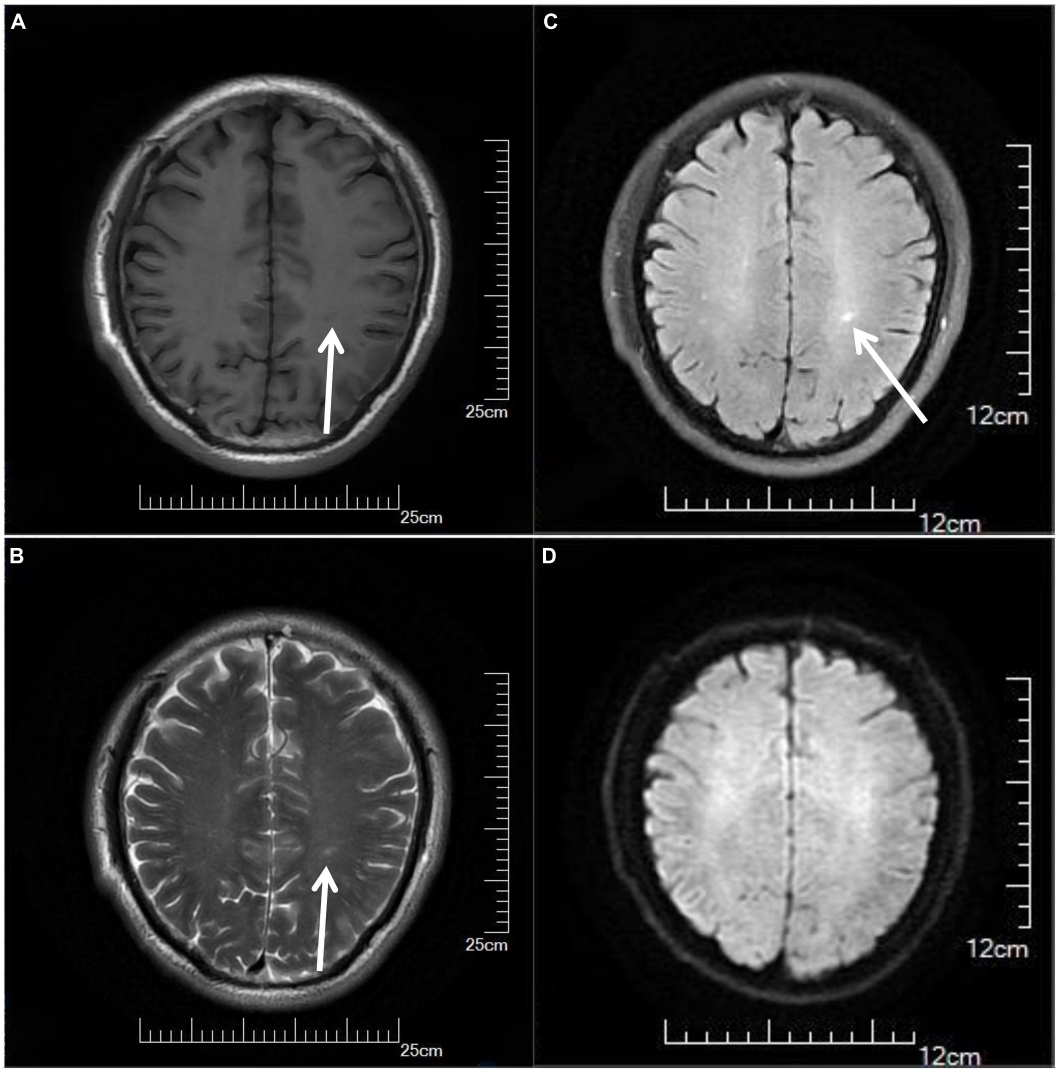

FIGURE 2
Cranial MRI results of the patient, showing multiple lesions in the center of the centrum semiovale with hypointensity on T1-weighted image **(A)** (arrows), hyperintensity on T2-weighted image **(B)** (arrows), on FLAIR **(C)** (arrows), and isointensity on DWI **(D)**. MRI, magnetic resonance imaging.

(10). RPD can be roughly categorized as primary or secondary. Primary RPD occurs in Creutzfeldt-Jakob disease (CJD), rapidly progressive types of other neurodegenerative dementias, encephalitis, and other diseases that typically cause severe neuronal damage in a relatively short period of time. Secondary rapid disease progression can occur in predominantly slowly progressive CNS diseases, such as Alzheimer's disease with cerebrovascular disease or Lewy body pathology. There are many secondary factors in RPD, and the rate and reversibility of RPD progression vary widely among factors. RPD caused by a viral infection, immune deficiency, toxicity, and metabolic diseases progresses fastest but is more effective in treatment (11). Although this patient demonstrated remarkably rapid progression, this patient had no clinical manifestations of ataxia, myoclonus, or other involuntary movements, no cranial MRI with high signal in the basal ganglia and thalamus, and no EEG showing triphasic waves. Therefore, the diagnosis of CJD was not supported in this patient. Additionally, the CSF was negative for AE and paraneoplastic markers, which could further exclude AE and paraneoplastic encephalitis.

The etiology of the elevated intracranial pressure can be divided into idiopathic and secondary. Idiopathic intracranial hypertension (IIH) is a clinical syndrome of unknown etiology that presents with headache, optic papilledema, and other intracranial pressure elevations as the main manifestations (12). IIH mostly occurs in obese women of childbearing age, excluding patients with parenchymal brain lesions and venous sinus system disease (13, 14). Approximately 70% of patients with idiopathic cranial hypertension have a visual impairment, and some patients may experience progressive

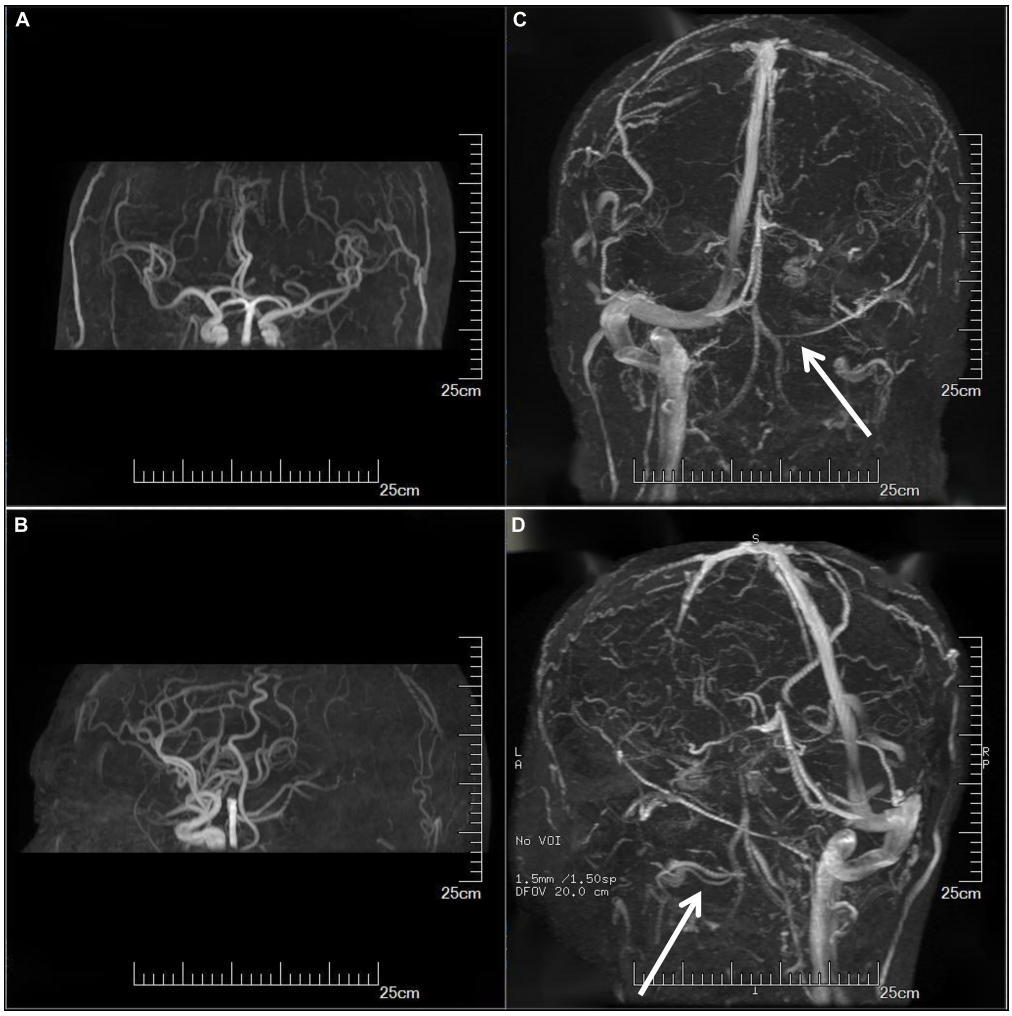

FIGURE 3
Imaging examination. Cranial MRA showed no significant intracranial aortic stenosis **(A,B)**. MRV showed thrombosis of the left transverse sinus, sigmoid sinus, and jugular venous bulb **(C,D)** (arrows). MRA, magnetic resonance angiography; MRV, magnetic resonance venography.

vision loss or even blindness (15, 16). Secondary intracranial hypertension usually refers to disorders of clear etiology such as occupying lesions, thrombotic disease, meningeal lesions, etc. (12, 17). Although lumbar puncture showed high CSF pressure, the patient had no clinical manifestations such as headache, vomiting, optic papilledema, and decreased visual acuity. Therefore, the diagnosis of IIH was also not supported. Finally, we performed MRV, which revealed thrombosis of the left transverse sinus, sigmoid sinus, and jugular venous bulb, with a possible diagnosis of CVST. This example emphasizes the challenges in detecting CVST because the diagnosis of CVST may have been delayed or prevented due to the unusual clinical characteristics. Diagnosing CVST can be extremely difficult because of the differences in clinical presentations and imaging findings. When the results of the cranial MRI and head CT are normal, CVST cannot be completely ruled out, necessitating further MRV screening. The cranial MRI and MRV examinations are simple to operate and can compensate for each other's shortcomings. Combined cranial MRI and MRV examinations have been used as an effective tool for the clinical diagnosis and prognosis of CVST (18).

The imbalance between venous thrombosis and fibrinolytic mechanisms is the main pathophysiological mechanism of CVST. Any condition that causes a hypercoagulable state of blood, abnormal venous blood flow, and inflammatory response of the venous wall may lead to CVST. The etiology of CVST is multifactorial and diverse, mainly including infectious and non-infectious factors (19). The infectious factors of CVST mainly include meningitis, mastoiditis, otitis, sinusitis, and skin and gum infections. The main non-infectious factors include hereditary prothrombotic conditions (e.g., protein S deficiency, protein C deficiency, and antithrombin III

deficiency), antiphospholipid antibody syndrome (APLS), autoimmune diseases (e.g., systemic lupus erythematosus, behçet's disease, and vasculitis), malignancies, nephrotic syndrome, hematological diseases, and oral contraceptives. Nevertheless, the exact cause of the disease is still unknown in about 30% of CVST patients (20). Recently, many cases of COVID-19 infection causing intracranial venous sinus thrombosis have been reported (21–23). Endothelial dysfunction occurs during COVID-2019 infection, leading to platelet adhesion, leukocyte aggregation, complement activation, and cytokine release, which results in microvascular thrombosis (24). Malignancy can cause the blood to be in a hypercoagulable state, which may be the cause of CVST. Since there was no weight loss, loss of appetite, anemia, or weakness, and routine examinations such as blood counts, biochemical markers, and tumor indicators came back negative, malignancy appeared improbable. We observed that this CVST patient had a mild elevation of D-dimer on day 5 after admission, which seemed to better corroborate the diagnosis of CVST. A meta-analysis indicated that D-dimer may be a useful diagnostic tool in the management of patients with suspected CVST (25). Therefore, if a patient presents CVST-related clinical manifestations, risk factors, and elevated D-dimer we need to be highly suspicious of CVST and should aggressively improve cranial imaging to clarify the diagnosis. However, a negative D-dimer does not completely exclude CVST, and further cranial imaging is warranted.

We also found that the patient had been diagnosed with nephrotic syndrome (NS) 1 month prior to the onset of the disease, and although the patient's family had described the disease was now cured. Furthermore, the patient was also not admitted for clinical manifestations associated with nephrotic syndromes, such as absence of massive proteinuria, hypoproteinemia, edema, and hyperlipidemia. Currently, there have been increasing reports of CVST in NS patients during treatment, and clinicians should be fully aware of this phenomenon (26–29). Recent studies have shown that hypercoagulability is one of the main pathogenic mechanisms for the development of CVST in patients (30, 31), and similarly, recent findings have shown that the blood of NS patients is in a hypercoagulable state (32). It is therefore hypothesized that the hypercoagulable state of blood in NS patients may be related to the occurrence of CVST. All reported risk factors for the hypercoagulable state in NS patients were summarized to draw the following conclusions (33, 34): (1) Hyperlipidemia: Increased synthesis of hepatic cholesterol, triglycerides, and lipoproteins in NS patients leads to hyperlipidemia and increased blood viscosity; (2) Hypoproteinemia: Decreased serum albumin results in systemic edema, fluid retention in the interstitial space, and insufficient effective blood volume, which leads to an increase in blood viscosity. In addition, because of the loss of protein, the liver compensates for the increase in protein synthesis, causing an imbalance in coagulation, anticoagulation, and fibrinolysis in the body; (3) Fibrinolytic system abnormalities: NS patients have a large amount of proteinuria, which reduces the protein content in the body and causes fibrinogen loss and hypoproteinemia, thus reducing the activity of the fibrinolytic system and further promoting a hypercoagulable state; (4) Increased fibrinogen: Higher fibrinogen can directly induce the aggregation of red blood cells and platelets, and increase blood viscosity, reducing blood flow, resulting in a hypercoagulable state of blood; (5) Thrombocytosis: NS patients have increased platelets and progressively reduced red blood cell deformability. In addition, the von Willebrand (vW) factor directs platelet aggregation to the vessel wall and increases platelet adhesion, and high platelet aggregation further aggravates the hypercoagulable state of the blood; (6) Iatrogenic factors: Patients with NS are hypovolemic due to massive diuretic use, which promotes thrombosis. Furthermore, NS patients receive long-term high-dose hormone therapy to treat primary disease, which can aggravate the hypercoagulable state of NS by stimulating platelet production, increasing the concentration of certain coagulation factors, aggravating the disorder of lipid metabolism, and decreasing fibrinolysis.

The patient's symptoms improved significantly on day 5 of anticoagulation therapy with an MMSE score of 22 and a MoCA score of 18. Subsequently, the patients were prescribed oral anticoagulants (warfarin) to maintain INR between 2 and 3 for 6 months or longer depending on the underlying cause. The patient's symptoms completely recovered 1 month after discharge, with an MMSE score of 28 and a MoCA score of 26. The treatment of CVST mainly consists of acute and long-term treatment, where the aim of the acute treatment is to prevent the progression of the thrombus and to recanalize the blocked sinus as much as possible, while the aim of the long-term treatment is to prevent the recurrence of the thrombus. Anticoagulation is the standard of care and the main therapy option for CVST patients throughout the acute period (35, 36). Anticoagulation therapy is effective in reducing the systemic hypercoagulability of CVST patients, reducing the spread and progression of thrombosis, and preventing the recurrence of CVST. Guidelines for CVST recommend that patients with CVST without contraindications to anticoagulation should be treated with anticoagulation as soon as possible, regardless of whether they have intracranial hemorrhage (36). Anticoagulants commonly administered to CVST patients in the acute phase included regular heparin and LMWH, with subcutaneous LMWH doses adjusted to patient weight being more effective and with a lower risk of bleeding than plain heparin (37). Patients with CVST should generally continue long-term treatment with oral anticoagulants, commonly warfarin, after the acute phase of anticoagulation; In principle, warfarin should be repeated with LMWH for 3–5 days, and LMWH should be discontinued after the maintenance of the INR between 2 and 3, and the warfarin dosage should be adjusted periodically to maintain the INR

between 2 and 3 in accordance with the INR (5). However, the duration of oral anticoagulant therapy should be considered on the basis of individual genetic factors, triggers, recurrence, follow-up, and possible bleeding risk.

This patient had an excellent response to treatment. As a result, the patient's extraordinarily good treatment success and full recovery from cognitive impairment constitute a significant strength of this study. Nevertheless, our study has several limitations. First, this is a case study of only one individual and therefore no broad conclusions or recommendations may be drawn. Second, although the patient's MRV demonstrated thrombosis, the exact cause of the thrombosis was not found. Third, we did not conduct a second MRV examination to determine whether the high signal defect of blood flow in the implicated venous sinus was reevaluated.

In summary, the clinical heterogeneity of CVST increased the difficulty and complexity of our diagnosis. This case provides an excellent example of the utility of diagnostic imaging in the diagnosis of rare diseases such as CVST, which should be taken into account for differential diagnosis of RPD. This case study also provides new information for the diagnosis of CVST and the classification of the clinical characteristics.

Data availability statement

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

Ethics statement

Written informed consent was obtained from the individual for the publication of any potentially identifiable images or data included in this article.

Author contributions

YL wrote the case report under the guidance of MZ. MX was the neurologist who treated the patient. MW from radiology was responsible for the interpretation of images. All authors were involved in making appropriate changes as needed and approved the final case report.

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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.

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References

1. Bousser MG, Ferro JM. Cerebral venous thrombosis: an update. *Lancet Neurol.* (2007) 6:162–70. doi: 10.1016/S1474-4422(07)70029-7

2. Ageno W, Beyer-Westendorf J, Garcia DA, Lazo-Langner A, McBane RD, Paciaroni M. Guidance for the management of venous thrombosis in unusual sites. *J Thromb Thrombolysis.* (2016) 41:129–43. doi: 10.1007/s11239-015-1308-1

3. Silvis SM, Lindgren E, Hiltunen S, Devasagayam S, Scheres LJ, Jood K, et al. Postpartum period is a risk factorfor cerebral venous thrombosis: a case-control study. *Stroke.* (2019) 50:501–3. doi: 10.1161/STROKEAHA.118.023017

4. Ferro JM, Canhão P, Stam J, Bousser MG, Barinagarrementeria F. Prognosis of cerebral vein anddural sinus thrombosis: results of the international study on cerebral vein and dural sinus thrombosis (ISCVT). *Stroke.* (2004) 35:664–70. doi: 10.1161/01.STR.0000117571.76197.26

5. Ferro JM, Canhão P, Stam J, Bousser MG, Barinagarrementeria F, Massaro A, et al. Delay in the diagnosis of cerebral veinand dural sinus thrombosis: influence on outcome. *Stroke.* (2009) 40:3133–8. doi: 10.1161/STROKEAHA.109.553891

6. Ferro JM, Lopes MG, Rosas MJ, Fontes J. Delay in hospital admission of patients with cerebral vein and dural sinus thrombosis. *Cerebrovasc Dis.* (2005) 19:152–6. doi: 10.1159/000083248

7. Green M, Styles T, Russell T, Sada C, Jallow E, Stewart J, et al. Non-genetic and genetic risk facors for adult cerebral venous thrombosis. *Thromb Res.* (2018) 169:15–22. doi: 10.1016/j.thromres.2018.07.005

8. Jin C, Pu J, Zhou Z, Chen X, Wu J, Zhang B. Rapidly progressive cognitive impairment: an unusual presentation of cerebral venous thrombosis caused by JAK2 V617F-positive primary myelofibrosis: a case report. *Medicine.* (2020) 99:e21757. doi: 10.1097/MD.0000000000021757

9. Goyal G, Charan A, Singh R. Clinical presentation, neuroimaging findings, and predictors of brain parenchymal lesions in cerebral vein and dural sinus thrombosis: a retrospective study. *Ann Indian Acad Neurol.* (2018) 21:203–8. doi: 10.4103/aian.AIAN_470_17

10. Hermann P, Zerr I. Rapidly progressive dementias-aetiologies, diagnosis and management. *Nat Rev Neurol.* (2022) 18:363–76. doi: 10.1038/s41582-022-00659-0

11. Zerr I, Hermann P. Diagnostic challenges in rapidly progressive dementia. *Expert Rev Neurother.* (2018) 18:761–72. doi: 10.1080/14737175.2018.1519397

12. Markey KA, Mollan SP, Jensen RH, Sinclair AJ. Understanding idiopathic intracranial hypertension: mechanisms, management, and future directions. *Lancet Neurol.* (2016) 15:78–91. doi: 10.1016/S1474-4422(15)00298-7

13. Biousse V, Bruce BB, Newman NJ. Update on the pathophysiology and management of idiopathic intracranial hypertension. *J Neurol Neurosurg Psychiatry.* (2012) 83:488–94. doi: 10.1136/jnnp-2011-302029

14. Madriz PG, Cestari DM. An update of idiopathic intracranial hypertension. *Curr Opin Ophthalmol.* (2018) 29:495–502. doi: 10.1097/ICU.0000000000000518

15. Wall M, Kupersmith MJ, Kieburtz KD, Corbett JJ, Feldon SE, Friedman DI, et al. The idiopathic intracranial hypertension treatment trial: clinical profile at baseline. *JAMA Neurol.* (2014) 71:693–701. doi: 10.1001/jamaneurol.2014.133

16. Farb RI, Vanek I, Scott JN, Mikulis DJ, Willinsky RA, Tomlinson G, et al. Idiopathic intracranial hypertension: theprevalence and morphology of sinovenous stenosis. *Neurology.* (2003) 60:1418–24. doi: 10.1212/01.wnl.0000066683.34093.e2

17. Liguori C, Romigi A, Albanese M, Marciani MG, Placidi F, Friedman D, et al. Revised diagnostic criteria for the pseudotumor cerebri syndrome in adults and children. *Neurology.* (2014) 82:1752–3. doi: 10.1212/01.wnl.0000449937.36671.08

18. Yiğit H, Turan A, Ergün E, Koşar P, Koşar U. Time-resolved MR angiography of the intracranial venous system: an alternative MR venography technique. *Eur Radiol.* (2012) 22:980–9. doi: 10.1007/s00330-011-2330-0

19. Capecchi M, Abbattista M, Martinelli I. Cerebral venous sinus thrombosis. *J Thromb Haemost.* (2018) 16:1918–31. doi: 10.1111/jth.14210

20. Zuber M, Meder JF. Cerebral venous and sinus thrombosis. *Rev Prat.* (2006) 56:829–37. doi: 10.4103/0976-3147.120193

21. Hinduja A, Nalleballe K, Onteddu S, Kovvuru S, Hussein O. Impact of cerebral venous sinus thrombosis associated with COVID-19. *J Neurol Sci.* (2021) 425:117448. doi: 10.1016/j.jns.2021.117448

22. Ahmad SA, Kakamad FH, Mohamad HS, Salih BK, Mohammed SH, Abdulla BA, et al. Post COVID-19 cerebral venous sinus thrombosis; a case report. *Ann Med Surg (Lond).* (2021) 72:103031. doi: 10.1016/j.amsu.2021.103031

23. Anipindi M, Scott A, Joyce L, Wali S, Morginstin M. Case report: cerebral venous sinus thrombosis and COVID-19 infection. *Front Med.* (2021) 8:741594. doi: 10.3389/fmed.2021.741594

24. Gavriilaki E, Anyfanti P, Gavriilaki M, Lazaridis A, Douma S, Gkaliagkousi E. Endothelial dysfunction in COVID-19: lessons learned from coronaviruses. *Curr Hypertens Rep.* (2020) 22:63. doi: 10.1007/s11906-020-01078-6

25. Dentali F, Squizzato A, Marchesi C, Bonzini M, Ferro JM, Ageno W. D-dimer testing in the diagnosis of cerebral vein thrombosis: a systematic review and a meta-analysis of the literature. *J Thromb Haemost.* (2012) 10:582–9. doi: 10.1111/j.1538-7836.2012.04637.x

26. Navarro D, Ferreira AC, Viana H, Carvalho F, Nolasco F. Cavernous sinus thrombosis in a patient with nephrotic syndrome. *CEN Case Rep.* (2017) 6:136–9. doi: 10.1007/s13730-017-0260-7

27. Wang Y, Meng R, Duan J, Liu G, Chen J, Li S, et al. Nephrotic syndrome may be one of the important etiologies of cerebral venous sinus thrombosis. *J Stroke Cerebrovasc Dis.* (2016) 25:2415–22. doi: 10.1016/j.jstrokecerebrovasdis.2016.06.013

28. Hacifazlioglu C, Arslan E, Arslan EA, Buyukserbetci G. Cerebral venous sinus thrombosis with internal jugular venous thrombosis in a male patient with nephrotic syndrome. *Turk Neurosurg.* (2015) 25:980–3. doi: 10.5137/1019-5149.JTN.11355-14.1

29. Torres RA, Torres BR, de Castilho AS, Honorato R. Venous sinus thrombosis in a child with nephrotic syndrome: a case report and literature review. *Rev Bras Ter Intensiva.* (2014) 26:430–4. doi: 10.5935/0103-507X.2014006

30. Silvis SM, Middeldorp S, Zuurbier SM, Cannegieter SC, Coutinho JM. Risk factors for cerebral venous thrombosis. *Semin Thromb Hemost.* (2016) 42:622–31. doi: 10.1055/s-0036-1584132

31. Sidhom Y, Mansour M, Messelmani M, Derbali H, Fekih-Mrissa N, Zaouali J, et al. Cerebral venous thrombosis:clinical features, risk factors, and long-term outcome in a Tunisian cohort. *J Stroke Cerebrovasc Dis.* (2014) 23:1291–5. doi: 10.1016/j.jstrokecerebrovasdis.2013.10.025

32. Xu H, Chen K, Lin D, Dai L, Chen H, Xu Z. Cerebral venous sinus thrombosis in adult nephrotic syndrome. *Clin Nephrol.* (2010) 74:144–9. doi: 10.5414/cnp74144

33. Kato S, Chernyavsky S, Tokita JE, Shimada YJ, Homel P, Rosen H, et al. Relationship between proteinuria and venous thromboembolism. *J Thromb Thrombolysis.* (2010) 30:281–5. doi: 10.1007/s11239-010-0442-z

34. Yang Y, Lv J, Zhou F, Chen M, Wang R, Zhao M, et al. Risk factors of pulmonary thrombosis/embolism in nephrotic syndrome. *Am J Med Sci.* (2014) 348:394–8. doi: 10.1097/MAJ.0000000000000315

35. Saposnik G, Barinagarrementeria F, Brown RD Jr., Bushnell CD, Cucchiara B, Cushman M, et al. Diagnosis and management of cerebral venous thrombosis: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke.* (2011) 42:1158–92. doi: 10.1161/STR.0b013e31820a8364

36. Ferro JM, Bousser MG, Canhão P, Coutinho JM, Crassard I, Dentali F, et al. European Stroke Organization guideline for the diagnosis and treatment of cerebral venous thrombosis-endorsed by the European Academy of Neurology. *Eur J Neurol.* (2017) 24:1203–13. doi: 10.1111/ene.13381

37. Misra UK, Kalita J, Chandra S, Kumar B, Bansal V. Low molecular weight heparin versus unfractionated heparin in cerebral venous sinus thrombosis: a randomized controlled trial. *Eur J Neurol.* (2012) 9:1030–6. doi: 10.1111/j.1468-1331.2012.03690.x

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CASE REPORT
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Idiopathic Spontaneous Intraperitoneal Hemorrhage Due to Vascular Malformations in the Muscularis of the Stomach: A Case Report

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Idiopathic spontaneous intraperitoneal hemorrhage (ISIH) is a phenomenon caused by spontaneous rupture of intra-abdominal visceral vessels, and vascular malformations (VMs) leading to ISIH are rare in previously reported cases. VMs of the gastric wall, which are commonly located in the mucosa and submucosa, mostly lead to upper gastrointestinal bleeding rather than intraperitoneal hemorrhage. To our knowledge, this is the first report of ISIH caused by VMs in gastric muscularis. In the current case, a 22-year-old male patient presented with sudden abdominal pain for 4 h, accompanied by tachycardia and hypotension. CT revealed a hematoma in the omental bursa and fluids in abdominopelvic cavities. Then intraperitoneal hemorrhage was confirmed after abdominal paracentesis. Furthermore, ultrasonic gastroscopy indicated that vascular malformation in the muscularis of the stomach probably led to intraperitoneal hemorrhage. The patient recovered after conservative treatment based on fluid resuscitation and remained stable for 12 months of follow-up. This case suggests that VMs located in the gastrointestinal tract may lead to ISIH and ultrasonic gastroscopy is helpful in the diagnosis of VMs in the gastrointestinal tract.

Keywords: idiopathic spontaneous intraperitoneal hemorrhage, abdominal apoplexy, vascular malformation, endoscopic ultrasonography, case report

INTRODUCTION

Idiopathic spontaneous intraperitoneal hemorrhage (ISIH), which was once labeled abdominal apoplexy, is a rare and potentially fatal condition caused by a spontaneous tear of intraperitoneal visceral vessels (1, 2). The presentation varies from non-specific abdominal pain to hemodynamic instability depending on the location and severity of bleeding (3). Surgical exploration remains a major diagnostic and therapeutic modality (4). In previously reported cases, ISIHs were commonly

caused by atherosclerosis, aneurysms, vasculitis, etc. (2, 4). ISIH caused by vascular malformations (VMs) is rarely seen (5). VMs are congenital anomalies that can affect each part of the vasculature (6). About 90% of gastrointestinal VMs occur in the small intestine, leading to gastrointestinal bleeding (7, 8). To our knowledge, this is the first case of ISIH due to VMs in the muscularis of the stomach, which has some guidance for the etiology, diagnosis, and treatment of ISIH.

CASE PRESENTATION

A 22-year-old Asian man was admitted with "severe mid-upper abdomen pain for 4 h." He presented persistent, severe pain in the upper abdomen and pain radiating to the left shoulder, accompanied by dizziness, palpitations, amaurosis, and cold sweats. He did not receive any treatment before the admission and denied surgery, trauma, alcohol intake, strenuous exercise, and use of non-steroidal anti-inflammatory drugs before the onset. No family history of related illness was reported. On physical examination, he was noted to have tachycardia (heart rate: 106 beats per min) and hypotension (blood pressure: 81/50 mmHg). Mild abdominal distension, epigastric tenderness, and rebound pain were also noted during physical examination.

Initial laboratory evaluation revealed a white cell count (WBC) of 16.82×10^9/L, hemoglobin (HGB) of 15 g/dL, and hematocrit (HCT) of 46.3% (**Table 1**). Abdominal enhanced computed tomography (CT) showed a 10×5.2 cm^2 hemoperitoneum in the gastro-pancreatic gap in addition to the massive hemoperitoneum around the spleen and in the rectovesical space, which was connected with the hemoperitoneum around the liver through the Winslow foramen (**Figure 1A**); and the size and density of each solid organ were normal. Subsequently, the patient underwent diagnostic abdominal paracentesis, which further confirmed that the intraperitoneal fluid was blood. Gastroscopy showed that the gastric mucosa was smooth, and no ulcer or bleeding spot was observed in the upper digestive tract (**Figure 1B**). Fluid resuscitation was conducted immediately after admission. Although blood routine tests revealed a downward trend of hemoglobin and hematocrit (HGB 13.1 g/dL, HCT of 37.8%), the patient's condition tended to be stabilized (heart rate: 88 beats per min; blood pressure: 130/75 mmHg). Given the success of rehydration therapy and ambiguous source of hemorrhage, conservative medical treatment (Fluid resuscitation, Octreotide Acetate, Pantoprazole, etc.) was initially adopted. Meanwhile, the patient was under close observation, and emergency surgical exploration would be conducted if necessary.

To clarify the reason for the hemorrhage, enhanced magnetic resonance imaging (MRI) was performed, which revealed a hematoma in the gastro-pancreatic gap, without signs of space-occupying lesion and other indications about the source of bleeding (**Figure 2A**). Later, we performed endoscopic ultrasonography (EUS) on him. A mixed hypoechoic and anechoic lesion was seen between the pancreatic tail and the gastric wall, which was closely related to the peripheral blood vessels and was considered to be a hematoma combined with the previous imaging reports. Part of the muscularis propria was connected to the hematoma. In addition, Doppler ultrasonography showed that the blood flow signal was continuous between the muscularis propria and hematoma, which was considered as the vascular malformation in the muscularis of the stomach (**Figure 2B**). He was ultimately diagnosed with "intraperitoneal hemorrhage due to VMs in the muscularis of the stomach" based on the CT images and the EUS results, combined with the clinical manifestation. The patient was young and had fertility demands shortly. He was reluctant to accept CTA and interventional therapy due to the radiation dose of CTA and endovascular treatment. Also, considering that no obvious bleeding site was found on enhanced CT, we were concerned that the intervention would not be able to locate the lesion, so we did not conduct invasive treatment, such as endovascular management or surgery. The patient recovered gradually after conservative treatment without signs of continuous bleeding, and he was discharged after 4 days (the final laboratory data are shown in **Table 1**). We performed follow-ups for the patient at the hospital outpatient department at 6 months intervals. The follow-up after 1 year showed that the patient had no signs of recurrence (**Figure 3**). The timeline from emergency to follow-up is presented in **Figure 3**.

TABLE 1 | Labs during hospitalization.

Laboratory test (Normal range)	Value^1	Value^2	Value^3	Value^4	Value^5
WBC (3.5–9.5 × 10^9/L)	16.82	12.18	10.24	8.57	7.64
RBC (4.3–5.8 × 10^{12}/L)	4.73	4.14	3.87	3.52	3.76
HGB (13.0–17.5 g/dL)	15.0	13.1	12.2	11.1	11.9
HCT (40.0–50.0%)	46.3%	37.8%	34.9%	31.6%	33.9%

WBC, white blood cells; RBC, red blood cells; HGB, hemoglobin; PLT, platelet; HCT, hematocrit; Value^1: Initial labs on admission; Value^{2,3,4}: Labs of decompensation period every 5 h after expanding blood volume; Value^5: Final labs at discharge.

DISCUSSION

Idiopathic spontaneous intraperitoneal hemorrhage (ISIH), originally known as abdominal apoplexy, is used to describe atypical and non-traumatic spontaneous intraperitoneal or retroperitoneal bleeding, excluding typical intraperitoneal bleeding caused by ectopic pregnancy, malignant tumor, aortic aneurysm or dissection, visceral rupture, trauma, and iatrogenic injury (9, 10). Abdominal aneurysm is the main reason of ISIH, of which splenic aneurysm is the most common (60%) and the gastric and gastroepiploic aneurysms are responsible for only 3% (2). The rupture of a splenic aneurysm is mostly caused by the erosion of the vessel wall owing to pancreatic enzymes released in pancreatitis (in about 50% of cases) and results in over 90% mortality (11). Besides abdominal aneurysms, atherosclerosis is considered to be another important cause of ISIH. The presumed mechanism

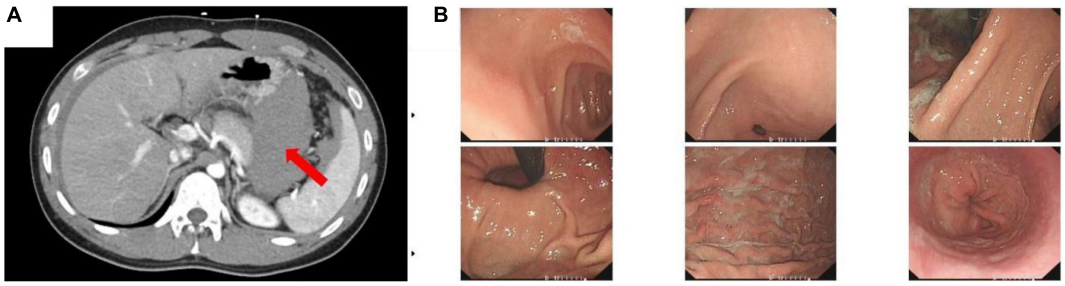

FIGURE 1 | (A) Enhanced computed tomography (CT) of the abdomen. Red arrow showed a 10 × 5.2 cm^2 high-density shadow in the gastro-pancreatic gap. **(B)** Gastroscopy showed that the gastric mucosa was smooth and had no ulcer or bleeding spot in the upper digestive tract.

is the weakness of the tunica media in the injured vessel, which ruptures when blood pressure suddenly rises (2). As described in the report, a patient was presented to our hospital with sudden, non-specific abdominal pain and, was finally diagnosed with ISIH caused by VMs in gastric muscularis mainly relying on EUS.

VMs are diseases with unpredictable clinical evolution and manifestations, which lead to life-threatening conditions in severe cases (12). The splenic artery is the most common vessel responsible for ISIH, while the gastric or gastroepiploic artery accounts for only 4% (13). The VMs of the gastric wall generally occur in the gastric mucosa and submucosa, so it usually causes intragastric hemorrhage (14–16). We checked the relevant literature and found that this is the first case of a vessel in the muscularis of the stomach inducing intraperitoneal bleeding, which suggests that the blood vessels of the gastric serosal surface, and even the entire stomach wall, should be considered when looking for the bleeding sites of ISIH.

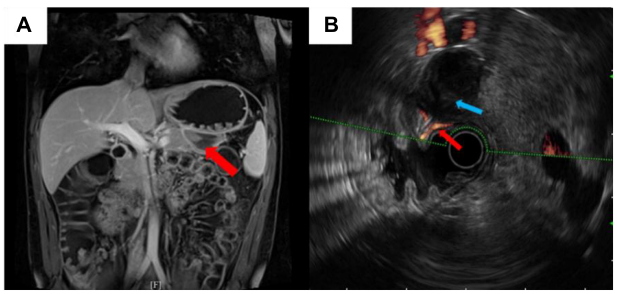

FIGURE 2 | (A) Enhanced magnetic resonance imaging (MRI) revealed no sign of space-occupying lesion. Red arrow showed a hematoma in the gastro-pancreatic gap. **(B)** Endoscopic ultrasonography (EUS) illustrated the vascular malformation in the muscularis of the stomach. Blue arrow showed a hypoechoic shadow. Red arrow showed a 1.3-mm diameter blood flow signal was continuous between the muscularis propria and the hypoechoic shadow.

According to the available literature, optimal diagnostic and therapeutic evidence of ISIH remain controversial (12). CT angiography (CTA) or digital subtraction angiography (DSA) is considered to be a primary tool for diagnosing ISIH, localizing the bleeding vessel if the hemodynamic and clinical status of patients enables it (3, 17). The non-invasiveness and short acquisition time of CTA give it more advantages for acute bleeding and a higher priority than DSA (18, 19). However, some literature points out that CTA may have false-negative results which can occur if the bleeding is not obvious at the time of the scan due to the short acquisition time (19–21). Considering that the patient had no indication of active bleeding, and it was difficult to locate the culprit vessel using CTA, we did not perform CTA in this case. EUS can provide real-time images of the gastrointestinal wall and blood vessels of adjacent tissues and has been applied to diagnostic interventions (22, 23). VMs can appear as a persistent blood flow signal in the parietal layer of the gastrointestinal tract under EUS guidance (24).

Surgery and embolization are methods for the treatment of VMs. Endovascular embolization is less invasive and recommended in most cases (9). However, it is difficult to locate culprit vessels in cases without active bleeding, so endovascular embolization was not selected in this case. Exploratory surgery is usually employed when CTA cannot be performed or failed to identify the culprit vessel (17). Exploratory laparotomy or laparoscopic exploration can detect the bleeding site, achieve initial hemostasis (25), and take tissue for pathological diagnosis. However, nearly 40% of operations failed in finding the bleeding site of the patient (9, 26). Therefore, due to the lack of continuous bleeding, we did not surgically explore and treat the patient. For younger patients without immediate surgical management, we suggest close attention and a conservative administration, including somatostatin or somatostatin analogs (such as octreotide), which have been concluded as an effective therapy for hemorrhage of gastrointestinal VMs (27, 28). However, for patients with decompensated or life-threatening conditions, we recommend emergency surgery; for patients with recurrent bleeding, surgery may be also finally required (29).

The limitations of our study include the lack of objectivity of imaging and the ambiguous assessment of therapeutic effect. Different from CT or MRI, EUS is operator dependent, and the analysis of the images is subjective. There is a risk of recurrence because the patient did not receive surgical treatment and EUS revealed that VMs still existed, though he is currently in stable condition. The length of follow-up time is not sufficient to accurately evaluate the effect of conservative treatment up to now.

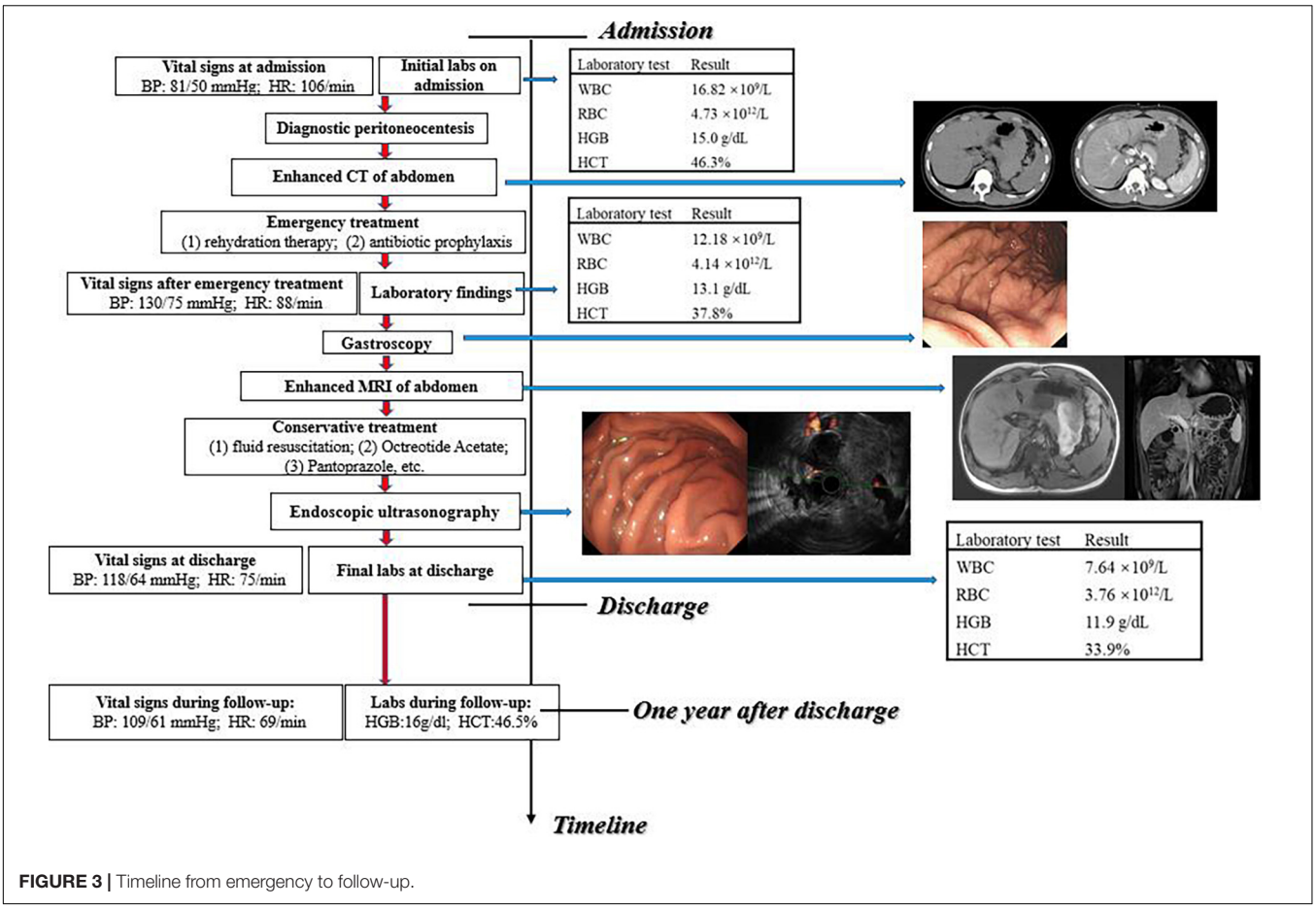

FIGURE 3 | Timeline from emergency to follow-up.

CONCLUSION

For patients with spontaneous intraperitoneal hemorrhage, particularly ISIH, VM rupture should be considered when the disease cannot be identified after excluding the common causes. VMs in the gastric wall may lead to intraperitoneal hemorrhage in addition to intragastric bleeding. If it is speculated that the vascular malformation is located in the gastric wall, EUS may be a new alternative diagnostic approach for CTA. Due to the lack of evidence, it is difficult to standardize the treatment of such patients. Conservative treatment can be chosen temporarily for patients without immediate surgery or indication of embolism.

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 author/s.

ETHICS STATEMENT

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

YHZ, YCZ, WL, and SL participated in the diagnosis and treatment of patients. YHZ and YCZ wrote the manuscript. WL provided professional opinions on diagnosis. SL reviewed and revised the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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REFERENCES

1. Green WT, Powers JH. Intra-abdominal apoplexy. *Ann Surg.* (1931) 93:1070–4. doi: 10.1097/00000658-193105000-00013
2. Wang H, Xiu D. Abdominal apoplexy because of the rupture of gastroduodenal artery and inferior pancreaticoduodenal artery: a case report. *Medicine.* (2017) 96:e8264. doi: 10.1097/MD.0000000000008264
3. Qaraqe TM, Abou Daher A, Alami RS. Abdominal apoplexy: a rare case of spontaneous middle colic artery rupture with transverse colectomy. *Int J Surg Case Rep.* (2021) 81:105835. doi: 10.1016/j.ijscr.2021.105835
4. Zeinalpour A, Aghili A, Gholizadeh B. Abdominal apoplexy due to rupture of inferior pancreaticoduodenal artery: a rare case of acute abdomen. *Caspian J Intern Med.* (2021) 12(Suppl 2):S479–81. doi: 10.22088/cjim.12.0.479
5. Saeed Y, Farkas Z, Azeez S. Idiopathic spontaneous intraperitoneal hemorrhage due to rupture of short gastric artery presenting as massive gastrointestinal bleeding: a rare case presentation and literature review. *Cureus.* (2020) 12:e11499. doi: 10.7759/cureus.11499
6. Della Rosa N, Bertozzi N, Adani R. Vascular malformation and their unpredictable evolution: a true challenge for physicians. *Acta Biomed.* (2020) 91:e2020067. doi: 10.23750/abm.v91i3.8298
7. Redondo-Cerezo E, Gomez-Ruiz CJ, Sanchez-Manjavacas N, Vinuelas M, Jimeno C, Perez-Vigara G, et al. Long-term follow-up of patients with small-bowel angiodysplasia on capsule endoscopy. determinants of a higher clinical impact and rebleeding rate. *Rev Esp Enferm Dig.* (2008) 100:202–7. doi: 10.4321/s1130-01082008000400002
8. Chen H, Fu S, Feng N, Chen H, Gao Y, Zhao Y, et al. Bleeding recurrence in patients with gastrointestinal vascular malformation after thalidomide. *Medicine.* (2016) 95:e4606. doi: 10.1097/MD.0000000000004606
9. Cawyer JC, Stone CK. Abdominal apoplexy: a case report and review. *J Emerg Med.* (2011) 40:e49–52. doi: 10.1016/j.jemermed.2007.11.080
10. Harbour LN, Koch MS, Louis TH, Fulmer JM, Guileyardo JM. Abdominal apoplexy: two unusual cases of hemoperitoneum. *Proceedings.* (2012) 25:16–9. doi: 10.1080/08998280.2012.11928772
11. Corvino F, Giurazza F, Ierardi AM, Lucatelli P, Basile A, Corvino A, et al. Splenic artery pseudoaneurysms: the role of Ce-Ct for diagnosis and treatment planning. *Diagnostics.* (2022) 12:1012. doi: 10.3390/diagnostics12041012
12. Cucuruz B, Koller M, Pfleiderer R, Geisthoff U, Meyer L, Kapp F, et al. Towards a better treatment of patients with vascular malformations: certified interdisciplinary centers are mandatory. *Z Evid Fortbild Qual Gesundhwes.* (2022) 168:1–7. doi: 10.1016/j.zefq.2021.11.003
13. Negmadjanov U, Ohanisian L, Rubay D, Hristov B, Belizon A. Abdominal apoplexy: a case study of idiopathic spontaneous lesser sac hematoma. *Cureus.* (2019) 11:e4937. doi: 10.7759/cureus.4937
14. Athanasoulis CA, Galdabini JJ, Waltman AC, Novelline RA, Greenfield AJ, Ezpeleta ML. Angiodysplasia of the colon: a cause of rectal bleeding. *Cardiovasc Radiol.* (1977) 1:3–13. doi: 10.1007/BF02551967
15. Gordon FH, Watkinson A, Hodgson H. Vascular malformations of the gastrointestinal tract. *Best Pract Res Clin Gastroenterol.* (2001) 15:41–58. doi: 10.1053/bega.2000.0155
16. Handra-Luca A, Montgomery E. Vascular malformations and hemangiolymphangiomas of the gastrointestinal tract: morphological features and clinical impact. *Int J Clin Exp Pathol.* (2011) 4:430–43.
17. Law EK, Lee RK, Hung EH, Ng AW. Radiological diagnosis and management of idiopathic spontaneous intra-abdominal haemorrhage (abdominal apoplexy): a case series. *Abdom Imaging.* (2015) 40:343–51. doi: 10.1007/s00261-014-0220-z
18. Jansen IGH, Berkhemer OA, Yoo AJ, Vos JA, Lycklama ANGJ, Sprengers MES, et al. Comparison of Cta- and Dsa-based collateral flow assessment in patients with anterior circulation stroke. *AJNR Am J Neuroradiol.* (2016) 37:2037–42. doi: 10.3174/ajnr.A4878
19. Wortman JR, Landman W, Fulwadhva UP, Viscomi SG, Sodickson AD. Ct angiography for acute gastrointestinal bleeding: what the radiologist needs to know. *Br J Radiol.* (2017) 90:20170076. doi: 10.1259/bjr.20170076
20. O'Brien AC, Healy GM, Rutledge N, Patil A, McCann JWJ, Cantwell CP. Conventional angiography findings in hemodynamically unstable patients with acute abdominal hemorrhage and a negative Ct bleeding study. *CVIR Endovasc.* (2020) 3:22. doi: 10.1186/s42155-020-00112-7
21. Wong H, Hodgson L, Banfield J, Shankar JJS. Digital subtraction angiography for Ct angiogram negative haemorrhages. *Can J Neurol Sci.* (2018) 45:522–6. doi: 10.1017/cjn.2018.75
22. Chapman CG, Waxman I. Eus-guided portal vein sampling. *Endosc Ultrasound.* (2018) 7:240–5. doi: 10.4103/eus.eus_28_18
23. Yang J, Zhou Y. Combined Ercp and endoscopic ultrasonography: a new treatment for rare hemorrhage from a duodenal papillary vascular malformation. *Endoscopy.* (2021) 53:E108–9. doi: 10.1055/a-1202-9858
24. Vila JJ, Perez-Miranda M, Basterra M, Gomez M, Fernandez-Urien I, Jimenez FJ. Endoscopic ultrasound-guided therapy of a rectal dieulafoy lesion. *Endoscopy.* (2014) 46(Suppl 1):E84–5. doi: 10.1055/s-0033-1344776
25. Jakob DA, Liasidis P, Schellenberg M, Matsushima K, Lam L, Demetriades D, et al. Intra-abdominal hemorrhage control: the need for routine four-quadrant packing explored. *World J Surg.* (2021) 45:1014–20. doi: 10.1007/s00268-020-05906-3
26. Hassani KI, Bounekar A, Gruss JM. Spontaneous rupture of the right gastroepiploic artery: unusual cause of acute abdomen and shock. *World J Emerg Surg.* (2009) 4:24. doi: 10.1186/1749-7922-4-24
27. Bauditz J. Effective treatment of gastrointestinal bleeding with thalidomide–chances and limitations. *World J Gastroenterol.* (2016) 22:3158–64. doi: 10.3748/wjg.v22.i11.3158
28. Iannone A, Principi M, Barone M, Losurdo G, Ierardi E, Di Leo A. Gastrointestinal bleeding from vascular malformations: is octreotide effective to rescue difficult-to-treat patients? *Clin Res Hepatol Gastroenterol.* (2016) 40:373–7. doi: 10.1016/j.clinre.2016.02.003
29. Sami SS, Al-Araji SA, Ragunath K. Review article: gastrointestinal angiodysplasia - pathogenesis, diagnosis and management. *Aliment Pharmacol Ther.* (2014) 39:15–34. doi: 10.1111/apt.12527

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Case report: Successful thromboprophylaxis with enoxaparin in a pregnant woman with internal jugular vein agenesis

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Internal jugular agenesis is a vascular malformation that is often associated with a history of recurrent headache. Due to the resulting abnormalities in intracranial venous drainage, it may be complicated by neurological dysfunction, such as intracranial hypertension, intracranial micro-thromboses, and neurodegenerative diseases such as multiple sclerosis. The simultaneous presence of jugular vein agenesis and thrombosis is possible in cases of acute illness, hormonal treatment, pregnancy, hypomobility, or venous drainage abnormalities (VDA) (e.g., May-Thurner syndrome). In particular, the literature still lacks data on thromboprophylaxis in pregnant women with jugular vein agenesis. Here, we report a positive experience with prophylaxis using enoxaparin during pregnancy in a patient with internal jugular agenesis.

KEYWORDS

venous thromboembolism, internal jugular agenesis, thromboprophylaxis, enoxaparin, pregnancy

Introduction

The internal jugular vein is crucial for the drainage of intracranial veins (1). The literature contains numerous reports of venous drainage abnormalities (1–3), each of which is associated with the possibility of increased intracranial venous pressure and, thus, with relevant symptoms and signs such as headache, intracranial micro-thrombosis, intracranial hypertension, and seizures, as well as with neurodegenerative diseases (e.g., multiple sclerosis).

The presence of venous malformations, such as May-Thurner syndrome, internal jugular agenesis, or other types of vascular malformation associated with prothrombotic conditions such as pregnancy, may increase the risk of venous thrombosis. However, data concerning the association of venous malformation with venous thrombosis in pregnancy are lacking in the literature.

We may postulate that internal jugular vein agenesis may be associated with an increased risk of developing venous thromboembolism (VTE) in the context of additional prothrombotic risk factors such as pregnancy. An association between venous malformation and venous thrombosis in the presence of other prothrombotic conditions such as those reported above has, in fact, already been found in May-Thurner syndrome and vena cava agenesis (4, 5).

Here, we report an intriguing case of internal jugular vein agenesis in a pregnant young woman with a history of recurrent headache who underwent successful thromboprophylaxis treatment throughout her pregnancy.

Case history

A 30-year-old pregnant woman was admitted to the outpatient clinic for thrombotic disorders at the Hospital Rizzoli in Lacco Ameno, Italy, due to an abnormal varicose vein located in her neck and a history of recurrent first-trimester miscarriage. During clinical evaluation, she specified that she was pregnant at week 8 (i.e., was experiencing pregnancy-induced amenorrhea). She was taking 200 mg of progesterone twice daily for miscarriage prevention (6), 15 mg calcium folinate, and 100 mg of aspirin because she was homozygous for MTHFR c677t (7). The patient's laboratory data are summarized in Table 1.

A differential diagnosis was made because of a positive history of recurrent miscarriage (Table 2) (8). Mild thrombophilia due to the predisposition to develop hyperhomocysteinemia (caused by the MTHFR 677 TT genotype) was the only detectable risk factor for recurrent miscarriage (defined as three spontaneous abortions during the first trimester of pregnancy without a detectable cause).

TABLE 1 Laboratory findings of reported patient.

Test	Result	Normal values
Factor V Leiden polymorphism	Wild type	Wild type
Prothrombin A20210G polymorphism	Wild type	Wild type
Protein S levels (%)	63%	50–120
Protein C levels (%)	65%	50–120
AT III levels (%)	71 (%)	60–120
d-dimer (ng/dL)	495	<500
Fibrinogen (mg/dL)	396	200–400
Haematocrit (%)	31	<45
Platelets (mmcube)	320,000	140,000–400,000
Homocysteine (mMol/L)	8	<15

mg, milligrams; ng, nanograms; AT III, antithrombin III; LAC, lupus anticoagulant; antiβ2GPI, antibeta2 glycoprotein I antibodies; mm^3, cubic millimeters; mMol, millimole. Anticardiolipin antibodies were dosed using fluoroenzyme immunoassay methods and kits, while antibeta2GP1 antibodies were tested *via* ELISA.

An elicitation of personal history to evaluate the presence of the unusual varicose vein did not reveal any previous surgical approaches, trauma to the neck, or the recent positioning of venous lines that could explain its development. Furthermore, no acute or chronic medical illness or acute infection was detected. For this reason, the probability of detecting an internal jugular vein thrombosis was considered low, and pharmacological antiplatelet treatment with 100 mg daily aspirin was confirmed.

The patient underwent a blood count and homocysteine check every month, as well as obstetric follow-up to prevent intrauterine growth retardation (IUGR).

However, 3 weeks after the first clinical evaluation, the patient was referred for an increase in the volume of the varicose vein in the neck, associated with cough (Figure 1). An color Doppler ultrasound examination was then scheduled and performed after a few hours.

The ultrasound scan revealed an enlarged left internal jugular vein with a large collateral venous circle involving the superior thyroidal vein, ipsilateral external jugular vein, and vertebral vein; on the right side, only the vertebral vein was visible, and it appeared to be of increased size. Therefore, the hypothesis of hypoplasia or agenesis of the right internal jugular

TABLE 2 Differential diagnosis performed by the patient for recurrent miscarriages.

Hysteroscopy for uterine malformation
Hormonal dosages to detect ovarian and thyroideal oligosynptomatic diseases
Uterine swab to detect uterine infections
Kariotype to look for chromosomal abnormalities
Immunological evaluation for antiphospholipid syndrome
Inherited thrombophilia

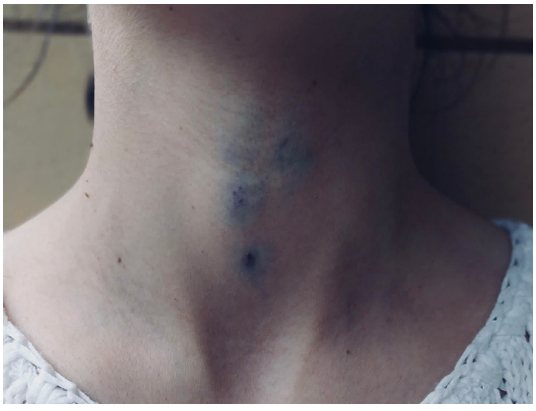

FIGURE 1
Collateral variceal venous circulation of the neck due to the presence of internal jugular agenesis.

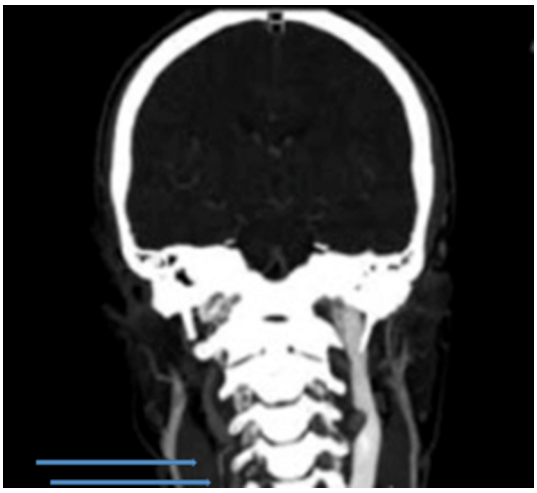

FIGURE 2
Magnetic resonance imaging with evidence of internal jugular agenesis.

vein was suggested. The blood flow in those veins was regular, and there was no ultrasonographic evidence of other vascular diseases. An MRI of the head and neck confirmed the hypothesis of venous agenesis (Figure 2).

Given the risk of jugular vein thrombosis, the use of a different method of thromboprophylaxis was considered. No guidelines are available in the literature, in fact, to suggest the proper approach in this clinical context, even though an increased thrombotic risk during pregnancy has been found for other venous malformations, such as May-Thurner syndrome and vena cava agenesis (4, 5). Our patient had several contemporaneous (if mild) thrombotic risk factors: a history of recurrent miscarriage, ongoing pregnancy and the associated hormonal treatment (although progesterone is less prothrombotic than other hormonal treatments), and venous malformation. For this reason, we stopped the administration of aspirin and we suggested starting a pharmacologic thromboprophylaxis with enoxaparin 40 mg daily until childbirth, to prevent the onset of venous thrombosis and also to prevent possible miscarriage.

Given the patient's atypical condition, periodic clinical and instrumental monitoring was planned, consisting of a monthly ultrasound of the veins of the leg and neck and an obstetric ultrasound to monitor uterine vascular pressure and gestational growth. Blood cell count, fibrinogen, prothrombin time, and activated partial thromboplastin time were also evaluated monthly (coagulation parameters and platelet count are often monitored during long-term prophylaxis with enoxaparin, particularly for pregnant women or patients with kidney failure, because of the modification of the half-life of low-molecular-weight heparin in these contexts).

No vascular complications were detected during the remainder of the pregnancy. The patient delivered a live-birth baby (weighing 3.0 kg) *via* cesarean section. Enoxaparin was prolonged for 4 weeks after delivery because of the surgical approach; the patient was then dismissed from our practice, having experienced no complications.

Written informed consent was obtained from the patient to describe her clinical picture.

Discussion

Venous drainage abnormality is a silent condition that is not life-threatening for the patient. From a hemodynamic point of view, it is associated with low-flow and low-resistance veins, and it therefore confers a low risk of complications such as rupture or microthrombosis (9). With the increased use of MRI, microthromboses are more frequently being diagnosed (10, 11), as is reflected in the literature (12–14). However, when these abnormalities are associated with other prothrombotic conditions such as hormone intake, pregnancy, and acute medical illness, the potential risk for thrombotic complications in the cerebral vessels increases, as was recently demonstrated by the association between cerebral venous thrombosis and anti–SARS-CoV-2 vaccines (15, 16). At these sites, where the veins take a narrower and less linear course, conditions for the formation of venous thrombosis are more favorable. Nevertheless, after cerebral venous thrombosis, the hypothesis of an undiagnosed occult venous drainage abnormality (17) is frequently considered.

Prophylactic strategies to prevent venous thrombosis in the setting of venous malformation have been inconsistently explored in clinical practice and in the literature; prophylactic strategies to prevent thrombosis in cases of internal jugular vein hypoplasia or agenesis are even more limited. In our case, the venous drainage abnormality resulted from the absence of the right jugular vein, which hemodynamically favored increased inflow into the contralateral. This is even more interesting in the context of our patient, who possessed numerous mild risk factors for venous thromboembolism, such as pregnancy, obstetrical history of recurrent miscarriage, and hormone treatment. We therefore deduced an urgent need for thromboprophylaxis treatment, even though there were no precise guidelines or recommendations for a special case such as this one. We know, however, that pregnancy is a condition with a specific thromboembolic risk (18), and for this reason, there are special guidelines for the prevention, diagnosis, and treatment of venous thromboembolism in pregnancy (19, 20). Deep venous thrombosis (DVT) during pregnancy is associated with high mortality, morbidity, and health care costs (21). In particular, the last trimester of pregnancy seems to be the most dangerous in that it carries the highest risk of thrombosis, especially

sinus thrombosis (22). During pregnancy, all three components of Virchow's triad are strongly represented: venous stasis, hypercoagulability, and endothelial dysfunction. Furthermore, increased uterine size results in slowed venous flow and blood stasis. This in turn also promotes endothelial damage. For this reason, after 30 weeks of gestation, there is a reduction in flow velocity, favoring the onset of deep venous thrombosis in the lower extremities and pelvis (23). Moreover, pregnancy-induced hypercoagulability, with its increased levels of coagulation factors II, VII, VIII, and X, as well as decreased levels of proteins S and C, contributes to thrombotic risk in addition to any pre-existing inherited predisposition to thrombosis (24).

Low-molecular-weight heparin is most commonly recommended for the prevention of venous thromboembolism in pregnancy (19). In contrast, there is no indication for direct oral anticoagulant therapy (20) during the gestational months, while low-dose aspirin prophylaxis (81 mg/day) is recommended only for women at high risk of pre-eclampsia and should be initiated between 12 and 28 weeks of gestation (25).

Conclusions

We reported a singular case of a pregnant woman with a rare vascular malformation, mild thrombophilia, and a risk of developing venous thrombosis. This anatomical venous malformation is only rarely described in case reports, and has not yet featured in randomized clinical trials. Because the patient was at risk of developing thrombotic complications, such as miscarriage or venous thrombosis, due to her medical history, we performed a thorough clinical evaluation of all clinical conditions and risk factors that may favor the adoption of a prolonged pharmacological thromboprophylaxis. As we await larger clinical trials to confirm the validity of this method, we hope that our precautional medical approach can be of use in similar cases.

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. The patients/participants provided their written informed consent to participate in this study.

Author contributions

PD wrote the draft. EI reviewed the draft. DC and LO took clinical information. 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.

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References

1. Mumtaz S, Singh M. Surgical review of the anatomical variations of the internal jugular vein: an update for head and neck surgeons. *Ann R Coll Surg Engl.* (2019) 101:2–6. doi: 10.1308/rcsann.2018.0185

2. Müller HR. Ultrasonic imaging of the internal jugular vein. *J Neuroimaging.* (1991) 1:74–8. doi: 10.1111/jon19911274

3. Lv X, Wu Z. Anatomic variations of internal jugular vein, inferior petrosal sinus and its confluence pattern: Implications in inferior petrosal sinus catheterization. *Interv Neuroradiol.* (2015) 21:769–73. doi: 10.1177/1753425915590067

4. Harbin MM, Lutsey PL. May-Thurner syndrome: History of understanding and need for defining population prevalence. *J Thromb Haemost.* (2020) 18:534–42. doi: 10.1111/jth.14707

5. Tufano A, López-Jiménez L, Bikdeli B, García-Bragado F, Mazzolai L, Amitrano M, et al. Inferior vena cava agenesis in patients with lower limb deep vein thrombosis in the RIETE registry When and why to suspect. *Int J Cardiol.* (2020) 305:115–9. doi: 10.1016/j.ijcard.2020.01.013

6. Wise J. NICE recommends progesterone to prevent early miscarriage. *BMJ.* (2021) 375:n2896. doi: 10.1136/bmj.n2896

7. Zhao X, Zhao Y, Ping Y, Chen L, Feng X. Association between gene polymorphism of folate metabolism and recurrent spontaneous abortion in Asia: A Meta-analysis. *Medicine (Baltimore).* (2020) 99:e21962. doi: 10.1097/MD.0000000000021962

8. Recurrent Pregnancy Loss. *ESHRE Early Pregnancy Guideline Development Group.* Version 2. Recurrent pregnancy loss (eshre.eu). (2017).

9. Lasjaunias P, Burrows P, Planet C. Developmental venous anomalies (DVA): the so-called venous angioma. *Neurosurg Rev.* (1986) 9:233–42. doi: 10.1007/BF01743138

10. Field LR, Russell EJ. Spontaneous hemorrhage from a cerebral venous malformation related to thrombosis of the central draining vein: demonstration with angiography and serial MR. *AJNR Am J Neuroradiol.* (1995) 16:1885–8.

11. Agarwal A, Kanekar S, Kalapos P, Vijay K. Spontaneous thrombosis of developmental venous anomaly (DVA) with venous infarct and acute cerebellar ataxia. *Emerg Radiol.* (2014) 21:427–30. doi: 10.1007/s10140-014-1216-2

12. Entezami P, Boulos A, Yamamoto J, Adamo M. Paediatric presentation of intracranial haemorrhage due to thrombosis of a developmental venous anomaly. *BMJ Case Rep.* (2019) 12:227362. doi: 10.1136/bcr-2018-227362

13. Kiroglu Y, Oran I, Dalbasti T, Karabulut N, Calli C. Thrombosis of a drainage vein in developmental venous anomaly (DVA) leading venous infarction: a case report and review of the literature. *J Neuroimaging.* (2011) 21:197–201. doi: 10.1111/j.1552-6569.2009.00399.x

14. Amuluru K, Al-Mufti F, Hannaford S, Singh IP, Prestigiacomo CJ, Gandhi CD. Symptomatic Infratentorial Thrombosed Developmental Venous Anomaly: Case Report and Review of the Literature. *Interv Neurol.* (2016) 4:130–7. doi: 10.1159/000444028

15. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. *N Engl J Med.* (2021) 384:2124–30. doi: 10.1056/NEJMoa2104882

16. Chevassut T, Hunt BJ, Pavord S. VITT COVID-19 and the Expert Haematology Panel: The story of how the UK responded to emerging cases of vaccine-induced immune thrombocytopenia and thrombosis during the vaccination programme. *Clin Med (Lond).* (2021) 21:e600–2. doi: 10.7861/clinmed.2021-0488

17. Chang SJ, Rebchuk AD, Teal P, Honey CR, Field TS. COVID-19-Associated Cerebral Developmental Venous Anomaly Thrombosis With Hemorrhagic Transformation. *Stroke.* (2022) 53:e255–6. doi: 10.1161/STROKEAHA.122.039534

18. McLEAN KC, James AH. Diagnosis and Management of VTE in Pregnancy. *Clin Obstet Gynecol.* (2018) 61:206–218. doi: 10.1097/GRF.0000000000000354

19. Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO. VTE, thrombophilia, antithrombotic therapy, and pregnancy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest.* (2012) 141:e691S–e736S. doi: 10.1378/chest.11-2300

20. Konstantinides SV, Meyer G, Becattini C, Bueno H, Geersing GJ, Harjola VP, et al. 2019 ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS). *Eur Heart J.* (2020) 41:543–603. doi: 10.1093/eurheartj/ehz405

21. Devis P, Knuttinen MG. Deep venous thrombosis in pregnancy: incidence, pathogenesis and endovascular management. *Cardiovasc Diagn Ther.* (2017) 7:S309–19. doi: 10.21037/cdt.2017.10.08

22. Cantú C, Barinagarrementeria F. Cerebral venous thrombosis associated with pregnancy and puerperium. Review of 67 cases. *Stroke*. (1993) 24:1880–4. doi: 10.1161/01.STR.24.12.1880

23. Kujovich JL. Hormones and pregnancy: thromboembolic risks for women. *Br J Haematol.* (2004) 126:443–54. doi: 10.1111/j.1365-2141.2004.05041.x

24. Kovac MK, Lalic-Cosic SZ, Dmitrovic JM, Djordjevic VJ, Radojkovic DP. Thrombin generation, D-dimer and protein S in uncomplicated pregnancy. *Clin Chem Lab Med.* (2015) 53:1975–9. doi: 10.1515/cclm-2014-1030

25. ACOG Committee Opinion No. 743: Low-dose aspirin use during pregnancy. *Obstet Gynecol.* (2018) 132:e44–e52. doi: 10.1097/AOG.0000000000002708

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Predictors of use of direct oral anticoagulants in patients with venous thromboembolism: Findings from the Registro Informatizado Enfermedad Tromboembólica registry

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Background: Current guidelines recommend the use of direct oral anticoagulants (DOACs) for patients with venous thromboembolism (VTE). However little is known about the use of DOACs in daily practice.

Methods: We used the RIETE registry to identify predictors of use of DOACs for initial and/or long-term therapy of VTE based on patient-related factors, institution-related factors or over time.

Results: Among 41,678 patients from March 2013 to September 2021, 12,286 (29%) used DOACs: for initial therapy 6,456; for long-term therapy 12,046. On multivariable analysis, independent predictors were: age < 65 years (odds ratio [OR]: 1.30; 95% CI: 1.23–1.38), body weight <50 kg (OR: 0.54; 95% CI: 0.45–0.65) or >120 kg (OR: 0.64; 95% CI: 0.53–0.77), initial VTE presentation as pulmonary embolism (OR: 1.18; 95% CI: 1.13–1.25), recent bleeding (OR: 0.53; 95% CI: 0.45–0.63), renal insufficiency (OR: 0.44; 95% CI: 0.38–0.51), liver cirrhosis (OR: 0.32; 95% CI: 0.20–0.52), thrombocytopenia (OR: 0.40; 95% CI: 0.34–0.49), atrial fibrillation (OR: 1.58; 95% CI: 1.42–1.75) and prior

VTE (OR: 1.14; 95% CI: 1.06–1.22). The DOACs were more likely used in other European countries (OR: 8.97; 95% CI: 8.49–9.49), America (OR: 6.35; 95% CI: 5.67–7.11) or in other countries of the world (OR: 2.99; 95% CI: 2.70–3.31) than in Spain, and progressively increased from 2013–2015 to 2016–2018 (OR: 2.78; 95% CI: 2.62–2.95) and 2019–2021 (OR: 6.36; 95% CI: 5.95–6.80).

Conclusion: In this large multinational VTE registry, variations were observed in the use of DOACs according to patient or country factors, and over time. The safety, costs, and influence of the DOACs on VTE-related outcomes in daily practice warrant further investigation.

KEYWORDS

venous thromboembolism, direct oral anticoagulants, anticoagulant therapy, predictors, RIETE, different countries

Introduction

Current guidelines of anticoagulant therapy recommend the use of direct oral anticoagulants (DOACs) for initial and long-term therapy of patients with venous thromboembolism (VTE) (1, 2). The risk reduction of recurrent VTE with DOACs is similar to the risk reduction with low-molecular-weight heparin (LMWH) and vitamin K antagonists (VKAs), while the risk of bleeding is less with DOACs than with standard therapy (3). However, the use of DOACs has not completely replaced the use of standard therapy. There are patient-related factors, and also institutional or logistical reasons that may limit the use of DOACs in daily practice. Patient-related factors include older age, extreme body weights (where there may be doubts about the optimal dose) or concomitant diseases (where there may be concern about the risk of bleeding) (4–12). In addition, resource availability may also drive the choice of therapies. A better knowledge of the reasons why physicians prescribe the use of DOACs for the initial and/or long-term therapy of VTE could lead to design randomized trials for subgroups of patients where its use is lower than expected (to reassure on the efficacy and safety of DOACs) or higher than expected (to avoid undesirable outcomes).

The RIETE (Registro Informatizado Enfermedad TromboEmbólica) registry is an international, ongoing registry of consecutive patients with symptomatic, objectively confirmed, acute VTE (ClinicalTrials.gov identifier: NCT02832245). Since its inception in 2001, data from this registry have been used to evaluate outcomes after acute VTE, such as the frequency of recurrent VTE, bleeding and mortality, and risk factors for these outcomes (13). In the current study, we aimed to determine the potential variations in the use of DOACs in patients with confirmed VTE, based on patient-related factors, institution-related factors, and over time.

Abbreviations: DOACs, direct oral anticoagulants; VTE, venous thromboembolism; OR, odds ratio; CI, confidence intervals; LMWH, low-molecular-weight heparin; VKAs, vitamin K antagonists; PE, pulmonary embolism; DVT, deep vein thrombosis; CT-scan, computed tomography scan; CrCl, creatinine clearance.

Patients and methods

Inclusion criteria

Consecutive patients with acute deep vein thrombosis (DVT) or pulmonary embolism (PE) confirmed by objective tests (compression ultrasonography for suspected DVT; helical CT-scan, ventilation-perfusion lung scintigraphy or conventional angiography for suspected PE) were enrolled in RIETE. Patients were excluded if they were currently participating in a therapeutic clinical trial with a blinded therapy. All patients (or their legal power of attorney) provided written or oral consent for participation in the registry, in accordance with local ethics committee requirements.

Study design

Data were collected from March 2013 (corresponding to the time when the prescription of DOACs was allowed) to July 2021. The primary goal of this study was to determine the potential variations in the use of DOACs in patients with symptomatic, objectively confirmed VTE, based on patient-related factors, or institution-related factors. As such, the main outcome was the proportion of patients using DOACs vs. those using other anticoagulant drugs. Secondary outcomes were the proportion of patients using each DOAC (vs. the other DOACs), and the proportion of patients using lower-than recommended doses of DOACs. Recommended dosing was defined as dosing

consistent with FDA-labeled dosing for treatment of VTE as of September 2021.

Patient-related factors explored in this study included demographics (sex, age, body weight), initial VTE presentation (PE with or without concomitant DVT vs. isolated DVT), concomitant diseases that could contraindicate the use of DOACs [including recent (<30 days before) major bleeding, biopsy-proven liver cirrhosis, creatinine clearance (CrCl) levels <30 mL/min and platelet count <100,000/μLat baseline], and concomitant disorders that could lead to prolong the duration of anticoagulant therapy (prior VTE and atrial fibrillation). We also evaluated the proportion of fragile patients that used DOACs (fragile patients defined as those aged ≥75 years, with CrCl levels ≤50 mL/min or body weight ≤50 kg) (14). Institutional factors assessed in the current study included the country of enrolment. Further, we explored the trends in the use of DOACs over the study years.

Treatment

Patients were managed according to the clinical practice of each participating hospital (i.e., there was no standardization of treatment). The decision on the type and duration of therapy was left to the attending physicians. Patients were followed-up for at least 3 months in the outpatient clinic or physician's office.

Statistical analysis

Categorical variables were compared using the chi-square test (two-sided) and Fisher's Exact Test (two-sided). Continuous variables were compared using Student *t* test. To identify predictors of prescription of drugs we used logistic regression analyses. All the analyses were adjusted for sex, age (65 years; 65–79; >79 years), body weight (<50 kg; 50–120; >120 kg), initial VTE presentation (symptomatic PE; isolated DVT), recent major bleeding, liver cirrhosis, CrCl levels at baseline <30 mL/min, platelet count <100,000/μL, atrial fibrillation, prior VTE, the country where the VTE was diagnosed (Spain; other European countries; America; rest of the world) and years of VTE diagnosis (2013–2015; 2016–2018; 2019–2021). Odds ratios (OR) and corresponding 95% confidence intervals (CI) were calculated, and a p value < 0.05 was considered to be statistically significant. Statistical analyses were conducted with SPSS for Windows Release 25.0 (SPSS, Inc.).

Role of the funding source

The sponsors of the RIETE registry (Sanofi, Leo Pharma and Rovi) had no role in study design, data collection, data analysis, data interpretation or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Among 41,678 patients with VTE recruited from January 2013 to September 2021 in RIETE, 12,286 (29%) used DOACs: 6,456 for initial therapy and 12,046 for long-term therapy. Among the 41,678 patients, 20,896 (50%) were men; mean age was 65 ± 17 years; 23,458 (56%) initially presented with PE; 970 (2.3%) had recent major bleeding; CrCl levels <30 mL/min 2,097 (5.0%); liver cirrhosis 209 (0.5%); platelet count < 100,000/μL 1,055 (2.5%); atrial fibrillation 2,361 (5.7%) and prior VTE 5,818 (14%). In total, 16,767 patients (40%) were fragile.

Most patients (65%) were attended in Spanish centers, 25% in other European countries, 3.7% in America and 6.0% in the rest of the world (**Table 1**). For initial therapy, 5,141 patients (12%) used rivaroxaban and 1,315 (3.2%) apixaban. For long-term therapy, 6,631 patients (16%) used rivaroxaban, apixaban 3,548 (8.5%), edoxaban 1,473 (3.5%), and dabigatran 394 (0.9%). The proportion of patients using DOACs progressively increased over time (**Figure 1**).

Predictors of use of direct oral anticoagulants vs. other drugs

Overall, 12,286 patients (29%) used DOACs for initial and/or for the long-term therapy of VTE. The proportion of patients using DOACs was highest among those aged <65 years (33%), with atrial fibrillation (33%) or prior VTE (34%), and lowest in patients with CrCl levels < 30 mL/min (15%), liver cirrhosis (11%) or thrombocytopenia (16%) (**Table 2**) The use of DOACs was lowest in Spain (17%) and highest in other European countries (59%) or America (50%), and progressively increased over time: from 17% in 2013–2015 to 47% in 2019–2021. Among 12,286 patients using DOACs, 2,154 (18%) used lower-than recommended doses. The subgroups of patients that were more likely to use lower-than recommended doses of DOACs were: patients aged >79 years (30%), with CrCl levels <30 mL/min (44%) or with atrial fibrillation (30%). Only 4,105 of the 17,767 fragile patients with VTE (24%) used DOACs: 25% at lower than recommended doses.

On multivariable analysis, independent predictors for the use of DOACs (vs. other anticoagulants) were: age <65 years (OR: 1.30; 95% CI: 1.23–1.38) body weight <50 kg (OR: 0.54; 95% CI: 0.45–0.65) or > 120 kg (OR: 0.64; 95% CI: 0.53–0.77) initial VTE presentation as PE (OR: 1.18; 95% CI: 1.13–1.25) recent bleeding (OR: 0.53; 95% CI: 0.45–0.63) CrCl levels <30 mL/min (OR: 0.44; 95% CI: 0.38–0.51) liver cirrhosis (OR: 0.32; 95% CI: 0.20–0.52) platelet count <100,000/μL (OR: 0.40;

TABLE 1 Prescription of DOACs over time in different countries.

	Total	2013–2014	2015–2016	2017–2018	2019–2021
All patients	***41,678***	***10,526***	***10,798***	***10,653***	***9,701***
Rivaroxaban initially	5,141 (12.3%)	1,041 (9.9%)	1,481 (13.7%)	1,288 (12.1%)	1,331 (13.7%)
Apixaban initially	1,315 (3.2%)‡	5 (0.1%)‡	226 (2.1%)‡	402 (3.8%)‡	682 (7.0%)‡
Rivaroxaban long-term	6,631 (15.9%)	1,367 (13.0%)	1,929 (17.9%)	1,658 (15.6%)	1,677 (17.3%)
Apixaban long-term	3,548 (8.5%)‡	74 (0.7%)‡	727 (6.7%)‡	1,025 (9.6%)‡	1,722 (17.7%)
Edoxaban long-term	1,473 (3.5%)‡	9 (0.1%)‡	54 (0.5%)‡	451 (4.2%)‡	959 (9.9%)‡
Dabigatran long-term	394 (0.9%)‡	21 (0.2%)‡	111 (1.0%)‡	140 (1.3%)‡	122 (1.3%)‡
Spain	***27,245***	***6,542***	***7,066***	***7,122***	***6,515***
Rivaroxaban initially	991 (3.6%)	198 (3.0%)	228 (3.2%)	256 (3.6%)	309 (4.7%)
Apixaban initially	341 (1.2%)‡	**1 (0.02%)**‡	43 (0.6%)‡	76 (1.1%)‡	221 (3.4%)‡
Rivaroxaban long-term	1,879 (6.9%)	**326 (5.0%)**	460 (6.5%)	498 (7.0%)	595 (9.1%)
Apixaban long-term	1,659 (6.1%)‡	**53 (0.8%)**‡	305 (4.3%)‡	437 (6.1%)*	864 (13.3%)‡
Edoxaban long-term	873 (3.2%)‡	8 (0.1%)‡	32 (0.4%)‡	184 (2.6%)‡	649 (10.0%)
Europe, other	***10,414***	***2,647***	***2,873***	***2,419***	***2,475***
Rivaroxaban initially	3,308 (31.8%)	624 (23.6%)	1,068 (37.2%)	794 (32.8%)	822 (33.2%)
Apixaban initially	730 (7.0%)‡	2 (0.1%)‡	156 (5.4%)‡	264 (10.9%)‡	308 (12.4%)‡
Rivaroxaban long-term	3,795 (36.4%)	793 (30.0%)	1,232 (42.9%)	901 (37.2%)	869 (35.1%)
Apixaban long-term	1,488 (14.3%)‡	10 (0.4%)‡	351 (12.2%)‡	484 (20.0%)‡	643 (26.0%)‡
Edoxaban long-term	586 (5.6%)‡	0	21 (0.7%)‡	266 (11.0%)‡	299 (12.1%)‡
America	***1,530***	***446***	***294***	***543***	***247***
Rivaroxaban initially	454 (29.7%)	165 (37.0%)	118 (40.1%)	85 (15.6%)	86 (34.8%)
Apixaban initially	116 (7.6%)‡	1 (0.2%)‡	9 (3.1%)‡	33 (6.1%)‡	73 (29.5%)
Rivaroxaban long-term	549 (35.9%)	190 (42.6%)	153 (52.0%)	112 (20.6%)	94 (38.1%)
Apixaban long-term	169 (11.0%)‡	3 (0.7%)‡	17 (5.8%)‡	60 (11.1%)‡	89 (36.0%)
Edoxaban long-term	2 (0.1%)‡	0	1 (0.3%)‡	1 (0.2%)‡	0
Rest of the world	***2,489***	***891***	***565***	***569***	***464***
Rivaroxaban initially	388 (15.6%)	54 (6.1%)	67 (11.9%)	153 (26.9%)	114 (24.6%)
Apixaban initially	128 (5.1%)‡	1 (0.1%)‡	18 (3.2%)‡	29 (5.1%)‡	80 (17.2%)*
Rivaroxaban long-term	408 (16.4%)	58 (6.5%)	84 (14.9%)	147 (25.8%)	119 (25.6%)
Apixaban long-term	232 (9.3%)‡	8 (0.9%)‡	54 (9.6%)*	44 (7.7%)‡	126 (27.2%)
Edoxaban long-term	12 (0.5%)‡	1 (0.1%)‡	0	0	11 (2.4%)‡

Differences between patients receiving rivaroxaban vs. other drugs: *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$. Bold words are the main subjects to study. Years of prescription, number of patients Italic words apply for the total number of patients in each period of study and in the different parts of the world.

95% CI: 0.34–0.49) atrial fibrillation (OR: 1.58; 95% CI: 1.42–1.75) and prior VTE (OR: 1.14; 95% CI: 1.06–1.22) (Table 3) The use of DOACs was more likely in other European countries (OR: 8.97; 95% CI: 8.49–9.49) America (OR: 6.35; 95% CI: 5.67–7.11) or in the rest of the world (OR: 2.99; 95% CI: 2.70–3.31) than in Spain, and progressively increased from 2013–2015 to 2016–2018 (OR: 2.78; 95% CI: 2.62–2.95) and 2019–2021 (OR: 6.36; 95% CI: 5.95–6.80).

Predictors of use of one direct oral anticoagulant vs. the rest of direct oral anticoagulants

Among patients using DOACs, rivaroxaban was more likely used in men (OR: 1.26; 95% CI: 1.18–1.36) in patients aged <65 years (OR: 1.51; 95% CI: 1.39–1.63) weighing > 120 kg (OR: 1.61; 95% CI: 1.21–2.15) or with prior VTE (OR: 1.23; 95% CI: 1.12–1.36) (Table 4). Apixaban was the preferred DOAC among patients >79 years (OR: 1.59; 95% CI: 1.43–1.76) in those initially presenting with PE (OR: 1.17; 95% CI: 1.08–1.27) with recent bleeding (OR: 1.80; 95% CI: 1.37–2.35) renal insufficiency (OR: 1.89; 95% CI: 1.51–2.38) atrial fibrillation (OR: 1.62; 95% CI: 1.39–1.88) or in fragile patients (OR: 1.79; 95% CI: 1.65–1.94) Edoxaban was much more likely used in Spain than in other countries.

Independent predictors for the use of rivaroxaban (vs. other DOACs) were: male gender (OR: 1.20; 95% CI: 1.10–1.30), age <65 years (OR: 1.47; 95% CI: 1.34–1.61) initial presentation as PE (OR: 1.16; 95% CI: 1.07–1.26) and VTE diagnosis in non-Spanish European countries (OR: 2.17; 95% CI: 1.99–2.36) America (OR: 2.80; 95% CI: 2.32–3.37) or in other countries

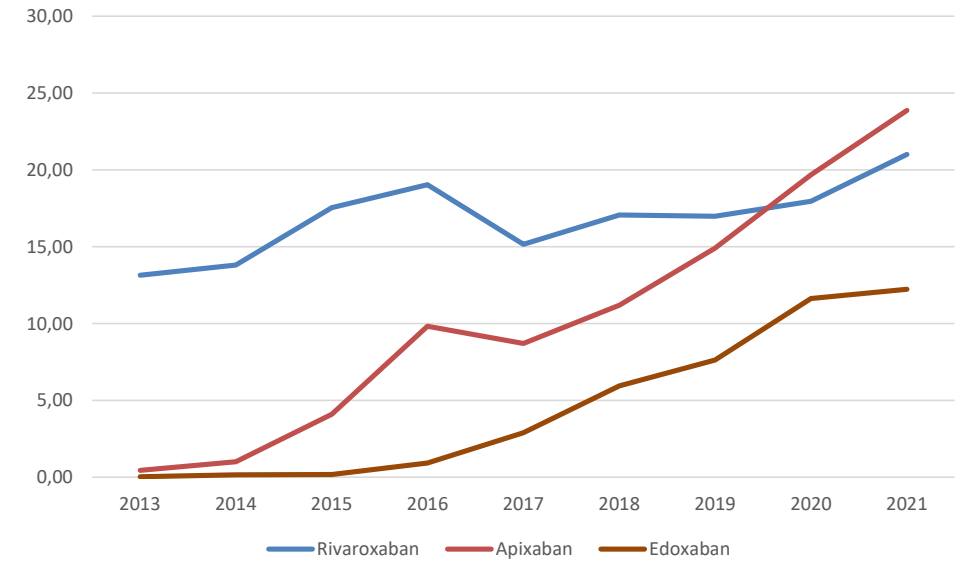

FIGURE 1
Cumulative rates of patients receiving DOACs for initial and/or long-term therapy of VTE over time.

(OR: 2.67; 95% CI: 2.24–3.18) Rivaroxaban was less prescribed for >79 years (OR: 0.70; 95% CI: 0.62–0.78) recent bleeding (OR: 0.58; 95% CI: 0.43–0.79) and atrial fibrillation (OR: 0.72; 95% CI: 0.61–0.85) (**Table 4**) Independent predictors for the use of apixaban were: male gender (OR: 0.88; 95% CI: 0.81–0.95) >79 years (OR: 1.46; 95% CI: 1.30–1.64) recent major bleeding (OR: 1.77; 95% CI: 1.33–2.36) atrial fibrillation (OR: 1.22; 95% CI: 1.04–1.44) Apixaban was less used in age <65 years (OR: 0.72; 95% CI: 0.65–0.79) and VTE diagnosis in non-Spanish European countries (OR: 0.67; 95% CI: 0.61–0.73) or in America (OR: 0.82; 95% CI: 0.68–0.98) Independent predictors for the use of edoxaban were: body weight >120 kg (OR: 0.53; 95% CI: 0.29–0.97) initial VTE presentation as PE (OR: 0.64; 95% CI: 0.57–0.72) liver cirrhosis (OR: 3.86; 95% CI: 1.21–12.3) prior VTE (OR: 0.80; 95% CI: 0.67–0.95) and being diagnosed in Spain. Interestingly, the use of rivaroxaban (comparatively with the other two DOACs) progressively decreased over time.

Discussion

Our findings, obtained from a large cohort of patients with acute VTE in up to 30 countries over the world, reveal large variations in the use of DOACs according to patient factors, institutional factors and also over time. As it could have been expected, the DOACs were more likely used in young patients, those with normal body weight and with no exclusion criteria to be enrolled in the pivotal trials where their indication was based (i.e., recent bleeding, renal insufficiency, liver cirrhosis or thrombocytopenia) Studies about patients preferences usually report more satisfied patients with DOAC than VKA drugs (15, 16) but it seems it is not a reason from prescription in some countries as Spain. Also, its use was much lower in Spain (where the DOACs are not reimbursed) and progressively increased over time. However, there were surprising findings in some subgroups of patients. For example, while the use of DOACs was lower than expected in the subgroups of patients where they had demonstrated to be superior to standard therapy, they were not infrequently used in patients with contraindications to their use.

Subgroup analyses from randomized trials revealed that the DOACs had advantages over standard anticoagulation in fragile patients with VTE. In the EINSTEIN trials, the risk for major bleeding in fragile patients using rivaroxaban was significantly lower than in those on standard therapy (17–19). This difference was not found in non-fragile patients. In the HOKUSAI trial, fragile patients using edoxaban had a significantly higher efficacy than those on VKAs (19). The superiority of the DOACs over standard therapy in fragile patients with VTE was subsequently confirmed in real-life conditions (20, 21). However, only 24% of the 17,767 fragile patients in our cohort used DOACs. We hypothesize that a higher use of DOACs in fragile patients with VTE (40% of the whole series) might have been associated with improved outcomes.

On the other hand, the use of DOACs is contraindicated in patients with severe liver or renal insufficiency, in pregnant or breast-feeding women, and in patients perceived to be at high risk for bleeding (4). Because most of these patients with were excluded from the clinical trials, data regarding their effectiveness and safety are only available through non-randomized studies of which statistical type I/type II errors could play a role (6, 22). Despite this knowledge

TABLE 2 Univariable and multivariable analyses for predictors of use of DOACs vs. other drugs.

	Total patient	Patients with DOACs	Patients with DOACs at low doses	Any DOACs (vs. other drugs)	
				Univariable OR (95% CI)	Multivariable OR (95% CI)
Patients, N	***41,678***	***12,286 (29%)***	***2,154 (18%)***		
Male gender	20,896	6,323 (30%)	1,011 (16%)	1.08 (1.03–1.12)*	1.01 (0.96–1.06)
Age 65–79 years	14,110	3,957 (28%)	627 (16%)	Ref.	Ref.
Age < 65 years	17,944	6,009 (33%)	825 (14%)	1.29 (1.23–1.36)‡	1.30 (1.23–1.38)‡
Age > 79 years	9,624	2,320 (24%)	702 (30%)	0.81 (0.77–0.86)‡	0.96 (0.89–1.03)
Body weight 50–120 kg	39,960	11,882 (30%)	2,073 (17%)	Ref.	Ref.
Body weight < 50 kg	977	190 (19%)	54 (28%)	0.57 (0.49–0.67)‡	0.54 (0.45–0.65)‡
Body weight > 120 kg	740	213 (29%)	27 (13%)	0.96 (0.81–1.12)	0.64 (0.53–0.77)‡
Initial VTE presentation					
Isolated DVT	18,220	5,020 (27%)	904 (18%)	Ref.	Ref.
Pulmonary embolism	23,458	7,266 (31%)	1,250 (17%)	1.18 (1.13–1.23)†	1.18 (1.13–1.25)‡
Concomitant disorders					
Recent major bleeding	970	224 (23%)	57 (25%)	0.71 (0.61–0.83)†	0.53 (0.45–0.63)‡
CrCl levels < 30 mL/min	2,097	310 (15%)	137 (44%)	0.40 (0.35–0.45)†	0.44 (0.38–0.51)‡
Liver cirrhosis	209	23 (11%)	6 (26%)	0.29 (0.19–0.45)†	0.32 (0.20–0.52)‡
Platelet count < 100,000/μL	1,055	168 (16%)	46 (27%)	0.45 (0.38–0.53)†	0.40 (0.34–0.49)‡
Atrial fibrillation	2,361	783 (33%)	237 (30%)	1.20 (1.10–1.31)†	1.58 (1.42–1.75)‡
Prior VTE	5,818	1,994 (34%)	357 (18%)	1.30 (1.22–1.37)†	1.14 (1.06–1.22)‡
Fragile patients	16,767	4,105 (24%)	1,034 (25%)	0.66 (0.63–0.69)†	–
Countries					
Spain	27,245	4,645 (17%)	859 (18%)	Ref.	Ref.
Rest of Europe	10,414	6,152 (59%)	956 (16%)	7.02 (6.68–7.38)‡	8.97 (8.49–9.49)‡
America	1,530	768 (50%)	130 (17%)	4.90 (4.41–5.45)‡	6.35 (5.67–7.11)‡
Rest of the world	2,489	721 (29%)	209 (29%)	1.98 (1.81–2.18)‡	2.99 (2.70–3.31)‡
Years					
2013–2015	16,114	2,784 (17%)	502 (18%)	Ref.	Ref.
2016–2018	15,863	4,954 (31%)	968 (20%)	2.17 (2.06–2.29)‡	2.78 (2.62–2.95)‡
2019–2021	9,701	4,548 (47%)	684 (15%)	4.23 (3.99–4.47)‡	6.36 (5.95–6.80)‡

*$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$. DOACs, direct oral anticoagulants; CI, confidence intervals; VTE, venous thromboembolism; DVT, deep vein thrombosis; CrCl, creatinine clearance; Ref., reference. Bold words are the main subjects to study. Years of prescription, number of patients Italic words apply for the total number of patients in each period of study and in the different parts of the world.

gap, 23% of patients with recent major bleeding, 15% with CrCl levels <30 mL/min, 11% with cirrhosis, and 16% with thrombocytopenia in our cohort used DOACs. There are few data about resuming anticoagulation after major bleeding with DOAC. In the study from Little (23), reassumption of DOAC in extracranial non-gastrointestinal bleeding was accompanied by reduction in thrombosis. We haven't studied DOAC in pregnancy in RIETE as opposite to GARFIELD study (24). Data of DOAC used in patient with recently bleeding are an interesting finding since the lack of a monitoring assays say and reversal agents (25) have been important safety concerns for clinicians. A substantial proportion of these patients (25, 44, 26, and 27%, respectively) received lower than recommended doses. This is also of concern, since under-dosing of DOACs has been associated with decreased efficacy and no benefit in safety (26–29). Apixaban was seen to be the most frequent DOAC with dose modification. It could be argued that attending physician has used the same adjustment for dose that has to be made in atrial fibrillation, as it's supposed in the study from, about changing pattern of type of anticoagulant use and in off-label use a cohort from Switzerland (30). Also low doses are used in atrial fibrillation (31, 32).

Finally, because patients at extremes of body weight were underrepresented in DOAC clinical trials and randomized trials for these patient subgroups are currently unavailable, the International Society of Thrombosis and Hemostasis Scientific and Standardization Committee recently suggested that rivaroxaban and apixaban can be adequate for VTE therapy

TABLE 3 Multivariable analyses for predictors of use of every DOAC (vs. the rest of DOACs).

	Rivaroxaban	Apixaban	Edoxaban
Patients, N	***6,886***	***3,601***	***1,473***
Clinical characteristics			
Male gender	1.20 (1.10–1.30)‡	0.88 (0.81–0.95)†	0.89 (0.79–1.00)
Age 65–79 years	Ref.	Ref.	Ref.
Age < 65 years	1.47 (1.34–1.61)‡	0.72 (0.65–0.79)‡	0.88 (0.77–1.01)
Age > 79 years	0.70 (0.62–0.78)‡	1.46 (1.30–1.64)‡	0.99 (0.84–1.17)
Body weight 50–100 kg	Ref.	Ref.	Ref.
Body weight < 50 kg	1.04 (0.75–1.43)	1.06 (0.77–1.47)	0.91 (0.56–1.50)
Body weight > 120 kg	1.21 (0.88–1.67)	1.04 (0.75–1.44)	0.53 (0.29–0.97)*
Initial VTE presentation			
Isolated DVT	Ref.	Ref.	Ref.
Pulmonary embolism	1.16 (1.07–1.26)‡	1.08 (0.99–1.17)	0.64 (0.57–0.72)‡
Concomitant disorders			
Recent major bleeding	0.58 (0.43–0.79)‡	1.77 (1.33–2.36)‡	0.88 (0.54–1.43)
CrCl levels < 30 mL/min	0.88 (0.67–1.14)	1.10 (0.85–1.41)	1.17 (0.82–1.67)
Liver cirrhosis	1.23 (0.48–3.19)	0.24 (0.05–1.05)	3.86 (1.21–12.3)*
Platelet count < 100,000/μL	0.93 (0.66–1.30)	1.18 (0.84–1.65)	0.84 (0.48–1.48)
Atrial fibrillation	0.72 (0.61–0.85)‡	1.22 (1.04–1.44)*	0.81 (0.63–1.05)
Prior VTE	1.03 (0.92–1.14)	1.10 (0.99–1.23)	0.80 (0.67–0.95)*
Countries			
Spain	Ref.	Ref.	Ref.
Rest of Europe	2.17 (1.99–2.36)‡	0.67 (0.61–0.73)‡	0.56 (0.50–0.63)‡
America	2.80 (2.32–3.37)‡	0.82 (0.68–0.98)*	0.02 (0.00–0.06)‡
Rest of the world	2.67 (2.24–3.18)‡	0.97 (0.82–1.16)	0.07 (0.04–0.12)‡
Years			
2013–2015	Ref.	Ref.	Ref.
2016–2018	0.20 (0.17–0.22)‡	3.54 (3.09–4.05)‡	15.9 (9.89–25.5)‡
2019–2021	0.10 (0.09–0.12)‡	4.74 (4.14–5.43)‡	36.6 (22.9–58.6)‡

Results are expressed as odds ratio and 95% confidence intervals (in brackets). *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$. VTE, venous thromboembolism; DVT, deep vein thrombosis; CrCl, creatinine clearance; Ref., reference. Bold words are the main subjects to study. Years of prescription, number of patients Italic words apply for the total number of patients in each period of study and in the different parts of the world.

regardless of body weight, and suggested not using dabigatran, edoxaban or betrixaban in patients weighing >120 kg (12). In our cohort, 12 patients weighing >120 kg used edoxaban (5.6% of the obese patients using DOACs).

Among patients receiving DOACs in our cohort, there was some preference for apixaban over rivaroxaban or edoxaban in the elderly (33) and in patients with recent major bleeding or atrial fibrillation. On the other hand, rivaroxaban was preferred in the young, and edoxaban in those with liver cirrhosis. However, in the absence of clinical trials comparing the DOACs each other, there is no evidence to support that one specific DOAC is superior to any other in terms of efficacy or safety in any clinical scenario.

TABLE 4 Univariable analyses for predictors of use of each DOAC vs. the rest of DOACs.

	Rivaroxaban		Apixaban		Edoxaban	
	N	**OR (95% CI)**	***N***	**OR (95% CI)**	***N***	**OR (95% CI)**
Patients, N	***6,886***		***3,601***		***1,473***	
Clinical characteristics						
Male gender	3,721	1.26 (1.18–1.36)†	1,709	0.80 (0.74–0.86)†	723	0.90 (0.80–1.00)
Age 65–79 years	2,109	Ref.	1,223	Ref.	502	Ref.
Age < 65 years	3,799	1.51 (1.39–1.63)‡	1,415	0.69 (0.63–0.75)‡	660	0.85 (0.75–0.96)†
Age > 79 years	978	0.64 (0.58–0.71)‡	963	1.59 (1.43–1.76)‡	311	1.07 (0.92–1.24)
Body weight 50–120 kg	6,646	Ref.	3,478	Ref.	1,439	Ref.
Body weight < 50 kg	96	0.80 (0.60–1.07)	69	1.38 (1.02–1.86)*	22	0.95 (0.61–1.49)
Body weight > 120 kg	143	1.61 (1.21–2.15)†	54	0.82 (0.60–1.12)	12	0.43 (0.24–0.78)†
Initial VTE presentation						
Isolated DVT	2,845	Ref.	1,373	Ref.	672	Ref.
Pulmonary embolism	4,041	0.96 (0.89–1.03)	2,228	1.17 (1.08–1.27)†	801	0.80 (0.72–0.89)†
Concomitant disorders						
Recent major bleeding	103	0.66 (0.51–0.86)†	95	1.80 (1.37–2.35)†	20	0.72 (0.45–1.14)
CrCl levels < 30 mL/min	122	0.50 (0.40–0.63)†	135	1.89 (1.51–2.38)†	47	1.32 (0.96–1.81)
Liver cirrhosis	15	1.47 (0.62–3.47)	2	0.23 (0.05–0.98)*	5	2.04 (0.76–5.51)
Platelet count < 100,000/μL	92	0.95 (0.70–1.29)	56	1.21 (0.87–1.67)	15	0.72 (0.42–1.22)
Atrial fibrillation	337	0.57 (0.49–0.66)†	308	1.62 (1.39–1.88)†	83	0.86 (0.68–1.09)
Prior VTE	1,202	1.23 (1.12–1.36)†	569	0.96 (0.86–1.06)	179	0.69 (0.58–0.81)†
Fragile patients	1,896	0.55 (0.51–0.59)†	1,541	1.79 (1.65–1.94)†	538	1.17 (1.04–1.31)†
Countries						
Spain	1,919	Ref.	1,669	Ref.	873	Ref.
Rest of Europe	3,935	2.52 (2.33–2.73)‡	1,509	0.58 (0.53–0.63)‡	586	0.45 (0.41–0.51)‡
America	569	4.06 (3.42–4.82)‡	181	0.55 (0.46–0.66)‡	2	0.01 (0.00–0.05)‡
Rest of the world	463	2.55 (2.17–3.00)‡	242	0.90 (0.76–1.06)	12	0.07 (0.04–0.13)‡
Years						
2013–2015	2,409	Ref.	305	Ref.	18	Ref.
2016–2018	2,724	0.19 (0.17–0.21)‡	1,550	3.70 (3.24–4.23)‡	496	17.1 (10.7–27.4)‡
2019–2021	1,753	0.10 (0.09–0.11)‡	1,746	5.06 (4.43–5.79)‡	959	41.1 (25.7–65.6)‡

*$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$. VTE, venous thromboembolism; DVT, deep vein thrombosis; CrCl, creatinine clearance; Ref., reference. Bold words are the main subjects to study. Years of prescription, number of patients Italic words apply for the total number of patients in each period of study and in the different parts of the world.

The main strength of this study is the large size of the RIETE registry, which enabled us to explore the variations across multiple settings, including across patient-related factors, across geographic regions as well over time. As such, the data related to temporal, institutional, and particularly patient-level variations per clinical subgroups provide real-world evidence about contemporary practice and could be helpful for practice management, policy making, and designing future research studies. However, a number of limitations of this study must be acknowledged. First, we did not evaluate the role of patient income and sociodemographic variables as they relate to patient's willingness to pay for DOACs. Second, factors related to DOACs therapy choice may change over time as prescribers and patients gain more familiarity and experience with the newer DOACs, and additional research will be needed to identify predictors of treatment and changes in DOAC treatment patterns in the future. Third, some countries had fewer participating centers or enrolled only a few patients. As such, although findings from this multicenter, multinational study demonstrate regional variations in diagnostic practices, accurate comparisons for point estimates are not feasible for some countries. Finally, future research needs to consider the impact of patient preferences in DOAC therapy decisions.

This is the first of a series of studies to explore the use of DOACs in patients with VTE and their potential consequences. The focus of the current study was on the assessment and description of potential variations in the choice of drugs for VTE therapy. Future studies are required to explore the reasons behind the variations, the accuracy of each approach, and to assess the impact of these variations on VTE-related and non-VTE-related outcomes in adjusted analyses.

In conclusion, in a large multicenter, multinational registry of patients with VTE, we observed noticeable variations in the

choice of DOACs according to the underlying patient factors and institutional factors.

Data availability statement

The original contributions presented in this study are included in the article further inquiries can be directed to the corresponding author.

Author contributions

PB, SO, JT, TS, LM, and AB-R: review of draft. All authors contributed to the article, approved the submitted version, and contributed to the patients' enrollment.

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.

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References

1. Ortel TL, Neumann I, Ageno W, Beyth R, Clark NP, Cuker A, et al. American Society of Hematology 2020 guidelines for management of venous thromboembolism: treatment of deep vein thrombosis and pulmonary embolism. *Blood Adv.* (2020) 4:4693–738. doi: 10.1182/bloodadvances.2020001830

2. Stevens SM, Woller SC, Baumann-Kreuziger L, Bounameaux H, Doerschug K, Geersing G, et al. Antithrombotic therapy for VTE disease: second update of the CHEST guideline and expert panel report. *Chest.* (2021) 160:e545–608. doi: 10.1016/j.chest.2021.07.055

3. Van Es N, Coppens M, Schulman S, Middeldorp S, Büller H. Direct oral anticoagulants compared with vitamin K antagonists for acute venous thromboembolism: evidence from phase 3 trials. *Blood.* (2014) 124:1968–75. doi: 10.1182/blood-2014-04-571232

4. Burnett AE, Mahan CE, Vazquez SR, Oertel LB, Garcia DA, Ansell J. Guidance for the practical management of the direct oral anticoagulants (DOACs) in VTE treatment. *J Thromb Thrombolys.* (2016) 41:206–32. doi: 10.1007/s11239-015-1310-7

5. Toorop MMA, Lijfering WM, Scheres LJJ. The relationship between DOAC levels and clinical outcomes: the measures tell the tale. *J Thromb Haemost.* (2020) 18:3163–8. doi: 10.1111/jth.15104

6. Weber J, Olyaei A, Shatzel J. The efficacy and safety of direct oral anticoagulants in patients with kidney disease. *Eur J Haematol.* (2019) 102:312–8. doi: 10.1111/ejh.13208

7. Cheung CYS, Parikh J, Farrell A, Lefebvre M, Summa-Sorgini C, Battistella M. Direct oral anticoagulant use in chronic kidney disease and dialysis patients with venous thromboembolism: a systematic review of thrombosis and bleeding outcomes. *Ann Pharmacother.* (2021) 55:711–22. doi: 10.1177/1060028020967635

8. Ting C, Rhoten M, Dempsey J, Nichols H, Fanikos J, Ruff CT. Evaluation of direct oral anticoagulant prescribing in patients with moderate to severe renal impairment. *Clin Appl Thromb Hemost.* (2021) 27:1076029620987900. doi: 0.177/1076029620987900

9. Elhosseiny S, Al Moussawi H, Chalhoub JM, Lafferty J, Deeb L. Direct oral anticoagulants in cirrhotic patients: current evidence and clinical observations. *Can J Gastroenterol Hepatol.* (2019) 2019:4383269. doi: 10.1155/2019/4383269

10. Speed V, Green B, Roberts LN, Woolcombe S, Bartoli-Abdou J, Barsam S, et al. Fixed dose rivaroxaban can be used in extremes of body weight: a population pharmacokinetic analysis. *J Thromb Haemost.* (2020) 18:2296–307. doi: 10.1111/jth.14948

11. Katel A, Aryal M, Neupane A, Gosain R, Pathak R, Bhandari Y, et al. Efficacy and safety of direct oral anticoagulants in venous thromboembolism compared to traditional anticoagulants in morbidly obese patients: a systematic review and meta-analysis. *Cureus.* (2021) 13:e14572. doi: 10.7759/cureus.14572

12. Martin KA, Beyer-Westendorf J, Davidson BL, Huisman MV, Sandset PM, Moll S. Use of direct oral anticoagulants in patients with obesity for treatment and prevention of venous thromboembolism: updated communication from the ISTH SSC subcommittee on control of anticoagulation. *J Thromb Haemost.* (2021) 19:1874–82. doi: 10.1111/jth.15358

13. Bikdeli B, Jiménez D, Hawkins M, Ortíz S, Prandoni P, Brenner B, et al. Rationale, design and methodology of the computerized registry of patients with venous thromboembolism (RIETE). *Thromb Haemost.* (2018) 118:214–24. doi: 10.1160/TH17-07-0511

14. Einstein-Pe Investigators, Büller HR, Prins MH, Lensin AW, Decousus H, Jacobson BF, et al. Oral rivaroxaban for symptomatic venous thromboembolism. *N Engl J Med.* (2010) 363:2499–510.

15. Afzal SK, Hasan SS, Babar ZU. A systematic review of patient-reported outcomes associated with the use of direct-acting oral anticoagulants. *Br J Clin Pharmacol.* (2019) 85:2652–67. doi: 10.1111/bcp.13985

16. Keita I, Aubin-Auger I, Lalanne C, Aubdert JR, Chassany O, Durancisky M, et al. Assessment of quality of life, satisfaction with anticoagulation therapy, and adherence to treatment in patients receiving long-course vitamin K antagonists or direct oral anticoagulants for venous thromboembolism. *Patient Prefer Adherence.* (2017) 11:1625–34. doi: 10.2147/PPA.S131157

17. Einstein Investigators, Bauersachs R, Berkowitz SD, Brenner B, Buller HR, Decousus H, et al. Oral rivaroxaban for the treatment of symptomatic pulmonary embolism. *N Engl J Med.* (2012) 366:1287–97. doi: 10.1056/NEJMoa1113572

18. Prins MH, Lensing A, Bauersachs R, van Bellen B, Bounameaux H, Brighton TA, et al. Oral rivaroxaban versus standard therapy for the treatment of symptomatic venous thromboembolism: a pooled analysis of the EINSTEIN-DVT an PE randomized studies. *Thromb J.* (2013) 11:21–31. doi: 10.1186/1477-9560-11-21

19. Hokusai-Vte Investigators, Büller HR, Décousus H, Grosso MA, Mercuri M, Middeldorp S, et al. Edoxaban versus warfarin for the treatment of symptomatic venous thromboembolism. *N Engl J Med.* (2013) 369:1406–15. doi: 10.1056/NEJMoa1306638

20. López-Núñez JJ, Pérez-Andrés R, Di Micco P, Schellong S, Gómez Cuervo C, Sahuquillo JC. Direct oral anticoagulants or standard anticoagulant therapy in fragile patients with venous thromboembolism. *TH Open.* (2019) 3:e67–76. doi: 10.1055/s-0039-1683970

21. Trujillo-Santos J, Beroiz P, Alonso A, Morejón E, López-Reyes R, Casado I, et al. Rivaroxaban or apixaban in fragile patients with acute venous thrombo-embolism. *Thromb Res.* (2020) 193:160–5. doi: 10.1016/j.thromres.2020.06.035

22. Derebail VK, Rheault MN, Kerlin BA. Role of direct oral anticoagulants in patients with kidney disease. *Kidney Int.* (2020) 97:664–75. doi: 10.1016/j.kint.2019.11.027

23. Little DHW, Sutradhar R, Cerasuolo JO, Perez R, Douketis J, Holbrook A, et al. Rates of rebleeding, thrombosis and mortality associated with resumption of anticoagulant therapy after anticoagulant-related bleeding. *CMAJ.* (2021) 193:E304–9. doi: 10.1503/cmaj.201433

24. Jerjes-Sánchez C, Rodriguez D, Farjat AE, Kayani G, MacCallum P, Lopes RD, et al. Pregnancy-associated venous thromboembolism: insights from GARFIELD-VTE. *TH Open.* (2021) 5:e24–34. doi: 10.1055/s-0040-1722611

25. Shih AW, Crowther MA. Reversal of direct oral anticoagulants: a practical approach. *Hematology Am Soc Hematol Educ Program.* (2016) 2016:2012–9. doi: 10.1182/asheducation-2016.1.612

26. Trujillo-Santos J, Di Micco P, Dentali F, Douketis J, Díaz-Peromingo JA, Núñez MJ, et al. Real-life treatment of venous thromboembolism with direct oral anticoagulants: the influence of recommended dosing and regimens. *Thromb Haemost.* (2017) 117:382–9. doi: 10.1160/TH16-07-0494

27. Dentali F, Fantoni C. Is it reasonable to use a lower DOAC dose in some patients with VTE? No. *Intern Emerg Med.* (2017) 12:565–7. doi: 10.1007/s11739-017-1695-8

28. Chopard R, Serzian G, Humbert S, Falvo N, Morel-Aleton M, Bonnet B, et al. Non-recommended dosing of direct oral anticoagulants in the treatment of acute pulmonary embolism is related to an increased rate of adverse events. *J Thromb Thrombolysis.* (2018) 46:283–91. doi: 10.1007/s11239-018-1690-6

29. Deitelzweig S, Keshishian A, Li X, Kang A, Dhamane AD, Luo X, et al. Comparisons between oral anticoagulants among older nonvalvular atrial fibrillation patients. *J Am Geriatr Soc.* (2019) 67: 1662–71. doi: 10.1111/jgs.15956

30. Eschler C, Antelo A, Funk GC, Exadaktylos AK, Lindner G. High fluctuation between anticoagulants, frequent off-label dosing, and no difference concerning outcomes: results of a real-life cohort study. *Am J Med.* (2021) 134:e165–70. doi: 10.1016/j.amjmed.2020.09.018

31. Navarro-Almenzara B, Cerezo-Manchado JJ, Caro-Martinez C, García-Candela C, Flores Blanco PJ, Elvira Ruiz G, et al. Real-life behaviour of direct oral anticoagulants in a Spanish cohort with non-valvular atrial fibrillation: refase registry. *Curr Med Res Opin.* (2019) 35:2035–41. doi: 10.1080/03007995.2019.1647735

32. Ruiz Orti M, Muñiz J, Raña Míguez P, Roldán I, Marín F, Esteve-Pastor A, et al. Inappropriate doses of direct oral anticoagulants in real-world clinical practice: prevalence and associated factors. A subanalysis of the FANTASIIA registry. *Europace.* (2018) 20:1577–83. doi: 10.1093/europace/eux316

33. Geldhof V, Vandenbriele C, Verhamme P, Vanassche T. Venous thromboembolism in the elderly: efficacy and safety of non-VKA oral anticoagulants. *Thromb Jour.* (2014) 12:21. doi: 10.1186/1477-9560-12-21

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Multicentered analysis of percutaneous sclerotherapies in venous malformations of the face

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Objectives: To evaluate the safety and outcome of image-guided sclerotherapy for treating venous malformations (VMs) of the face.

Materials and methods: A multicenter cohort of 68 patients with VMs primarily affecting the face was retrospectively investigated. In total, 142 image-guided sclerotherapies were performed using gelified ethanol and/or polidocanol. Clinical and imaging findings were assessed to evaluate clinical response, lesion size reduction, and complication rates. Sub-analyses of complication rates depending on type and injected volume of the sclerosant as well as of pediatric versus adult patient groups were conducted.

Results: Mean number of procedures per patient was 2.1 (±1.7) and mean follow-up consisted of 8.7 months (±6.8 months). Clinical response (n = 58) revealed a partial relief of symptoms in 70.7% (41/58), 13/58 patients

(22.4%) presented symptom-free while only 4/58 patients (6.9%) reported no improvement. Post-treatment imaging (n = 52) revealed an overall objective response rate of 86.5% (45/52). The total complication rate was 10.6% (15/142) including 4.2% (7/142) major complications, mostly (14/15, 93.3%) resolved by conservative means. In one case, a mild facial palsy persisted over time. The complication rate in the gelified ethanol subgroup was significantly higher compared to polidocanol and to the combination of both sclerosants (23.5 vs. 6.0 vs. 8.3%, p = 0.01). No significant differences in complications between the pediatric and the adult subgroup were observed (12.1 vs. 9.2%, p = 0.57). Clinical response did not correlate with lesion size reduction on magnetic resonance imaging (MRI).

Conclusion: Image-guided sclerotherapy is effective for treating VMs of the face. Clinical response is not necessarily associated with size reduction on imaging. Despite the complex anatomy of this location, the procedures are safe for both adults and children.

KEYWORDS

VM, slow-flow, face, sclerotherapy, outcome

Introduction

According to the classification of the International Society for the Study of Vascular Anomalies (ISSVA) (1, 2) vascular malformations are divided in slow-flow and high-flow lesions, related to the underlying flow pattern, which is crucial both for treatment decision and the prognosis (3). Slow-flow malformations represent the majority (>90%) of vascular malformations and among these, venous malformations (VMs) are the most common type (4). VMs are composed of a dilated, dysplastic, and hemodynamically non-functional venous-like network presenting as compressible, bluish, non-pulsatile, and soft with expanding skin and subcutaneous tissue (5). In many cases, VMs grow proportionally during childhood remaining unnoticed for years prior to symptomatic clinical presentation (6). While possibly occurring at any part of the body, VMs frequently affect the head and neck area including the face (7). As the complex anatomy of the face is based on multiple small functional units containing dense innervation and limited soft tissue coverage, therapy of these lesions often has a relevant risk for nerve injury, necrosis, and aesthetic disfigurement (8).

The purpose of this multicenter study was to evaluate the safety and outcome of image-guided sclerotherapy with ethanol gel and/or polidocanol for the treatment of VMs affecting the face.

Abbreviations: CIRSE, Cardiovascular and Interventional Radiological Society of Europe; CR, complete response; ISSVA, International Society for the Study of Vascular Anomalies; MRI, magnetic resonance imaging; PR, partial response; RECIST, response evaluation criteria in solid tumors; SD, stable disease; VM, venous malformation; vs., versus; WMA, World Medical Association.

Materials and methods

Study design

The present retrospective multicenter study was approved by the local ethics committee (University Hospital, LMU Munich, protocol No. 21-0943, 10/06/2021) and was performed in accordance with the Declaration of the World Medical Association (WMA). All patients were recruited from Interdisciplinary Vascular Anomalies Centers at six tertiary care university hospitals in Germany, Switzerland, and Tanzania. Data collection was performed using electronic patient records as well as the Picture Archiving and Communication System (PACS) searching for corresponding diagnosis related groups (DRGs). VMs were diagnosed based on the combination of patient history, physical examination, and imaging using magnetic resonance imaging (MRI) as well as duplex ultrasound (9). The angiographic classification was performed according to Puig et al. (10). All malformations predominantly affected the face, patients with lesions involving the neck or head without the face were excluded. In addition, patients who underwent diagnostic workup only without invasive treatment were excluded. The indications for image-guided sclerotherapy were pain, swelling, bleeding, recurring infections, repetitive thrombosis and/or other blood coagulopathy, aesthetic disfigurement as well as accompanying functional impairment

(e.g., insufficient breathing, jaw motional restriction, epiphora, exophthalmos, and facial paresthesia).

Procedural details

Interventional treatment was conducted under general anesthesia. Sclerotherapy was performed under real-time ultrasound and fluoroscopic guidance using gelified ethanol (Sclerogel®, Balt Germany GmbH & Co. KG, Düsseldorf, Germany) and/or 2–3% polidocanol foam (Aethoxysclerol®, Kreussler & Co. GmbH, Wiesbaden, Germany; ratio of polidocanol to sterile air 1:4). Gelified alcohol was used in cases of rapid venous drainage toward larger draining veins due to its higher viscosity. For sclerotherapy ultrasound guidance was applied to gain access to the VM. After placing a 20/21G needle within the lesion, venous blood was aspirated for verification of intralesional position and contrast injection under fluoroscopy was performed in order to detect rapid venous drainage into the deep venous system and classify the lesion. Subsequently the sclerosant was injected in majority of cases under ultrasound control, either until the lesion was properly covered or dislocation of sclerosant toward the deep venous system occurred. Only in case of lesions with fast venous drainage or drainage toward susceptible areas like the brain, the filling defect technique using fluoroscopy was additionally used. E.g., for orbital malformations, additional fluoroscopic was routinely applied. Patients were discharged at day 2–5 following the procedure, with low molecular weight heparin routinely administered for 7 days, in order to prevent potential acute postinterventional symptoms, especially those caused by local thrombophlebitis. Repetitive sclerotherapy procedures were performed depending on the extent of the lesion, response to therapy, and course of clinical symptomatology.

Follow-up

In the six centers involved the patients were seen within a standardized follow-up regime. The first clinical follow-up was conducted at 1–3 months after each sclerotherapy session including repeated contrast-enhanced MRI examination. In case of insufficient improvement of symptoms or residually perfused lesion(s) being present, an additional sclerotherapy session was performed. In case of no additional treatment the next follow-up was scheduled at 6 months, again comprising a clinical examination as well as contrast-enhanced MRI. Hereafter, additional follow-ups were performed annually.

Outcome evaluation

Data analysis was conducted to evaluate demographics and to define lesion classification, clinical response, objective response (imaging), and complication rates. Clinical response at follow-up was measured using the following grading scale: symptom-free, partial relief of symptoms, no improvement of symptoms, and clinical progression despite sclerotherapy. Objective response was assessed by changes in lesion size using pre-procedural MR images compared to those obtained at terminal follow-up after the last sclerotherapy. Imaging findings were classified into the following four categories: complete response (CR, 100% lesion size reduction), partial response (PR, 30% lesion size reduction), stable disease (SD, neither PR nor PD criteria met), progressive disease (PD, 20% lesion size increase) (11, 12). For the size assessment of the VMs on delayed-phase contrast-enhanced fat-saturated T1-weighted images, the largest lesion diameter in one imaging plane was used, comparable to the response evaluation criteria in solid tumors (RECIST) (11). Peri- and post-procedural complications were classified into minor and major adverse events (AE) according to the Cardiovascular and Interventional Radiological Society of Europe (CIRSE) classification (13) as well as analyzed depending on the type and the injected volume of the sclerosant and between the adult and pediatric subgroup. A comparison of clinical and objective response was performed.

Statistical analyses

Descriptive statistics were used to analyze the distribution of patients among the different categories. Kolmogorov–Smirnov (K-S) test was used for the assessment of normality. Data are presented as means (±SD) in case of normal distribution or as medians (range, minimum–maximum) for skewed distribution. Sub-analyses were performed using the Pearson's Chi-squared test for categorial data and the Mann–Whitney U test for metric data. Statistical testing was conducted using SPSS (version 26.0, IBM Corp., USA), with $p < 0.05$ considered as significant.

Results

Patients characteristics

A total of 68 consecutive patients, 30 males and 38 females, with VMs of the face underwent a total of 142 image-guided sclerotherapies between 2010 and 2021 (**Table 1**). The median age was 18.0 years (range, 0.7–64 years) at treatment initiation while 31/68 (45.6%) have been pediatric cases (<18 years). In general, the 68 VMs presented with frontal (4/68, 5.9%), temporal (11/68, 16.2%), orbital (5/68, 7.4%), auricular (4/68, 5.9%), nasal (4/68, 5.9%), buccal (54/68, 79.4%), mental (12/68, 17.6%), and labial (17/68, 25.0%) involvement. Part of the cohort (24/68, 35.3%) presented with extensive lesions extending to more than 1 of these anatomical areas. Thereof, one VM (1/68, 1.5%) included 6 areas and one 4 areas, while 12/68 patients

TABLE 1 Patient characteristics of the study cohort.

Characteristic		Cohort (total, $n = 68$)	Cohort (at terminal follow-up, $n = 52^1/58^2$)
Age at diagnosis	Median (range)	10.5 (0–59)	
Men		30 (44.1%)	
Lesion size (ml)	Median (range)	18.5 (1.5–582.9)	7.0 (1.0–359.1)[1]
Max. lesion diameter (mm)	Median (range)	40.0 (5.0–82.0)	21.5 (3.0–65.0)[1]
Puig classification			
Type I		28 (41.2%)	
Type II		27 (39.7%)	
Type III		13 (19.1%)	
Type IV		0 (0.0%)	
Involved anatomical areas			
Frontal		4 (5.9%)	
Orbital		5 (7.4%)	
Nasal		4 (5.9%)	
Temporal		11 (16.2%)	
Buccal		54 (79.4%)	
Labial		17 (25.0%)	
Mental		12 (17.6%)	
Auricular		4 (5.9%)	
Treatment rationales			
Pain		41 (60.3%)	
Swelling		63 (92.6%)	
Thrombosis		7 (10.3%)	
Cosmetic disfigurement		41 (60.3%)	
Functional limitation		10 (14.7%)	
Impaired swallowing[3]		7 (10.3%)	
Impaired breathing[3]		3 (4.4%)	
Accompanying sequelae		5 (7.4%)	
Exophthalmus		3 (4.4%)	
Blurry visualization		1 (1.5%)	
Sialadenitis		1 (1.5%)	
Symptom graduation			
None		0 (0.0%)	13 (19.1%)[2]
Light		3 (4.4%)	26 (38.2%)[2]
Moderate		22 (32.4%)	19 (27.9%)[2]
Strong		36 (52.9%)	0 (0.0%)[2]
Very strong		7 (10.3%)	0 (0.0%)[2]

Max., maximum; SD, standard deviation; define $n = 52^1/58^2$, related to the available cohort size with MRI (1) and clinical (2) data at terminal follow-up; [3]related to swelling of the cheek/lips, however, without objective obstruction of the upper airways.

(17.6%) presented lesions extending to 3 areas and 10/68 patients (14.7%) to 2 areas. None of the lesions extended into retroorbital areas and none of the patients presented with VMs associated with other anomalies (such as Klippel-Trenaunay syndrome). Puig et al.'s classification (10) showed mostly type I (28/68, 41.2%) and type II (27/68, 39.7%) while 13/68 lesions (19.1%) were categorized as type III. Both therapy-naive patients (36/68, 52.9%) and patients having undergone previous invasive treatments (32/68, 47.1%) by debulking surgery (16/68, 23.5%), sclerotherapy (16/68, 23.5%), or laser-therapy (6/68, 8.8%) without sufficient symptom improvement, were included. Clinical follow-up and MR imaging was obtained for 52/68 patients (76.5%). Regarding the remaining 16 patients, 10/68 (14.7%) have been lost to follow-up while 6/68 patients (8.8%) were children <6 years of age; in the latter cases, only a clinical follow-up was performed, as the aim was to avoid the anesthesia required for MRI and the clinical responses had been sufficient. The mean follow-up period after the last treatment session was 8.7 (±6.8) months for the total cohort.

TABLE 2 Procedural data of the study cohort.

Characteristic		Cohort (total, $n = 68$)
Age at treatment initiation	median (range)	18.0 (0.7–64)
Total number of serial procedures		
1		35 (51.5%)
2		17 (25.0%)
3		6 (8.8%)
4		4 (5.9%)
5		1 (1.5%)
6		2 (2.9%)
7		2 (2.9%)
8		1 (1.5%)

SD, standard deviation.

Procedural characteristics

The mean number of image-guided sclerotherapies per patient was 2.1 (±1.7), for details see Table 2. Thereby, 66/142 procedures (46.5%) were performed in children. A total of 84/142 sclerotherapies (59.2%) were performed using polidocanol foam with a mean injected volume of 4.9 ml (±3.7) while 34/142 treatments (23.9%) were performed using gelified ethanol with a mean injected volume of 1.5 ml (±0.9). Of the 34 latter, lipidiol was mixed to gelified ethanol (1:1) in 9 cases resulting in a mean injected volume of 0.7 ml (±0.6). With regard to the 25/142 (17.6%) treatments with pure gelified ethanol, the mean injected volume was 1.8 ml (±0.9). A sequential combination of both sclerosants, polidocanol foam, and gelified ethanol, with a mean injected volume of 4.5 ml (±4.9) and 2.2 ml (±0.8), respectively, was used in 24/142 procedures (16.9%). The median duration of hospitalization was 3.0 days (range, 2–5 days).

Clinical response

Final clinical follow-up (after last sclerotherapy) revealed an overall response of 54/58 patients (93.1%) including mainly partial relief of symptoms (41/58, 70.7%) and symptom-free presentation (13/58, 22.4%). There was no improvement of

symptoms in 4/58 patients (6.9%) at the last obtained follow-up. No patient presented with clinical progression or relapse of symptoms following sclerotherapy.

Imaging response

After the last sclerotherapy session, the lesion size evaluation of the post-treatment MRI at terminal follow-up compared to pre-treatment imaging revealed CR in 6/52 patients (11.5%), PR in 39/52 patients (75.0%), and SD in 7/52 patients (13.5%). This resulted in an overall objective response rate of 86.5% (45/52), see Figure 1.

Safety and complications

Peri- and postprocedural complications were reported after 15/142 sclerotherapies (10.6%, CIRSE grade 1–4). During one procedure (1/130, 0.7%, CIRSE grade 1), persistent venous bleeding occurred which was resolved after application of suprarenin and a sponge sealant patch coated with human fibrinogen/thrombin as well as manual compression. The postprocedural complications included local skin necroses/ulcerations (2/142, 1.4%, CIRSE grade 3) at the treated area of malformation, in one patient with temporary bleeding, both conservatively resolved by intensified wound management. Further adverse events involved prolonged swelling at injection side (4/142, 2.8% CIRSE grade 2) entailing elongated postprocedural observation, abscess formations successfully treated with antibiotics during prolonged hospitalization (2/142, 1.4%, CIRSE grade 3) as well as mild superficial inflammations (4/142, 2.8%, CIRSE grade 2), the latter not resulting in additional measures. One patient suffered from thrombophlebitis beyond the treated area of malformation (1/142, 0.7%, CIRSE grade 3) 1 week after sclerotherapy. In one case a mild facial palsy occurred postprocedural (1/142, 0.7%, CIRSE grade 4) basically manifested as mouth asymmetry which partially improved but did not recover entirely. Except for the latter case, all complications were self-limited and entirely resolved with conservative means (14/15, 93.3%). Overall, the major complication rate (CIRSE grade >2) was 4.2% (6/142). In our patient cohort, no systemic procedure-related complications have been reported such as allergic reactions due to administered drugs, postinterventional syncope, nausea/vomiting, or fever. No late complications (>30 days) were reported.

Sub-analysis of sclerosant type and volume

The sub-analysis revealed significant differences between the procedures performed with gelified ethanol compared to polidocanol foam concerning the incidence of post-procedural complications (Chi-squared test, $p = 0.01$): regarding the polidocanol subgroup ($n = 84$) a total complication rate of 6.0% (5/84) was found while in the gelified ethanol subgroup ($n = 34$) complications occurred in 23.5% (8/34). In patients with both sclerosant type used in combination ($n = 24$) a complication rate of 8.3% (2/24) was calculated. No significant differences between these subgroups according to the sclerosant used and clinical as well as imaging response were evaluated (Chi-squared test, $p > 0.05$). No significant differences between the sclerosant volume injected and the complication rates were found (Mann–Whitney U test, $p = 0.11$).

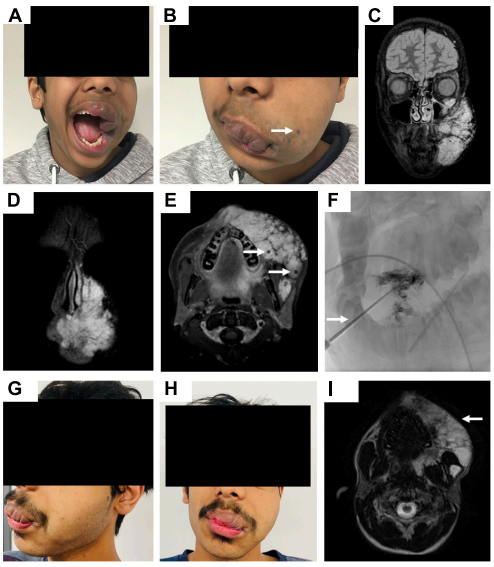

FIGURE 1
Fourteen-year-old male patient with a venous malformation (VM) primarily affecting the nasal, buccal, mental, and labial area of the left face. **(A,B)** Clinical photographs demonstrate the appearance of the patient with extensive swelling in the areas affected by the VM and enormous labial deformation on the left side. Superficial bluish discoloration of the skin is marked by an arrow. **(C–E)** Coronar and axial fat-saturated T2-weighted MR images present typical hyperintense signal of the facial VM with multiple hypointense spots representing phleboliths (arrows). **(F)** Periprocedural digital subtraction angiography (DSA) images while successful percutaneous sclerotherapy of oropharyngeal compartments, the arrow marks the oral inserted angiocatheter. **(G,H)** Clinical photographs show the appearance of the patient 3 years post sclerotherapy with reduced swelling of the affected areas. The patients' lip closure can be performed completely as well as no functional problems with food intake occur. **(I)** In parallel, post-treatment axial T2-weighted MR image reveals a reduced volume of the VM (arrow).

Correlation of clinical and imaging response

The evaluation (of all patients with clinical follow-up and MRI, $n = 52$) revealed no statistically significant differences

between the lesion size reduction on MRI compared to the grade of clinical response (Chi-squared test, $p = 0.15$), even though the percentage of malformations achieving CR on MRI was higher in the symptom-free group (2/11, 18.2%) than in the group with partial relief of symptoms (4/37, 10.8%), see Table 3.

Comparison of children and adults

Comparison of the sclerotherapies performed in children and adults revealed no significant differences in clinical response (Chi-squared test, $p = 0.40$) or imaging response (Chi-squared test, $p = 0.93$), respectively, for details see Table 4. In addition, there were no statistically significant differences between the complication rates (Chi-squared test, $p = 0.57$) in both groups: regarding the pediatric subgroup ($n = 66$) a total complication rate of 12.1% (8/66) was found while complications occurred in 7/76 adult patients (9.2%).

Discussion

In this study of VMs primarily affecting the face, a high overall clinical and objective response rate for image-guided sclerotherapy is being reported, accompanied by a low rate of major complications.

In general, several studies reported on the outcome of image-guided therapies of VMs of the head and neck (14, 15) but were rarely restricted to the face (16); consequently, the comparison of our data with the literature is limited. With respect to most of the studies in this field (17), we evaluated a large cohort while the mean number of sclerotherapies per patient of 2.1 is similar to that reported in the literature.

We were able to demonstrate that percutaneous sclerotherapy of VMs of the face using gelified ethanol and/or polidocanol foam is effective regarding clinical and imaging response which results were also comparable in the adult and pediatric subgroups, respectively. Our findings of at least a partial relief of symptoms in 93% of the patients are similar to those reported after sclerotherapy of head and neck VMs in general (17). Likewise, equivalent results were reported in the few cohorts that focused specifically on facial lesions as exemplarily shown by Spence et al. treating 32 facial VMs using bleomycin and reporting subjective improvement in 91% (16). In this cohort, in which the risk of pneumonitis and pulmonary fibrosis as well as the maximum dose limitation with repeated use of bleomycin had to be considered, a mean dose of 10.5 U was used per session in an average of 3.5 sessions, consisting of a moderate total dose of bleomycin. In the present study a similar clinical outcome could be achieved with comparable complications regarding the minor complication rate of 13% in the cohort of Spence et al. (16). Further, there are currently novel approaches such as combining directly injected bleomycin with reversible electroporation (electrosclerotherapy) to increase the effectiveness of the sclerosants thereby reducing the dose and the risk for relevant adverse events (18). In our study, postprocedural MRI revealed an overall response rate of 87%, supported by the results of previously published studies of head and neck VMs (19, 20). Regarding the correlation of symptom improvement with objective response on MRI, different results have been published. Several studies reported a positive correlation between clinical and objective findings, such as Alexander et al. in 37 venous and lymphatic head and neck malformations (21). Other data suggested that clinical improvement is not always associated with size reduction on MRI (16). Regarding our results, the percentage of malformations achieving complete a response on MRI was higher (18%) in the symptom-free group compared to the group with partial relief of symptoms (11%), though this was not confirmed by statistical significance. Thus, in our opinion, a fraction of patients presents with substantial clinical benefit after sclerotherapy despite a lack of size reduction on MRI, and MRI at follow-up should never be considered as the primary

TABLE 3 Lesion size reduction by clinical response.

	Clinical response ($n = 52$)		
	Symptom-free ($n = 11$)	**Partial relief of symptoms ($n = 37$)**	**No improvement of symptoms ($n = 4$)**
Lesion size reduction ($n = 52$)			
Complete response ($n = 6$)	2 (3.8%)	4 (7.7%)	0 (0.0%)
Partial response ($n = 39$)	9 (17.3%)	28 (53.8%)	2 (3.8%)
Stable disease ($n = 7$)	0 (0.0%)	5 (9.6%)	2 (3.8%)

SD, standard deviation; define complete response, 100% lesion size reduction; partial response, 30% lesion size reduction; stable disease, neither partial response nor progressive disease criteria met.

TABLE 4 Comparison of outcomes between pediatric and adult group.

	Pediatric group ($n = 19^1/25^2$)	**Adult group ($n = 33$)**	***P*-value**
Lesion size reduction ($n = 52$)			$p = 0.93$
Complete response ($n = 6$)	2 (3.8%)[1]	4 (7.7%)	
Partial response ($n = 39$)	14 (26.9%)[1]	25 (48.1%)	
Stable disease ($n = 7$)	3 (5.8%)[1]	4 (7.7%)	
Clinical response ($n = 58$)			$p = 0.40$
Symptom-free ($n = 13$)	5 (8.6%)[2]	8 (13.8%)	
Partial relief ($n = 41$)	17 (29.3%)[2]	24 (41.4%)	
No improvement ($n = 4$)	3 (5.2%)[2]	1 (1.7%)	

SD, standard deviation; define complete response, 100% lesion size reduction; partial response, 30% lesion size reduction; stable disease, neither partial response nor progressive disease criteria met; define $n = 19^1/25^2$, related to the available pediatric cohort with MRI (1) and clinical (2) data at terminal follow-up.

or sole measure of treatment success. Nevertheless, it should be taken into account that due to the relatively short follow-up in our cohort, MRI may not (yet) have been able to show the possibly ongoing remodeling of connective tissue in the treated lesion. In addition, when considering the outcome of this study, it should also be noted that this cohort tended to present fewer complex lesions (Puig I and II = 81% vs. Puig III and IV = 19%) even if similar distributions have been described in the literature as well (10, 22). In parallel to the correlating of clinical outcome with post-treatment MRI, there have also been valuable approaches to obtain predictive data for upcoming treatment planning from pre-treatment imaging. Goyal et al. developed their own MR classification in their series and found that the number of sclerotherapy sessions, the amounts of ethanol for each lesion, and the number of access sites increased with increasing lesion grade (23).

The present study showed a low overall complication rate of 11%, which was also similar in the adult and pediatric subgroups considered separately, with most sequalae having resolved by conservative means. This is rather low compared to published data of sclerotherapy of extracranial VMs such as summarized in the meta-analysis by De Maria et al. involving 37 head and neck studies with a local temporary complication rate of 27–57% (17). Consequently, the presented approach confirms the acceptable risk profile of both sclerosants used and makes them particularly attractive for repeated sclerotherapy sessions even in challenging anatomical locations. Nevertheless, comparing these sclerosants in relation to the incidence of complications, we found significantly more complications in the group being treated with gelified ethanol compared to polidocanol. The latter is a frequently descripted sclerosant causing lysis of vessel endothelium while showing low complication rates (24). Gelified ethanol, a composition of ethanol, is supplemented with water-insoluble cellulose derivative and embedded by a cotton wool-like network. Local adverse events, such as necrosis, temporary nerve palsy, and ethylcellulose fistulas, are reported in 12–48% of patients (25, 26), therefore, this is generally similar to our findings (24%). The hospitalization period of a median of 3 days may be considered long in some countries where sclerotherapies may even be carried out as day-care procedures, which however may also reflect differences in reimbursement systems across countries.

Further, it should be noted that the classification of complications referring to CIRSE used here for facial VMs may not specifically reflect essential relevant consequences related to this specific localization, particularly nerve damage or aesthetic disfigurement, which significantly affect patients in the long term. In our study, a singular case of mild facial palsy occurred which did not resolve completely. The mean injected volume of sclerosant in our study was rather low, which may additionally account for the overall low complication rate. More aggressive approaches can be more effective but may potentially be accompanied with more adverse events, which in our eyes should particularly be considered in anatomically challenging areas such as the face. Though there is some evidence that higher sclerosant volumes may increase the peri- and postprocedural complication rate (27), we could not confirm this relationship in our cohort. This may be due to the fact that by using several access needles, it is possible to distribute the sclerosant over larger lesion volumes avoiding local peak concentrations at the injection sites. Even if we did not analyze the exact number, the use of several puncture sites might help in the reduction of complications.

This multicenter analysis has several limitations: first, it represents a retrospective design including a lack of standardized follow-up data available for the reported patient cohort. Second, standardized disease-related questionnaires to evaluate the specific symptomatology and functional impairments were not routinely used as systematic tools for clinical response. Exemplarily, visual analog scales (VAS) to classify pain as a symptom would have been desirable for more standardized pain assessment. In general, standardized evaluation of quality of life (QoL) may be an appropriate measurement here that should be investigated in further studies using prospective study designs. There was recently published a prospective study protocol presenting QoL as primary study objective after treatment of arteriovenous malformations (28). Additionally, further approaches are being developed to standardize treatment outcome measures, such as the international core outcome set developed by Horbach et al. (29). Third, the objective response was measured by postprocedural changes in VM size as assessed by MRI. For this purpose, no standardized protocol or guidelines/recommendations exist and the oncological RECIST criteria may not be the best option for assessing vascular malformations. In this regard, new functional imaging modalities and advanced analysis tools may prove more versatile for treatment response evaluation in the future (30, 31). These aspects emphasize the general complexity in studying this rare disease, as there are currently no established criteria for the analysis of both clinical and objective response. Fourth, in regard of the high recurrence rate of VMs commonly manifesting after a longer-term, the mean follow-up time of about 9 months after the last sclerotherapy was relatively short. Therefore, it was not yet reasonable to evaluate the recurrence rate as an essential outcome parameter in vascular anomalies. Consequently, conclusions with respect to long-term efficacy of the proposed approach were not feasible.

Conclusion

Image-guided sclerotherapy is effective for treating venous malformations of the face. Clinical response is not always associated with lesion size reduction on imaging. Despite the challenging and complex anatomy of this location, the

procedures carry low complication rates for both adults and children.

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 the Local Ethics Committee (University Hospital, LMU Munich, protocol No. 21-0943, 10/06/2021). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

VS, MM, and MW contributed to the conception and design of the study. VS contributed to the organization of the database, performed the statistical analysis, and wrote the first draft of the manuscript. MM, CG, PS, LS, VS-Z, SD, OÖ, NM, MÜ, MDS, BB, EM, AN, and OU contributed to the acquisition of the data. RB, MK, MS, JR, BG, and WW contributed to the interpretation of the data. All authors revised the work critically for important intellectual content and provided approval for publication of the content, 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.

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References

1. Monroe E. Brief description of ISSVA classification for radiologists. *Tech Vasc Interv Radiol.* (2019) 22:100628. doi: 10.1016/j.tvir.2019.100628

2. Wassef M, Borsik M, Cerceau P, Faucon B, Laurian C, Le Clerc N, et al. [Classification of vascular tumours and vascular malformations. contribution of the ISSVA 2014/2018 classification]. *Ann Pathol.* (2020) 41:58–70. doi: 10.1016/j.annpat.2020.11.004

3. Greene A, Liu A, Mulliken J, Chalache K, Fishman S. Vascular anomalies in 5,621 patients: guidelines for referral. *J Pediatr Surg.* (2011) 46:1784–9. doi: 10.1016/j.jpedsurg.2011.05.006

4. Sadick M, Muller-Wille R, Wildgruber M, Wohlgemuth W. Vascular anomalies (part I): classification and diagnostics of vascular anomalies. *Fortschr Röntgenstr.* (2018) 190:825–35.

5. Dompmartin A, Vikkula M, Boon L. Venous malformation: update on aetiopathogenesis, diagnosis and management. *Phlebology.* (2010) 25:224–35. doi: 10.1258/phleb.2009.009041

6. Hassanein A, Mulliken J, Fishman S, Alomari A, Zurakowski D, Greene A. Venous malformation: risk of progression during childhood and adolescence. *Ann Plast Surg.* (2012) 68:198–201.

7. Colletti G, Ierardi A. Understanding venous malformations of the head and neck: a comprehensive insight. *Med Oncol.* (2017) 34:42. doi: 10.1007/s12032-017-0896-3

8. Schmidt V, Masthoff M, Brill R, Sporns P, Köhler M, Schulze-Zachau V, et al. Image-guided embolotherapy of arteriovenous malformations of the face. *Cardiovasc Intervent Radiol.* (2022) 45:992–1000. doi: 10.1007/s00270-022-03169-0

9. Schmidt V, Masthoff M, Czihal M, Cucuruz B, Häberle B, Brill R, et al. Imaging of peripheral vascular malformations – current concepts and future perspectives. *Mol Cell Pediatr.* (2021) 8:19. doi: 10.1186/s40348-021-00132-w

10. Puig S, Aref H, Chigot V, Bonin B, Brunelle F. Classification of venous malformations in children and implications for sclerotherapy. *Pediatr Radiol.* (2003) 33:99–103. doi: 10.1007/s00247-002-0838-9

11. Miller A, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer.* (1981) 47:207–14.

12. Schmidt V, Masthoff M, Goldann C, Deniz S, Öcal O, Häberle B, et al. Percutaneous sclerotherapy of venous malformations of the hand: a multicenter analysis. *Cardiovasc Intervent Radiol.* (2021) 44:1543–50. doi: 10.1007/s00270-021-02926-x

13. Filippiadis D, Binkert C, Pellerin O, Hoffmann R, Krajina A, Pereira P. Cirse quality assurance document and standards for classification of complications: the cirse classification system. *Cardiovasc Intervent Radiol.* (2017) 40:1141–6. doi: 10.1007/s00270-017-1703-4

14. Songsaeng D, Churojana A, Khumthong R, Mahiwan L. Comparative outcomes for sclerotherapy of head and neck venous vascular malformation between alcohol and bleomycin. *J Med Assoc Thai.* (2015) 98:408–13.

15. Vollherbst D, Gebhart P, Kargus S, Burger A, Kühle R, Günther P, et al. Image-guided percutaneous sclerotherapy of venous malformations of the head and neck: clinical and MR-based volumetric mid-term outcome. *PLoS One.* (2020) 15:e0241347. doi: 10.1371/journal.pone.0241347

16. Spence J, Krings T, terBrugge K, da Costa L, Agid R. Percutaneous sclerotherapy for facial venous malformations: subjective clinical and objective MR imaging follow-up results. *AJNR Am J Neuroradiol.* (2010) 31: 955–60.

17. De Maria L, De Sanctis P, Balakrishnan K, Tollefson M, Brinjikji W. Sclerotherapy for venous malformations of head and neck: systematic review and meta-analysis. *Neurointervention.* (2020) 15:4–17.

18. Wohlgemuth W, Müller-Wille R, Meyer L, Wildgruber M, Guntau M, Heydt S, et al. Bleomycin electrosclerotherapy in therapy-resistant venous malformations of the body. *J Vasc Surg Venous Lymphat Disord.* (2021) 9:731–9. doi: 10.1016/j.jvsv.2020.09.009

19. Baek H, Hong J, Choi J, Suh D. Direct percutaneous alcohol sclerotherapy for venous malformations of head and neck region without fluoroscopic guidance: technical consideration and outcome. *Neurointervention.* (2011) 6:84–8. doi: 10.5469/neuroint.2011.6.2.84

20. Wang Y, Zheng J, Zhu H, Ye W, He Y, Zhang Z. Sclerotherapy of voluminous venous malformation in head and neck with absolute ethanol under digital subtraction angiography guidance. *Phlebology.* (2010) 25:138–44. doi: 10.1258/phleb.2009.009019

21. Alexander M, McTaggart R, Choudhri O, Pandit R, Wu A, Ross M, et al. Quantitative volumetric analysis of head and neck venous and lymphatic malformations to assess response to percutaneous sclerotherapy. *Acta Radiol.* (2016) 57:205–9. doi: 10.1177/0284185115575779

22. Anh T, Nguyen Q, Thi Q, Minh T. Digital subtraction angiography-guided foam sclerotherapy with polidocanol for treating superficial venous malformation. *Ann Vasc Dis.* (2021) 14:231–5. doi: 10.3400/avd.oa.20-00164

23. Goyal M, Causer P, Armstrong D. Venous vascular malformations in pediatric patients: comparison of results of alcohol sclerotherapy with proposed MR imaging classification. *Radiology.* (2002) 223:639–44. doi: 10.1148/radiol.2233010025

24. Ali S, Mitchell S. Outcomes of venous malformation sclerotherapy: a review of study methodology and long-term results. *Semin Intervent Radiol.* (2017) 34:288–93.

25. Schumacher M, Dupuy P, Bartoli J, Ernemann U, Herbreteau D, Ghienne C, et al. Treatment of venous malformations: first experience with a new sclerosing agent–a multicenter study. *Eur J Radiol.* (2011) 80:e366–72. doi: 10.1016/j.ejrad.2010.12.074

26. Dompmartin A, Blaizot X, Théron J, Hammer F, Chene Y, Labbé D, et al. Radio-opaque ethylcellulose-ethanol is a safe and efficient sclerosing agent for venous malformations. *Eur Radiol.* (2011) 21:2647–56. doi: 10.1007/s00330-011-2213-4

27. Rabe E, Pannier F. Sclerotherapy in venous malformation. *Phlebology.* (2013) 28(Suppl. 1):188–91.

28. Schmidt V, Masthoff M, Vielsmeier V, Seebauer C, Cangir Ö, Meyer L, et al. Clinical outcome and quality of life of multimodal treatment of extracranial arteriovenous malformations: the APOLLON study protocol. *Cardiovasc Intervent Radiol.* (2022). [Epub ahead of print]. doi: 10.1007/s00270-022-03296-8

29. Horbach S, van der Horst C, Blei F, van der Vleuten C, Frieden I, Richter G, et al. Development of an international core outcome set for peripheral vascular malformations: the OVAMA project. *Br J Dermatol.* (2018) 178:473–81. doi: 10.1111/bjd.16029

30. Masthoff M, Helfen A, Claussen J, Karlas A, Markwardt N, Ntziachristos V, et al. Use of multispectral optoacoustic tomography to diagnose vascular malformations. *JAMA Dermatol.* (2018) 154:1457–62. doi: 10.1001/jamadermatol.2018.3269

31. Gerwing M, Schindler P, Schneider K, Sundermann B, Köhler M, Stamm A, et al. Diffusion-weighted imaging prior to percutaneous sclerotherapy of venous malformations-proof of concept study for prediction of clinical outcome. *Diagnostics.* (2022) 12:1430. doi: 10.3390/diagnostics12061430

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Coagulation status and determinants of possible aspirin resistance in patients with essential thrombocythemia

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Objectives: The currently recommended aspirin regimen appears inadequate for thromboprophylaxis in essential thrombocythemia (ET). This study aimed not only to evaluate the curative effect of aspirin but also to explore the coagulation status and determinants of aspirin resistance (AR) of ET patients.

Methods: A total of 80 ET patients who underwent coagulation tests, thromboelastography (TEG), and next-generation sequencing (NGS) were involved in the study. Patients were divided into the aspirin sensitivity (AS) group and AR group according to the arachidonic acid inhibition rate. Their clinical features and coagulation function were analyzed.

Results: The incidence of AR was 53.75% (43/80) in 80 ET patients. Fbg was significantly higher in coagulation tests in AR patients compared with AS patients ($P < 0.05$), while the differences in other variables (D-D, PT, PTA, INR, APTT, TT, FDP, and AT-III) were not statistically significant ($P > 0.05$). Compared with AS patients, the *K* values, α angles, MA values, and CI values of TEG in AR patients were statistically smaller ($P < 0.05$), but there was no significant difference in *R* value between them ($P > 0.05$). Univariate and multivariate logistic regression analysis showed that age, irregular use of aspirin, smoking, dyslipidemia, and hypertension increased the risk of AR ($P < 0.05$). In the routine NGS, the driver gene and non-driver gene had no effect on AR in ET patients.

Conclusion: Compared with AS patients, AR patients have enhanced platelet aggregation function, are in a relatively hypercoagulable state, and haveelevated fibrinogen function/levels, all of which cause a worse

coagulation status. ET patients with increasing age, irregular use of aspirin, smoking, dyslipidemia, and hypertension are possibly at higher risk of AR. The routine NGS may not be helpful for the prediction of AR, therefore we recommend adding relevant drug-resistance genes to NGS.

KEYWORDS

essential thrombocythemia, aspirin resistance, clinical features, coagulation status, next-generation sequencing

1 Introduction

Essential thrombocythemia (ET) is a Philadelphia chromosome-negative myeloproliferative neoplasm (MPN) characterized by highly proliferative megakaryocytes in the bone marrow and markedly elevated platelet counts in peripheral blood (1). The arterial and venous thrombosis rate in MPN patients has been estimated as 3-fold and 10-fold increased, respectively, compared with the general population (2). Therefore, it is very important to prevent thrombosis in the treatment of these patients (3).

Low-dose aspirin (75–100 mg/day) is widely used to prevent thrombosis in patients with ET (4, 5). In recent years, studies have shown that patients' responses to aspirin are different (6–8). In the clinic, even if some patients regularly take aspirin for a long time, thrombosis will still occur. This may be because aspirin has insufficient inhibitory effect on platelets. This phenomenon is called aspirin resistance (AR), while the opposite is called aspirin sensitivity (AS) (9, 10). The purpose of this study was not only to evaluate the curative effect of aspirin in ET patients, but also to explore the coagulation status and determinants of AR.

2 Materials and methods

2.1 Patients

We collected data from 80 ET patients in the Xiyuan Hospital from June 2019 to December 2021. All patients were diagnosed according to World Health Organization (WHO) diagnostic criteria (11) and underwent the next-generation sequencing (NGS), coagulation test, and thromboelastography (TEG). All patients were over 18 years old and had been taking aspirin (100 mg once a day) for at least 1 month. Patients could not take other drugs that affect coagulation function within one month before being examined. This study was approved by the medical ethics committee of the hospital (Reference number: 2019XLA024-3) and by the Chinese Clinical Trial Registry (Registry number: ChiCTR2200057736).

2.2 Clinical and laboratory data

Laboratory data included sex, age, aspirin use, cardiovascular risk factors (smoking, dyslipidemia, hypertension, and diabetes), history of thrombosis, presence or absence of splenomegaly, driver gene types (*JAK2*, *CALR*, *MPL*, and Triple negative), presence or absence of non-driver genes, routine blood test (WBC, HGB, PLT, NLR, and PLR), coagulation test (Fbg, D-D, PT, PTA, INR, APTT, TT, FDP, and AT-III), and TEG (R, K, α angle, MA, and CI). Referring to the relevant evaluation criteria (12–16), AR is defined as the inhibition rate of arachidonic acid (AA) <50%, and AS is defined as the inhibition rate of AA ≥50%.

2.3 Statistical analysis

SPSS 26.0 statistical software was used for analysis. The measurement data conforming to the normal distribution adopted the mean ± standard deviation ($x \pm s$) and two-sample *t*-tests. If the measurement data did not conform to the normal distribution, *M* (P25 and P75) was used to express it, and the rank sum test was adopted. The risk factors of AR were analyzed by univariate and multivariate logistic regression. The enumeration data were statistically analyzed with the Chi-squared test. $P < 0.05$ meant statistically significant.

Abbreviations: APTT, activated partial thromboplastin time; AR, aspirin resistance; AS, aspirin sensitivity; AT-III, antithrombin III; CALR, calreticulin; CI, coagulation index; COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; CRP, C-reactive protein; D-D, D-dimer; ET, essential thrombocythemia; Fbg, fibrinogen; FDP, fibrin degradation products; GPIba, glycoprotein 1b, alpha polypeptide; HGB, hemoglobin; INR, international normalized ratio; JAK2, janus kinase 2; K, clot kinetics; MA, maximum amplitude; MPL, myeloproliferative leukemia virus; MPN, myeloproliferative neoplasm; NGS, next-generation sequencing; NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio; PLT, platelet; PT, prothrombin time; PTA, prothrombin time activity; R, reaction time; TEG, thromboelastography; TT, thrombin time; TXA2, thromboxane A2; WBC, white blood cell; WHO, World Health Organization; N/A, not applicable.

3 Results

3.1 The incidence of AR in ET patients

Among the 80 ET patients, 43 (53.75%) developed AR, with an average AA inhibition rate of 25.91%. There were 37 patients with AS, and the average inhibition rate of AA was 65.05%.

3.2 Comparison of clinical characteristics between AR and AS patients

Compared with AS patients, AR patients were significantly older, took aspirin irregularly, and had smoking, dyslipidemia, and hypertension ($P < 0.05$). There was no significant difference ($P > 0.05$) between the two groups in gender, thrombosis history, splenomegaly, driver gene type, white blood cell count (WBC), hemoglobin (HGB), platelet count (PLT), neutrophil-lymphocyte ratio (NLR), and platelet-lymphocyte ratio (PLR), as shown in Table 1.

TABLE 1 Comparison of clinical characteristics between AR and AS patients.

Variable	AR patients $n = 43$	AS patients $n = 37$	P
Sex			0.215
Male, *n* (%)	15 (34.88)	18 (48.65)	
Female, *n* (%)	28 (65.12)	19 (51.35)	
Age at enrollment ($x \pm s$ years)	55.26 ± 11.21	46.08 ± 14.04	0.002
Irregular use of aspirin	20 (46.51)	8 (21.62)	0.021
Cardiovascular risk factors	31 (72.09)	10 (27.03)	0.000
Smoking	9 (20.93)	2 (5.41)	0.046
Dyslipidemia	19 (44.19)	5 (13.51)	0.003
Hypertension	16 (37.21)	3 (8.11)	0.002
Diabetes	1 (2.33)	0	0.354
Thrombosis history	8 (18.60)	6 (16.22)	0.972
Splenomegaly	13 (30.23)	14 (37.84)	0.476
Driver gene			
JAK2+	26 (60.47)	24 (64.86)	0.687
CALR+	10 (23.26)	9 (24.32)	0.911
MPL+	1 (2.33)	0	0.354
Triple negative	6 (13.95)	4 (10.81)	0.674
Routine blood test			
WBC (×109/L)	7.70 (5.94, 9.85)	6.83 (5.29, 9.78)	0.559
HGB (g/L)	140.42 ± 18.89	146.27 ± 18.78	0.170
PLT (×109/L)	593.00 (488.00, 723.00)	599.00 (498.50, 708.50)	0.919
NLR	2.60 (1.69, 3.80)	2.70 (2.06, 3.91)	0.493
PLR	376.44 (278.99, 446.09)	377.89 (277.57, 471.85)	0.717

WBC, white blood cell; HGB, hemoglobin; NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio.

3.3 Comparison of coagulation status between AR patients and AS patients

In the coagulation test, the fibrinogen (Fbg) of 5% (4/80, 3 cases of AR and 1 case of AS) ET patients exceeded the normal upper limit, while other indexes (D-D, PT, PTA, INR, APTT, TT, FDP, and AT-III) did not. On TEG, 7.5% (6/80, 5 cases of AR and 1 case of AS) of patients had enhanced coagulation factor function ($R < 4$ min), 30.0% (24/80, 18 cases of AR and 6 cases of AS) of patients had increased fibrinogen function/level ($K < 1$ min or α angle >72°), 45.0% (36/80, 24 cases of AR and 12 cases of AS) of patients had enhanced platelet aggregation function (MA >70 mm), and 26.3% (21/80, 17 cases

TABLE 2 Comparison of coagulation status between AR patients and AS patients.

Variable	AR patients ($n = 43$)	AS patients ($n = 37$)	P
Coagulation test			
D-D (mg/L)	0.260 (0.170, 0.380)	0.340 (0.160, 0.405)	0.727
PT (s)	11.800 (11.100, 12.100)	11.900 (11.400, 12.550)	0.115
PTA (%)	98.600 (95.400, 105.700)	97.500 (92.000, 103.250)	0.191
INR	1.030 (0.960, 1.050)	1.040 (0.990, 1.080)	0.176
APTT (s)	30.000 (27.800, 31.400)	30.800 (29.100, 32.750)	0.131
TT (s)	18.430 ± 1.422	18.787 ± 1.328	0.253
Fbg (g/L)	2.710 (2.310, 3.220)	2.430 (2.060, 2.745)	0.006
FDP (μg/ml)	2.000 (2.000, 2.000)	2.000 (1.050, 2.000)	0.072
AT-III (%)	98.630 ± 8.866	96.058 ± 10.520	0.239
TEG			
R (min)	5.200 (4.600, 6.700)	5.600 (5.150, 6.650)	0.123
K (min)	1.300 (1.000, 1.600)	1.600 (1.300, 1.850)	0.008
α angle (°)	70.093 ± 6.665	66.603 ± 5.153	0.012
MA (min)	71.412 ± 6.170	67.105 ± 5.220	0.001
CI	2.174 ± 2.172	1.119 ± 1.478	0.015

D-D, D-dimer; PT, prothrombin time; PTA, prothrombin time activity; INR, international normalized ratio; APTT, activated partial thromboplastin time; TT, thrombin time; Fbg, fibrinogen; FDP, fibrin degradation products; AT-III, antithrombin III; R, reaction time; K, clot kinetics; MA, maximum amplitude; CI, coagulation index.

of AR and 4 cases of AS) of patients were in hypercoagulable state (CI >3).

Specifically, in the coagulation test, the Fbg of AR patients was significantly higher than that of AS patients ($P < 0.05$), while the differences in other variables (D-D, PT, PTA, INR, APTT, TT, FDP, and AT-III) were not statistically significant ($P > 0.05$). Compared with AS patients, the *K* values, α angles, MA values, and CI values of TEG in AR patients were lower ($P < 0.05$). But the difference of *R* values was not statistically significant ($P > 0.05$). See Table 2 for greater detail.

3.4 Risk factors for AR: Univariate logistic regression analysis

Aspirin resistance was used as the dependent variable, as shown in Table 3, and 19 influencing factors were analyzed

TABLE 3 Univariate logistic regression analysis of AR.

Variable	Group	*B*	Standard error	Wald	*P*	OR	95% CI
Sex	Female	0.570	0.459	1.543	0.214	1.768	0.719–4.347
	Male*						
Age at enrollment		0.058	0.020	8.612	0.003	1.059	1.019–1.101
Use of aspirin	Irregular	1.148	0.503	5.211	0.022	3.152	1.176–8.447
	Regular*						
Smoking	Yes	1.533	0.818	3.513	0.061	4.632	0.932–23.018
	No*						
Dyslipidemia	Yes	1.623	0.571	8.088	0.004	5.067	1.656–15.502
	No*						
Hypertension	Yes	1.905	0.680	7.846	0.005	6.716	1.772–25.460
	No*						
Diabetes	Yes	21.076	40,192.969	0.000	1.000	1,423,156,409.178	N/A
	No*						
Thrombosis history	Yes	0.166	0.594	0.078	0.779	1.181	0.369–3.781
	No*						
Splenomegaly	Yes	−0.340	0.475	0.513	0.474	0.712	0.281–1.804
	No*						
JAK2+	Yes	−0.188	0.465	0.164	0.685	0.828	0.333–2.059
	No*						
CALR+	Yes	−0.059	0.526	0.013	0.911	0.943	0.336–2.645
	No*						
MPL+	Yes	21.076	40,192.969	0.000	1.000	1,423,156,409.178	N/A
	No*						
Triple negative	Yes	0.291	0.688	0.179	0.672	1.338	0.347–5.157
	No*						
Non-driving gene	Yes	−0.345	0.467	0.547	0.460	0.708	0.284–1.768
	No*						
WBC		−0.046	0.058	0.631	0.427	0.955	0.853–1.070
HGB		−0.017	0.012	1.878	0.171	0.983	0.960–1.007
PLT		0.000	0.001	0.042	0.838	1.000	0.998–1.002
NLR		−0.134	0.141	0.902	0.342	0.875	0.663–1.153
PLR		−0.001	0.001	0.900	0.343	0.999	0.997–1.001

*Control group. N/A, not applicable.

TABLE 4 Multivariate logistic regression analysis of the influencing factors of AR.

Variable	Group	*B*	Standard error	Wald	*P*	OR	95% CI
Age at enrollment		0.040	0.027	2.265	0.132	1.041	0.988–1.097
Use of aspirin	Irregular	1.968	0.657	8.963	0.003	7.158	1.973–25.964
	Regular*						
Smoking	Yes	2.178	0.932	5.465	0.019	8.830	1.422–54.833
	No*						
Dyslipidemia	Yes	1.599	0.758	4.454	0.035	4.949	1.121–21.850
	No*						
Hypertension	Yes	1.728	0.794	4.731	0.030	5.629	1.186–26.710
	No*						

*Control group.

by univariate logistic regression. The results showed that patients with older age (OR = 1.059, 95% CI 1.019–1.101, $P = 0.003$), irregular aspirin use (OR = 3.152, 95% CI 1.176–8.447, $P = 0.022$), dyslipidemia (OR = 5.067, 95% CI 1.656–15.502, $P = 0.004$), and hypertension (OR = 6.716, 95% CI 1.772–25.460, $P = 0.005$) had a higher risk of AR.

3.5 Risk factors for AR: Multivariate logistic regression analysis

Variables with $P < 0.10$ in the univariate logistic regression analysis were included in the multivariate logistic regression analysis. A total of five variables were eligible (age, irregular aspirin use, smoking, dyslipidemia, and hypertension). The results showed that patients who took aspirin irregularly (OR = 7.158 95% CI 1.973–25.964, $P = 0.003$), smoked (OR = 8.830, 95% CI 1.422–54.833, $P = 0.019$), had dyslipidemia (OR = 4.949, 95% CI 1.121–21.850, $P = 0.035$), and had hypertension (OR = 5.629, 95% CI 1.186–26.710, $P = 0.030$) had a higher risk of AR, as shown in Table 4.

Age was statistically significant in the univariate logistic regression analysis, but not in the multivariate logistic regression analysis. The main reason could be the presence of intermediate or confounding variables. We used the two-factor model approach to explore the reasons for the inconsistent results. We established several regression models, each with the dependent variable "aspirin resistance," the independent variable "age" and one other independent variable. Only in the two-factor regression model of "age + dyslipidemia," age was found to be statistically insignificant, so "dyslipidemia" was considered to be an interfering factor affecting age. Studies have shown that age is a risk factor for dyslipidemia (17, 18), and a directed acyclic graph (DAG) showed that dyslipidemia is an intermediate variable of age (Figure 1). After excluding dyslipidemia, the other four variables (age, irregular aspirin use, smoking, and hypertension) were analyzed by multivariate logistic regression. The results showed that the older the patients were, the higher the risk of AR was (OR = 1.067, 95% CI 1.017–1.119, $P = 0.008$). The Chi-square test was used to further analyze the relationship between different age segments and AR, and there were significant differences ($\chi^2 = 11.410$, $P = 0.022$). It could be seen that the AR resistance rate gradually increased with age increase, especially after the age of 40, the proportion of AR patients was significantly higher than that of AS patients (as shown in Table 5).

3.6 Comparison of driver genes mutational load and non-driver genes types between AR and AS patients based on NGS

Table 1 demonstrated that there was no significant difference in the type of driver genes between AR and AS patients. As shown in Table 6, our further analysis showed that there was no significant difference in driver gene mutational load and non-driver gene types between AR and AS patients ($P > 0.05$).

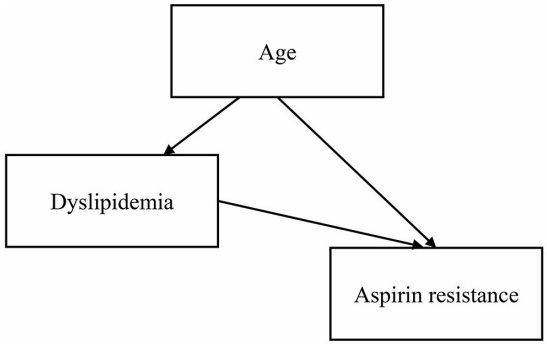

FIGURE 1
The directed acyclic graph among age, dyslipidemia, and aspirin resistance.

TABLE 5 The Chi-square test analysis of the relationship between different age segments and AR.

Variable	Group	Age (%)					*n*	χ^2	*P*
		20–30 years	30–40 years	40–50 years	50–60 years	>60 years			
AR	Yes	1 (14.29)	4 (28.57)	7 (53.85)	16 (66.67)	15 (68.18)	43 (53.75)	11.410	0.022*
	No	6 (85.71)	10 (71.43)	6 (46.15)	8 (33.33)	7 (31.82)	37 (46.25)		
n		7	14	13	24	22	80		

*$p < 0.05$.

TABLE 6 Comparison of driver genes mutational load and non-driver genes types between AR and AS patients.

Variable	AR patients (*n* = 43)	AS patients (*n* = 37)	*P*
Driver gene mutational load			
JAK2 mutational load (%)	20.860 (14.570, 42.238)	20.485 (11.050, 40.573)	0.801
CALR mutational load (%)	24.864 ± 12.812	32.369 ± 13.487	0.231
MPL mutational load (%)	N/A	N/A	N/A
Non-driver gene type	14 (32.56)	15 (40.54)	0.462
DNA methylation (*TET2, DNMT3A, IDH1, IDH2*)	8 (18.60)	8 (21.62)	0.738
Histone modification (*ASXL1, EZH2, KMT2D, KMT2B*)	4 (9.30)	3 (8.11)	0.851
mRNA splicing (*SF3B1, SRSF2, U2AF1, ZRSR2*)	0	2 (5.41)	0.125
Signaling pathways (*LNK/SH2B3, CBL, NRAS/KRAS, PTPN11, ATG2B, ABCB1, NF1*)	3 (6.98)	1 (2.70)	0.385
Transcription factor (*RUNX1, NFE2, PPM1D, TP53, BCOR, WT1, ETV6*)	2 (4.65)	3 (8.11)	0.527

N/A, not applicable.

4 Discussion

Thrombosis prevention is an important therapeutic goal of ET. Aspirin is widely used for the primary and secondary prevention of thrombosis in ET patients. A study showed that the widely promoted low-dose (100 mg mg/day) aspirin regimen could not effectively reduce platelet activation (19). Therefore, exploring coagulation status and determinants of AR has important clinical significance for thromboprophylaxis of ET.

Thrombosis occurs as a result of a combination of changes in the vascular endothelial cells, platelets, coagulation, fibrinolytic system, and blood rheology. Studies have shown that all these factors have changed to varying degrees before thrombosis (3, 20, 21). TEG can dynamically monitor the process of coagulation, and it can help identify the prethrombotic state of patients in combination with a coagulation test (22–24).

In the study, the incidence of AR was 53.75% in 80 patients. Our study found that 45.0% of ET patients had enhanced platelet aggregation (MA >70 mm), and 26.3% had hypercoagulability (CI >3). Specifically, the incidence of enhanced platelet aggregation was higher in AR patients than in AS patients (55.81 > 32.43%, $P < 0.05$), and the incidence of hypercoagulable state was also higher in AR patients than in AS patients (39.53 > 10.81%, $P < 0.05$). This means that even in AS patients, 1/3 of them still have enhanced platelet aggregation function, and 1/10 of them are in a hypercoagulable state. This indicates that the current antiplatelet treatment scheme is really inadequate and needs to be improved. And compared to AS patients, AR patients had significantly higher fibrinogen values, significantly lower *K* values, and significantly larger alpha angles ($p < 0.05$). Therefore, it is considered that hypercoagulability is not only related to the enhancement of platelet aggregation function, but also related to the function/level of fibrinogen. The *R* value can reflect the activity of coagulation factors. In this study, 7.5% of patients had *R* values below normal, which means that there is no general abnormality of coagulation factors in ET patients. And there was no difference in *R* values between AR patients and AS patients, which implies that aspirin has little effect on coagulation factors.

Aspirin can prevent the production of thromboxane A2 (TXA2) in platelets by irreversibly acetylating a serine residue at position 529 of the cyclooxygenase-1 (COX-1) isoform (25). Aging affects AR probably associated with some reduction in the first-pass metabolism and bioavailability of aspirin, which is due to decreased liver mass and perfusion (26, 27). Taking aspirin irregularly will weaken its efficacy, which is an important factor of AR (25). In smokers, the biosynthesis of TXA2 is increased. Their serum C-reactive protein (CRP) also increases, while in the inflammatory state, the risk of AR increases (28, 29). Hypercholesterolemia and hypertension can lead to overexpression of COX-1, which enhances AR (30–32). After univariate and multivariate logistic regression analysis, the risk of AR was higher in ET patients with increasing age, irregular aspirin use, smoking, dyslipidemia, and hypertension. It suggests that ET patients should take aspirin regularly, quit

smoking, and control blood lipid and blood pressure. However, there are too few diabetic patients among these patients to accurately judge the relationship between diabetes and AR, which is still worthy of attention.

Polymorphisms in some genes (COX-1, COX-2, GPIba, etc.) are strongly associated with AR, but it is still unknown whether the ET's driver and non-driver genes affect it (33, 34). Unfortunately, our study showed that the driver and non-driver genes do not assist in predicting AR in ET patients. This reminds us of the need to add relevant drug-resistance genes to the routine NGS.

5 Conclusion

Compared with AS patients, AR patients have enhanced platelet aggregation function, are in a relatively hypercoagulable state, and have elevated fibrinogen function/levels, all of which cause a worse coagulation status. ET patients with increasing age, irregular aspirin use, smoking, dyslipidemia, and hypertension may have a higher risk of AR. The routine NGS may not be helpful for the prediction of AR, therefore we recommend adding relevant drug-resistance genes to NGS.

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 human participants were reviewed and approved by the Medical Ethics Committee of China Academy of Chinese Medical Sciences Xiyuan Hospital. The patients/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

EY and XH designed the study. ZW collected the data. YuL, DW, YS, and YZ performed the analysis. JN and ZC normalized the pictures. EY, YaL, and WL wrote the original draft. All authors contributed to the article and approved the submitted version.

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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. Tefferi A, Pardanani A. Essential thrombocythemia. *N Engl J Med.* (2019) 381:2135–44. doi: 10.1056/NEJMcp1816082

2. Hultcrantz M, Björkholm M, Dickman P, Landgren O, Derolf ÅR, Kristinsson S, et al. Risk for arterial and venous thrombosis in patients with myeloproliferative neoplasms: a population-based cohort study. *Ann Intern Med.* (2018) 168:317–25. doi: 10.7326/M17-0028

3. Falanga A, Marchetti M, Schieppati F. Prevention and management of thrombosis in BCR/ABL-negative myeloproliferative neoplasms. *Hamostaseologie.* (2021) 41:48–57. doi: 10.1055/a-1334-3259

4. Landolfi R, Marchioli R, Kutti J, Gisslinger H, Tognoni G, Patrono C, et al. Efficacy and safety of low-dose aspirin in polycythemia vera. *N Engl J Med.* (2004) 350:114–24. doi: 10.1056/NEJMoa035572

5. Barbui T, Vannucchi A, Buxhofer-Ausch V, De Stefano V, Betti S, Rambaldi A, et al. Practice-relevant revision of IPSET-thrombosis based on 1019 patients with WHO-defined essential thrombocythemia. *Blood Cancer J.* (2015) 5:e369. doi: 10.1038/bcj.2015.94

6. Perrier-Cornet A, Ianotto J, Mingant F, Perrot M, Lippert E, Galinat H. Decreased turnover aspirin resistance by bidaily aspirin intake and efficient

cytoreduction in myeloproliferative neoplasms. *Platelets.* (2018) 29:723–8. doi: 10.1080/09537104.2017.1361018

7. Gillet B, Ianotto J, Mingant F, Didier R, Gilard M, Ugo V, et al. Multiple Electrode Aggregometry is an adequate method for aspirin response testing in myeloproliferative neoplasms and differentiates the mechanisms of aspirin resistance. *Thromb Res.* (2016) 142:26–32. doi: 10.1016/j.thromres.2016.04.006

8. Hankey G, Eikelboom J. Aspirin resistance. *Lancet.* (2006) 367:606–17. doi: 10.1016/S0140-6736(06)68040-9

9. Jing Y, Yue X, Yang S, Li S. Association of aspirin resistance with increased mortality in ischemic stroke. *J Nutr Health Aging.* (2019) 23:266–70. doi: 10.1007/s12603-019-1168-z

10. Yi X, Zhou Q, Lin J, Chi L. Aspirin resistance in Chinese stroke patients increased the rate of recurrent stroke and other vascular events. *Int J Stroke.* (2013) 8:535–9. doi: 10.1111/j.1747-4949.2012.00929.x

11. Arber D, Orazi A, Hasserjian R, Thiele J, Borowitz M, Le Beau M, 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

12. Schöchl H, Nienaber U, Hofer G, Voelckel W, Jambor C, Scharbert G, et al. Goal-directed coagulation management of major trauma patients using thromboelastometry (ROTEM)-guided administration of fibrinogen concentrate and prothrombin complex concentrate. *Crit Care.* (2010) 14:R55. doi: 10.1186/cc8948

13. Kang L, Jian L, Cheng-bin W, Hai W, Li Y, Yu-long C. Application of PFA-100 and thromboelastograph for monitoring clinical efficacy of aspirin in elderly patients with cardiovascular disease. *Chin J Misdiagnostics.* (2011) 11:3789–91.

14. Temperilli F, Rina A, Massimi I, Montemari A, Guarino M, Zicari A, et al. Arachidonic acid-stimulated platelet tests: identification of patients less sensitive to aspirin treatment. *Platelets.* (2015) 26:783–7. doi: 10.3109/09537104.2014.1003291

15. Olechowski B, Ashby A, Mariathas M, Khanna V, Mahmoudi M, Curzen N. Is arachidonic acid stimulation really a test for the response to aspirin? Time to think again? *Expert Rev Cardiovasc Ther.* (2017) 15:35–46. doi: 10.1080/14779072.2017.1266255

16. Gurbel P, Bliden K, DiChiara J, Newcomer J, Weng W, Neerchal N, et al. Evaluation of dose-related effects of aspirin on platelet function: results from the aspirin-induced platelet effect (ASPECT) study. *Circulation.* (2007) 115:3156–64. doi: 10.1161/CIRCULATIONAHA.106.675587

17. Yu J, Cunningham J, Thouin S, Gurvich T, Liu D. Hyperlipidemia. *Prim Care.* (2000) 27:541–87. doi: 10.1016/S0095-4543(05)70164-0

18. Eaton C. Hyperlipidemia. *Prim Care.* (2005) 32:1027–55. doi: 10.1016/j.pop.2005.09.002

19. Rocca B, Tosetto A, Betti S, Soldati D, Petrucci G, Rossi E, et al. A randomized double-blind trial of 3 aspirin regimens to optimize antiplatelet therapy in essential thrombocythemia. *Blood.* (2020) 136:171–82. doi: 10.1182/blood.2019004596

20. Sixma J. The prethrombotic state. *Br J Haematol.* (1980) 46:515–22. doi: 10.1111/j.1365-2141.1980.tb06007.x

21. Bauer K, Rosenberg R. The pathophysiology of the prethrombotic state in humans: insights gained from studies using markers of hemostatic system activation. *Blood.* (1987) 70:343–50. doi: 10.1182/blood.V70.2.343.343

22. Yao Y, Zhang J, Tang X, He C, Ma Y, Xu J, et al. Head to head comparison of two point-of-care platelet function tests used for assessment of on-clopidogrel platelet reactivity in chinese acute myocardial infarction patients undergoing percutaneous coronary intervention. *Chin Med J.* (2016) 129:2269–74. doi: 10.4103/0366-6999.190664

23. Tang N, Yin S, Sun Z, Xu X, Qin J. The relationship between on-clopidogrel platelet reactivity, genotype, and post-percutaneous coronary intervention outcomes in Chinese patients. *Scand J Clin Lab Invest.* (2015) 75:223–9. doi: 10.3109/00365513.2014.993696

24. Trelinski J, Okonska M, Robak M, Chojnowski K. Assessment of rotation thromboelastometry parameters in patients with essential thrombocythemia at diagnosis and after hydroxyurea therapy. *Blood Coagul Fibrinolysis.* (2016) 27:205–9. doi: 10.1097/MBC.0000000000000421

25. Floyd C, Ferro A. Antiplatelet drug resistance: molecular insights and clinical implications. *Prostaglandins Other Lipid Mediat.* (2015) 120:21–7. doi: 10.1016/j.prostaglandins.2015.03.011

26. Zeeh J, Platt D. The aging liver: structural and functional changes and their consequences for drug treatment in old age. *Gerontology.* (2002) 48:121–7. doi: 10.1159/000052829

27. Shi S, Klotz U. Age-related changes in pharmacokinetics. *Curr Drug Metab.* (2011) 12:601–10. doi: 10.2174/138920011796504527

28. Aksu K, Donmez A, Keser G. Inflammation-induced thrombosis: mechanisms, disease associations and management. *Curr Pharm Des.* (2012) 18:1478–93. doi: 10.2174/138161212799504731

29. Poudel K, Poudel-Tandukar K, Bertone-Johnson E, Pekow P, Vidrine D. Inflammation in relation to intensity and duration of cigarette smoking among people living with HIV. *AIDS Behav.* (2021) 25:856–65. doi: 10.1007/s10461-020-03048-0

30. Gendron M, Thorin-Trescases N, Villeneuve L, Thorin E. Aging associated with mild dyslipidemia reveals that COX-2 preserves dilation despite endothelial dysfunction. *Am J Physiol Heart Circ Physiol.* (2007) 292:H451–8. doi: 10.1152/ajpheart.00551.2006

31. Wong W, Tian X, Huang Y. Endothelial dysfunction in diabetes and hypertension: cross talk in RAS, BMP4, and ROS-dependent COX-2-derived prostanoids. *J Cardiovasc Pharmacol.* (2013) 61:204–14. doi: 10.1097/FJC.0b013e31827fe46e

32. Gong X, Wang X, Xu Z, Zhu T, Zhang Q, Zhang J, et al. Over-expression of cyclooxygenase-2 in increased reticulated platelets leads to aspirin resistance after elective off-pump coronary artery bypass surgery. *Thromb Res.* (2017) 160:114–8. doi: 10.1016/j.thromres.2017.11.003

33. Li X, Cao J, Fan L, Wang Q, Ye L, Cui C, et al. Genetic polymorphisms of HO-1 and COX-1 are associated with aspirin resistance defined by light transmittance aggregation in Chinese Han patients. *Clin Appl Thromb Hemost.* (2013) 19:513–21. doi: 10.1177/1076029612444002

34. Al-Azzam S, Alzoubi K, Khabour O, Tawalbeh D, Al-Azzeh O. The contribution of platelet glycoproteins (GPIa C807T and GPIba C-5T) and cyclooxygenase 2 (COX-2G-765C) polymorphisms to platelet response in patients treated with aspirin. *Gene.* (2013) 526:118–21. doi: 10.1016/j.gene.2013.04.083

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Case report: Umbilical vessel aneurysm thrombosis and factor V Leiden mutation leading to fetal demise

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Complicated pregnancies are nowadays a major public health concern, with possible lethality or sequelae both for the mother and the fetus. Blood coagulation disorders (including antiphospholipid syndrome, factor V Leiden mutation and antithrombin deficiency) and hypertensive gestational disorders are very well-known contributors of complicated pregnancies with poor fetal outcome, such as intrauterine growth retardation (IUGR) and fetal demise. Less commonly, vascular malformations of the placenta can also potentially lead to serious complications such as IUGR and fetal death. These malformations include hypercoiled umbilical cord, umbilical cord knot, umbilical cord varix, umbilical cord arterial or venous aneurysm, and velamentous insertion of the umbilical cord potentially leading to Benckiser's hemorrhage. Here, we report the case of a 29-year-old Gravida 2 Para 0 mother with previous history of stillbirth and smoking, admitted to the obstetrics department for the absence of fetal movement at 38 weeks of amenorrhea (WA). First-trimester and second-trimester routine ultrasounds were otherwise normal. Ultrasound performed at 38 WA revealed a 83 × 66 × 54 mm cystic heterogenous mass at the umbilical cord insertion. After delivery, fetal and placental pathology as well as maternal blood testing were performed. Fetal pathology was otherwise normal, except for diffuse congestion and meconial overload suggesting acute fetal distress. Fetal karyotype was normal (46 XX). Placental pathology revealed an umbilical artery aneurysm (UAA) at the base of the insertion of the umbilical cord, lined with a $CD34^+$ $CD31^+$ endothelium. After dissection, the aneurysm was filled with hemorrhagic debris, indicating aneurysm thrombosis. Histopathology revealed associated maternal vascular malperfusion (MVM) and increased peri-villous fibrin (IPF). Maternal blood tests revealed heterozygous factor V Leiden mutation, without other associated auto-immune conditions (such as antiphospholipid syndrome). Umbilical artery aneurysms remain extremely rare findings in the placenta, with <20 reported cases. Umbilical artery aneurysms have tendency to be located at the base of the insertion of the placenta, and lead to fetal demise in more than 60% of cases, mainly due to aneurysmal thrombosis, hematoma, possible vascular compression and/or rupture. Umbilical vessel aneurysms can be associated with trisomy 18 or 13. In our case, the association of factor V Leiden mutation, a hypercoagulable state, with UAA could explain massive thrombosis of the

aneurysmal lumen and sudden fetal demise. Further consideration of current guidelines for surveillance and management of UAA would allow appropriate planned delivery in maternal care settings.

KEYWORDS

umbilical vessel aneurysm, thrombosis, thrombophilia, fetal demise, stillbirth, umbilical artery aneurysm

1. Introduction

Fetal demise remains a major concern in the course of a pregnancy, with an important psychological impact on mothers, necessitating precise identification and careful postpartum follow-up (1–3). In high-income countries, advanced maternal age, maternal smoking, obesity and primiparity are well-known risk factors of fetal demise (4). Etiology of stillbirth include placental anomalies and/or associated lesions, chromosomic, genetic, infectious, and inflammatory causes (2, 5–7). The wide spectrum of etiologies accounting for fetal demise, requires accurate clinical history taking, laboratory tests, ultrasound assessment, and most importantly pathological evaluation of the fetus and placenta (2, 5, 6). Among placental causes, vascular insufficiency inducing maternal vascular malperfusion (MVM), fetal vascular malperfusion (FVM) and increased peri-villous fibrin (IPF) remain an important cause of fetal anoxia and death (8, 9). In some cases, vascular insufficiency is associated with maternal thrombophilia, such as factor V Leiden mutation, especially in the context of recurrent pregnancy loss (10, 11). In some cases, umbilical cord anomalies can be the single explanation accounting for fetal IUGR, acute fetal asphyxia and stillbirth (8, 12, 13). Among umbilical cord abnormalities, the presence of a single umbilical artery (SUA), umbilical knots (UK), hypercoiled umbilical cord (HUC), umbilical cord thrombosis (UCT) or umbilical vessel aneurysm (UVA) account for most of the etiologies of IUGR and stillbirth in developing fetuses (12–15). Umbilical vessel aneurysms remain very rare yet potentially lethal abnormalities of the umbilical cord, especially in association with disturbed blood flow, aneurysm rupture, or intra-vascular thrombosis (15–17). In this article, we report a unique case of umbilical artery aneurysm thrombosis in a mother suffering from thrombophilia (factor V Leiden mutation) leading to stillbirth at 38 weeks of amenorrhea (WA) in an otherwise healthy woman.

2. Case description

A 29-year-old Caucasian Gravida 2 Para 0 mother admitted to the Department of Obstetrics and Gynecology (University Hospital of Dijon—France) for the absence of fetal movement at 38 WA. Past medical history includes previous early miscarriage associated with previous maternal smoking. The mother was not under medication during pregnancy. Maternal body mass index was otherwise normal (23.8 kg/m^2). Concerning family history, the patient's mother and grandmother suffered from recurrent thrombophlebitis. Maternal serologies remained negative (mother naive for toxoplasmosis and viral infections) except for elevated IgG against rubella virus. First-trimester maternal serum screening was otherwise normal, with free β-human chorionic gonadotrophin (free β-hCG) at 39.200 IU/L −1,2 Multiple of the Median (MoM), pregnancy associated plasma protein A (PAPP-A) at 3.77 IU/L −1.07 MoM, and nuchal translucency at 1.3 mm −0.83 MoM. Combined first-trimester screening for trisomy 21 remained beyond 1/10.000, which indicated the absence of fetal aneuploidy. During pregnancy, first-trimester (12 WA) and second-trimester routine ultrasounds (22 WA) were otherwise normal. Third-trimester ultrasound performed at 37 WA revealed a 73 mm (major axis) cystic heterogenous mass at the umbilical cord insertion (Figure 1). The patient was referred to the Prenatal Diagnostic Center of the University Hospital of Dijon for further investigation. Ultrasonography performed at the Prenatal Diagnostic Center at 37 WA confirmed the presence of the cystic mass at the umbilical cord insertion, which revealed normal blood flow. The mother was discharged from the hospital, with appropriate instructions in case of abnormal fetal movements and/or signs of labor. The mother was later admitted to the Gynecology Emergency Services (University Hospital of Dijon) at 38 WA for abdominal pain and absence of fetal movements. Ultrasonography confirmed the absence of fetal movements and fetal cardiac activity. At time of fetal death, maternal blood testing was performed to rule out coagulation disorders or associated infection. The Kleihauer and antiglobin test were negative, indicating the absence of fetal-maternal hemorrhage or fetal hemolytic anemia. Elevated C-Reactive protein (20.3 mg/L) was associated with hyperleukocytosis (18.5 G/L), thus raising suspicion for chorioamnionitis. Immune assays revealed positive anti-nuclear antibodies (titers 1/160). Fibrinogen (3.3 g/L) and prothrombin factors (factor II: 112%, factor V: 90%, factor VII: 90%) were within normal ranges. Testing for SARS-CoV-2, CMV and HSV infections were negative at time of fetal death.

Vaginal delivery and fetal expulsion were then performed. After delivery, the placenta and the fetus were referred to the Department of Pathology (University Hospital of Dijon)

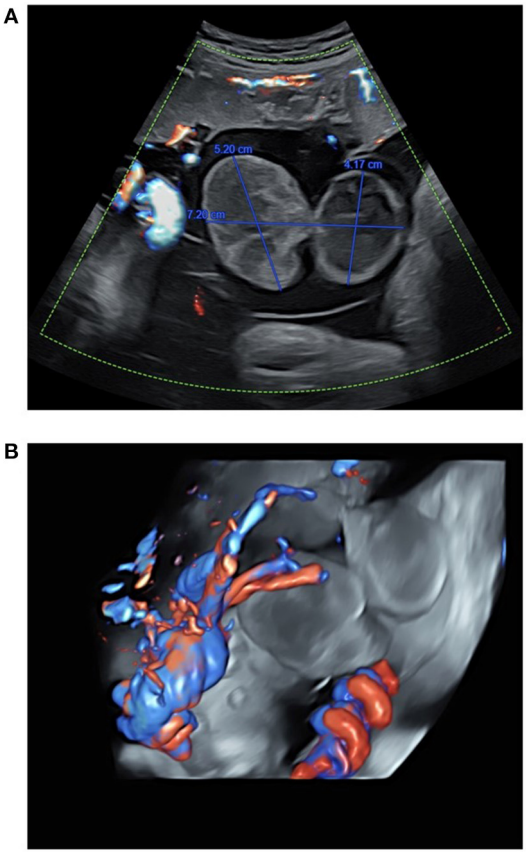

FIGURE 1
Ultrasound assessment of the umbilical cord in a 29-year-old mother at 37 WA, before stillbirth. **(A)** Ultrasound showed a bilocular cyst measuring 72 mm of major axis, and 31–52 mm of minor axis. **(B)** Umbilical vessel assessment of blood flow using tridimensional high definition Doppler ultrasound showing blood flow around the umbilical cord cyst.

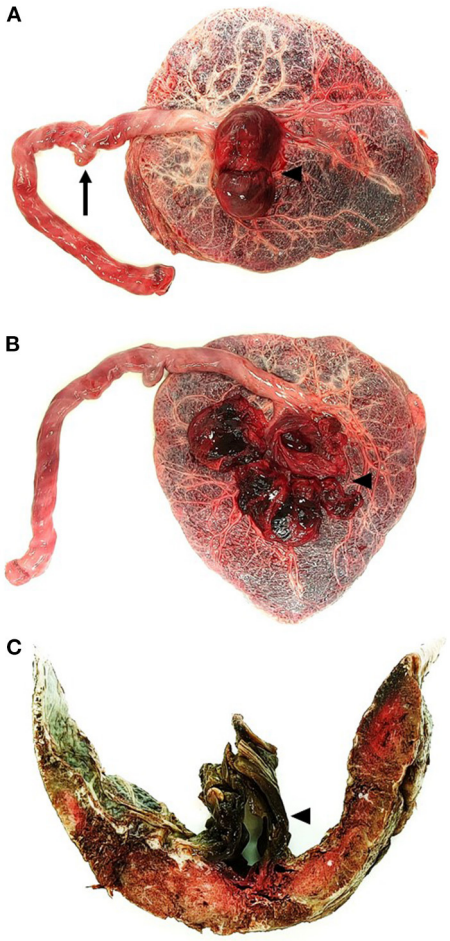

FIGURE 2
Gross examination of the placenta of a 29-year-old with stillbirth at 38 WA. **(A)** Placental examination of the fetal plate revealed a false knot on the umbilical cord (arrow) associated with a large aneurysmal cyst at the insertion of the umbilical cord (arrowhead). **(B)** At dissection, the aneurysmal cyst was filled with large blood clots (arrowhead). **(C)** At cut-section, the placental parenchyma showed subchorionic whitish nodules and confirmed the presence of the aneurysm, in relation to the umbilical arteries (arrowhead).

for further analysis. Management of stillbirth was performed according to the 2016 French guidelines (18). Placental analysis was performed in accordance with the 2016 Amsterdam consensus (9). Fetal autopsy was performed according to the current French guidelines (19). At autopsy, fetal pathology revealed a non-macerated, female eutrophic fetus (weight: 2,952 g −40th percentile), showing cyanosis of the lips and fingers, devoid of dysmorphic traits (including facial dysmorphism or limb anomalies) (20). Fetal measurements were within normal ranges, including crown-heel length (49 cm −40th percentile), crown-rump length (33 cm −30th percentile) and head circumference (33 cm −50th percentile) (21). At dissection, formalin-fixed organ weights remained within normal ranges, without evidence of malformation (22). Histopathological analysis revealed, apart from diffuse visceral congestion, meconial and keratin pigments in lung alveoli, thus suggesting previous meconium aspiration syndrome in the context of acute fetal distress. Fetal karyotype performed using thymic tissue was normal (negative for aneuploidy or chromosomal anomalies), with a 46 XX formula. Gross examination of the placenta revealed a eutrophic placenta (496 g −50th percentile), of normal configuration (oval shape), measuring 24 cm of length, 18 cm of width and 2 cm of thickness, with normal membrane insertion (23, 24). The umbilical cord measured 36 cm of length and 2 cm of diameter. At the fetal plate, a large bilocular cystic lesion was observed at the insertion of the umbilical cord (Figure 2) measuring ∼8 cm of diameter. At dissection, the cystic lesion was filled up with hemorrhagic debris and large blood clots (Figure 2).

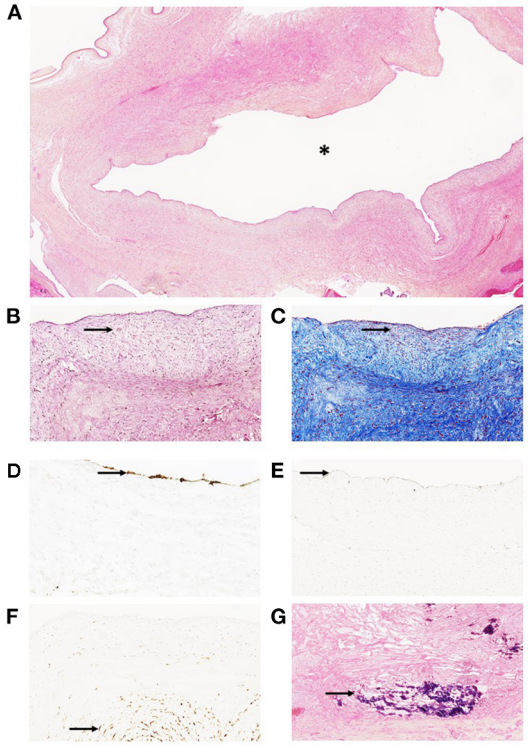

FIGURE 3
Histopathological analysis of the aneurysmal cyst of the umbilical cord in a 29-year-old mother with stillbirth at 38 WA. **(A)** (Hematoxylin Eosin Saffron—HES, ×100): histopathology showed a thick-walled large cyst (asterisk). **(B)** (HES, ×200)–**(C)** (Trichrome Blue, ×200): The wall of the cyst is mainly composed of mesenchymal cells and few scattered smooth muscle cells (arrows). **(D)** (CD34, ×200): Immunodetection of CD34 revealed few endothelial cells at the surface of the cyst (arrow). **(E)** (D2-40, ×200): Few mesenchymal cells exhibited cytoplasmic positivity after immunodetection (arrow). **(F)** (CD31, × 200): immunodetection of CD31 revealed few endothelial cells at the surface of the cyst (arrow). **(G)** (HES, × 200): calcified hemorrhagic debris (arrow) were seen after dissection and sampling of the aneurysmal cyst.

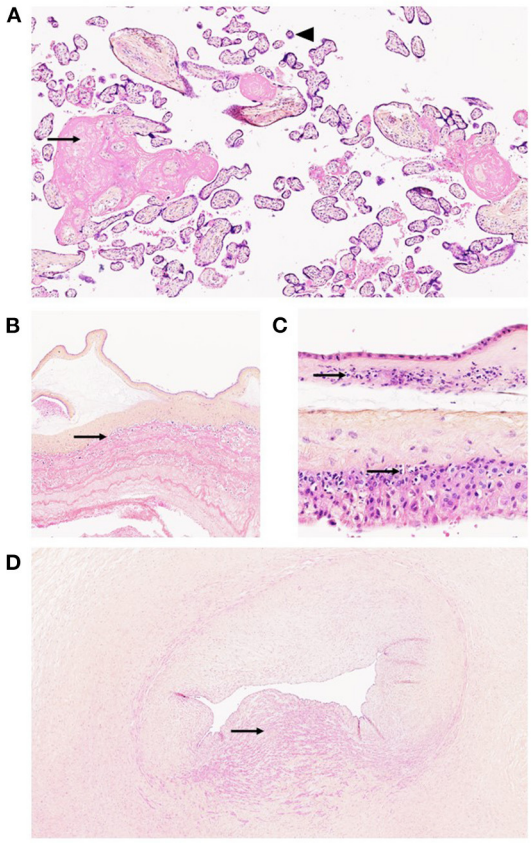

FIGURE 4
Histopathological analysis of the placenta in a 29-year-old mother with stillbirth at 38 WA. **(A)** (HES, ×200): Increased perivillous fibrin (arrow) and distal villous hypoplasia (arrowhead) were seen, indicating maternal vascular malperfusion. **(B)** (HES, ×200): Subchorionic thrombosis was observed (arrow), characterized by linear deposition of fibrin and red blood cells in the intervillous space adjacent to the fetal plate. **(C)** (HES, ×400): Histopathology showed evidence of stage 1 grade 2 chorioamnionitis, characterized by neutrophilic infiltration of the decidua parietalis of the membranes (arrows), without amniotic necrosis. **(D)** (HES, ×200): Histopathology showed rarefaction of smooth muscle cells (arrow) at the level of umbilical vessels adjacent to the aneurysmal cyst, inserted at the fetal plate.

Placental cut section revealed whitish subchorionic nodules compatible with subchorionic thromboses (SCT; Figure 2). Histopathological analysis of the cystic lesion revealed a large cavity filled with hemorrhagic debris at the base of the umbilical cord (Figure 3). The wall of the cyst was mainly composed of elastic fibers intermingled with scattered smooth muscle cells (Figure 3). Few scattered $CD34^+$ $CD31^+$ $D2\text{-}40^+$ endothelial cells were observed close to the lumen of the cyst (Figure 3). Histopathological analysis of the subchorionic nodules confirmed SCT (Figure 4). Concerning the placental villi, MVM and focal IPF were also observed (Figure 4). Histopathological analysis of the membranes revealed stage 1 grade 2 chorioamnionitis (Figure 4). Furthermore, histopathological analysis showed rarefaction of smooth muscle cells at the level of umbilical vessels adjacent to the aneurysmal cyst (Figure 4).

Considering placental pathological examination (MVM, SCT, IPF, aneurysmal thrombosis), previous stillbirth, and previous family history of thromboembolism, the mother was referred to the Department of Hemostasis (University Hospital of Dijon—France) for thrombophilia testing. Blood analysis indicated an antithrombin activity within normal ranges (122%). The protein C resistance test revealed an increased coagulation time of maternal blood with adjunction of activated C protein (35 s before adjunction, vs. 62.9 s after adjunction of activated C protein). Protein S activity was

measured at 70% of normal activity. Lupus anticoagulant testing was negative. Further genetic analysis of the maternal blood revealed the presence of heterozygous factor V Leiden mutation (c.1691G>A; p.Arg506Gln), which confirmed thrombophilia (LightCycler® 480 System, Roche—Switzerland). After the episode, the presence of maternal thrombophilia would indicate the necessity for preventive anticoagulant therapy (100 mg of aspirin per day) during pregnancy, associated with low-molecular weight heparin (LMWH) for 6 weeks postpartum.

3. Discussion

Umbilical artery aneurysms remain a very rare yet lethal finding in the placenta, with only six live births (16, 25, 26). To date, including our case, only 18 cases were reported in the literature (16). Two thirds (12/18) of the published cases were associated with a single umbilical artery, and one quarter (4/18) with placental trisomy 18 mosaicism (17, 26–32). The pathophysiology of UAA might be explained by the increased weakness of umbilical arteries at their insertion on the fetal plate, where Wharton's jelly is relatively less abundant, thus favoring the appearance of aneurysms (16, 25, 28). The presence of an increased fetal cardiac output during development might explain the increase in umbilical artery intravascular pressure and the genesis of an aneurysm, in areas of greater elasticity where Wharton's jelly is absent (25, 26, 28). Including our case, 12 out of 18 UAA were located at the insertion of the umbilical cord (16, 25, 28). However, in our case, no evidence of trisomy mosaicism or single umbilical artery was noted.

In all cases of UAA, the cystic appearance of UAA during ultrasonography routine checkups can potentially lead to a misdiagnosis of a non-lethal umbilical cord pseudocyst, patent urachus or omphalocele (29, 33–35). Current guidelines for management of umbilical cord cysts in the second and third trimester imply to perform fetal karyotype testing in order to rule out aneuploidy, due to the frequent association of umbilical cord cysts and chromosomal anomalies such as trisomy 13 or 18 (29, 33). Nevertheless, in the absence of chromosomal anomalies, the presence of a potentially lethal vascular malformation of the umbilical cord should be considered. The potential lethality of such rare lesions of the umbilical cord should raise awareness for the discussion of new up-to-date guidelines on the management of umbilical cord cystic lesions in otherwise healthy fetuses. Scheduled induction of labor and preventive anticoagulant therapy should therefore be considered in large umbilical cord cysts, regardless of the presence of reassuring signs at ultrasonography.

In our case, history of previous stillbirth motivated thrombophilia testing in this patient. Previous studies showed that mothers carrying Factor V Leiden mutation had higher rates of early and late fetal loss during pregnancy (36–38). Histopathological findings in placentas of mothers with thrombophilia, including placental infarcts, MVM, IPF, and avascular villi, provide a partial explanation for chronic placental malperfusion, IUGR, fetal hypoxia and fetal demise (39, 40). In our case, the presence of MVM and IPF could be attributed to maternal Factor V Leiden mutation, without IUGR (eutrophic fetus-−40th percentile). The presence of UAA alone provides an explanation for fetal demise, as very high rates of stillbirth in mothers carrying UAA were observed in the literature. Of note, the occurrence of intra-aneurysmal thrombosis remain poorly explained in the literature. Data concerning UAA showed that compression of surrounding umbilical vessels following the formation of a large-sized aneurysm could lead to vascular thrombosis following altered blood flow (27, 41). In our case, we can hypothesize that the presence of maternal thrombophilia might have facilitated aneurysm thrombosis and acute fetal asphyxia. Few studies have focused on the possible outcomes of umbilical artery thrombosis, which remain a very rare event during pregnancy (42, 43). The association of umbilical artery thrombosis with Factor V Leiden mutation remain controversial in the literature and poorly described (42, 43). Without enough clear evidence of the association between maternal Factor V Leiden mutation and umbilical artery thrombosis, further studies will be required in order to explore putative links between UAA and thrombophilia. As an example, polymorphisms of the angiotensin-converting enzyme gene, involved in preeclampsia, have been demonstrated as risk factors of aneurysm formation and potentially identified as a cause of thrombophilia (44–46).

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 authors.

Ethics statement

Ethical approval was not provided for this study on human participants because patient consent was obtained for the case report. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Writing and editing: GT, CO, and LM. Resources: ED, FH, OM, NI, LR, and LM. Investigation: GT, CO, ED, OM, NI, and LR. All authors

contributed to the article and approved the submitted version.

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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.

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References

1. Serfaty A. Stillbirth in France. *Lancet.* (2014) 384:1672. doi: 10.1016/S0140-6736(14)62032-8

2. Page JM, Silver RM. Stillbirth: evaluation and follow-up. *Obstet Gynecol Clin North Am.* (2020) 47:439–51. doi: 10.1016/j.ogc.2020.04.008

3. Burden C, Bradley S, Storey C, Ellis A, Heazell AEP, Downe S, et al. From grief, guilt pain and stigma to hope and pride - a systematic review and meta-analysis of mixed-method research of the psychosocial impact of stillbirth. *BMC Pregnancy Childbirth.* (2016) 16:9. doi: 10.1186/s12884-016-0800-8

4. Flenady V, Koopmans L, Middleton P, Frøen JF, Smith GC, Gibbons K, et al. Major risk factors for stillbirth in high-income countries: a systematic review and meta-analysis. *Lancet.* (2011) 377:1331–40. doi: 10.1016/S0140-6736(10)62233-7

5. Stanley KE, Giordano J, Thorsten V, Buchovecky C, Thomas A, Ganapathi M, et al. Causal genetic variants in stillbirth. *N Engl J Med.* (2020) 383:1107–16. doi: 10.1056/NEJMoa1908753

6. Miller ES, Minturn L, Linn R, Weese-Mayer DE, Ernst LM. Stillbirth evaluation: a stepwise assessment of placental pathology and autopsy. *Am J Obstet Gynecol.* (2016) 214:115.e1–6. doi: 10.1016/j.ajog.2015.08.049

7. Da Silva FT, Gonik B, McMillan M, Keech C, Dellicour S, Bhange S, et al. Stillbirth: case definition and guidelines for data collection, analysis, and presentation of maternal immunization safety data. *Vaccine.* (2016) 34:6057–68. doi: 10.1016/j.vaccine.2016.03.044

8. Burke CJ, Tannenberg AET. Intrapartum stillbirths in hospital unrelated to uteroplacental vascular insufficiency. *Pediatr Dev Pathol.* (2007) 10:35–40. doi: 10.2350/06-02-0042.1

9. Khong TY, Mooney EE, Ariel I, Balmus NCM, Boyd TK, Brundler M-A, et al. Sampling and definitions of placental lesions: Amsterdam placental workshop group consensus statement. *Arch Pathol Lab Med.* (2016) 140:698–713. doi: 10.5858/arpa.2015-0225-CC

10. Redline RW. Thrombophilia and placental pathology. *Clin Obstet Gynecol.* (2006) 49:885–94. doi: 10.1097/01.grf.0000211957.68745.6b

11. Kinzler WL, Prasad V, Ananth CV, New Jersey-Placental Abruption Study Investigators. The effect of maternal thrombophilia on placental abruption: histologic correlates. *J Matern Fetal Neonatal Med.* (2009) 22:243–8. doi: 10.1080/14767050802551795

12. Tantbirojn P, Saleemuddin A, Sirois K, Crum CP, Boyd TK, Tworoger S, et al. Gross abnormalities of the umbilical cord: related placental histology and clinical significance. *Placenta.* (2009) 30:1083–8. doi: 10.1016/j.placenta.2009.09.005

13. Pinar H, Carpenter M. Placenta and umbilical cord abnormalities seen with stillbirth. *Clin Obstet Gynecol.* (2010) 53:656–72. doi: 10.1097/GRF.0b013e3181eb68fe

14. Hammad IA, Blue NR, Allshouse AA, Silver RM, Gibbins KJ, Page JM, et al. Umbilical cord abnormalities and stillbirth. *Obstet Gynecol.* (2020) 135:644–52. doi: 10.1097/AOG.0000000000003676

15. Vandevijver N, Hermans RH, Schrander-Stumpel CC, Arends JW, Peeters LL, Moerman PL. Aneurysm of the umbilical vein: case report and review of literature. *Eur J Obstet Gynecol Reprod Biol.* (2000) 89:85–7. doi: 10.1016/S0301-2115(99)00167-0

16. Vyas NM, Manjeera L, Rai S, Devdas S. Prenatal diagnosis of umbilical artery aneurysm with good fetal outcome and review of literature. *J Clin Diagn Res.* (2016) 10:QD01–3. doi: 10.7860/JCDR/2016/14800.7030

17. Doehrman P, Derksen BJ, Perlow JH, Clewell WH, Finberg HJ. Umbilical artery aneurysm: a case report, literature review, and management recommendations. *Obstet Gynecol Surv.* (2014) 69:159–63. doi: 10.1097/OGX.0000000000000051

18. Huchon C, Deffieux X, Beucher G, Capmas P, Carcopino X, Costedoat-Chalumeau N, et al. Pregnancy loss: French clinical practice guidelines. *Eur J Obstet Gynecol Reprod Biol.* (2016) 201:18–26. doi: 10.1016/j.ejogrb.2016.02.015

19. Haute Autorité de santé. [Standard protocol for fetal or perinatal autopsy Haute Autorité de santé] *Ann Pathol.* (2014) 34:415–33. doi: 10.1016/j.annpat.2014.10.005

20. Guihard-Costa A-M, Menez F, Delezoide AL. Standards for dysmorphological diagnosis in human fetuses. *Pediatr Dev Pathol.* (2003) 6:427–34. doi: 10.1007/s10024-003-1004-6

21. Guihard-Costa AM, Larroche JC, Droullé P, Narcy F. Fetal biometry. Growth charts for practical use in fetopathology and antenatal ultrasonography. Introduction. *Fetal Diagn Ther.* (1995) 10:211–78. doi: 10.1159/000264243

22. Guihard-Costa A-M, Ménez F, Delezoide A-L. Organ weights in human fetuses after formalin fixation: standards by gestational age and body weight. *Pediatr Dev Pathol.* (2002) 5:559–78. doi: 10.1007/s10024-002-0036-7

23. Almog B, Shehata F, Aljabri S, Levin I, Shalom-Paz E, Shrim A. Placenta weight percentile curves for singleton and twins deliveries. *Placenta.* (2011) 32:58–62. doi: 10.1016/j.placenta.2010.10.008

24. Vogler C, Petterchak J, Sotelo-Avila C, Thorpe C. Placental pathology for the surgical pathologist. *Adv Anat Pathol.* (2000) 7:214–29. doi: 10.1097/00125480-200007040-00004

25. Matsuki R, Nakago S, Kato H, Shibata T, Kotera T, Kotsuji F. Management strategy of umbilical artery aneurysm complicated by cardiac anomaly: case study and literature review. *J Matern Fetal Neonatal Med.* (2017) 30:1809–12. doi: 10.1080/14767058.2016.1226796

26. Szadok P, Kubiaczyk F, Koscielniak T. Umbilical artery aneurysm. *Ginekol Pol.* (2020) 91:777–8. doi: 10.5603/GP.a2020.0128

27. Fortune DW, Ostör AG. Umbilical artery aneurysm. *Am J Obstet Gynecol.* (1978) 131:339–40. doi: 10.1016/0002-9378(78)90610-5

28. Hill AJ, Strong TH, Elliott JP, Perlow JH. Umbilical artery aneurysm. *Obstet Gynecol.* (2010) 116(Suppl 2):559–62. doi: 10.1097/AOG.0b013e3181e7d280

29. Sepulveda W, Corral E, Kottmann C, Illanes S, Vasquez P, Monckeberg MJ. Umbilical artery aneurysm: prenatal identification in three fetuses with trisomy 18. *Ultrasound Obstet Gynecol.* (2003) 21:292–6. doi: 10.1002/uog.69

30. Weber MA, Sau A, Maxwell DJ, Mounter NA, Lucas SB, Sebire NJ. Third trimester intrauterine fetal death caused by arterial aneurysm of the umbilical cord. *Pediatr Dev Pathol.* (2007) 10:305–8. doi: 10.2350/06-07-0136.1

31. Olog A, Thomas JT, Petersen S, Cattanach S, Lourie R, Gardener G. Large umbilical artery aneurysm with a live healthy baby delivered at 31 weeks. *Fetal Diagn Ther.* (2011) 29:331–3. doi: 10.1159/000322960

32. Shen O, Reinus C, Baranov A, Rabinowitz RR. Prenatal diagnosis of umbilical artery aneurysm: a potentially lethal anomaly. *J Ultrasound Med.* (2007) 26:251–3. doi: 10.7863/jum.2007.26.2.251

33. Zangen R, Boldes R, Yaffe H, Schwed P, Weiner Z. Umbilical cord cysts in the second and third trimesters: significance and prenatal approach. *Ultrasound Obstet Gynecol.* (2010) 36:296–301. doi: 10.1002/uog.7576

34. Whipple NS, Bennett EE, Kaza E, O'Connor M. Umbilical cord pseudocyst in a newborn. *J Pediatr.* (2016) 177:333. doi: 10.1016/j.jpeds.2016.06.060

35. Chien C-W, Chen K-J, Lai J-Y, Chao A-S. Patent urachus or bladder exstrophy occulta? A case of prenatally disappeared umbilical cord cyst. *Urol Case Rep.* (2021) 39:101772. doi: 10.1016/j.eucr.2021.101772

36. Meinardi JR, Middeldorp S, de Kam PJ, Koopman MM, van Pampus EC, Hamulyák K, et al. Increased risk for fetal loss in carriers of the factor V Leiden mutation. *Ann Intern Med.* (1999) 130:736–9. doi: 10.7326/0003-4819-130-9-199905040-00013

37. Simchen MJ, Ofir K, Moran O, Kedem A, Sivan E, Schiff E. Thrombophilic risk factors for placental stillbirth. *Eur J Obstet Gynecol Reprod Biol.* (2010) 153:160–4. doi: 10.1016/j.ejogrb.2010.07.031

38. Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: a meta-analysis. *Lancet.* (2003) 361:901–8. doi: 10.1016/S0140-6736(03)12771-7

39. Many A, Schreiber L, Rosner S, Lessing JB, Eldor A, Kupferminc MJ. Pathologic features of the placenta in women with severe pregnancy complications and thrombophilia. *Obstet Gynecol.* (2001) 98:1041–4. doi: 10.1097/00006250-200112000-00010

40. Rogers BB, Momirova V, Dizon-Townson D, Wenstrom K, Samuels P, Sibai B, et al. Avascular villi, increased syncytial knots, and hypervascular villi are associated with pregnancies complicated by factor V Leiden mutation. *Pediatr Dev Pathol.* (2010) 13:341–7. doi: 10.2350/09-05-0657-OA.1

41. Siddiqi TA, Bendon R, Schultz DM, Miodovnik M. Umbilical artery aneurysm: prenatal diagnosis and management. *Obstet Gynecol.* (1992) 80:530–3.

42. Wei J, Li Q, Zhai H. Umbilical artery thrombosis diagnosed at different gestational ages and fetal outcomes: a case series. *BMC Pregnancy Childbirth.* (2021) 21:788. doi: 10.1186/s12884-021-04264-9

43. Heifetz SA. Thrombosis of the umbilical cord: analysis of 52 cases and literature review. *Pediatr Pathol.* (1988) 8:37–54. doi: 10.3109/15513818809022278

44. Castellano M, Muiesan ML, Rizzoni D, Beschi M, Pasini G, Cinelli A, et al. Angiotensin-converting enzyme I/D polymorphism and arterial wall thickness in a general population. The Vobarno Study. *Circulation.* (1995) 91:2721–4. doi: 10.1161/01.CIR.91.11.2721

45. von Depka M, Czwalinna A, Wermes C, Eisert R, Scharrer I, Ganser A, et al. The deletion polymorphism in the angiotensin-converting enzyme gene is a moderate risk factor for venous thromboembolism. *Thromb Haemost.* (2003) 89:847–52. doi: 10.1055/s-0037-1613472

46. Tamanna S, Lumbers ER, Morosin SK, Delforce SJ, Pringle KG. ACE2: a key modulator of the renin-angiotensin system and pregnancy. *Am J Physiol Regul Integr Comp Physiol.* (2021) 321:R833–43. doi: 10.1152/ajpregu.00211.2021

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Establishment of an interdisciplinary vascular anomalies program in Tanzania, East Africa

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Purpose: The aim of this project is the sustainable implementation of a vascular anomalies (VA) program in Tanzania.

Materials and methods: In 2021 the first interdisciplinary VA program was initiated at Muhimbili National Hospital (MNH), Dar Es Salaam, Tanzania in a stepwise approach. During the planning phase the clinical need for minimally-invasive therapies of VAs and the preexisting structures were assessed by the local Interventional Radiology (IR) team at MNH. During the initiation phase, an IR team from two German VA centers joined the interdisciplinary team at MNH for clinical workup, image-guided procedures and follow-up. VA patients were recruited from existing patient records or seen at clinics as *de novo* presentations following nationwide advertisement. In the post-processing phase joined online conferences for follow-up and support in management of new patients were established. Further follow-up was supported by attending providers from other established VA centers, traveling to bolster the primary operators of MNH.

Results: The first interdisciplinary VA program was successfully launched in Tanzania. Minimally-invasive treatments were successfully trained, by performing ultrasound-guided sclerotherapy with polidocanol and bleomycin in twelve patients with slow-flow malformations, one endovascular embolization of a high-flow malformation, and medical treatment of an aggressive infantile hemangioma. Regular online follow-up presentations have been initiated. Follow-up evaluation and required treatment was sustained when appropriate.

Conclusion: The presented "hands-on" training set the ground for the first interdisciplinary VA program in Tanzania. This framework is expected to establish comprehensive and sustainable care of patients with VAs in East Africa and can serve as a blueprint for other sites.

KEYWORDS

radiology, interventional/education, health services needs and demand, developing countries, Tanzania, interventional/pediatrics, vascular malformations

1. Introduction

Interventional radiology (IR) has evolved rapidly in high-income countries, offering a broad spectrum of minimally-invasive diagnostic and therapeutic options. However, IR is not available to the majority of patients in low and middle-income countries (1). While already severe in adult IR, this disparity becomes even more evident in pediatric IR. Tanzania, an East African country with 60 million people, has a growth rate of approximately 3% and a birth rate of 4.9 births per woman and about 50% of the population being below the age of 18 years (2). Considering these demographics, vascular anomalies (VAs) can commonly be found in Tanzania. In general, the majority of vascular malformations are venous malformations (VM, ∼70%) with 1-2 in 10,000, followed by lymphatic malformations (LM, ∼12%), arterio-venous malformations (AVM, ∼8%) and others, such as capillary or mixed types (3, 4). Overall, 16-48% of VAs can be found in the head or neck region (5). Incorrect classification of VAs as hemangiomas and ineffective treatment is common even in high-income countries, including the risk of harmful treatments of VA (6, 7). In Tanzania, treatment options of VA until now were limited to propranolol, surgery, or interstitial bleomycin injection. Traditional treatments of VAs can be found in rural areas of Tanzania as reported by VA patients, in some cases including incision of the skin and subcutaneous tissues, which puts patients at high risk for infection or bleeding as well as disfiguring scarring. Interdisciplinary minimally-invasive IR treatment strategies of these malformations have not been established in Tanzania and neighboring countries until now (4, 8–11). Therefore, after the successful implementation of the first IR service and training program in Tanzania in October 2018 (12), a multi-departmental training camp to initiate an interdisciplinary VA program in East Africa at MNH was undertaken in November 2021. MNH is a national referral hospital in Daressalam, Tanzania, with a capacity of 1600 beds including surgical, internal medicine, pediatric, neurology and neurosurgery as well as radiology and interventional radiology service.

The goal of this program is to establish an interdisciplinary framework for the diagnosis and treatment of VAs on site, to train the IR team at MNH in interventional therapies of the common types of VA, and to create an interdisciplinary platform for follow up and future therapies.

2. Materials and methods

2.1. Planning phase

In preparation for the on-site training, monthly virtual meetings with the interventional radiology trainees and graduates at MNH, the program coordinator, former visiting IR physicians, and the visiting pediatric IR team were started six months prior.

Videoconferences focused on the following topics:

1. Education in VA diagnostics, treatment (medical, interventional, surgical) and follow-up with lectures and journal club.
2. Recruitment of VA patients from the entire nation – phone calls with regional and district hospitals in Tanzania, review of VA patient records from OMFS.

3. Establishment of a structured VA reporting form for initial patient presentations, treatment documentation and follow-up (**Supplementary material**).
4. Organizing materials for VA treatment (sclerosants, liquid embolics, catheters) due to very limited access to IR materials in Tanzania.
5. Case discussions for treatment planning during the visit. For case discussions patients were completely anonymized.

For the first implementation of the program, the focus was set to the treatment of facial vascular anomalies. Therefore, the local interdisciplinary VA team was established as followed: (1) interventional radiologists performing pre-interventional clinical assessments and sonography in clinics and the image-guided sclerotherapy or embolization, respectively, (2) oral and maxillofacial surgeons for patient recruitment, clinical assessment, support during image guided therapy, pre- and post-interventional care of patients on the ward and surgical treatment in case of necrosis or other complications, (3) pediatricians and pediatric surgeons for patient recruitment, pre- and post-interventional care of patients on the ward, management of potential complications or second-step resection after complete occlusion of the malformation, and (4) neonatologists for medicamentous and conservative treatment of patients with congenital vascular anomalies, especially hemangiomas.

The visiting IR team consisted of three interventional radiologists and one IR technologist from two academic VA centers in Germany. Recruitment of VA patients for this project was initiated three months prior to the teaching visit in August 2021 in close coordination with the collaborating departments (**Figure 1**).

2.2. IR devices

IR devices are expensive and up to date there is no dedicated market for IR material in Tanzania. Therefore, this program largely depends on devices brought along by the visiting teams which are product donations from cooperating companies in the respective home countries. For the future, lower price devices and cooperation with companies are crucial to establish an official selling of material to Tanzania for long term sustainability.

2.3. On-site training phase

In November 2021 the visiting IR team joined the local IR team at MNH. Several lectures including grand rounds on diagnosis and treatment of VA were conducted to educate and integrate IR in the local interdisciplinary setting for this topic. Patients recruited during the planning phase were seen in interdisciplinary clinic, which included the local IR team and collaborating departments. Thorough patient history, clinical examination, review of cross-sectional imaging, and ultrasound assessment of vascular anomalies were performed under supervision of the visiting team with a focus on differentiating characteristics of slow-flow versus high-flow vascular malformations. Imaging protocols and characteristics of VAs on CT and MRI were discussed and trained with the local IR team and radiographers. For diagnosis in accordance with ISSVA classification, consensus was reached between two visiting IR attendings experienced in VA diagnosis and treatment and two local trainees and/or attending IR physicians. Individual cases were discussed by the local IR team, collaborating disciplines (e.g., oral and maxillofacial surgery, OMFS), and the visiting IR team for specific treatment planning. Based on the final recommendations of the interdisciplinary VA clinic, interventions were performed by the local IR team in collaboration with the various surgical disciplines under close supervision of the visiting IR team. Informed consent was obtained from the patient or parent before undergoing treatment.

2.4. Technical implementation

VMs were treated primarily with an ultrasound-guided approach (due to limited availability of fluoroscopy). In case of VMs, ultrasound needles were placed intravascularly within the dysplastic venous network under general anesthesia and a sclerosant was injected under continuous ultrasound visualization. All sclerotherapies were performed by local IR team members (trainees and faculty members) under supervision of the visiting IR team. Embolization of fast-flow malformations was performed in a dedicated angiosuite, equipped with an angiography unit (Artis Zee, Siemens Healthineers, Forchheim, Germany). Embolization of a high flow arteriovenous malformation (AVM) was trained using liquid embolic material.

2.5. Post-treatment phase

All patients were admitted to the hospital and observed for at least one day after the procedure to guarantee sufficient pain management and to control swelling in the head and neck region as the infrastructure for specialized outpatient care is not available in Tanzania. Training included interdisciplinary ward rounds for follow-up management of VA patients. An early follow up was performed after 4 weeks in clinic or remotely via telephone in cases where patients came from far away. Further, management of patients for follow-up appointments and/or additional procedures such as repeated sclerotherapy or surgery after embolization was planned. After completion of

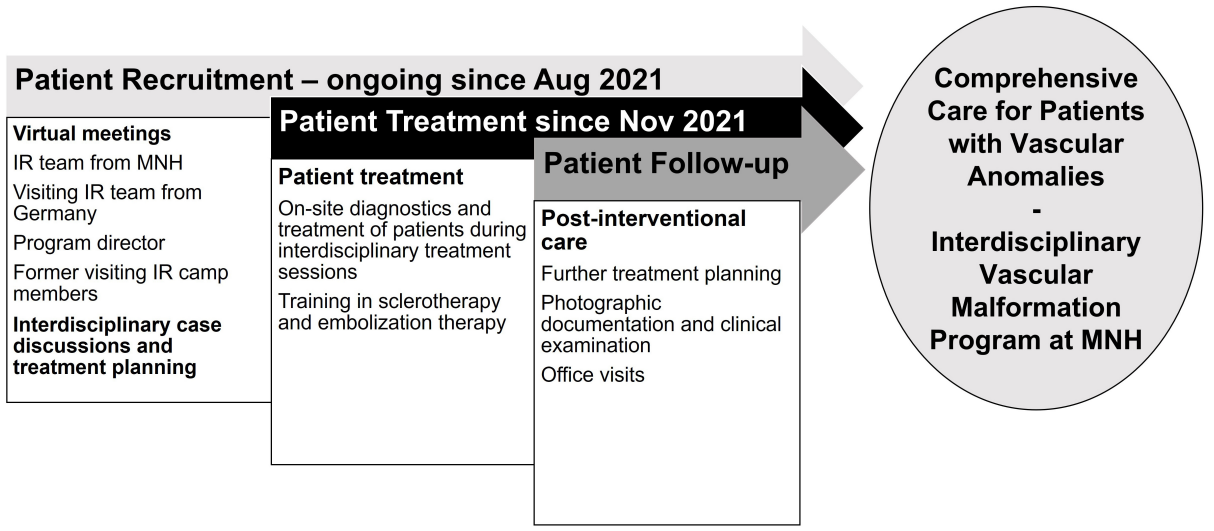

FIGURE 1
Timeline of the establishment of a vascular anomalies treatment program in Tanzania: preparation for the two-week camp, goals during hands-on training, and post-interventional patient care. IR = Interventional Radiology; MNH = Muhimbili National Hospital.

the onsite training with the visiting IR team, sustainability was ensured by regular conferences of the MNH local and visiting IR team regarding patients' follow-up or, if needed, support in management of new patients.

2.6. Follow-up and retreatment phase

All patients treated during initial implementation were in regular contact with a VA team member (OMFS team or IR team). Follow-up of patients at clinics at MNH was planned 4 weeks after the initial therapy including clinical examination and ultrasound assessment. If patients came from far away also telemedicine-type follow up (video calls) was used. Follow-up evaluation was done in conjunction with the multidisciplinary team, and primarily managed by MNH local IR team centering on physical exam (development of the size of the malformation, areas of post-interventional necrosis?) and ultrasound (residual perfused parts of the malformation, low flow vs. high flow). In addition to these findings, foundational outcomes such as pain, cosmesis and function were central to each evaluation and need for follow up. Follow-up presentation was also used to assess the need for further treatment sessions. The majority of these patients for which follow-up treatment was required were recalled, at the time when the next VA trained IR (this time from the USA) was based at MNH. For patients requiring follow-up treatments, the treatment plan was determined, and the patient was either admitted or referred for return during dedicated vascular anomaly operating theater block time. Similar to established VA programs in high-income countries, sclerotherapy is best prescribed as serial therapy in order to gain maximum results and minimize complications. Planned follow-up may require a different sclerosant or method for safety, or in the case of LMIC settings - availability of sclerosants. Continuity of care and management was primarily performed by MNH IR team members with consultation by the visiting IR team. The follow-up plan for each patient was determined prior to discharge from the post-operative ward. Imaging and gross pictures are kept and managed by MNH the local IR team as the primary operator.

3. Results

The first interdisciplinary VA program in East Africa was successfully launched, including establishment of protocols guiding patient referral, evaluation, state of the art treatment, and follow-up. Pre-treatment case discussions and patient recruitment was key for the success of the interdisciplinary VA camp. During the first days of the VA camp patients were seen in clinics and cross-sectional imaging was reviewed. In parallel, the treatments started for low flow malformations.

During this initial training visit a total of eight children and five adults with VAs were treated with an image-guided, minimally invasive approach at MNH. All malformations treated during this first VA camp were localized in the head, face, or neck. Eleven patients presented with VMs (84.6%), two with venous-lymphatic malformations (15.4%), and one with a large high flow AVM of the forehead. The different nature of these vascular anomalies contributed significantly to the learning experience as different treatment approaches were trained (e.g., draining of LMs with subsequent percutaneous image-guided

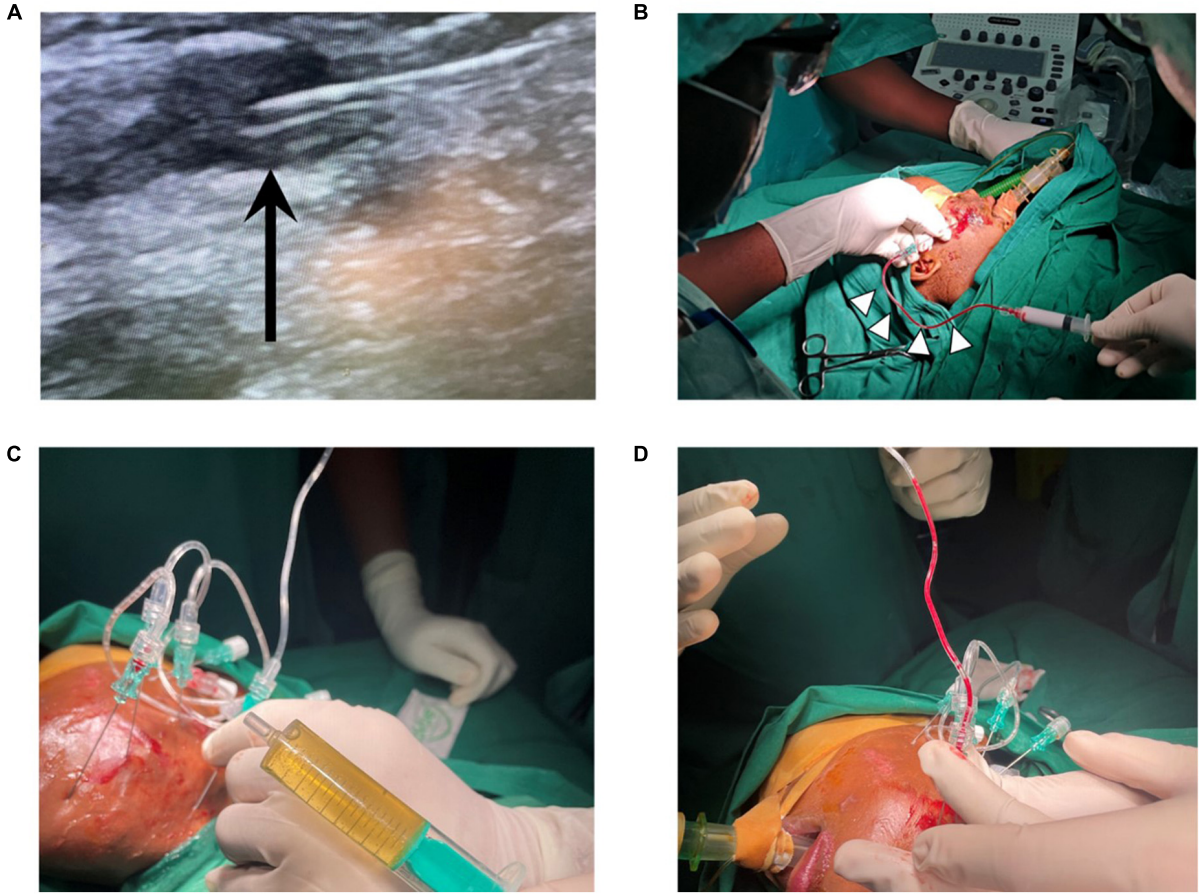

FIGURE 2
(A) Ultrasound-guided needle placement in dysplastic veins of a VM, black arrow points at needle tip. **(B)** Aspiration of blood (white arrow heads) confirms needle position within dysplastic VM vessels prior to sclerotherapy with polidocanol foam. **(C)** Aspiration of lymph fluid and **(D)** aspiration of blood in a mixed-type lymphatic and venous malformation, treatment with bleomycin (for lymphatic cysts) and polidocanol (for venous components). VM = venous malformation.

bleomycin sclerotherapy, endovascular embolotherapy for AVM). These cases illustrated well the aim and benefits of image-guided sclerotherapy. All 13 patients received the first session of intravascular percutaneous, image-guided sclerotherapy during this initial teaching visit, with a plan for subsequent sessions during future VA camps with supporting visiting teams. Teaching points such as the correct needle placement and distribution of the sclerosing agent was imaged in real-time on ultrasound Figures 2A, B). The key teaching points were safe intravascular ultrasound-guided needle placement within the dysplastic vessels, aspiration of blood/lymphatic fluid to ensure stable intravascular position of the needle, and subsequent injection of sclerosant with real-time monitoring under ultrasound. Two patients had malformations with lymphatic and venous components (LVM). The lymphatic cysts were drained and bleomycin (up to 10 mg in a concentration of 1 mg/ml) was injected into the cavities (Figure 2C). In these cases, the patient's charts were reviewed carefully before therapy to ensure that the bleomycin lifetime dose was not violated. The administered bleomycin dose was meticulously documented to assure correct dose calculations within the allowed lifetime dose during follow up treatments. These teaching points aimed for sustainability and safe follow up treatments for the patients. One patient (age: 10 months) presented with a high-flow arteriovenous malformation on the forehead with growth progression. In this case, successful embolization with liquid embolics was performed under general anesthesia followed by surgical resection in March 2022. Teaching points were safe arterial access (4F) through the femoral artery, cautious navigation into the supraaortic arteries avoiding any risks of air embolism, superselective positioning of a microcatheter within the maxillary artery, and embolization of the nidus via the arterial feeders of the AVM in plug-and-push technique with a liquid embolic agent, while avoiding non-target embolization.

TABLE 1 Patient characteristics and procedural details.

ID	Age [y]	Sex	Diagnosis	Localization	Previous therapy	Kind of previous therapy	Treatment	Sclerosing agent	Complications	Plt (x10^3/μ L) WBC (x10^3/μ L)	Days in hospital	Follow up in months
1	1	F	VM	Upper lip	Yes	Unguided bleomycine injection	PS	Polidocanol (2 ml)	Small localized necrosis	467/7.4	2	10
2	2	F	VM	Left cheek	Yes	Unguided bleomycine injection	PS	Polidocanol (6 ml)	None	273/3.6	2	10
3	2	F	VM	Right cheek	Yes	Unguided bleomycine injection and traditional medicine	PS	Polidocanol (4 ml)	None	244/8.3	2	10
4	8	F	VLM	Mandible/chin	Yes	Unguided bleomycine injection	PS	Polidocanol (4 ml) Bleomycine (28 mg)	None	306/6.2	2	10
5	4	M	VM	Left cheek	Yes	Unguided bleomycine injection	PS	Bleomycin (10 mg)	None	116/10.3	2	10
6	5	F	Tufted angioma/VM	Left cheek	Yes	Unguided bleomycine injection	PS	Polidocanol (4 ml)	None	382/7.4	2	10
7	52	M	VM	Right cheek	No	-	PS	Polidocanol (8 ml)	Necrosis	262/8.2	2	10
8	20	M	VM	Left cheek	Yes	Unguided bleomycine injection	PS	Polidocanol (1.7 ml)	None	241/4.3	2	10
9	15	F	VM	Right cheek	No	-	PS	Polidocanol (8 ml)	None	354/5.1	3	10
10	64	F	VLM	Right cheek and lip	No	-	PS	Polidocanol (0.7 ml)	None	171/6.5	2	10
11	32	M	VM	Right cheek	Yes	Unguided bleomycine injection	PS	Polidocanol (6 ml)	None	104/3.4	2	10
12	30	F	VM	Chin and both cheeks	Yes	Unguided bleomycine injection	PS	Polidocanol (6 ml)	None	253/3.2	2	10
13	0 (11 mo)	F	AVM	Forehead	No	-	Embolization	EVOH (1.2 ml)	Pneumonia	67/6.8	4	10

F: female; M: male; mo: months; y: years; VM: venous malformation; AVM: arteriovenous malformation; PS: percutaneous sclerotherapy; EVOH: ethylene vinyl alcohol; Plt: platelet count; WBC: white blood cell count.

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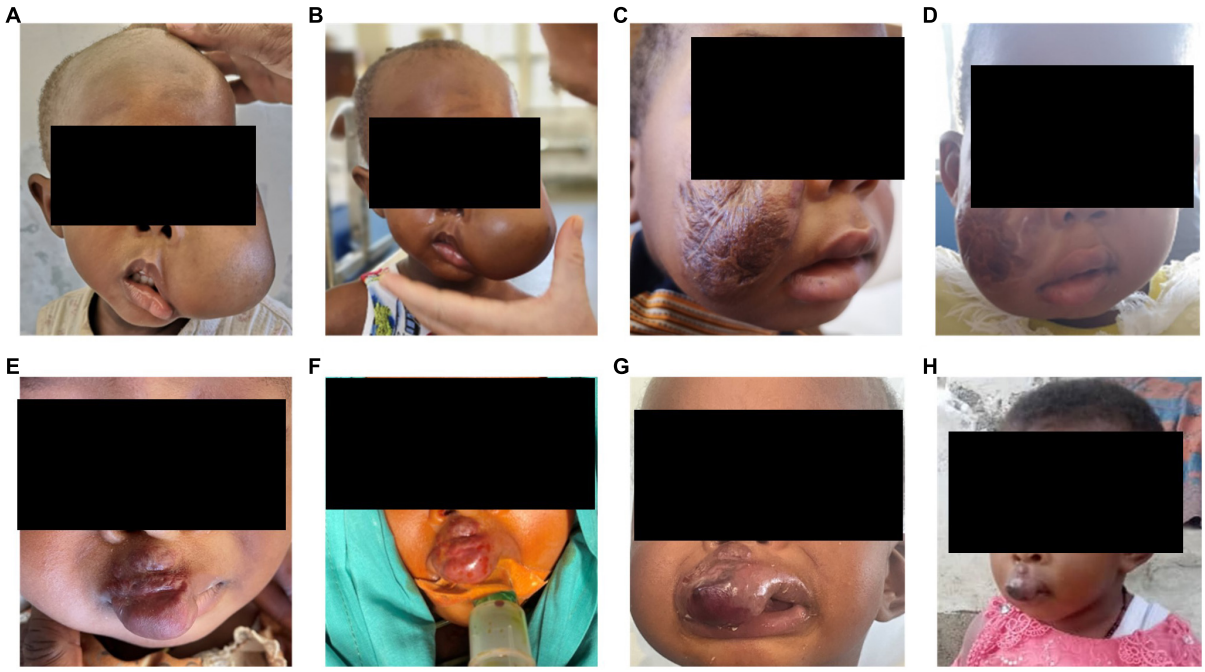

FIGURE 3
(A) Pre-procedural images of a 2-year-old girl who presented to the VA clinic with left cheek swelling due to a VM of the left cheek and face with involvement of the lateral border of the left eye. **(B)** Post-interventional swelling occurred in this patient as expected. **(C)** Pre-procedural images of a 3-year-old girl with a VM of the right cheek, the patient underwent prior traditional medicine treatment leaving extensive scars on the right cheek. **(D)** Post-interventional swelling occurred as expected in this patient. **(E,F)** Pre- and intra-procedural images of a 2-year-old girl with a VM on the upper lip extending up to the right nostril. **(G)** After the procedure a swelling occurred, and a slight dark discoloration demarcated on the lip. **(H)** The small necrosis healed quickly and the proportion below the right nostril already shrunk significantly. All patients need further treatment sessions and close follow-up. VA = vascular anomalies.

Patients were admitted to the hospital for minimally-invasive treatment and in-patient treatment was provided for 2-4 days after the interventional therapy, depending on the localization and nature of the vascular malformation. In-patient follow up included laboratory and clinical examinations. No infectious complications and no fever occurred in patients treated with percutaneous sclerotherapy. On the day after sclerotherapy, post-interventional swelling was observed in all patients as expected (pre- and post-treatment examples shown in Figure 3). One patient underwent sclerotherapy of a venous malformation on the upper lip and developed a small, localized necrosis, which did not require further treatment other than close follow up and careful wound management (Figures 3E–H). Another patient developed more severe necrosis of the cheek and nose tip with the need of wound management and plastic surgery. This complication likely could have been avoided with fluoroscopy control (which was not available for sclerotherapy at that time at MNH). However, this offered an opportunity to train management of typical complications with the local interdisciplinary VA team. Access to fluoroscopy for the MNH IR team has since improved and will continue to expand in coming years. No embolization-related complications were observed after the high flow AVM embolization on the forehead. Pre-therapeutic images and angiography are shown in Figure 4. However, the child developed a pneumonia in the first week after the therapy which was most likely attributed to the difficult intubation and prolonged mask ventilation in preparation of the procedure. The child had to be treated on ICU for 3 days, had a quick recovery and was discharged after 4 days. No other complications such as reflex syncope occurred in patients treated with percutaneous sclerotherapy or endovascular embolization, respectively. Details on observed adverse events can be found in Table 1.

One patient on the neonatology ward presented with an aggressive infantile hemangioma of the cheek and periorbital region leading to a localized steal-effect with subsequent necrosis on the upper lip (Figure 5). Conservative treatment with propranolol p.o. (hemangiol starting dose 0.5 ml for 3 kg child) was initiated and further active surveillance was chosen as treatment strategy for this patient.

Of the initial 13 patients treated, 12 (92.3%) patients reported improvement of symptoms and only one (7.7%) showed no change in symptoms after therapy at 10 months

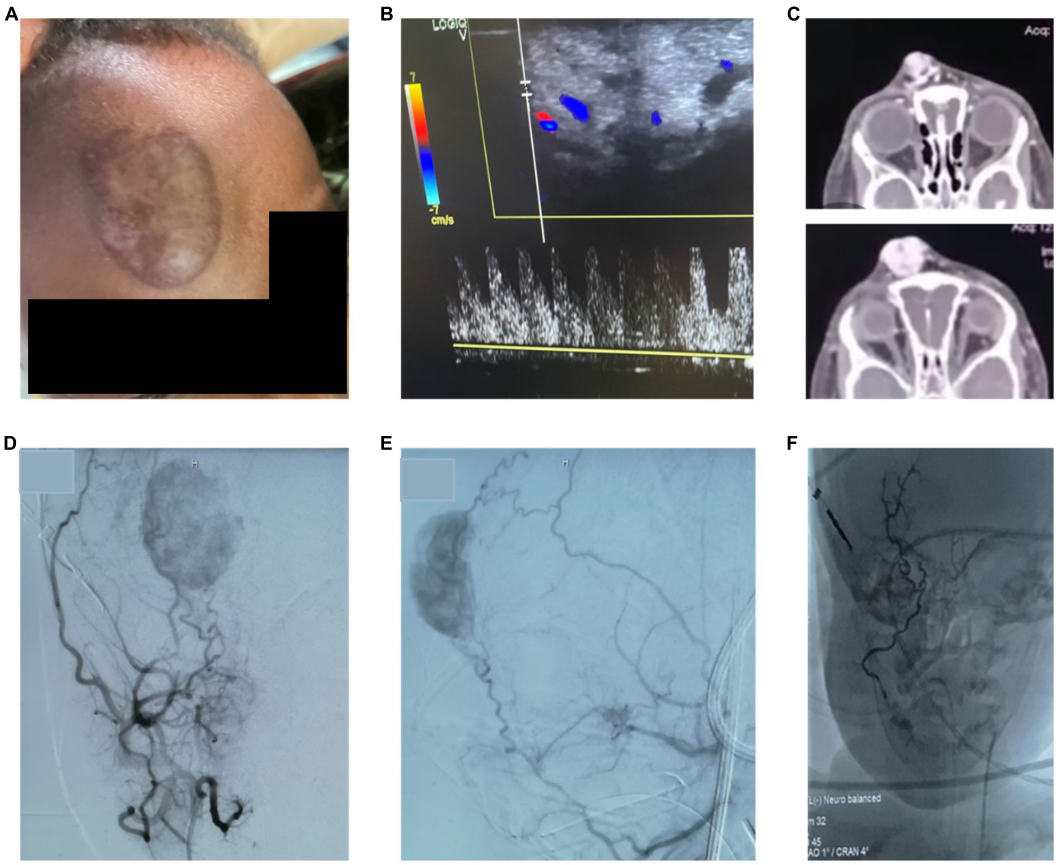

FIGURE 4
(A,B) Pre-procedural picture of a clinical examination showing a pulsatile, warm AVM of the forehead with corresponding high flow on doppler ultrasound. **(C)** CT-images with contrast show a highly vascularized lesion with arterial feeders and venous drainage via the ophthalmic veins. **(D,E)** Angiographic images of the AVM by contrast administration through the external carotid artery confirm the facial artery as the major feeding vessel. Further, the frontal branch of the temporal artery has small feeding branches. **(F)** After embolization of the main feeding vessels with liquid embolic material occluding the arterial vascular network, the intravascular cast formed by the liquid embolic agent can be appreciated. The patient underwent subsequent surgical resection of the lesion.

follow-up. Long-term sustainability of the VA program in Tanzania has been secured by addressing several points: First, treated patients were followed up by the local IR team. Second, ongoing close communication between the visiting IR team, other pediatric IR worldwide and the local IR team via digital (video-) platforms to discuss further treatments and presentation of new patients was ensured. Third and most importantly, on site visits of further specialized pediatric IR teams every two to three months were organized. Until today (10/2022) three further pediatric IR teams have continued to build the VA program at Tanzania onsite. Within the first 12 months after the visit of the first pediatric IR team, a total of 36 further VA procedures were performed at MNH. Fourth, a cooperation with the Society of Pediatric Interventional Radiology (SPIR) was established including reduced membership fees and annual meeting discount for conference participation to MNH IR trainees and graduates.

4. Discussion

Considering the increasing demand for IR procedures in low and middle income countries, strategies to provide structured, accredited, and sustainable on-site training programs aiming to supply fully trained interventional radiologists in these settings are needed (1, 12, 13). While few such programs have been initiated over the past several years, demonstrating on a small scale that this can be achieved, there is need for massive upscaling of these efforts if IR care is to be provided worldwide (12, 14, 15). This especially holds true for pediatric IR, which struggles with even more challenges than adult IR, given the need for more specialized training and equipment to meet the needs of a young patient population (16). Thus, especially low- and middle-income countries and their overall younger populations are confronted with a near-total lack of cost-effective minimally-invasive care, resulting

in major long-term consequences for individual patients and their societies at large. In this context, vascular anomalies, as common congenital disease affecting mostly children and adolescents, which can be treated by a range of IR procedures, provide a suitable starting point for implementation of pediatric interventional care along with a variety of other important pediatric IR treatments (e.g., biopsies, nephrostomy tube placement, abscess drainage). The presented study reports on such a program, establishing the first VA treatment center in Tanzania. Emphasis was placed on training the full cycle of care from clinical presentation to diagnosis, treatment, and follow-up. The most common types of VAs were diagnosed and treated by local IR team members during this half-year (and ongoing) theoretical and case-based educational program including a two-week VA training camp under the supervision of an experienced visiting IR team. The interdisciplinary framework for VA treatment was successfully launched for sustainable integrated VA treatment in close collaboration with OMFS, pediatric surgery, pediatrics and neonatology, thereby enabling Tanzanian IR team members to independently build up specialized VA-care over the total cycle of care. To ensure homogeneity of the patient cohort for teaching purposes, the focus of this first implementation was set to the treatment of facial VA, since most VA are located there (7). However, a majority of vascular malformations is also located at the extremities causing pain, swelling and immobility emphasizing the need to expand the vascular anomalies team in the future (7). In detail, the interdisciplinarity of the program will be further extended with the aim to include plastic and reconstructive surgery, ENT/head and neck surgery, orthopedics, dermatology and medical oncology as the next step. Exemplarily, first patients with VAs of the extremities have been treated at MNH in 2022. Thereby, the presented strategy on the one hand increases sustainability and independence from visiting IR specialists and on the other hand aims to foster IR not only as a diagnostic but also treating specialty in the center of interdisciplinary teams improving patient care for this complex condition. IR is recognized for providing minimally-invasive, cost-effective care (17). This holds especially true for VA treatment and investment in IR personnel and equipment may help to reduce costs in the long run in low- and middle-income countries such as Tanzania, as current strategies include sending VA patients to India for treatment on government expenses with costs of up to 10,000 USD per treatment. Moreover, it should be emphasized that having strong IR in one country will have positive effects on IR everywhere, but particularly in neighboring countries (13). For instance, the local IR team in Tanzania also consists of IR team members from other African countries such as Nigeria and Rwanda, established or plan to establish IR in their home countries after finishing the training program in Tanzania, making the program a nucleus for spreading IR in Africa (18). Maintenance of digital communication of experienced IR teams from the US and Europe with the local IR team members ensures ongoing education and consultation for new patients. Inclusion and active involvement of African IR team members in international societies such as the Society of Pediatric Interventional Radiology (SPIR) ensures access to scientific and educational content with the goal of keeping VA treatment algorithms up to date.

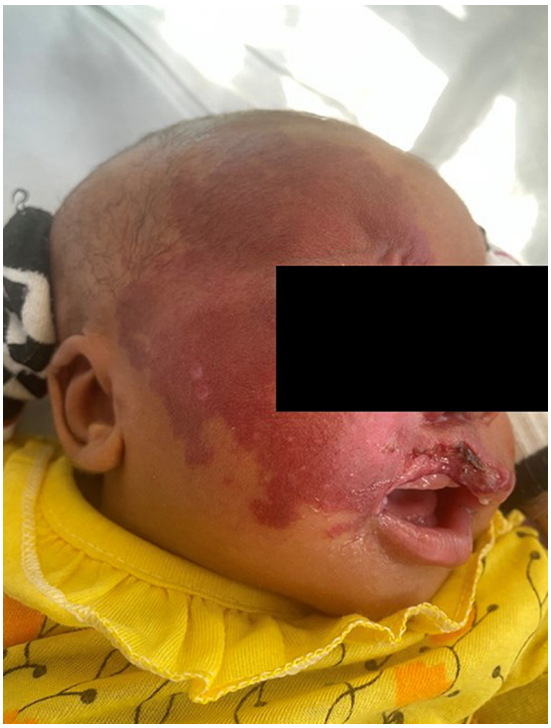

FIGURE 5
Neonate with an aggressive infantile hemangioma of the cheek and periorbital region leading to a localized steal-effect with subsequent necrosis on the upper lip. Conservative treatment with propranolol p.o. (hemangiol starting dose 0.5 ml for 3 kg child) was initiated and further active surveillance was chosen as treatment strategy for this patient.

One major limiting factor for sustainability is the lack of a dedicated market for IR devices for Tanzania. Due to this fact, the IR program largely depends on devices brought along by the visiting teams which are product donations from cooperating companies in the respective home countries. For the future, lower price devices and cooperation with companies are crucial to establish an official selling of material to Tanzania for long term sustainability.

In summary, we report on the successful establishment of a sustainable, highly specialized IR guided interdisciplinary VA team in Tanzania, which can serve as a model for further expansion of pediatric and adult IR care in low- and middle-income countries. Establishment of minimally-invasive image-guided treatments in the field of congenital anomalies contributes to the major goal of providing equal care worldwide.

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 author.

Author contributions

All authors contributed significantly to establish the VA program, planning the VA camp, therapy of patients, and writing of the manuscript.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.1056539/full#supplementary-material

References

1. Mollura D, Soroosh G, Culp M, Averill S, Axelrod D, Baheti A, et al. 2016 RAD-AID conference on internastional radiology for developing countries: gaps, growth, and United Nations sustainable development goals. *J Am Coll Radiol.* (2017) 14:841–7. doi: 10.1016/j.jacr.2017.01.049

2. Worldometer. *Tanzania demographics 2020 (population, age, sex, trends).* (2022). Available online at: https://www.worldometers.info/demographics/tanzania-demographics/#urb (accessed on March 13, 2022).

3. Behravesh S, Yakes W, Gupta N, Naidu S, Chong B, Khademhosseini A, et al. Venous malformations: clinical diagnosis and treatment. *Cardiovasc Diagn Ther.* (2016) 6:557–69. doi: 10.21037/cdt.2016.11.10

4. Sadick M, Müller-Wille R, Wildgruber M, Wohlgemuth W. Vascular anomalies (Part I): classification and diagnostics of vascular anomalies. *Fortschr Röntgenstr.* (2018) 190:825–35. doi: 10.1055/a-0620-8925

5. Kobayashi K, Nakao K, Kishishita S, Tamaruya N, Monobe H, Saito K, et al. Vascular malformations of the head and neck. *Auris Nasus Larynx.* (2013) 40:89–92. doi: 10.1016/j.anl.2012.02.002

6. Hassanein A, Mulliken J, Fishman S, Greene A. Evaluation of terminology for vascular anomalies in current literature. *Plastic Reconstr Surg.* (2011) 127:347–51. doi: 10.1097/PRS.0b013e3181f95b83

7. Greene A, Liu A, Mulliken J, Chalache K, Fishman S. Vascular anomalies in 5621 patients: guidelines for referral. *J Pediatr Surg.* (2011) 46:1784–9. doi: 10.1016/j.jpedsurg.2011.05.006

8. Müller-Wille R, Wildgruber M, Sadick M, Wohlgemuth W. Vascular anomalies (Part II): interventional therapy of peripheral vascular malformations. *Fortschr Röntgenstr.* (2018) 190:927–37. doi: 10.1055/s-0044-101266

9. Legiehn G, Heran M. Classification, diagnosis, and interventional radiologic management of vascular malformations. *Orthopedic Clin North Am.* (2006) 37:435–74. doi: 10.1016/j.ocl.2006.04.005

10. Legiehn G, Heran M. A step-by-step practical approach to imaging diagnosis and interventional radiologic therapy in vascular malformations. *Semin intervent Radiol.* (2010) 27:209–31. doi: 10.1055/s-0030-1253521

11. Gibson C, Barnacle A. Vascular anomalies: special considerations in children. *CVIR Endovasc.* (2020) 3:60. doi: 10.1186/s42155-020-00153-y

12. Laage Gaupp F, Solomon N, Rukundo I, Naif A, Mbuguje E, Gonchigar A, et al. Tanzania IR initiative: training the first generation of interventional radiologists. *J Vasc Intervent Radiol.* (2019) 30:2036–40. doi: 10.1016/j.jvir.2019.08.002

13. Kaufman J, Sacks D, Stainken B. Denied in Canada: why we need a global strategic plan for interventional radiology. *J Vasc Intervent Radiol.* (2008) 19:13–4. doi: 10.1016/j.jvir.2007.11.001

14. Kesselman A, Gaupp F. RAD-AID global curriculum for interventional radiology. *Endovasc Today.* (2018) 17.

15. Kline A, Dixon R, Brown M, Culp M. Interventional radiology readiness assessment tool for global health. *JGR.* (2017) 3:2. doi: 10.7191/jgr.2017.1035

16. Shah S, Binkovitz L, Ho M, Trout A, Adler B, Andronikou S. Pediatric radiology mission work: opportunities, challenges and outcomes. *Pediatr Radiol.* (2018) 48:1698–708. doi: 10.1007/s00247-018-4221-x

17. Masthoff M, Schneider K, Schindler P, Heindel W, Köhler M, Schlüchtermann J, et al. Value improvement by assessing IR care via time-driven activity-based costing. *J Vasc Intervent Radiol.* (2021) 32:262–9. doi: 10.1016/j.jvir.2020.09.017

18. Novasys.io E ku. Rwanda conducts first vascular interventional radiology procedure at king faisal hospital. Kigali: King Faisal Hospital. (2022).

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Genetically predicted green tea intake and the risk of arterial embolism and thrombosis

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Background: In previous observational studies, green tea intake has been demonstrated to protect against arterial embolism and thrombosis. However, whether there is a causative connection between green tea intake and arterial embolism and thrombosis is currently unclear.

Methods: A two-sample Mendelian randomization (MR) study has been designed to explore whether there is a causal association between green tea intake and arterial embolism and thrombosis by acquiring exposure and outcome data from previously published research. Data from the MRC-IEU (data on green tea intake, 64,949 participants) consortium and the FinnGen project (data on arterial embolism and thrombosis, 278 cases of arterial thrombosis and 92,349 control participants) has been utilized to determine the causal impact of green tea intake on arterial embolism and thrombosis.

Results: We found that genetically predicted green tea intake was causally associated with a lower risk of arterial embolism and thrombosis (IVW odds ratio [OR] per SD decrease in green tea intake=0.92 [95% confidence interval, 0.85–0.99]; p=0.032). Moreover, the sensitivity analysis (both MR Egger regression and weighted median) yielded comparable estimates but with low precision. No directional pleiotropic effect between green tea intake and arterial embolism and thrombosis was observed in both funnel plots and MR-Egger intercepts.

Conclusions: Our study provided causal evidence that genetically predicted green tea intake may be a protective factor against arterial embolism and thrombosis.

KEYWORDS

green tea intake, arterial embolism and thrombosis, Mendelian randomization, ischemic heart disease, stroke, genome-wide association study

1. Introduction

Arterial embolism and thrombosis is the formation of a blood clot inside an arterial blood vessel or the arterial thrombus coming from the heart, proximal arterial wall, or other sources, leading to the obstruction of blood flow in the arterial circulatory system, (1) acting as the cause of ischemic heart disease and stroke and the most common causes of death in the developing and developed countries (2, 3). Despite numerous advances in diagnosis and treatment, arterial embolism, and thrombosis remain a challenge for clinicians. Many lifestyle factors are involved in the occurrence and development of arterial embolism and thrombosis, such as diet, smoking, and physical inactivity (4). Therefore, the primary prevention for the risk of arterial thrombosis may be crucial.

The effects of green tea intake on reducing arterial thromboembolic risk have been proved by many observational studies. However, the findings and conclusions of those studies are partly contradictory. The results of a meta-analysis of observational evidence from prospective studies

(5) revealed a reduced risk of coronary heart disease (CHD) associated with an increased green tea consumption of three cups per day (risk ratio 0.73, 95% CI 0.53–0.99), cardiac death (0.74, 95% CI 0.63–0.86), stroke (0.82, 95% CI 0.73–0.92), and mortality (0.76, 95% CI 0.63–0.91). Similarly, another meta-analysis, which enrolled both observational and randomized trials, showed a lower risk of stroke and myocardial infarction in patients with higher tea consumption (6). However, some other studies found that green tea intake indeed increases cardiovascular disease risk. Zahra Gaeini et al. suggested that each 1 cup/day increased habitual consumption of tea was related to a 4 and 14% increased risk of cardiovascular disease (7). Nevertheless, other unmeasured lifestyle components, like other underlying disorders, physical activity, or smoking habits, may have impacted these findings. Therefore, it remains unclear whether green tea consumption is causally associated with arterial embolism and thrombosis.

Mendelian randomization (MR), (8) which employs genetic variants as instrumental variables, is an epidemiological approach that avoids potential confounders or reversing causality. According to Mendel's second law, this method is not susceptible to reverse causality or confounding, analogous to a randomized trial where randomization to genotype occurs at conception. Consequently, MR is a powerful predictive tool for assessing causal associations, as shown in Figure 1 (9).

The SNPs associated with hypertension were selected from the MRC-IEU and the corresponding effect for these SNPs was estimated based on the effect of ED. MR is a powerfully predictive tool to test causal associations without any bias inherent to observational study designs due to the randomization and independence of alleles at meiosis.

In this study, through two-sample MR analysis, we investigated the causal association between green tea intake and arterial thrombosis using data from a genome-wide association study (GWAS) (10). We measured the association of 11 single nucleotide polymorphisms (SNPs) for green tea consumption with an arterial embolism and thrombosis study of 278 cases and 92,349 controls from the FinnGen project.

2. Methods

2.1. Overall study design

The MR data analyzed in this study were taken from our previous work and the institutional review board had given its approval to each of the published studies. Therefore, there was no need for further approval in the present study (11). Here, two-sample MR was used to investigate the causal association between gene-risk factors (e.g., Green tea intake) and gene outcome (e.g., arterial embolism and thrombosis) (12).

Abbreviations: CHD, Coronary heart disease; MR, Mendelian randomization; GWAS, Genome-wide association study; SNP, Single-nucleotide polymorphisms; CI, Confidence interval; OR, Odds ratio; SD, Standard deviation; FMD, Flow-mediated dilation; EGCG, Epi-gallocatechin-3-gallate.

2.2. Data sources

2.2.1. SNPs identification associated with green tea intake

The SNPs associated with green tea intake were identified from 64,949 individuals from the MRC-IEU Traits consortium based on the European populations (Output from GWAS pipeline using Phesant derived variables from UKBiobank), which was the most recent GWAS on green tea intake when we started the MR analysis. In the original GWAS study, the green tea intake was reported as a categorical ordered variable (cups of green tea) with no covariates considered in their model in the UK Biobank (data field: 100420). The summary statistics were generated following the exclusion of samples of poor quality and the original questionnaire divided the number of cups of tea into 0, 1, 2, 3, 4, 5, and ≥ 6 cups. As mentioned in the original study, one cup means 250 mL. A detailed description of the study design, including quality control procedures and statistical analyses, is available at http://www.nealelab.is/uk-biobank/.

2.2.2. Study outcome: Arterial thrombosis

The SNPs of arterial embolism and thrombosis were obtained from the FinnGen project (FinnGen), which can be found at https://gwas.mrcieu.ac.uk/datasets/finn-a-I9_ARTEMBTHR. There were 278 cases of arterial thrombosis and 92,349 people who served as controls in this study, which has been permitted by their institutional review board, and all participants gave their informed consent as part of their original study.

2.3. Statistical analysis

We selected SNPs independent with a genome-wide significance, by following these criteria: First, the selected SNPs were significantly associated with green tea intake based on a genome-wide significance threshold ($p < 5.0 \times 10^{-8}$); (13). Second, SNPs in linkage disequilibrium with $r^2 < 0.001$ and distance >10,000 kb were excluded (14, 15). Third, those SNPs weakly associated with instruction variants and green tea intake were excluded.

Due to no available individual-level GWAS data, the two-sample MR was used to estimate the effect of green tea on arterial thrombosis. Assumptions of MR are violated by horizontal pleiotropy (16), which is a mechanism by which genetic variants can influence the outcome rather than just exposure. Therefore, three methods (IVW, Weighted

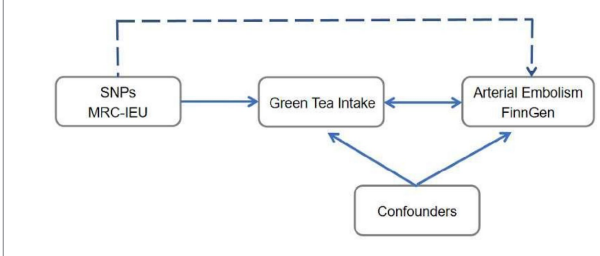

FIGURE 1
Schematic representation of our MR analysis.

median, and MR Egger regression) were used as a safeguard against this in the present MR analysis (17).

Different horizontal pleiotropy models underlie each analytical approach. The consistency of all three methods can help us ensure that our conclusions are solid (18). The study's statistical coding and related data are available from the corresponding author based on your reasonable request. R version 4.0.3 (2020-10-10; The R Foundation for Statistical Computing, Vienna, Austria) was used for all statistical analyses (19).

3. Results

3.1. Genetic factors for green tea consumption and arterial embolism and thrombosis

The SNPs associated with green tea consumption and arterial embolism and thrombosis are summarized in Table 1. Eleven new genetic instruments had never been used in any previous research. The effects of the variant in green tea consumption on the risk of arterial embolism and thrombosis are shown in Figures 2, 3.

To evaluate the causal relationships between genetically predicted green tea consumption and arterial embolism and thrombosis, weighted median regression, IVW, and MR-Egger were utilized, as shown in Figures 2, 3. All three MR methods found broadly consistent support for a negative correlation between arterial thrombosis and consumption of green tea, suggesting that green tea consumption was causally associated with arterial embolism and thrombosis (IVW odds ratio = 0.92 per SD increase in green tea consumption [95% CI, 0.85–0.99]; $p = 0.032$). The MR Egger regression likewise yielded estimates that were also directionally similar (MR—Egger OR per SD increase in green tea consumption, 0.85 [95% CI, 0.75–0.98], $p = 0.045$).

3.2. Analysis of horizontal pleiotropy

In the present MR analysis, the Funnel plot, MR-Egger intercept, and MR pleiotropy test were used for the detection of horizontal pleiotropy. The individual Wald ratios for every SNP plotted against their precision are displayed by Funnel plots, with asymmetry exhibiting directional horizontal pleiotropy. Nevertheless, it should be highlighted that assessing funnel plots for symmetry is challenging when only a few genetic instruments are utilized, as shown in Figure 4. In this research, there is no evidence of considerable directional pleiotropy for arterial thrombosis using the MR-Egger intercept and MR pleiotropy test (both $p > 0.05$), suggesting the association between green tea consumption and arterial embolism and thrombosis has no directional pleiotropic impacts.

TABLE 1 List of genetic instruments.

SNP	Position	chr.	EA	OA	eaf.exposure	beta. exposure	se.exposure	*p* Value exposure
rs12144868	41,539,745	1	C	T	0.015146	3.07273	0.527463	5.70E−09
rs145313301	79,537,576	2	C	G	0.006149	4.99373	0.812513	7.90E−10
rs144954030	213,836,477	2	C	T	0.005665	4.88015	0.821066	2.80E−09
rs142811251	22,985,272	2	C	G	0.010296	3.45976	0.617814	2.10E−08
rs115952340	40,875,392	3	A	G	0.01334	3.00972	0.550266	4.50E−08
rs78547201	61,713,522	5	G	C	0.011607	3.27496	0.577735	1.40E−08
rs116985617	133,084,903	6	T	C	0.008531	3.89273	0.674419	7.80E−09
rs79774709	117,931,871	6	T	C	0.005782	5.14056	0.853785	1.70E−09
rs189140232	35,517,966	7	A	G	0.010006	3.56083	0.625652	1.30E−08
rs11976995	157,531,582	7	G	T	0.030423	2.01942	0.348689	7.00E−09
rs116035596	28,832,005	9	T	C	0.006559	4.90357	0.742882	4.10E−11
rs79638269	14,841,242	10	T	C	0.016328	2.67635	0.472895	1.50E−08
rs644205	89,745,814	10	A	G	0.196485	0.848874	0.150756	1.80E−08
rs183788045	64,006,281	11	T	G	0.008749	3.54822	0.64978	4.70E−08
rs117251267	95,673,607	13	C	T	0.01159	3.55014	0.617762	9.10E−09
rs142373582	104,757,781	14	C	T	0.014186	3.24633	0.565762	9.60E−09
rs113898417	21,614,895	16	G	C	0.006957	3.97893	0.726631	4.40E−08
rs62059726	48,715,705	17	A	G	0.015194	2.8216	0.511814	3.50E−08
rs12958992	71,259,772	18	G	A	0.026945	2.16091	0.378903	1.20E−08
rs117077082	42,928,868	20	G	A	0.031795	1.92055	0.34217	2.00E−08
rs113322644	33,992,170	21	A	C	0.011694	3.20963	0.555077	7.40E−09

SNP, single nucleotide polymorphism; EA, effect allele; OA, other allele; chr., chromosome; and se, standard error.

FIGURE 2
Scatter plot to visualize the causal effect of hypertension on the risk of ED. The slope of the straight line shows the magnitude of the causal association. IVW indicates inverse-variance weighted; and MR, Mendelian randomization.

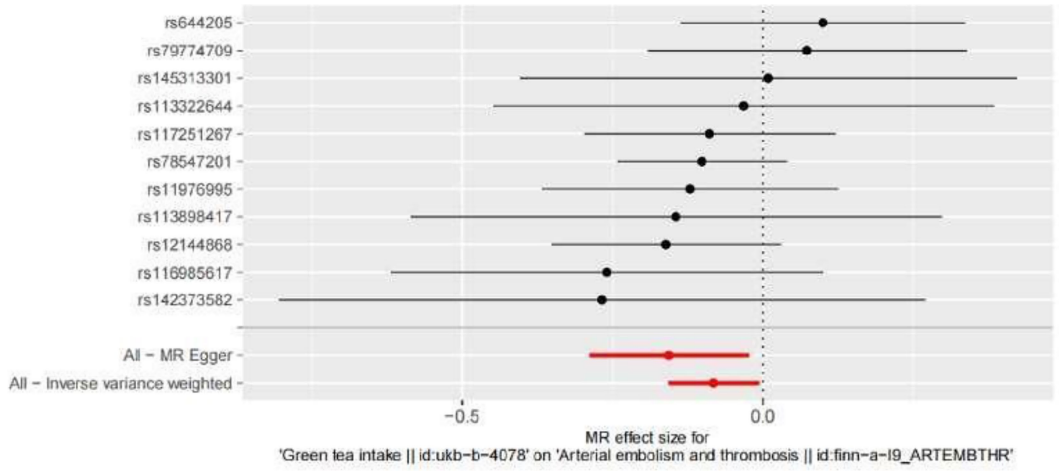

FIGURE 3
Forest plot to visualize the causal effect of every single SNP on ED. MR indicates Mendelian randomization.

3.3. Effects of individual genetic instruments on arterial embolism and thrombosis

Leave-one-out analyses were carried out to see how each SNP affected the overall causal estimate. When individual SNPs were systematically eliminated and the MR analyses were performed again, no significant disparity in the estimated causal effect was observed, as shown in Figure 5. Thus, the projected effects could not be attributed to any single genetic instrument.

3.4. Three assumptions of MR analysis

Three other assumptions should be satisfied. Firstly, the instruction variants must be associated with green tea intake (Assumption 1). In the present MR analysis, we only SNPs with a genome-wide significance threshold ($p < 5.0 \times 10^{-8}$) to satisfy assumption 1. Secondly, the instruction variants affect the risk of arterial embolism and thrombosis only *via* green tea intake (Assumption 2). We performed MR Egger regression and no evidence of directional pleiotropic effects was observed in our study. Thirdly, confounders were not involved in the IVs, including the measured or unmeasured ones (Assumption 3), which was satisfied that both the exposure and outcome GWAS were finished in the European ancestry populations. Therefore, the heterogeneity of our population is relatively low.

4. Discussion

In the present study, we used an MR method to investigate the causal association between green tea intake and arterial embolism and thrombosis in a European ancestry population. Our results show

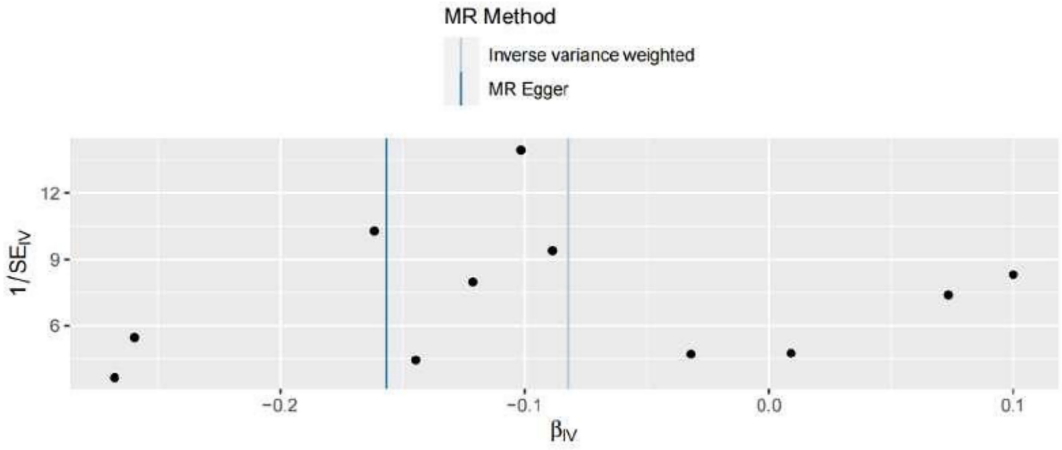

FIGURE 4
Funnel plots to visualize the overall heterogeneity of MR estimates for the effect of hypertension on the risk of ED. IVW indicates inverse-variance weighted, and MR indicates Mendelian randomization.

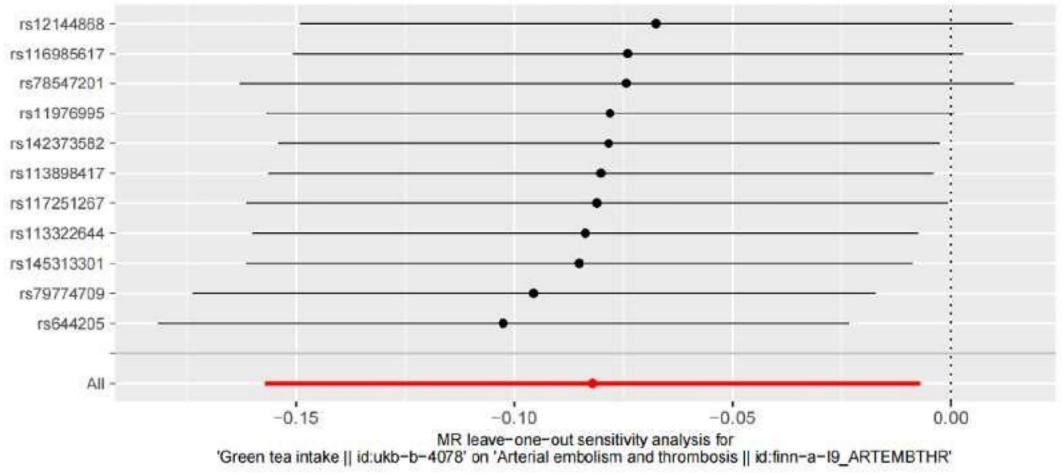

FIGURE 5
Leave-one-out plot to visualize the causal effect of hypertension on the risk of ED when leaving one SNP out MR indicates Mendelian randomization.

that genetically determined green tea intake is causally associated with decreased arterial thromboembolic risk, which is in line with previous observational research. Our findings suggest that green tea intake may be a modifiable protective factor for arterial embolism and thrombosis.

Multiple studies have shown that lifestyle interventions are effective in reducing arterial thrombotic diseases, especially for high-risk individuals (16). Recently, the Framingham Offspring Study (20) found similar results: participants with intermediate or ideal cardiovascular health were 33% less likely to develop hypertension and 25% less likely to develop cardiovascular diseases than individuals who had poor cardiovascular health in the past 5 years (21). Therefore, lifestyle intervention plays an important role in reducing the risk of cardiovascular diseases and has become a hotspot for recent research.

The preventive function of green tea on arterial thrombosis has been proved by many observational studies (22). Pang et al. evaluated the association between green tea consumption and the risk of ischemic-related diseases, and a total of nine studies on Japanese were included in their meta-analysis (6). Individuals who did not consume green tea had a 19, 24, and 15% increased risk of cardiovascular diseases, intracerebral hemorrhage, and cerebral infarction when compared with those who consume one cup of green tea per day, respectively. In addition, individuals who drank 1–3 cups of green tea per day had a 19 and 36% reduced risk of myocardial infarction and stroke when compared to those who drank 1 cup/per day, respectively. Furthermore, individuals who drank ≥4 cups/day had a 32% reduced risk of myocardial infarction when compared with those who drank 1 cup/day. However, all of those studies are observational and these results may have been affected by other confounding factors.

Our MR analysis is a contribution to make up for this deficiency of observational studies on the relationship between green tea intake and arterial thrombosis, avoiding being affected by confounding or

reverse causation (23). Therefore, in our present study, we used the two-sample MR method to find the causal associations based on the summary data from the biggest GWAS studies for green tea intake ($n = 64,949$) and arterial thrombosis (up to 278 arterial thrombosis cases and 92,349 control) in European ancestry people. In this sensitivity analysis, the directional pleiotropy was estimated using three distinct approaches: weighted median regression, IVW, and MR-Egger. The consistency of the three methodologies suggested that our results were credible. Since one's genetic variations are stable over a lifetime, the results of MR symbolize a lifetime protective impact of high green tea consumption against arterial embolism and thrombosis. Myocardial infarction and stroke are the most important and severe clinical manifestations of arterial thrombosis. Hence, identifying the potential protective factor of arterial thrombosis may be more effective in lowering the risk of myocardial infarction and stroke recurrence as well as occurrence. Exploring the causal association between green tea consumption and arterial embolism and thrombosis, in other words, could be very useful for clinical and social purposes.

Several possible mechanisms may explain the protective effect of green tea intake on the risk of arterial embolism and thrombosis. Grassi et al. measured brachial artery flow mediated dilation (FMD) in healthy individuals, which showed that drinking tea can improve endothelial function, suggesting that endothelial dysfunction may play a crucial part in arterial thrombosis pathogenesis (24). In addition to arterial thrombosis, green tea intake also plays an important part in other cardiovascular diseases. Widlansky et al. reported that acute epi-gallocatechin-3-gallate (EGCG) supplementation can improve brachial artery FMD in patients with cardiovascular disease and green tea is rich in acute EGCG (25). Redford demonstrated that the KCNQ5 voltage-gated potassium channel contributes activation to vasodilation by green tea, leading to lower blood pressure (26). A meta-analysis by Yarmolinsky et al., focusing on randomized controlled trials of at least 8 weeks and aiming for secondary prevention of hypertension among prehypertensive or hypertensive persons, showed statistically significant reductions in blood pressure with green tea or tea extract consumption (27). In addition, green tea has positive biological activities against chronic diseases such as cancer, metabolic syndrome, and type 2 diabetes, antibacterial and antiviral activity, protection against UV radiation, an increase of bone mineral density, and antifibrotic and neuroprotective properties (28–31). We still need to further study the potential mechanism of green tea intake for reducing arterial thrombosis. Thrombosis is a complex condition that arises from the interplay of genetic and environmental factors. The contribution of green tea intake to thrombus formation may be mediated by its impact on environmental factors, in addition to genetic factors. Therefore, it is important to consider both genetic and environmental factors when exploring the potential impact of green tea intake on thrombosis.

The major strength of our study is that the two-sample MR design can avoid potential confounding factors (9). To meet the assumptions of the MR analysis, some important measures were taken: Only SNPs at genome-wide significant levels showing a valid association with green tea intake were employed in our MR analysis for ensuring the legitimate link among risk variables and SNPs (such as green tea intake in our research). In our study, only SNPs were used if they showed sufficient genome-wide significance in European populations. Hence, we assume that the potential confounders in this research are very low. For ensuring that SNPs merely impact arterial thrombosis from green tea consumption (no pleiotropic effects), weighted medium and MR Egger regression were carried out, and no indication of directional pleiotropic effects was found in this finding.

However, it should be pointed out that excessive green tea consumption may have adverse health effects, including on the kidneys. In the present study, we have included individuals with a wide range of green tea intake, up to six or more cups per day. While we did not observe any significant associations between green consumption and adverse health outcomes within this range. It should be noted that our findings should not be extrapolated to higher levels of green tea intake.

We just utilized sum-level statistics in our analysis, not individual-level data, which could be a limitation. Therefore, we will not be able to investigate the causal relationship among subgroups, for instance, men, women, or one cup per day and two cups per day. Besides, it is well known that the risk factors are various in different races and ethnicities (32). And Finns have a unique genetic structure and profile compared to other European populations. Therefore, the conclusion should be further reconfirmed by using outcome data from the latest GWAS on European ancestry. Most importantly, the OR may always exaggerate the size of the effect compared with relative risk (33). However, the incidence of arterial embolism and thrombosis was only 0.3% [278/(278 + 92,349)] in the included populations, which is much lower than 5%. Therefore, we consider the OR and relative risk to be approximately equal in this study.

5. Conclusion

In summary, our study found that genetically predicted green tea intake may be causally associated with a lower risk of arterial embolism and thrombosis. And the conclusion also needs to be further reconfirmed in the future.

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.

Ethics statement

Ethical approval was not provided for this study on human participants because the MR data analyzed in this study were taken from our previous work and the institutional review board had given its approval to each of the published studies. Therefore, there was no need for further approval in the present study. The patients/

participants provided their written informed consent to participate in this study.

Author contributions

MJ and YC: conceptualization. LJ: methodology, writing—original draft preparation, and writing—review and editing. YL: software. CL: validation. MJ: visualization and supervision. All authors contributed to the article and approved the submitted version.

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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. Mackman N. Triggers, targets and treatments for thrombosis. *Nature*. (2008) 451:914–8. doi: 10.1038/nature06797

2. Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, et al. Heart disease and stroke Statistics-2021 update: a report from the American Heart Association. *Circulation*. (2021) 143:e254–743. doi: 10.1161/CIR.0000000000000950

3. Wendelboe AM, Raskob GE. Global burden of thrombosis: epidemiologic aspects. *Circ Res*. (2016) 118:1340–7. doi: 10.1161/CIRCRESAHA.115.306841

4. Meah MN, Dweck MR, Newby DE. Cardiovascular imaging to guide primary prevention. *Heart*. (2020) 106:1267–75. doi: 10.1136/heartjnl-2019-316217

5. Zhang C, Qin YY, Wei X, Yu FF, Zhou Y, He J. Tea consumption and risk of cardiovascular outcomes and total mortality: a systematic review and meta-analysis of prospective observational studies. *Eur J Epidemiol*. (2015) 30:103–13. doi: 10.1007/s10654-014-9960-x

6. Pang J, Zhang Z, Zheng T-z, Bassig BA, Mao C, Liu X, et al. Green tea consumption and risk of cardiovascular and ischemic related diseases: a meta-analysis. *Int J Cardiol*. (2016) 202:967–74. doi: 10.1016/j.ijcard.2014.12.176

7. Gaeini Z, Bahadoran Z, Mirmiran P, Azizi F. Tea, coffee, caffeine intake and the risk of cardio-metabolic outcomes: findings from a population with low coffee and high tea consumption. *Nutr Metab*. (2019) 16:28. doi: 10.1186/s12986-019-0355-6

8. Bowden J, Mv H. Meta-analysis and Mendelian randomization: a review. *Res Synth Methods*. (2019) 10:486–96. doi: 10.1002/jrsm.1346

9. Emdin CA, Khera AV, Kathiresan S. Mendelian randomization. *JAMA*. (2017) 318:1925–6. doi: 10.1001/jama.2017.17219

10. Smith JG, Newton-Cheh C. Genome-wide association study in humans. *Methods Mol Biol*. (2009) 573:231–58. doi: 10.1007/978-1-60761-247-6_14

11. Liu N, Js T, Liu L, Wang Y, Hua L, Qian Q. Genetic predisposition between COVID-19 and four mental illnesses: a bidirectional, two-sample Mendelian randomization study. *Front Psychol*. (2021) 12:746276. doi: 10.3389/fpsyt.2021.746276

12. Lawlor DA. Commentary: two-sample Mendelian randomization: opportunities and challenges. *Int J Epidemiol*. (2016) 45:908–15. doi: 10.1093/ije/dyw127

13. Jiang J, Shao M, Wu X. Vitamin D and risk of ankylosing spondylitis: a two-sample mendelian randomization study. *Hum Immunol*. (2022) 83:81–5. doi: 10.1016/j.humimm.2021.09.003

14. Jones HJ, Borges MC, Carnegie R, Mongan D, Rogers PJ, Lewis SJ, et al. Associations between plasma fatty acid concentrations and schizophrenia: a two-sample Mendelian randomisation study. The lancet. *Psychiatry*. (2021) 8:1062–70. doi: 10.1016/S2215-0366(21)00286-8

15. Ai S, Zhang J, Zhao G, Wang N, Li G, So HC, et al. Causal associations of short and long sleep durations with 12 cardiovascular diseases: linear and nonlinear Mendelian randomization analyses in UK biobank. *Eur Heart J*. (2021) 42:3349–57. doi: 10.1093/eurheartj/ehab170

16. Hwang LD, Davies NM, Warrington NM, Evans DM. Integrating family-based and Mendelian randomization designs. *Cold Spring Harb Perspect Med*. (2021) 11:a039503. doi: 10.1101/cshperspect.a039503

17. Tan JS, Liu NN, Guo TT, Hu S, Hua L. Genetic predisposition to COVID-19 may increase the risk of hypertension disorders in pregnancy: a two-sample Mendelian randomization study. *Pregnancy Hypertens*. (2021a) 26:17–23. doi: 10.1016/j.preghy.2021.08.112

18. Tan JS, Liu NN, Guo TT, Hu S, Hua L. Genetically predicted obesity and risk of deep vein thrombosis. *Thromb Res*. (2021b) 207:16–24. doi: 10.1016/j.thromres.2021.08.026

19. Jr T, Minelli C, Del Greco MF. Mendelian randomization using public data from genetic consortia. *Int J Biostat*. (2016) 12:12. doi: 10.1515/ijb-2015-0074

20. Corlin L, Short MI, Vasan RS, Xanthakis V. Association of the Duration of ideal cardiovascular health through adulthood with cardiometabolic outcomes and mortality in the Framingham offspring study. *JAMA Cardiol*. (2020) 5:549–56. doi: 10.1001/jamacardio.2020.0109

21. Álvarez-Bueno C, Cavero-Redondo I, Martínez-Andrés M, Arias-Palencia N, Ramos-Blanes R, Salcedo-Aguilar F. Effectiveness of multifactorial interventions in primary health care settings for primary prevention of cardiovascular disease: a systematic review of systematic reviews. *Prev Med*. (2015) 76:S68–75. doi: 10.1016/j.ypmed.2014.11.028

22. Arab L, Liu W, Elashoff D. Green and black tea consumption and risk of stroke: a meta-analysis. *Stroke*. (2009) 40:1786–92. doi: 10.1161/STROKEAHA.108.538470

23. Birney E. Mendelian randomization. *Cold Spring Harb Perspect Med*. (2022) 12:a041302. doi: 10.1101/cshperspect.a041302

24. Grassi D, Desideri G, Di Giosia P, De Feo M, Fellini E, Cheli P, et al. Tea, flavonoids, and cardiovascular health: endothelial protection. *Am J Clin Nutr*. (2013) 98:S1660–6. doi: 10.3945/ajcn.113.058313

25. Widlansky ME, Hamburg NM, Anter E, Holbrook M, Kahn DF, Elliott JG, et al. Acute EGCG supplementation reverses endothelial dysfunction in patients with coronary artery disease. *J Am Coll Nutr*. (2007) 26:95–102. doi: 10.1080/07315724.2007.10719590

26. Redford KE, Rognant S, Jepps TA, Abbott GW. KCNQ5 Potassium Channel activation underlies vasodilation by tea. *Cell Physiol Biochem*. (2021) 55:46–64. doi: 10.33594/000000337

27. Yarmolinsky J, Gon G, Edwards P. Effect of tea on blood pressure for secondary prevention of cardiovascular disease: a systematic review and meta-analysis of randomized controlled trials. *Nutr Rev*. (2015) 73:236–46. doi: 10.1093/nutrit/nuv001

28. Ohishi T, Goto S, Monira P, Isemura M, Nakamura Y. Anti-inflammatory action of green tea. *Antiinflamm Antiallergy Agents Med Chem*. (2016) 15:74–90. doi: 10.2174/1871523015666160915154443

29. Levy Y, Narotzki B, Az R. Green tea, weight loss and physical activity. *Clin Nutr*. (2017) 36:315. doi: 10.1016/j.clnu.2016.11.001

30. Shirakami Y, Shimizu M. Possible mechanisms of green tea and its constituents against cancer. *Molecules*. (2018) 23:2284. doi: 10.3390/molecules23092284

31. Jakubczyk K, Kochman J, Kwiatkowska A, Kałduńska J, Dec K, Kawczuga D, et al. Antioxidant properties and nutritional composition of Matcha green tea. *Foods*. (2020) 9:483. doi: 10.3390/foods9040483

32. JS T, Yan XX, Wu Y, Gao X, Xu XQ, Jiang X, et al. Rare variants in MTHFR predispose to occurrence and recurrence of pulmonary embolism. *Int J Cardiol*. (2021) 331:236–42. doi: 10.1016/j.ijcard.2021.01.073

33. Davies HT, Crombie IK, Tavakoli M. When can odds ratios mislead? *BMJ*. (1998) 316:989–91. doi: 10.1136/bmj.316.7136.989

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Evaluation of a gene signature related to thrombotic manifestations in antiphospholipid syndrome

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Thrombotic primary antiphospholipid syndrome (t-PAPS) is an acquired condition characterized by heterogeneous thrombotic manifestations, which is intriguing since venous and arterial thrombosis appear to have distinct pathogenesis. Gene expression analysis may constitute a new approach to evaluate potential similarities or differences between the clinical manifestations of t-PAPS. Recently, dysregulation of the *ANXA3, TNFAIP6, TXK, BACH2,* and *SERPINB2* genes has been associated with both arterial and venous thrombosis in the general population. Therefore, the aim of this study was to examine whether *ANXA3, TNFAIP6, TXK, BACH2,* and *SERPINB2* expression was associated with t-PAPS. Gene expression was quantified by qPCR of total leukocyte mRNA. In this case-control study, 102 t-PAPS patients, 17 asymptomatic antiphospholipid (aPL) carriers and 100 controls were evaluated. Increased expression of *ANXA3* (P = 0.008) and *TNFAIP6* (P = 0.001) and decreased expression of the *TXK* gene (P = 0.0001) were associated with an increased risk of t-PAPS compared to the control. *ANXA3* upregulation was more evident in cases of arterial thrombosis and multiple thrombotic events. There was no difference in the expression of these genes between triple and non-triple aPL positivity. *ANXA3, TNFAIP6, TXK, BACH2,* and *SERPINB2* expression levels were also similar between aPL carriers and controls (P = 0.77; P = 0.48; P = 0.08; P = 0.73, and P = 0.13, respectively). In conclusion, our results showed that genes related to hemostasis (*ANXA3*) and immunity (*TNFAIP6*, *TXK*) are dysregulated in t-PAPS compared to controls. Gene dysregulation was not detected in aPL carriers and was not related to the aPL profile, suggesting that this gene signature is related to thrombotic manifestations rather than to aPL burden. Our results suggest that innate immunity and hemostasis pathways are associated with t-PAPS at a molecular level and may play a role in disease severity.

KEYWORDS

antiphospholipid syndrome, thrombosis, gene expression, antiphospholipid antibody, mRNA

Introduction

Antiphospholipid syndrome (APS) is an acquired prothrombotic condition characterized by thrombosis or pregnancy complications (1) due to the presence of antiphospholipid antibodies (aPLs). These antibodies are directed against phospholipid-binding proteins, particularly beta2-glycoprotein I, found in cell membranes, including monocytes, platelets, and endothelial cells (2, 3). In primary APS (PAPS), there is no underlying systemic autoimmune disease.

The activation of platelets, monocytes, and endothelial cell membranes by aPLs leads to a hypercoagulable state (4), which is further enhanced by a secondary stimulus (second trigger), such as oral contraceptive use, infectious or inflammatory diseases, hypertension, and dyslipidemia (4, 5), resulting in thrombotic events. PAPS-associated thrombosis (t-PAPS) is one of the few thrombotic disorders that occurs indistinctly in veins, arteries or capillaries, affecting different organs and tissues (6).

Although t-PAPS is described as a single disease, arterial and venous complications differ in pathology, clinical course, treatment and prognosis, suggesting that different mechanisms underlie these thrombotic complications. Recently, studies aimed at evaluating the vascular impairment of t-PAPS have shown that aPLs can induce changes in the expression of genes associated with procoagulant and proinflammatory markers, leading to a prothrombotic state (7). Therefore, gene expression analysis may be a new approach to study the etiology of thrombosis in APS.

Recently, the profile of genes associated with venous and arterial thrombosis in the general population (not associated with APS) was evaluated by meta-analysis (8). In this study, five databases were analyzed and gene expression levels in whole blood from patients with venous thrombosis or arterial cardiovascular disease were compared with gene expression levels in controls. A total of 124 genes showed differential expression levels between these diseases. A further 473 genes had altered expression in the same direction in venous and arterial disease (168 upregulated and 305 downregulated). Of these, six genes were most strongly associated with both venous and arterial thrombosis: G0S2, BCL2A1, TNFAIP6 (upregulated), CLIC3, BACH2, and TXK (downregulated). The results of the meta-analysis suggest that a specific gene profile may be associated with the occurrence of both arterial and venous thrombosis in patients without APS.

Comparing the results of the meta-analysis described above (8) with the results of previous APS studies (9, 10), we found that the genes ANXA3, TNFAIP6, TXK, BACH2, and SERPINB2 are differentially expressed in both venous and arterial thrombosis as well as in APS compared to healthy subjects. Considering that the heterogeneity of t-PAPS manifestations cannot be explained by a single prothrombotic mechanism, it is possible that a gene signature underlies the occurrence and severity of thrombosis in PAPS.

In this context, the primary aim of this study was to determine the expression in patients with t-PAPS of genes previously associated with both arterial and venous thrombosis in the general population. We also investigated whether these genes are dysregulated in asymptomatic aPL carriers.

Materials and methods

Study design and participant selection

In this case-control study, we initially included consecutive patients with t-PAPS treated at the Hematology and Hemotherapy Center of the University of Campinas–Hemocentro-UNICAMP and individuals without a history of thrombosis (controls). Subsequently, asymptomatic aPL carriers were also included in the study. The study was approved by the local Ethics Committee (CAAE: 14902019.1.0000.5404), and the procedures were only performed after a signed informed consent form was obtained from patients.

We included patients with confirmed APS, a history of at least one previous thrombotic event within the previous 10 years. Cancer, pregnancy, isolated obstetric APS, systemic autoimmune diseases and infectious diseases were reasons for exclusion. APS diagnosis was confirmed based on the Sydney criteria (11).

Individuals with no previous history of thrombotic events and negative aPL results (called controls) were selected. Controls were recruited among people from the same geographic region as the patients and matched to them according to sex and age group ($\pm$ 5 years). Controls were selected among students, university staff and voluntary blood donors. Previous venous thrombosis, stroke, myocardial infarction, neoplasia, and pregnancy were reasons for exclusion. Individuals with persistently positive aPL results and no previous history of thrombosis or obstetric complications suggestive of APS were selected as asymptomatic aPL carriers. aPL carriers group was composed of asymptomatic patients under investigation of prolonged activated partial thromboplastin time (aPTT), immune thrombocytopenia (ITP) patients treated at the same center as t-PAPS or among healthy controls whose aPL tests were positive. Only individuals without signs of infection or acute inflammation were tested for aPL. As part of the diagnosis criteria (11), aPL positivity was confirmed after a 12-week interval. Exclusion criteria were the same as for healthy controls.

Patients, controls and aPL carriers answered a questionnaire about their demographic data, habits, health status and use of medication. Information regarding clinical data was also obtained by consulting the electronic medical record.

Laboratory procedures and mRNA expression of selected genes

Blood samples were collected upon inclusion into anticoagulant ethylenediaminetetraacetic acid (EDTA)-containing tubes. The samples were processed within 2 h of collection, the red blood cells were lysed with a buffer containing ammonium chloride and ammonium bicarbonate and centrifuged in a refrigerated centrifuge at 4°C, and the leukocyte layer was separated for RNA isolation. Samples were stored at −80°C until analysis.

As mentioned above, the relative expression of the five genes of interest was investigated in t-PAPS patients. These genes were selected among those described in a recent meta-analysis that evaluated genes with concordant regulation in arterial and venous thrombosis (8). The ones most associated with arterial and venous thrombosis, three upregulated (ANXA3, TNFAIP6,

and SERPINB2) and two downregulated (TXK and BACH2), were selected to be validated in thrombotic APS.

TRIzol™ reagent (Invitrogen™) was used to isolate mRNA from total leukocytes, following the manufacturer's instructions. Next, samples were transcribed into cDNA using the RevertAid First Strand cDNA Synthesis Kit® (Thermo Scientific™). The order and steps of incubation were performed according to manufacturer's instructions.

The primers used in the real-time quantitative PCR (qPCR) reaction were designed in the Gene Runner program (**Supplementary Table 1**). We verified the specificity, dissociation temperature, and formation of secondary structures of the primer pair. The slope of a standard curve is commonly used to estimate the amplification efficiency of a real-time qPCR. The estimated efficiency (EFF) calculation for a real-time PCR assay is EFF = $(10 - 1/\text{slope} - 1) \times 100$ (12). An *ANXA3* calibration curve was obtained at a primer concentration of 150 nM [slope −3.4, correlation coefficient (CC) = 0.99 and EFF 93%], *TNFAIP6* at 150 nM [slope −3.3, CC = 0.99, and EFF 99%], *TXK* at 150 nM [slope −3.3, CC = 0.99 and EFF 98%], *BACH2* at 150 nM [slope −3.4, CC = 0.99 and EFF 95%], *SERPINB2* at 300 nM [slope −3.3, CC = 0.98 and EFF 99%], *RHOA* at 150 nM [slope −3.2, CC = 0.99 and EFF 104%], and *EEF2* at 150 nM [slope −3.3, CC = 0.99 and EFF 99%]. Relative expression of genes was normalized using *EEF2* and *RHOA* as reference genes. Real-time amplification detection was performed using a real-time PCR thermocycler (QuantStudio 6 and 7–Thermo Fisher Scientific). Negative controls (NTC–No Template Control) were pipetted into all plates (wells containing only SYBR and primers) without the addition of cDNA to verify the absence of contamination. Reactions were performed in 96-well plates (Applied Biosystems) sealed with optical adhesives (Applied Biosystems). The relative expression was denoted as an "arbitrary unit" (AU).

Statistical analysis

Descriptive analyses were performed using frequency tables for categorical variables and position and dispersion measures for numerical variables, which were expressed as the mean and standard deviation (if the variable was normally distributed) or median and interquartile range [IQR] (if the variable was non-normally distributed).

To compare relative mRNA expression between two groups, we used either a parametric *t*-test or a non-parametric Mann-Whitney test, depending on the distribution of values (normal vs. non-normal). For the correlation between two variables, a non-parametric test of the Spearman rank correlation coefficient was used.

Logistic regression models were used to evaluate the association between relative *ANXA3*, *TNFAIP6*, *TXK*, *BACH2,* and *SERPINB2* mRNA expression and t-PAPS. Next, patients with t-PAPS were divided into 3 subgroups: (i) venous thrombosis or arterial thrombosis; (ii) single thrombotic event or multiple thrombotic events; and (iii) non-triple aPL-positive or triple aPL-positive. Logistic regression models were used to identify the association between mRNA expression and the subgroups, using the control group as a reference. The results were expressed as odds ratios (ORs) and 95% confidence intervals (CIs).

SPSS version 25.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. GraphPad Prism version 8.0 (GraphPad Software Inc., La Jolla, CA, USA) was used for graph plotting.

Results

Patient's selection and clinical characteristics

A total of 219 participants were included in the study: 102 t-PAPS, 100 healthy controls and 17 asymptomatic aPL carriers. **Figure 1** illustrates the flowchart of the selection process of controls and patients, as well as the reasons for exclusion from the study.

Participants' demographic, clinical and laboratory features are shown in **Table 1**. Cardiovascular risk factors were more prevalent in t-PAPS than in asymptomatic aPL carriers or controls. Most t-PAPS and asymptomatic aPL carriers were positive for lupus anticoagulant, while 22% of t-PAPS and 29% of asymptomatic aPL carriers were triple positive for aPLs. Details on APS-associated thrombotic events are presented in **Table 2**. The median age at the time of the first thrombosis was 35 years old [IQR 25–48], 60% of thrombotic events were unprovoked, 72% were venous and 28% arterial thrombosis. The first thrombotic event was deep vein thrombosis in 42% of the patients, ischemic stroke in 25%, pulmonary thromboembolism (PE) in 15%, and cerebral venous thrombosis in 12%. Portal vein thrombosis, acute myocardial infarction, intestinal thrombosis and retinal thrombosis occurred in 6% of the patients. A total of 36% of the patients had multiple thrombotic events. The median time elapsed between thrombotic events was 47 months [IQR 12.0–91.5].

ANXA3, *TNFAIP6,* and *TXK* are differentially expressed in patients with t-PAPS as compared to the control group

When comparing relative mRNA expressions, t-PAPS patients had higher levels of *ANXA3* and *TNFAIP6* and lower levels of *TXK* than healthy controls [$P = 0.005$; $P = 0.002$, and $P = < 0.0001$, respectively]. There were no statistically relevant differences between groups in *BACH2* and *SERPINB2* levels (**Figure 2**, panel A). Consequently, increased *ANXA3* and *TNFAIP6* mRNA expression and decreased *TXK* mRNA expression were associated with a higher risk of t-PAPS (**Figure 2**, panel B).

In addition, as shown in **Figure 3**, the mRNA expression levels of *ANXA3*, *TNFAIP6*, *TXK*, *BACH2*, and *SERPINB2* were similar between asymptomatic aPL carriers and controls. [$P = 0.77$; $P = 0.48$; $P = 0.08$; $P = 0.73$, and $P = 0.13$, respectively].

Elevated *ANXA3* expression tends to be associated with arterial thrombosis and multiple thrombotic events

The association of *ANXA3*, *TNFAIP6*, *TXK*, *BACH2,* and *SERPINB2* mRNA expression with the type of thrombosis (arterial

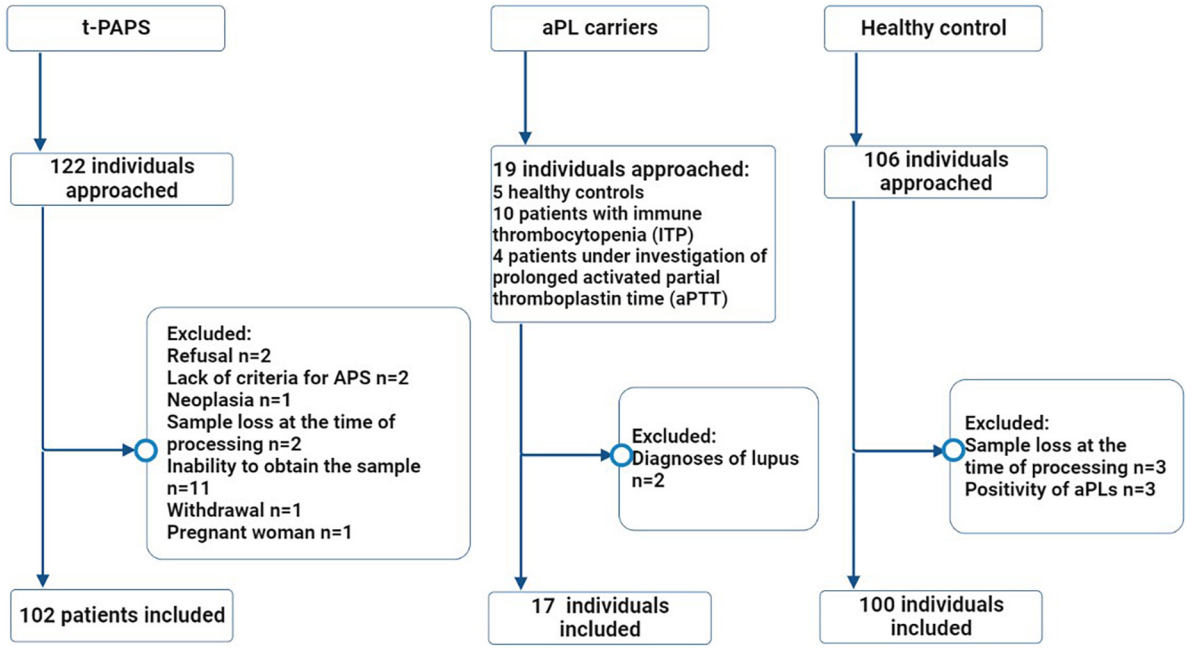

FIGURE 1
Flowchart of the selection process of study participants. Legend: The flowchart illustrates the number of individuals who were approached (*n* = 247), the excluded participants and the reasons for exclusions, the final number of individuals who were included in the study (*n* = 219) and the distribution between the groups. t-PAPS, primary thrombotic APS; aPL, antiphospholipid antibodies; *n, absolute number.

or venous) in t-PAPS is shown in Figure 4, Panel A. *ANXA3* mRNA upregulation was more evident in arterial thrombosis than in venous thrombosis when compared with controls. The relative expression of the other genes did not differ significantly between arterial and venous t-PAPS compared to controls.

Next, the association of *ANXA3*, *TNFAIP6*, *TXK*, *BACH2*, and *SERPINB2* mRNA expression with the number of previous thrombosis events (single or multiple thrombosis) is shown in Figure 4, Panel B. *ANXA3* mRNA upregulation was, again, more pronounced in multiple thrombosis than in single thrombosis when compared to controls. *BACH2* mRNA downregulation was more pronounced in single thrombosis than in multiple thrombosis compared to controls. The relative expression of the remaining genes did not differ between single and multiple thrombotic events compared to controls.

Finally, *ANXA3*, *TNFAIP6*, *TXK*, *BACH2*, and *SERPINB2* mRNA expression levels were similar between non-triple-positive and triple aPL-positive t-PAPS (Figure 4, Panel C).

Discussion

Antiphospholipid syndrome is a good clinical model to evaluate similarities and differences between venous and arterial thrombosis, as the disease is characterized by thrombotic manifestations in multiple vascular sites. This heterogeneous presentation is intriguing because venous and arterial thrombosis appear to have different pathogenesis. While stasis-related mechanisms, such as immobilization and congestive heart failure, and hypercoagulability-related mechanisms, such as familial thrombophilia and oral contraceptive use, play a fundamental role in the occurrence of venous thrombosis (13), arterial thrombosis occurs in high-flow sites and is mainly dependent on vascular integrity and platelet function (14, 15). However, some clinical aspects may be common to both arterial and venous thrombosis. Age, obesity and hypertension are known risk factors for atheromatous disease and arterial thrombosis. These factors may also contribute to the development of venous thrombosis (16, 17).

Much remains to be understood about common pathologic mechanisms leading to heterogeneous thrombotic manifestations in APS. One way to investigate common or diverse causes of the heterogeneous clinical presentation of APS is to study the genetic profile of the disease. Translational studies have allowed the identification of gene sets (or signatures) associated with PAPS (18) and secondary APS (SAPS) (19), as well as the expression profile of genes that differentiate thrombotic APS from APS with obstetric complications (9).

For example, obstetric APS is characterized by differentially expressed genes involved in cell adhesion, extracellular matrix, embryogenesis, and skeletal development, whereas thrombotic APS is characterized by differentially expressed genes involved in pro-inflammatory cytokine production, cellular response to stress, oxidative stress, and cellular homeostasis (9). PAPS and SAPS share the expression of genes involved in the type 1 interferon signature, inflammation and atherosclerosis (10), whereas PAPS differs from systemic lupus erythematosus (SLE) by the expression of genes related to mitochondrial function, oxidative stress and antioxidant capacity (10, 18, 19). We recently observed that genes related to the type 1 interferon signature were overexpressed in t-SAPS, but not in t-PAPS, compared to controls (20), suggesting that the inflammatory profile is different between these two presentations

TABLE 1 Demographic, clinical and laboratory features of the study participants.

Parameters	t-PAPS (*n* = 102)	aPL carriers (*n* = 17)	Controls (*n* = 100)
Age, median (IQR)	40 (31–53)	36 (24–59)	39 (29–49)
Woman, *n* (%)	65 (64)	14 (82)	67 (67)
Tabagism, *n* (%)	19 (19)	1 (6)	1 (1)
Hypertension, *n* (%)	33 (32)	2 (12)	6 (6)
Dyslipidemia, *n* (%)	34 (33)	1 (6)	11 (11)
Diabetes, *n* (%)	7 (7)	2 (12)	6 (6)
Obesity (BMI $\geq$ 30.0 kg/m^2), *n* (%)	39 (38)	1 (6)	9 (9)
Continuous medication use, *n* (%)	95 (93)	10 (59)	33 (33)
Statin use, *n* (%)	28 (27)	1 (6)	7 (7)
ASA use, *n* (%)	26 (25)	1 (6)	0 (0)
Use of anticoagulants*, *n* (%)	80 (78)	0	0
Family history**, *n* (%)	59 (58)	11 (65)	40 (40)
Gestational complication***, *n* (%)	17 (26)	0	0 (0)
Abortion, *n* (%)	19 (29)	1 (7)	2 (3)
LAC, *n* (%)	84 (82)	12 (71)	0
aCL IgM, *n* (%)	15 (15)	4 (24)	0
aCL IgG, *n* (%)	44 (43)	8 (47)	0
Anti-β2GPI IgM, *n* (%)	35 (34)	8 (47)	0
Anti-β2GPI IgG, *n* (%)	39 (38)	9 (53)	0
Triple positivity for aPL, *n* (%)	22 (22)	5 (29)	0

n, absolute number; t-PAPS, primary thrombotic antiphospholipid syndrome; aPL, antiphospholipid antibodies; ASA, acetylsalicylic acid; LAC, lupus anticoagulant; aCL, anticardiolipin; Antiβ2GPI, anti-beta2-glycoprotein-1 antibody; IgM, immunoglobulin M; IgG, immunoglobulin G.
*Most patients were using warfarin, and only two patients in the primary APS group were using rivaroxaban at the time of inclusion in the study.
**Family histories were deep venous thrombosis, arterial occlusion, stroke, and acute myocardial infarction.
***Pregnancy complications were fetal loss, intrauterine growth retardation (IUGR), preeclampsia, eclampsia or HELLP syndrome. Dyslipidemia was characterized as LDL-C levels above 100 mg/dL, HDL-C levels below 40 mg/dL (men), or 50 mg/dL (women), TG levels above 200 mg/dL, non-HDL-C levels above 130 mg/dL and TC levels above 190 mg/dL (31).

TABLE 2 Thrombotic manifestation in patients with t-PAPS.

	t-PAPS (*n* = 102)
Number of thrombotic events, median (IQR)	1 (1–2)
Age at thrombotic event, median (IQR)	35 (25–48)
Characterization of the thrombotic event	
Provoked, *n* (%)	41 (40)
Unprovoked, *n* (%)	61 (60)
Type of thrombosis	
Arterial, *n* (%)	29 (28)
Venous, *n* (%)	73 (72)
Thrombosis site (first event)	
Deep vein thrombosis, *n* (%)	43 (42)
Ischemic stroke, *n* (%)	25 (25)
PE, *n* (%)	15 (15)
Cerebral venous thrombosis, *n* (%)	12 (12)
Other sites*, *n* (%)	7 (6)
Multiple thromboses, *n* (%)	37 (36)
Time elapsed between thrombotic events in months, median (IQR)	47 (12.0–91.5)
Site of recurrence	
Deep vein thrombosis, *n* (%)	15 (14)
Ischemic stroke, *n* (%)	8 (8)
PE, *n* (%)	7 (7)
Other sites*, *n* (%)	7 (7)

n, absolute number; IQR, interquartile range; PE, pulmonary embolism.
*Other sites were portal vein thrombosis, acute myocardial infarction, intestinal thrombosis and retinal thrombosis.

of APS. A recent meta-analysis identified 16 genes differentially expressed in t-PAPS compared to controls that were expressed in 32 different tissues, which may explain the fact that thrombosis in APS occurs in any vascular site (18). Thus, measurement of gene expression has been used to develop new biological concepts, refine disease classification, improve diagnostic and prognostic accuracy, and identify new molecular targets for treatment.

We chose to quantify *ANXA3*, *TNFAIP6*, *TXK*, *BACH2*, and *SERPINB2* mRNA expression in t-PAPS based on the results of a meta-analysis using a bioinformatics panel to explore the differences and similarities between venous thromboembolism (VTE) and cardiovascular disease. The results of the meta-analysis represented the first comparison of venous and arterial thrombosis at the transcriptomic level, analyzing gene expression datasets from microarray studies involving human patients with cardiovascular disease or VTE in the Gene Expression Omnibus (GEO) public repository (8); however, they needed to be validated in a real-world cohort.

In this context, this study analyzed the expression of genes previously associated with venous and arterial thrombosis in the general population in t-PAPS and asymptomatic aPL carriers. Our results showed that the expression of *ANXA3* and *TNFAIP6* is upregulated and *TXK* is downregulated in t-PAPS compared to controls. Furthermore, increased *ANXA3* mRNA expression is more pronounced in patients with arterial thrombosis or multiple thromboses, suggesting that *ANXA3* is not only associated with thrombosis in PAPS but also with the severity of the thrombotic event.

ANXA3, *TNFAIP6*, *TXK*, *BACH2,* and *SERPINB2* are mainly associated with immunity and hemostasis. *ANXA3* plays a role in regulation of cell growth, regulation of endothelial cell migration, maintenance of cellular phospholipids and signal transduction pathways and has been associated with stroke in animal studies (21). *TNFAIP6* is involved in cell-cell and cell-matrix interactions in inflammation and cancer, and its increased expression in t-PAPS may be explained by the inflammatory process of the disease (8, 22). *TXK* acts on pathways related

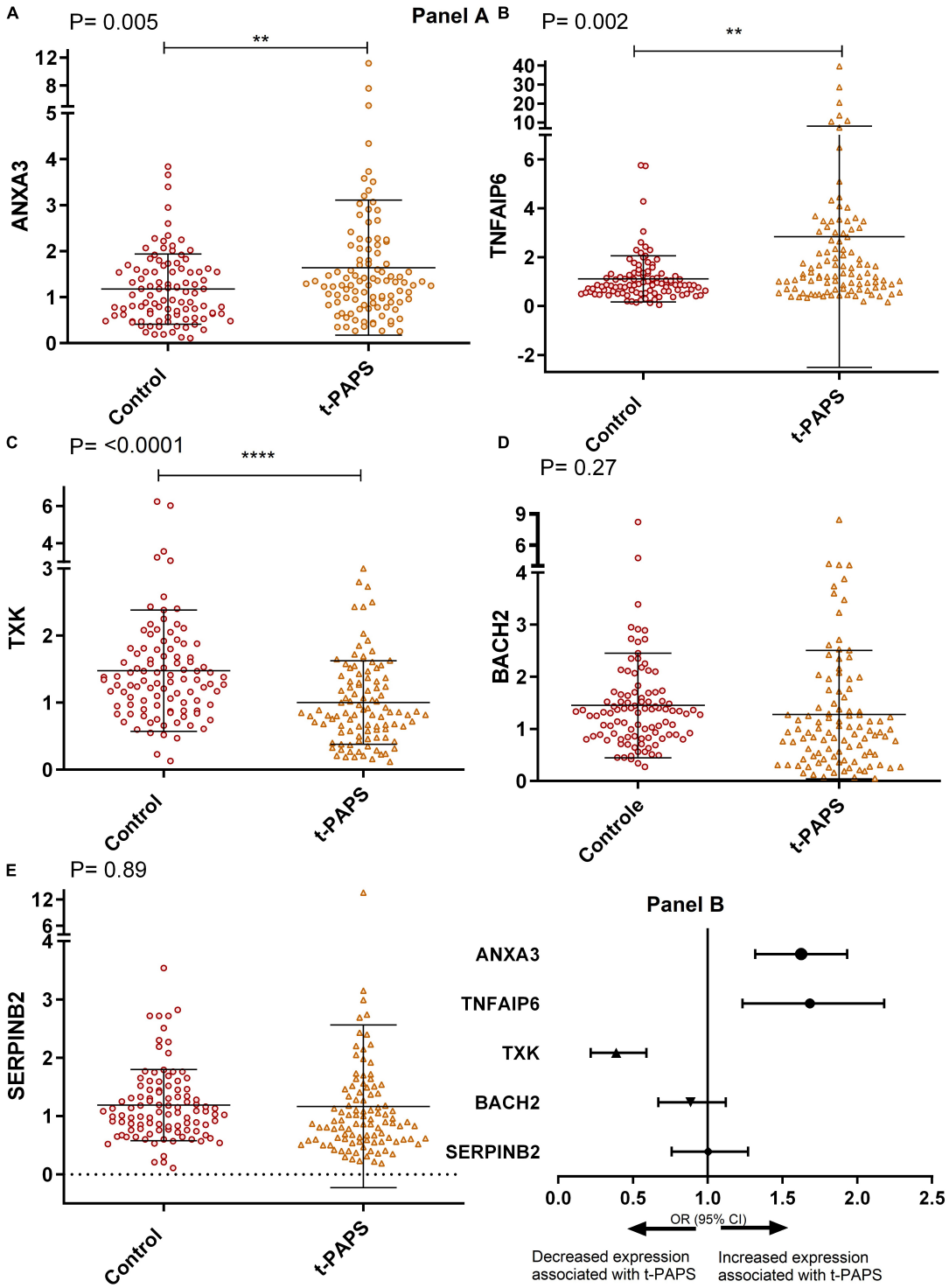

FIGURE 2
Panel **(A)** comparative graphs of the expression of genes related to thrombosis. Relative expression in total leukocytes of the genes: **(A)** *ANXA3*, **(B)** *TNFAIP6*, **(C)** *TXK*, **(D)** *BACH2*, and **(E)** *SERPINB2*. To illustrate the graphs above, mean and SD were used, and *P*-values were calculated using Student's *t*-test. t-PAPS = primary thrombotic APS. The mean *ANXA3* mRNA expression was 1.17 AU [95% CI 1.03–1.33] in controls and 1.64 AU [95% CI 1.36–1.93] in t-PAPS [$P = 0.005$]. The mean *TNFAIP6* expression was 1.10 AU [95% CI 0.92–1.29] in controls and 2.84 [95% CI 1.89–3.89] in t-PAPS [$P = 0.002$]. The mean *TXK* expression was 1.47 AU [95% CI 1.30–1.66] in controls and 1.00 AU [95% CI 0.88–1.12] in t-PAPS [$P = < 0.0001$]. The mean *BACH2* expression was 1.45 AU [95% CI 1.25–1.65] in controls and 1.27 AU [95% CI 1.03–1.52] in t-PAPS [$P = 0.27$]. Finally, the mean *SERPINB2* expression was 1.19 AU [95% CI 1.07–1.31] in controls and 1.17 AU [95% CI 0.89–1.44] in t-PAPS [$P = 0.89$]. Panel **(B)** association of *ANXA3*, *TNFAIP6*, *TXK*, *BACH2*, and *SERPINB2* relative expression with t-PAPS diagnosis. Increased *ANXA3* mRNA expression [OR = 1.57; 95% CI 1.13–2.18; $P = 0.008$] and *TNFAIP6* expression [OR = 1.64; 95% CI 1.23–2.18; $P = 0.001$] and decreased *TXK* mRNA expression [OR = 0.36; 95% CI 0.22–0.59; $P = 0.0001$] were associated with a higher risk of t-PAPS compared with controls. *BACH2* and *SERPINB2* mRNA expression was not associated with t-PAPS [OR = 0.87; 95% CI 0.67–1.12; $P = 0.28$ and OR = 0.98; 95% CI 0.75–1.3; $P = 0.89$, respectively]. OR, odds ratio; CI, confidence interval; t-PAPS, primary thrombotic APS. $^{**}P < 0.01$ and $^{****}P = < 0.0001$.

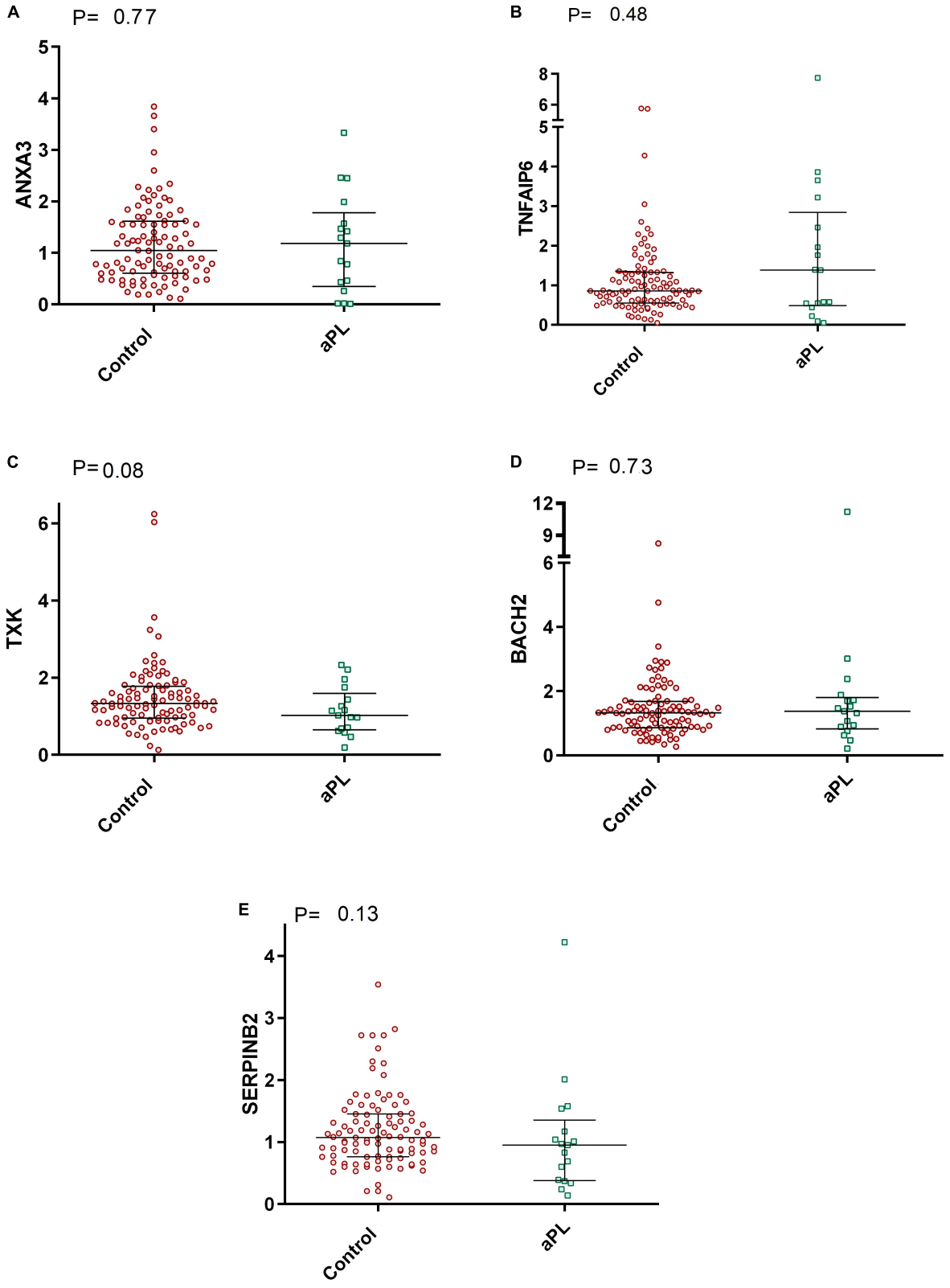

FIGURE 3
Comparative graphs of the expression of genes related to thrombosis. Legend: Relative expression of the genes **(A)** *ANXA3*, **(B)** *TNFAIP6*, **(C)** *TXK*, **(D)** *BACH2*, and **(E)** *SERPINB2* in total leukocytes was evaluated in aPL carriers compared to controls. Median and IQR were used by the Mann-Whitney test. aPL, asymptomatic aPL carriers (aPL +). The median relative expression of the *ANXA3* gene was 1.04 (IQR 0.60–1.61) in control subjects and 1.18 (IQR 0.34–1.78) in aPL + subjects. The relative expression of *TNFAIP6* was 0.86 (IQR 0.55–1.32) in controls and 1.38 (IQR 0.49–2.84) in aPL +. The relative expression of *TXK* was 1.33 (IQR 0.95–1.77) in controls and 1.02 (IQR 0.65–1.59) in aPL + patients. *BACH2* relative expression was 1.32 (IQR 0.86–1.68) in controls and 1.38 (IQR 0.82–1.80) in aPL +. Finally, the relative expression of *SERPINB2* was 1.07 (IQR 0.76–1.45) in controls and 0.95 (IQR 0.38–1.35) in aPL +.

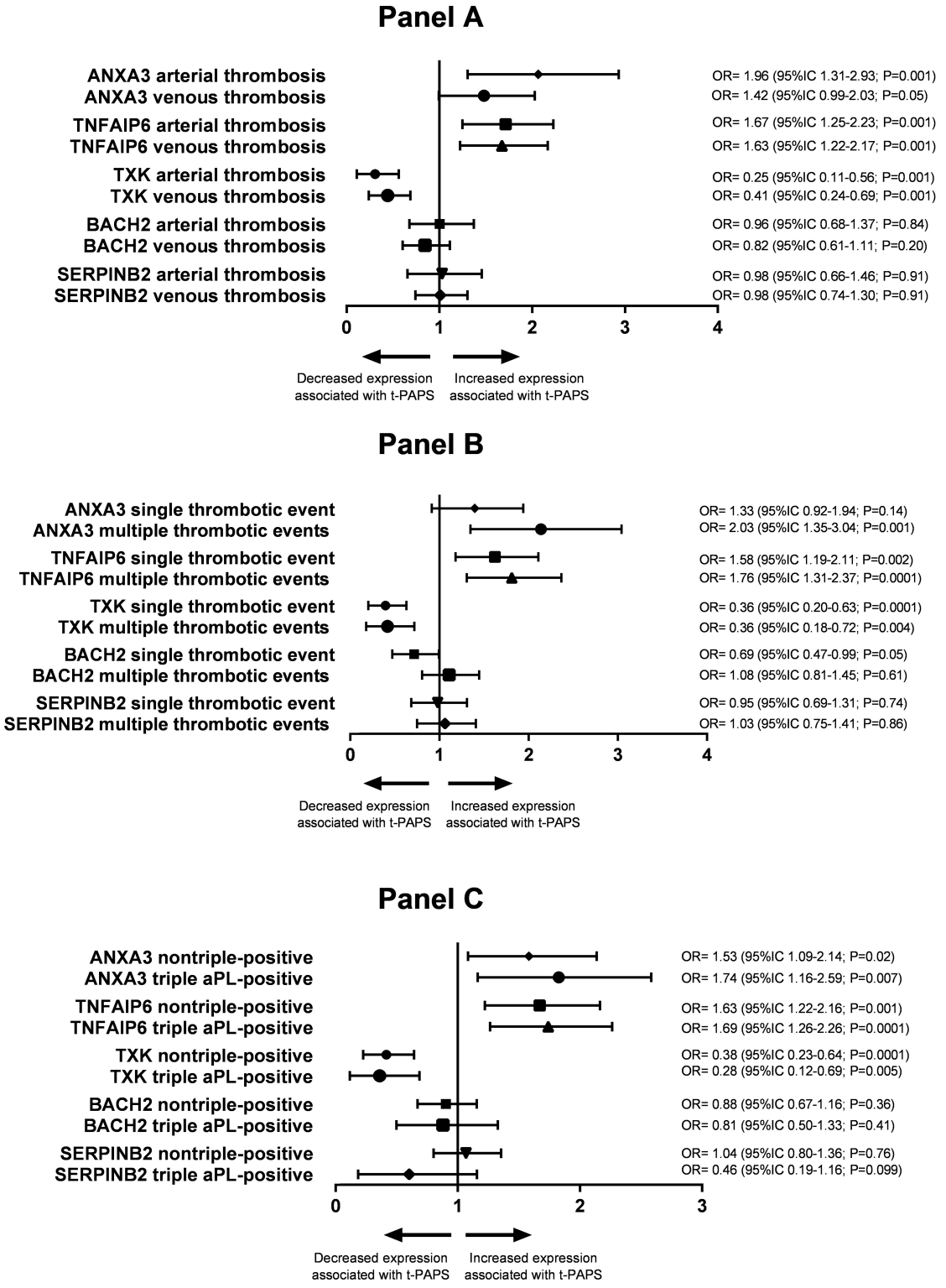

FIGURE 4
Panel **(A)** association between the relative expression of the study genes and arterial or venous thrombosis in t-PAPS. Panel **(B)** association between the relative expression of the study genes and a single thrombotic event or multiple events in t-PAPS. Panel **(C)** association between the relative expression of the study genes and non-triple-positive or triple aPL-positive subgroups. OR, odds ratio; CI, confidence interval; t-PAPS, primary thrombotic APS.

to the blood-brain barrier, transmigration of immune cells, and regulates the development, function, and differentiation of T-cells and NKT cells (23, 24). *BACH2* is responsible for immune regulation, mainly related to the adaptive immune response, and has been implicated in B-cell function (25, 26). *SERPINB2* regulates the production of plasminogen activator inhibitor-2

and is directly related to fibrinolysis pathways (8, 27). Our study showed that t-PAPS is associated with dysregulation of genes related to hemostasis (*ANXA3*) and inflammation/immunity (*TNFAIP6* and *TXK*). *ANXA3* has also been associated with the occurrence of arterial thrombosis and multiple thrombotic events. Interestingly, this gene has been shown to be upregulated in rodent models of ischemic stroke in animal studies (28). Therefore, our results suggest that innate immunity and hemostasis are associated with thrombotic manifestations of t-PAPS at the molecular level, with the potential to detect more severe forms of the disease.

In the meta-analysis that inspired this study, the association between *BACH2* and *SERPINB2* with arterial and venous thrombosis was weaker than that of the other genes. Moreover, from a biological point of view, *SERPINB2* regulates the plasminogen activator-2 inhibitor pathway, which is responsible for a fibrinolytic mechanism that is less associated with APS-related hypercoagulability. *BACH2* is a gene related to adaptive/humoral immunity, it has been described as a regulator of adaptive immunity through T-cell maintenance and B-cell maturation. In t-PAPS patients, we observed a dysregulation of genes associated with innate immunity.

The estimated incidence of thrombosis in asymptomatic aPL+ carriers is approximately 1% per year (29). Present in up to 5% of the general population, aPL antibodies may either be clinically irrelevant or confer an increased risk of thrombotic manifestations (30). In this context, we included the group of asymptomatic aPL carriers in this study to evaluate whether the molecular alterations observed in t-PAPS are also present in asymptomatic aPL carriers. Interestingly, the mRNA expression levels of *ANXA3*, *TNFAIP6*, *TXK*, *BACH2,* and *SERPINB2* were similar between asymptomatic aPL carriers and controls. Gene expression was not affected by aPL profile, particularly aPL triple positivity. Taken together, these results suggest that dysregulation of *ANXA3*, *TNFAIP6,* and *TXK*, although associated with thrombotic manifestations of PAPS, is not due to the presence or burden of aPL antibodies.

Limitations of our study include the fact that we included a group of patients treated in a tertiary care center and, therefore, it is possible that our patients presented with a more severe clinical profile. The cohort is heterogeneous because of the variable duration of the disease; however, there was no correlation between the time elapsed since diagnosis of t-PAPS and the relative expression of the genes (data not shown). Data collection was performed retrospectively by consulting electronic medical records, which may contain imprecise information.

Conclusion

The results demonstrated that genes related to both innate immunity and hemostasis were associated with thrombotic manifestations in PAPS and with disease severity (arterial thrombosis and multiple thrombotic events), independent of the aPL profile. Therefore, our findings suggest a molecular link between hemostasis, immune response and thrombosis in PAPS, providing new insights into the mechanisms underlying the development of thrombosis in PAPS.

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 author.

Ethics statement

The studies involving human participants were reviewed and approved by the CEP UNICAMP–Campus Campinas. The patients/participants provided their written informed consent to participate in this study.

Author contributions

BJ performed the laboratory and statistical analyses and drafted the manuscript. BM, BH, JO, AS, CV, and EP revised the manuscript. FO designed the study and the analyses and revised the manuscript. All authors contributed to the article and approved the submitted version.

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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.2023.1139906/full#supplementary-material

References

1. Gardiner C, Hills J, MacHin S, Cohen H. Diagnosis of antiphospholipid syndrome in routine clinical practice. *Lupus.* (2013) 22:18–25.

2. Espinosa G, Cervera R. Antiphospholipid syndrome: Frequency, main causes and risk factors of mortality. *Nat Rev Rheumatol.* (2010) 6:296–300. doi: 10.1038/nrrheum.2010.47

3. Meroni P, Borghi M, Raschi E, Tedesco F. Pathogenesis of antiphospholipid syndrome: understanding the antibodies. *Nat Rev Rheumatol.* (2011) 7:330. doi: 10.1038/nrrheum.2011.52

4. Ruiz-Irastorza G, Crowther M, Branch W, Khamashta M. Antiphospholipid syndrome. *Lancet.* (2010) 376:1498–509.

5. Lopes M, Danowski A, Funke A, Rêgo J, Levy R, de Andrade D. Update on antiphospholipid antibody syndrome. *Rev Assoc Med Bras.* (2017) 63:994–9.

6. Cervera R, Piette J, Font J, Khamashta M, Shoenfeld Y, Camps M, et al. Antiphospholipid syndrome: Clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. *Arthritis Rheum.* (2002) 46:1019–27. doi: 10.1002/art.10187

7. Lopez-pedrera C, Barbarroja N, Patiño-trives A. New Biomarkers for Atherothrombosis in Antiphospholipid Syndrome : Genomics and Epigenetics Approaches. *Front Immunol.* (2019) 10:764. doi: 10.3389/fimmu.2019.00764

8. Hounkpe B, de Oliveira Benatti R, de Sá Carvalho B, de Paula E. Identification of common and divergent gene expression signatures in patients with venous and arterial thrombosis using data from public repositories. *PLoS One.* (2020) 15:e0235501. doi: 10.1371/journal.pone.0235501

9. Ripoll V, Pregnolato F, Mazza S, Bodio C, Grossi C, Mcdonnell T, et al. Gene expression pro filing identi fies distinct molecular signatures in thrombotic and obstetric antiphospholipid syndrome. *J Autoimmun.* (2018) 93:114–23. doi: 10.1016/j.jaut.2018.07.002

10. Knight J, Meng H, Coit P, Yalavarthi S, Sule G, Gandhi A, et al. Activated signature of antiphospholipid syndrome neutrophils reveals potential therapeutic target. *JCI Insight.* (2017) 2:e93897. doi: 10.1172/jci.insight.93897

11. Miyakis S, Lockshin M, Atsumi T, Derksen R, Groot P, Koike T. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome. *J Thromb Haem.* (2006) 4:295–306. doi: 10.1111/j.1538-7836.2006.01753.x

12. Donia D, Divizia M, Pana' A. Use of armored RNA as a standard to construct a calibration curve for real-time RT-PCR. *J Virol Methods.* (2005) 126:157–63. doi: 10.1016/j.jviromet.2005.02.004

13. Mackman N. Triggers, targets and treatments for thrombosis. *Nature.* (2008) 451:914–8.

14. Aird W. Vascular bed-specific thrombosis. *J Thromb Haem.* (2007) 5(Suppl. 1):283–91.

15. Furie B, Furie B. Mechanisms of Thrombus Formation. *N Engl J Med.* (2008) 359:938–49.

16. Green D. Risk of future arterial cardiovascular events in patients with idiopathic venous thromboembolism. *Hematology.* (2009) 6:259–66. doi: 10.1182/asheducation-2009.1.259

17. Ageno W, Becattini C, Brighton T, Selby R, Kamphuisen P. Cardiovascular risk factors and venous thromboembolism: A meta-analysis. *Circulation.* (2008) 117:93–102.

18. Islam A, Saif S, Alam F. Genetic risk factors in thrombotic primary antiphospholipid syndrome : a systematic review with bioinformatic analyses. *Autoimmun Rev.* (2018) 17:226–43. doi: 10.1016/j.autrev.2017.10.014

19. Perez-Sanchez C, Barbarroja N, Messineo S, Ruiz-Limon P, Rodriguez-Ariza A, Jimenez-Gomez Y, et al. Gene profiling reveals specific molecular pathways in the pathogenesis of atherosclerosis and cardiovascular disease in antiphospholipid syndrome, systemic lupus erythematosus and antiphospholipid syndrome with lupus. *Ann Rheum Dis.* (2015) 74:1441–9. doi: 10.1136/annrheumdis-2013-204600

20. Rosa dos Santos A, de Oliveira Vaz C, Hounkpe B, Jacintho B, Oliveira J, Tripiquia Vechiatto Mesquita G, et al. Association between interferon-I producing plasmacytoid dendritic cells and thrombotic antiphospholipid syndrome. *Lupus.* (2022) 31:1067–77. doi: 10.1177/09612033221101731

21. Wang X, Li X, Xu L, Zhan Y, Yaish-Ohad S, Erhardt J, et al. Up-regulation of secretory leukocyte protease inhibitor (SLPI) in the brain after ischemic stroke: Adenoviral expression of SLPI protects brain from ischemic injury. *Mol Pharmacol.* (2003) 64:833–40. doi: 10.1124/mol.64.4.833

22. Danchuk S, Ylostalo J, Hossain F, Sorge R, Ramsey A, Bonvillain R, et al. Human multipotent stromal cells attenuate lipopolysaccharide-induced acute lung injury in mice via secretion of tumor necrosis factor-α-induced protein 6. *Stem Cell Res Ther.* (2011) 2:27. doi: 10.1186/scrt68

23. Kashiwakura J, Suzuki N, Nagafuchi H, Takeno M, Takeba Y, Shimoyama Y. Txk, a Nonreceptor Tyrosine Kinase of the Tec Family, Is Expressed in T Helper Type 1 Cells and Regulates Interferon γ Production in Human T Lymphocytes. *J Exp Med.* (2002) 190:1147–54. doi: 10.1084/jem.190.8.1147

24. Schneider H, Schwartzberg P, Rudd C. Resting lymphocyte kinase (Rlk/Txk) phosphorylates the YVKM motif and regulates PI 3-kinase binding to T-Cell antigen CTLA-4. *Biochem Biophys Res Commun.* (1998) 252:14–9. doi: 10.1006/bbrc.1998.9559

25. Yoshida C, Yoshida F, Sears D, Hart S, Ikebe D, Muto A, et al. Bcr-Abl signaling through the PI-3/S6 kinase pathway inhibits nuclear translocation of the transcription factor Bach2, which represses the antiapoptotic factor heme oxygenase-1. *Blood.* (2007) 109:1211–9. doi: 10.1182/blood-2005-12-040972

26. Afzali B, Grönholm J, Vandrovcova J, O'Brien C, Sun H, Vanderleyden I, et al. BACH2 immunodeficiency illustrates an association between super-enhancers and haploinsufficiency. *Nat Immunol.* (2017) 18:813–23. doi: 10.1038/ni.3753

27. Corsetti J, Salzman P, Ryan D, Moss A, Zareba W, Sparks C. Influences on plasminogen activator inhibitor-2 polymorphism-associated recurrent cardiovascular disease risk in patients with high HDL cholesterol and inflammation. *Atherosclerosis.* (2016) 250:1–8. doi: 10.1016/j.atherosclerosis.2016.04.017

28. Junker H, Suofu Y, Venz S, Sascau M, Herndon J, Kessler C, et al. Proteomic identification of an upregulated isoform of annexin A3 in the rat brain following reversible cerebral ischemia. *Glia.* (2007) 55:1630–7. doi: 10.1002/glia.20581

29. Arnaud L, Mathian A, Ruffatti A, Erkan D, Tektonidou M, Cervera R, et al. Efficacy of aspirin for the primary prevention of thrombosis in patients with antiphospholipid antibodies: An international and collaborative meta-analysis. *Autoimmun Rev.* (2014) 13:281–91. doi: 10.1016/j.autrev.2013.10.014

30. Clark E, Silver R, Branch D. Do Antiphospholipid Antibodies Cause Preeclampsia and HELLP Syndrome? *Curr Rheumatol Rep.* (2007) 9:219–25.

31. Rodrigues T, de Oliveira Vaz C, Miranda E, Pereira M, da Silva Saraiva S, Annichino-Bizzacchi J. Efficacy of a hypolipid diet in patients with primary antiphospholipid syndrome with dyslipidemia: a prospective study. *J Thromb Thrombolysis.* (2022) 53:390–8. doi: 10.1007/s11239-021-02542-z

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Magnitude and relevance of change in health-related quality of life in patients with vascular malformations treated with sirolimus

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Introduction: Vascular malformations are rare congenital anomalies of the vascular system, which can involve the capillaries, veins, arteries, lymphatics, or a combination of vessel types. Patients with vascular malformations experience an impaired health-related quality of life (HRQoL) because of their symptoms (e.g., pain, swelling, and bleeding) and psychosocial distress. Sirolimus is an effective drug used in the medical treatment of these patients; however, relatively little is known about the effect of sirolimus on specific changes in the HRQoL domains and its magnitude.

Methods: The magnitude of change (effect size) following intervention is more informative to clinical practitioners than statistically significant but clinically unimportant changes; therefore, this study aimed to examine the magnitude and meaningfulness of change in the HRQoL of children and adults with vascular malformations following sirolimus treatment using low target levels.

Results: In total, 50 patients with vascular malformations (19 children, 31 adults) were included in this study. These patients experienced a lower HRQoL than the general population, with the adults reporting a significantly lower score in almost all domains. A 6-month sirolimus treatment improved the HRQoL in 29 patients, including 77.8% of the children (Pediatric Quality of Life Inventory score [PedsQL]) and 57.7% of the adults (Short Form 36 [SF-36]). The effect sizes of sirolimus for each SF-36/PedsQL domain ranged from 0.19 to 1.02. The clinically relevant moderate magnitude of changes was seen in the domains of the children's reports: "Physical functioning" and "Social functioning" and in the domains of the parent reports: "Social functioning," "School functioning," and "Psychosocial." A high-magnitude change was seen in the domains "Emotional functioning" and "Psychosocial" in the children's reports and "Physical functioning" in the parent reports. In addition, the moderate magnitude of changes was also seen in the adults SF-36: in all domains except for "Role limitations—physical problems," "Role limitations—emotional problems," and "General health perception."

Conclusion: We believe this is the first study showing the magnitude of change in HRQoL after sirolimus treatment in patients with vascular malformations. Before treatment, these patients experienced an impaired HRQoL compared with the general Dutch population. A 6-month sirolimus treatment with low target levels led to moderate-to-high clinically relevant changes in multiple domains, which significantly improved the HRQoL.

Clinical trial registration: https://clinicaltrials.gov/ct2/show/NCT03987152?cond=Vascular+Malformations&cntry=NL&city=Nijmegen&draw=2&rank=1, identifier: NCT03987152.

KEYWORDS

vascular malformation, quality of life, sirolimus (rapamycin), SF-36: 36-item short form health survey, PedsQL: pediatric quality of life inventory

Introduction

Vascular malformations are rare congenital anomalies of the vascular system that grow proportionally with age. These malformations can involve the capillaries, veins, arteries, lymphatics, or any combination of these (1, 2). Of these, venous and lymphatic malformations are categorized as low-flow vascular malformations. Most of the frequently found somatic mutations in low-flow vascular malformations occur in genes involved in the mammalian target of rapamycin (mTOR) pathway; for example, PIK3CA and TEK/TIE-2 mutations lead to a gain of function and increased activity of mTOR (3–7). Patients experience symptoms of pain, swelling, bleeding, ulcerations, leakage, thrombotic complications, disfigurement, functional impairment, and psychosocial distress, which affects their health-related quality of life (HRQoL) (8–12).

The treatment options for low-flow vascular malformations are conservative, including compressive hosiery, analgesics, anti-inflammatory or anti-coagulation drugs, intralesional sclerotherapy or embolization, and surgery (13). Sclerotherapy alone or in combination with surgical resection is frequently applied in most vascular malformations (14, 15). Unfortunately, treatment is challenging and not always successful and can leave patients with a high clinical burden and subsequently a reduced HRQoL (16).

The treatment of pain and other symptoms in patients with vascular malformations can improve their HRQoL. Several studies have shown that sirolimus, an mTOR inhibitor, can reduce pain and, in some cases, may reduce the size of the vascular malformation (17–23). The reduction in symptoms due to sirolimus may improve the HRQoL. To measure HRQoL in patients with vascular malformations, the generic questionnaires including the Pediatric Quality of Life Inventory (PedsQL) questionnaire (PedsQL^{TM} 4.0 Generic Core Scales) and the 36-Item Short Form Health (SF-36) questionnaire are frequently used (17–20); however, the exact measures of the domains in these questionnaires have not yet been reported in the literature for patients treated with sirolimus.

The aim of this report was therefore to analyze the efficacy of sirolimus in terms of improving patients' HRQoL. The magnitude of the change (effect size) following intervention is more informative to clinical practitioners than statistically significant (whether the changes are likely to be caused by chance) but clinically unimportant changes. We, therefore, investigated the magnitude and meaningfulness of change in the PedsQL and SF-36 scores of patients with vascular malformations following a low-dose sirolimus treatment. We explored this after 6 months of treatment, which was implemented as part of the national phase IIB open-label single-arm clinical study (24). This report presents the magnitude of changes in the HRQoL of pediatric and adult patients who received doses of sirolimus with low target levels for vascular malformations. To interpret the size of the change, the results were compared with the age-adjusted norms of the Dutch population (25–28).

This report provides a detailed insight into the magnitude of changes for each domain, which are clinically relevant for the patients (and the parents of pediatric patients).

Materials and methods

Study design and patients

The HRQoL was measured in patients who participated in the phase IIB open-label clinical study "Treatment of Congenital Vascular Malformations Using Sirolimus: Improving Quality of Life," which is a nationwide study performed in the Netherlands. The study is registered in ClinicalTrials.gov (identifier: NCT03987152) and EudraCT (number: 2016-002157-38). Patients with low-flow malformations included in the clinical trial had no other remaining treatment options, and the medication they had taken with the intention to relieve pain had not produced the desired effect. In total, 74 patients were enrolled at Radboud University Medical Center (Radboudumc), Nijmegen, the Netherlands, between September 2017 and February 2021. Seven patients did not complete the Challenge phase (including one case series patient), leaving a total of 67 remaining patients in the Challenge phase of the trial using low target levels of sirolimus. The patients were treated over a 6-month period (Challenge phase), which was followed by a 12-month follow-up period (Dechallenge phase). To evaluate the efficacy of the treatment, the pain was scored daily using the visual analog scale and numeric pain rating scale. In addition, magnetic resonance imaging (MRI) and HRQoL assessments were performed at the baseline and after 6 months (at end of the Challenge phase). The study demonstrated that the target levels of 4–10 ng/ml sirolimus are comparably effective and were

accompanied by less severe adverse events than those reported in the literature using high target levels of 10 ng/ml or above (24).

Outcome measures

Patients aged 2 years and older were included in the study. Two HRQoL questionnaires were used; the PedsQL questionnaire was sent as per age category (2–4 years, 5–7 years, 8–12 years, or 13–16 years) based on the age of the participant at that moment, while the SF-36 questionnaire was used for adults (17 years and older). The HRQoL questionnaires were sent out digitally before and after the 6-month treatment (Challenge phase). In all these questionnaires, higher scores indicate a better HRQoL.

PedsQL

The PedsQL questionnaire (PedsQL™ 4.0 Generic Core Scales) is a widely used standardized generic instrument used to assess patients' and proxies' perceptions of HRQoL in pediatric patients with chronic health conditions (29, 30). This instrument contains 23 items measuring four domains of HRQoL: "Physical functioning," "Emotional functioning," "Social functioning," and "School functioning." Additionally, a "Psychosocial" score can be calculated. For each domain, a score can be calculated ranging from 0 to 100, with a higher score indicating a better HRQoL. The "total scale score" can be derived as the sum of all items over the number of items answered on all the scales (30).

SF-36

The SF-36 questionnaire is frequently used to explore HRQoL in adults in terms of eight domains: "Physical functioning," "Social functioning," "Role limitations—physical problems," "Role limitations—emotional problems," "Mental health," "Energy levels/vitality," "Pain," and "General health perception." A mental component summary (MCS) and physical component summary (PCS) can be derived from the scores. We used the RAND-36 version 1.0, and scoring was carried out using the RAND-36 scoring guidelines (27, 28, 31). For each domain and component, a score can be derived ranging from 0 to 100, with a higher score indicating a better HRQoL.

Statistical analyses

Statistical analyses were performed using SPSS version 25.0 (IBM). Descriptive statistics were used for the patients' demographic characteristics. When the majority of domains were normally distributed, the continuous variables and the proportions for nominal variables were presented as means with 95% confidence intervals (95% CI) or standard deviations (SD). When the majority of the domains had a skewed distribution, medians with IQR values were used.

Comparison with the general Dutch population

The HRQoL scores of the patients were compared with the general Dutch population. For this comparison, parent PedsQL reports were used for children under 7 years of age, while the children's reports were used in children aged older than 7 years corresponding to the general Dutch population scores. HRQoL mean scores of patients with vascular malformations were compared with HRQoL mean scores of the general Dutch population. If data showed a skewed distribution, the medians of the general Dutch population were used. For children above 7 years old, these median scores are not available for the general Dutch population; therefore, mean scores of the general Dutch population were used as the median values for this group, since median scores of the general Dutch population (older than 7 years) are not published (26). Note that in large groups (as a general Dutch population) in a normal distribution, the mean value corresponds to the median value. For the children aged 2–7 years, the median scores of the general Dutch population (Parent reports) were used, as these scores of the general Dutch parent's reports under 7 are published (25).

To compare the SF36 scores of patients with vascular malformation, the published mean SF-36 scores of the general Dutch population were used (26, 27).

The difference in HRQoL between patients with vascular malformations and the general population was analyzed using a *t*-test when normally distributed or using a one-sample Wilcoxon signed-rank test when the data were skewed. All *P*-values were two-sided, and the results were considered statistically significant if $P < 0.05$.

Changes after sirolimus treatment

The changes in HRQoL after the sirolimus treatment were analyzed. The guidelines of the particular survey were followed to calculate the correct score when values were missing. Surveys were excluded from the analysis when the surveys at the baseline and/or at the end of the Challenge phase were missing.

A change in the HRQoL of each child was quantified as a change in the total scale score of >4.4 in the self-reported PedsQL or >4.5 in the parent-reported PedsQL. These PedsQL thresholds were based on previous clinical trials within this patient category, and our phase IIB study as published previously (18, 20, 24).

In addition, the change of HRQoL per patient was quantified as a change of 3.5 in the MCS score or 4.1 in the PCS score of the SF-36 (24). The upper threshold of the minimal important difference (MID) of modest changes was used for the SF-36, based on the systematic review by Frendl et al. (32) who determined the size and meaningful changes of the SF-36 MCS and PCS and mean net of placebo changes with treatment across different diseases. This resulted in net mean modest changes of MCS [interquartile range (IQR) 0.8; 3.5] and PCS [IQR 1.6; 4.1]. The percentage of patients who experienced a change in HRQoL after 6 months of sirolimus

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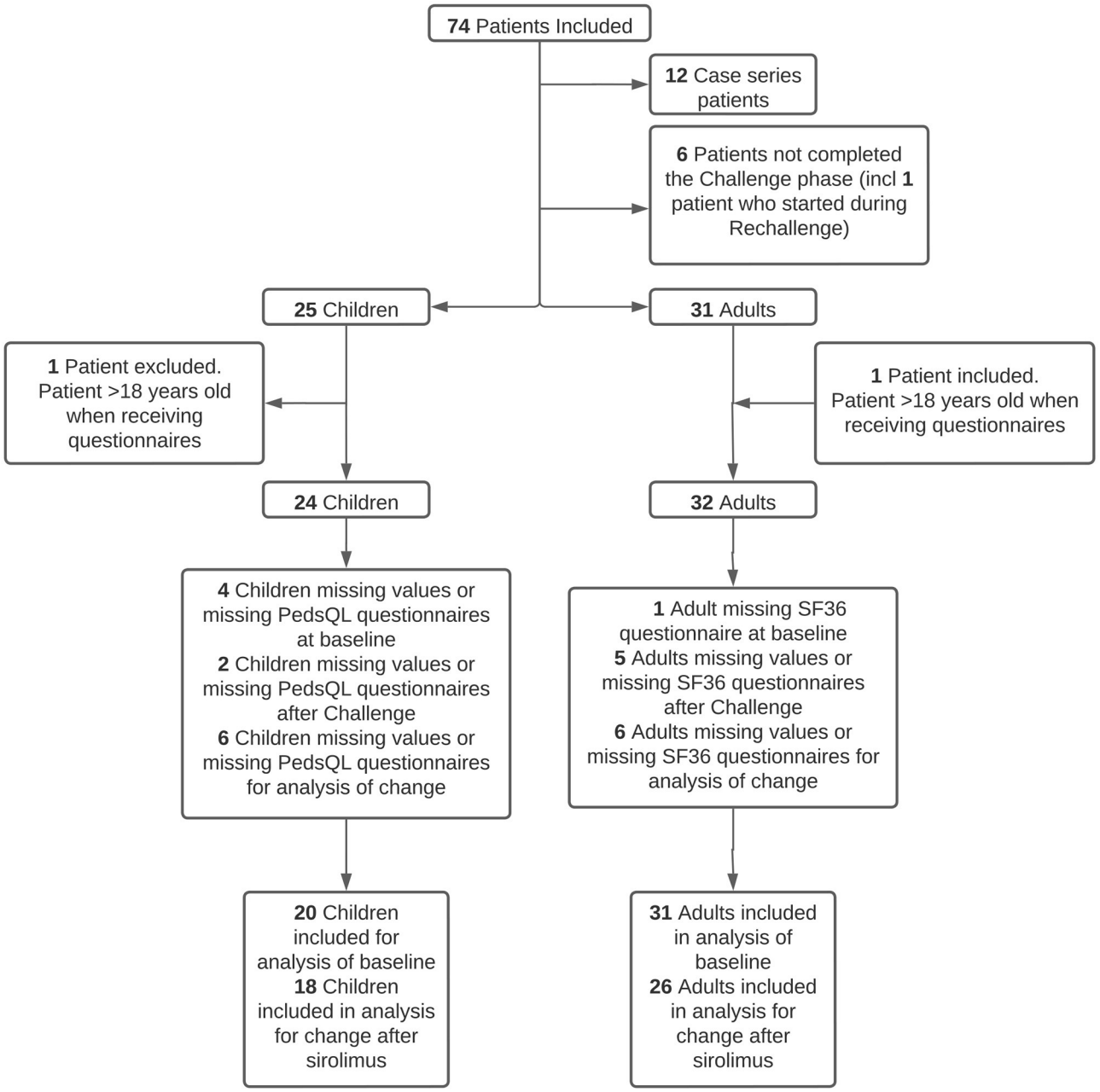

FIGURE 1
Patient distribution. In total, 12 case series patients did not receive the quality-of-life questionnaire due to different protocols (including one patient who did not complete the Challenge phase), five patients did not complete the Challenge phase, and one child was included in the study during the Rechallenge phase.

treatment was calculated (*n* patients with a change of HRQoL/*n* of evaluable patients).

The mean differences with 95% CI between the pre-treatment (baseline) and post-treatment (Challenge phase) HRQoL scores were calculated when the differences in scores between the baseline and the end of the Challenge phase were normally distributed in the majority of domains. When these data were skewed, the median with IQR difference was calculated. For the comparison of the HRQoL scores at the baseline and the end of the Challenge phase, paired *t*-tests were used. Non-parametric tests (Wilcoxon signed rank tests) were used when the data were skewed. The threshold for statistical significance was set a priori at $\alpha = 0.05$.

Magnitude of change

The responsiveness of the HRQoL was analyzed using the widely accepted method of "effect sizes" (33–35). The effect sizes were used to translate the baseline and changes after the end of the Challenge phase into a standard unit of measurement that should

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provide a clearer understanding of the HRQoL results. The effect sizes can supplement standard statistical testing to obtain a more complete and clinically relevant picture of health status change (36). The effect size was calculated using Cohen's *d* formula: *d* = mean difference/SD difference. When the data were skewed, the effect size was calculated using the *z*-value (37). The effect size was considered to be small (0.20–0.49), moderate (0.50–0.79), or high (>0.80). An effect size of more than 0.5 was considered clinically relevant.

Results

Patient characteristics

In total, 56 patients (24 children and/or parents and 32 adults) received the HRQoL surveys. At the baseline, 91.1% ($n = 51/56$) of patients (children and/or parents: 83.3%, $n = 20/24$; adults: 96.9%, $n = 31/32$) completed the surveys. The mean age of these patients was 25.8 years (SD = 16.5). Of the 56 patients, 87.5% ($n = 49/56$) of patients (children and/or parents: 91.7%, $n = 22/24$; adults: 84.4%, $n = 27/32$) completed the end of Challenge phase questionnaires. In total, 78.6% ($n = 44/56$) of patients (children: $n = 18$; adults: $n = 26$) were evaluable for the analyses of the HRQoL change after sirolimus treatment. See Figure 1 for patient distribution.

In total, 68 HRQoL questionnaires were completed (PedsQL: children [aged 5–16 years] $n = 17$ and parents [of children 2–16 years] $n = 20$; SF-36: adults $n = 31$) before the start of the sirolimus treatment (baseline). After 6 months of the sirolimus treatment, a total of 64 HRQoL questionnaires were completed (PedsQL: children [aged 5–16 years] $n = 17$ and parents [of children 2–16 years] $n = 20$; SF-36: adults $n = 27$). Despite repeated requests, data were missing for four children and one adult at the baseline, and for two children and five adults at the end of the Challenge phase. Due to this missing data before and after treatment, a total of six children and six adults were excluded from the analysis of change after the Challenge phase.

The characteristics of the included patients for the HRQoL analysis are presented in Table 1. The majority of both the pediatric and adult patients were women. At the baseline, the median total HRQoL score of the children's reports ($n = 17$) was 73.91 (IQR = 55.43; 77.17) and of the parent reports ($n = 20$) was 59.24 [IQR = 55.71; 75.54]. The means scores of Mental Component Summary (MCS) and Physical Component Summary (PCS) in adults ($n = 31$) were 47.5 [95% CI 43.4; 51.5] and 33.1 [95% CI 29.1; 37.1], respectively, at the baseline.

TABLE 1 Patient characteristics of the included patients.

Characteristic	Included children ($n = 20$)	Included adults ($n = 31$)
Age groups *n*		
Under 2	0 (0.0%)	–
2–4	2 (10.0%)	–
5–7	3 (15.0%)	–
8–12	11 (55.0%)	–
13–17	4 (20.0%)	–
18 and older	–	31 (100%)
Gender *n*		
Male	5 (25.0%)	10 (32.3%)
Female	15 (75.0%)	21 (67.7%)
Vascular malformation type *n*		
Lymphatic malformation[a]	9 (45.0%)	7 (22.6%)
Venous malformation[b]	9 (45.0%)	16 (51.6%)
Combined malformation[c]	1 (5.0%)	7 (22.6%)
Other[d]	1 (5.0%)	1 (3.2%)
Vascular malformation location *n*		
Head and neck	7 (35.0%)	4 (12.9%)
Thorax	0 (0.0%)	4 (12.9%)
Abdominal	1 (5.0%)	3 (9.7%)
Upper extremity	1 (5.0%)	3 (9.7%)
Lower extremity	8 (40.0%)	14 (45.2%)
Multiple locations	3 (15.0%)	3 (9.7%)

[a]Lymphatic malformation: CLOVES syndrome ($n = 1$), lymphangiomatosis ($n = 3$). [b]Venous malformation: Blue Rubber Bleb Nevus syndrome ($n = 1$) and angio-osteohypertrophy syndrome with venous malformation ($n = 1$). [c]Combined malformation: Klippel–Trenaunay syndrome ($n = 7$), veno-lymphatic malformation ($n = 3$), and capillary and venous malformation ($n = 1$). [d]Other vascular malformations: fibro adipose vascular malformation ($n = 1$) and multiple spindle-cell hemangioma ($n = 1$).

Health-related quality of life compared with the general Dutch population

At the baseline, the HRQoL scores of children with vascular malformations were lower than that of the general Dutch population (Figures 1, 2, Supplementary Table 1). After the Challenge phase, the PedsQL scores of the patient group increased, and the difference in HRQoL scores between this group and the general Dutch population was smaller.

Compared with the general Dutch population, the adult patients with vascular malformations experienced significantly lower SF-36 scores in all domains, except for "Mental health" (Figure 3, Supplementary Table 2). After the Challenge phase, the adult patient group reached the level HRQoL of the general Dutch population. The domains "Social functioning," "Role limitations—emotional problems," and "Energy levels/vitality" normalized after the 6-month treatment with low target levels of sirolimus.

Supplementary Tables 1, 2 show the scores of each HRQoL questionnaire in our study at the baseline and at the end of the Challenge phase compared with the general Dutch population.

An overall change in health-related quality of life

After the Challenge phase, 29 patients had an improved HRQoL, including 77.8% of the children ($n = 14/18$) and 57.7% of the adults ($n = 15/26$). In 11.1% of the children ($n = 2/18$) and 11.5% of the adults ($n = 3/26$), a worsened HRQoL was observed.

At the baseline, the mean total scale score of the PedsQL as reported by the children ($n = 16$) was 66.3 [SD 17.7], while the

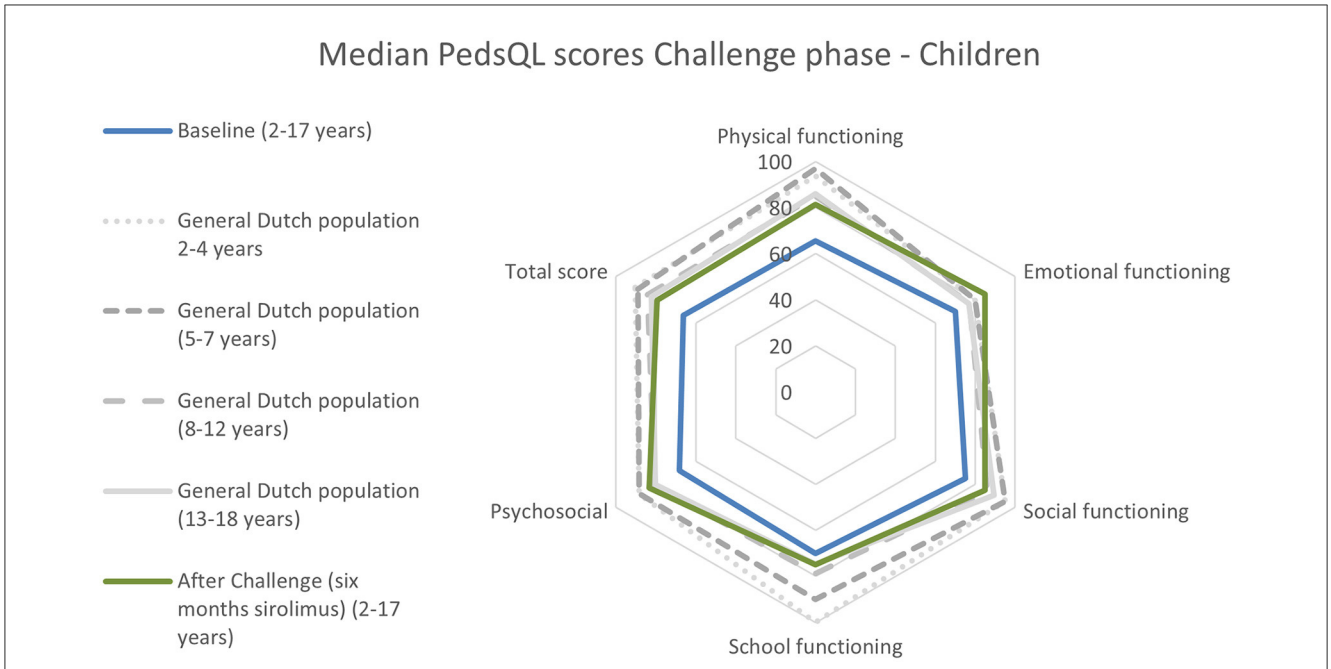


FIGURE 2
PedsQL scores of children before and after the Challenge phase for each domain. Results of the Dutch population aged 5–18 years are represented in gray (26). Baseline $n = 19$ patients with low-flow vascular malformations, and after the Challenge phase, $n = 21$ patients (parent reports for children aged 2–7 years and children's reports for patients aged 8–16 years). Exact data are presented in Supplementary Table 1. For the general Dutch population, parent reports obtained by Schepers et al. (25) are presented for children aged 2–7, while for children aged 8–16 years, the child reports obtained by van Engelen et al. (26) were used.

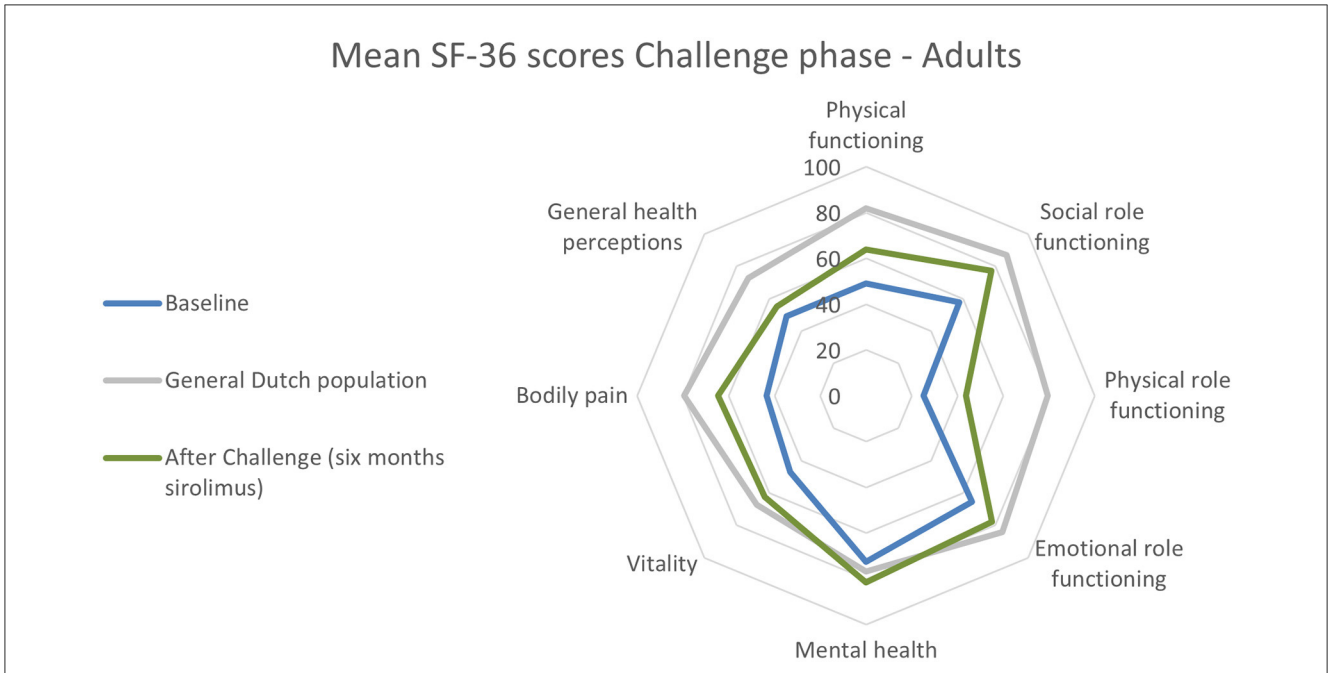


FIGURE 3
Quality-of-life scores for each domain during the Challenge phase in adults using the SF-36 questionnaire. Baseline $n = 31$ patients with low-flow vascular malformations and after Challenge phase $n = 27$. Exact data are presented in Supplementary Table 2. The RAND-36 scores for the general Dutch adult population were obtained from van der Zee et al. (27, 28).

parent reports ($n = 18$) gave a mean score of 64.0 [SD 16.2]. The mean total scale score of the children significantly increased by 9.9 points [SD 12.6] after the Challenge phase to 76.2 [SD 18.1], $P < 0.05$. The parental PedsQL scores showed an increase of 10.9 points [SD 10.7] after the Challenge phase to 74.9 [SD 17.0], $P < 0.05$. The PedsQL scores of each domain at the baseline and the changes

after the sirolimus treatment did not significantly differ between the children and parents.

In adults ($n = 26$), the mean MCS score at the baseline was 48.9 [SD 9.3], which significantly increased to 52.5 [SD 8.7] after the Challenge phase, with a mean increase of 3.6 points ([SD 8.3], $P < 0.005$). The mean PCS score at the baseline was 32.8 [SD 10.7], which significantly increased to 39.0 [SD 13.1] after the Challenge phase, with a mean increase of 6.2 ([SD 9.5], $P < 0.005$).

Figures 2, 3 present the results of the changes in HRQoL during the Challenge phase. The detailed data are provided in Supplementary Table 3.

Magnitude of change

The effect sizes of the sirolimus treatment on the PedsQL and SF-36 scores between the baseline and the end of the Challenge phase are shown in Figures 4, 5. In the PedsQL children's reports, a clinically relevant change was seen in all domains except for "School functioning," in which the effect size was small (0.20–0.49). A clinically relevant moderate effect size (effect size 0.50–0.79) was seen in the domains "Physical functioning," "Social functioning," and "Total scores." High (>0.80) effect sizes were observed in the domains of "Emotional functioning" and "Psychosocial functioning." Moderate-to-high effect sizes were observed in the PedsQL parent reports in almost all domains, except "Emotional functioning." It is noteworthy that the effect size of the "Total scale score" was 1.02. When the children's and parental reports were considered together, a clinically relevant effect size was observed in every domain.

Moderate clinically relevant effect sizes were seen in adults in the SF-36 domains "Physical functioning," "Social functioning," "Mental health," "Energy levels/vitality," "Pain," and "Physical Component Summary." In the other domains, a small effect size was seen.

Discussion

The present study indicates that Dutch patients with vascular malformation experience an impaired HRQoL compared with the general population. In addition to the heterogenic group of slow-flow vascular malformations, patients have similar symptoms leading to a reduced HRQoL. All domains were significantly impaired in adults, except for "Mental health." This result showed a worse HRQoL than that reported by Breugem et al. in patients with vascular malformations of the lower extremity, who reported that their HRQoL was not greatly impaired relative to the general Dutch population (10). Breugem et al. found impaired "Vitality" and higher levels of "Pain" in these patients; however, no differences were seen in the other dimensions of SF-36. By contrast, a systematic review of 11 studies showed that the bodily pain and mental health scores of patients with congenital vascular malformations were significantly worse than the general population of the USA, as determined using the SF-36 (38). This was also seen in a Dutch prospective cross-sectional study of 133 patients who completed the HRQoL surveys using Patient-Reported Outcome Measurement Information System (PROMIS) scales, performed by Stor et al. (39) who showed that the presence of pain negatively impacted the patients' HRQoL. In addition, the low HRQoL in our phase IIB study may be explained by the fact that the included patients had no (remaining effective) treatment options.

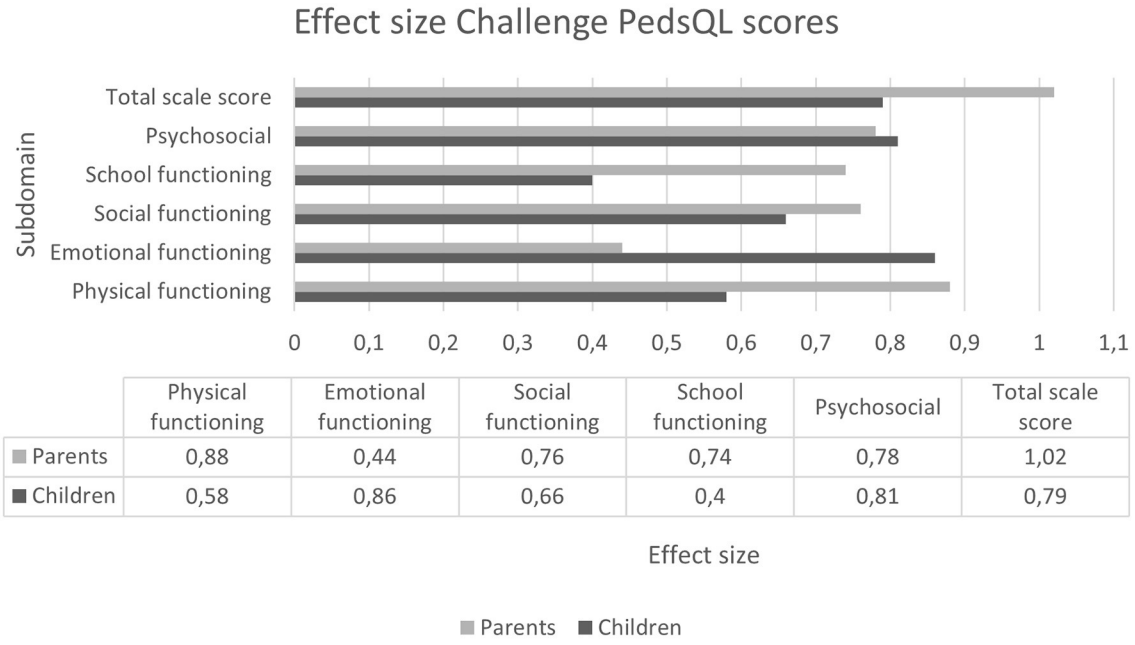
Because of the low number of patients per age category and the corresponding HRQoL scores of the general Dutch population, the significance of the difference in treatment effect on the HRQoL between ages was not calculated. Future research should therefore involve a larger cohort of patients to determine the changes and effect sizes in each domain for each patient age category. Additionally, it would be interesting to investigate the HRQoL effect size of different treatments, such as surgery, sclerotherapy, or (other) systemic treatments, and to compare the differences in magnitude and the meaningfulness of the changes between these treatments. In our recently published results of the clinical response to sirolimus, we showed that there was no difference in the general response to sirolimus for each vascular malformation type (24).

In the present study, we showed that the HRQoL significantly improved after the sirolimus treatment (total scale score improvement >4.4 in the self-reported PedsQL, >4.5 in the parent-reported PedsQL, >3.5 in the adult MCS score of the SF-36, and >4.1 in the PCS score of the SF-36) in 65.9% of the patients. This is comparable with the effects reported in other studies, including patients with vascular anomalies (20, 21). The SF-36 domains "Physical functioning," "Social functioning," "Role limitations—physical problems," "Mental health," "Energy levels/vitality," and "Pain" significantly improved after 6 months of treatment with low target levels of sirolimus. Pang et al. also used the SF-36 to identify changes in each domain after 6 months of treatment with sirolimus (40). Six adult patients with low-flow head and neck vascular malformations were included and treated with sirolimus using high target levels (5–15 ng/mL). In contrast to our results, Pang et al. found no statistically significant changes in the SF-36 domains after the sirolimus treatment. The low target levels of sirolimus in our cohort (4–10 ng/mL) might play a role in this; the low occurrence of toxicities alongside the maintenance of the effectiveness of sirolimus might have resulted in a more substantially improved HRQoL (24), although more research is needed to examine this hypothesis.

A limitation of the study is that the design used in the phase IIB clinical trial did not include a placebo group. The optimal design would be a randomized placebo clinical trial (RCT); however, to prove the efficacy of sirolimus in their HRQoL, the concept of Challenge, Dechallenge, and Rechallenge (CDR design) can be used. This CDR design, in which the patients are under their own control, is frequently used for rare diseases for N-of-1 clinical trials to assess efficacy (24, 41).

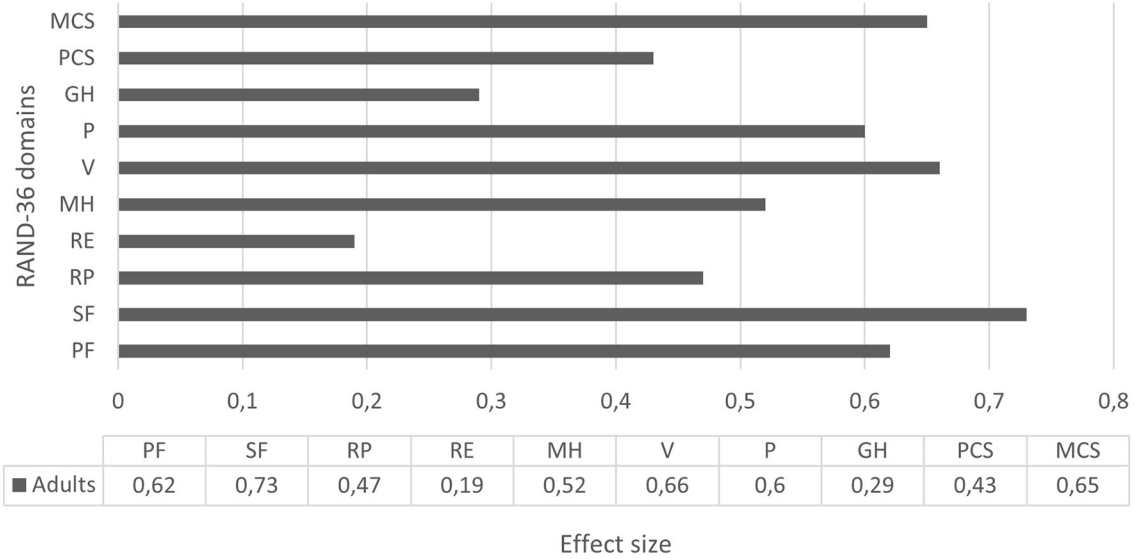
Other studies investigated the change in HRQoL after treatment; however, they did not analyze or show the number of patients who experienced changes in their HRQoL and/or their specific changes in each domain after treatment in both children and adults (18, 20, 21, 39, 42).

Lokhorst et al. developed recently a new questionnaire, the OVAMA questionnaire, which could be additionally used in future research (43). This is a disease-specific questionnaire, which is not suitable for comparing the HRQoL scores with the general (Dutch) population and/or other (chronic) diseases. The effect of sirolimus was not investigated in the study by Lokhorst et al., which might have influenced the clinical improvements that patients showed and the ability to pick up improvements using the



	Physical functioning	Emotional functioning	Social functioning	School functioning	Psychosocial	Total scale score
Parents	0,88	0,44	0,76	0,74	0,78	1,02
Children	0,58	0,86	0,66	0,4	0,81	0,79

FIGURE 4
Effect sizes on each domain of the PedsQL as reported by each child ($n = 16$) and parent ($n = 18$). The effect sizes were categorized as small (0.20–0.49), moderate (0.50–0.79), or high (>0.80). An effect size of more than 0.5 is considered clinically relevant.



	PF	SF	RP	RE	MH	V	P	GH	PCS	MCS
Adults	0,62	0,73	0,47	0,19	0,52	0,66	0,6	0,29	0,43	0,65

FIGURE 5
Effect sizes for the SF-36 domains at the end of the Challenge phase in 26 adult patients. The effect sizes were categorized as small (0.20–0.49), moderate (0.50–0.79), or high (>0.80). An effect size of more than 0.5 is considered clinically relevant. PF, physical functioning; SF, social functioning; RP, role limitations—physical problems; RE, role limitations—emotional problems; MH, mental health; V, energy levels/vitality; P, pain; GH, general health perception; PCS, physical component summary; and MCS, mental component summary.

SF-36. Additionally, the SF-36 can be used to calculate a utility score, which is necessary for a cost-effective analysis. For these reasons, the additional use of generic questionnaires such as SF-36 remains necessary.

Conclusion

This study shows that adult and pediatric patients with vascular malformation experience a decreased HRQoL compared with the

general Dutch population. Six months of treatment with low target levels of sirolimus significantly improved the HRQoL in adults, while in children, a clear tendency to improve was also observed. This study is one of the first studies to investigate the magnitude of change in the HRQoL resulting from sirolimus treatment in patients with vascular malformations. Sirolimus led to a moderate-to-high clinically relevant change in multiple domains in the adult population. In conclusion, the health-related quality of life of children and adults with vascular malformations improved after treatment with low target levels of sirolimus, leading to a more normalized HRQoL.

Author's note

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics statement

The studies involving human participants were reviewed and approved by Central Committee on Research Involving Human Subjects (CCMO). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

VH conducted the clinical trial, drafted the manuscript, and analyzed and interpreted the data. FB and IR conducted the clinical trial and critically reviewed the manuscript. BV, CV, LS, PL, CH, and WK critically reviewed the manuscript. WK analyzed the data. DL is the principal investigator of the clinical trial, critically reviewed the analysis, interpreted the data, and significantly contributed to writing the manuscript. All authors read and approved the final manuscript.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1155476/full#supplementary-material

References

1. Wassef M, Blei F, Adams D, Alomari A, Baselga E, Berenstein A, et al. Vascular anomalies classification: recommendations from the international society for the study of vascular anomalies. *Pediatrics.* (2015) 136:e203–14. doi: 10.1542/peds.2014-3673

2. Garzon MC, Huang JT, Enjolras O, Frieden IJ. Vascular malformations: part I. *J Am Acad Dermatol.* (2007) 56:353–70. doi: 10.1016/j.jaad.2006.05.069

3. Ten Broek RW, Eijkelenboom A, van der Vleuten CJM, Kamping EJ, Kets M, Verhoeven BH, et al. Comprehensive molecular and clinicopathological analysis of vascular malformations: a study of 319 cases. *Genes Chromosomes Cancer.* (2019) 58:541–50. doi: 10.1002/gcc.22739

4. Keppler-Noreuil KM, Rios JJ, Parker VE, Semple RK, Lindhurst MJ, Sapp JC, et al. PIK3CA-related overgrowth spectrum (PROS): diagnostic and testing eligibility criteria, differential diagnosis, and evaluation. *Am J Med Genet A.* (2015) 167A:287–95. doi: 10.1002/ajmg.a.36836

5. Freed D, Stevens EL, Pevsner J. Somatic mosaicism in the human genome. *Genes.* (2014) 5:1064–94. doi: 10.3390/genes5041064

6. Boscolo E, Limaye N, Huang L, Kang KT, Soblet J, Uebelhoer M, et al. Rapamycin improves TIE2-mutated venous malformation in murine model and human subjects. *J Clin Invest.* (2015) 125:3491–504. doi: 10.1172/JCI76004

7. Nathan N, Keppler-Noreuil KM, Biesecker LG, Moss J, Darling TN. Mosaic disorders of the PI3K/PTEN/AKT/TSC/mTORC1 signalling pathway. *Dermatol Clin.* (2017) 35:51–60. doi: 10.1016/j.det.2016.07.001

8. Nguyen LS, Vautier M, Allenbach Y, Zahr N, Benveniste O, Funck-Brentano C, et al. Sirolimus and mTOR inhibitors: a review of side effects and specific management in solid organ transplantation. *Drug Saf.* (2019) 42:813–25. doi: 10.1007/s40264-019-00810-9

9. Fahrni JO, Cho EY, Engelberger RP, Baumgartner I, von Kanel R. Quality of life in patients with congenital vascular malformations. *J Vasc Surg Venous Lymphat Disord.* (2014) 2:46–51. doi: 10.1016/j.jvsv.2013.09.001

10. Breugem CC, Merkus MP, Smitt JH, Legemate DA, van der Horst CM. Quality of life in patients with vascular malformations of the lower extremity. *Br J Plast Surg.* (2004) 57:754–63. doi: 10.1016/j.bjps.2004.05.006

11. van der Ploeg HM, van der Ploeg MN, van der Ploeg-Stapert JD. Psychological aspects of the Klippel-Trenaunay syndrome. *J Psychosom Res.* (1995) 39:183–91.

12. Cox JA, Bartlett E, Lee EI. Vascular malformations: a review. *Semin Plast Surg.* (2014) 28:58–63. doi: 10.1055/s-0034-1376263

13. Akita S, Houbara S, Hirano A. Management of vascular malformations. *Plast Reconstr Surg Glob Open.* (2014) 2:e128. doi: 10.1097/GOX.0000000000000079

14. Cahill AM, Nijs EL. Pediatric vascular malformations: pathophysiology, diagnosis, and the role of interventional radiology. *Cardiovasc Intervent Radiol.* (2011) 34:691–704. doi: 10.1007/s00270-011-0123-0

15. Boon LM, Vanwijck R. Medical and surgical treatment of venous malformations. *Ann Chir Plast Esthet.* (2006) 51:403–11. doi: 10.1016/j.anplas.2006.07.023

16. Mulligan PR, Prajapati HJ, Martin LG, Patel TH. Vascular anomalies: classification, imaging characteristics and implications for interventional radiology treatment approaches. *Br J Radiol.* (2014) 87:20130392. doi: 10.1259/bjr.20130392

17. Hammill AM, Wentzel M, Gupta A, Nelson S, Lucky A, Elluru R, et al. Sirolimus for the treatment of complicated vascular anomalies in children. *Pediatr Blood Cancer.* (2011) 57:1018–24. doi: 10.1002/pbc.23124

18. Adams DM, Trenor CC, Hammill AM, Vinks AA, Patel MN, Chaudry G, et al. Efficacy and safety of sirolimus in the treatment of complicated vascular anomalies. *Pediatrics.* (2016) 137:e20153257. doi: 10.1542/peds.2015-3257

19. Lackner H, Karastaneva A, Schwinger W, Benesch M, Sovinz P, Seidel M, et al. Sirolimus for the treatment of children with various complicated vascular anomalies. *Eur J Pediatr.* (2015) 174:1579–84. doi: 10.1007/s00431-015-2572-y

20. Hammer J, Seront E, Duez S, Dupont S, Van Damme A, Schmitz S, et al. Sirolimus is efficacious in treatment for extensive and/or complex slow-flow vascular malformations: a monocentric prospective phase II study. *Orphanet J Rare Dis.* (2018) 13:191. doi: 10.1186/s13023-018-0934-z

21. Ozeki M, Nozawa A, Yasue S, Endo S, Asada R, Hashimoto H, et al. The impact of sirolimus therapy on lesion size, clinical symptoms, and quality of life of patients with lymphatic anomalies. *Orphanet J Rare Dis.* (2019) 14:141. doi: 10.1186/s13023-019-1118-1

22. Maruani A, Tavernier E, Boccara O, Mazereeuw-Hautier J, Leducq S, Bessis D, et al. Sirolimus (rapamycin) for slow-flow malformations in children: the observational-phase randomized clinical PERFORMUS trial. *JAMA Dermatol.* (2021) 157:1289–98. doi: 10.1001/jamadermatol.2021.3459

23. Harbers VEM, Rongen G, van der Vleuten CJM, Verhoeven BH, de Laat PCJ, van der Horst C, et al. Patients with congenital low-flow vascular malformation treated with low dose sirolimus. *Adv Ther.* (2021) 38:3465–82. doi: 10.1007/s12325-021-01758-y

24. Harbers VEM, Zwerink L, Rongen GA, Klein WM, van der Vleuten CJM, van Rijnsoever IMP, et al. Clinical differences in sirolimus treatment with low target levels between children and adults with vascular malformations: a nationwide trial. *Clin Transl Sci.* (2023) 12:13488. doi: 10.1111/cts.13488

25. Schepers SA, van Oers HA, Maurice-Stam H, Huisman J, Verhaak CM, Grootenhuis MA, et al. Health related quality of life in Dutch infants, toddlers, and young children. *Health Qual Life Outcomes.* (2017) 15:81. doi: 10.1186/s12955-017-0654-4

26. Engelen V, Haentjens MM, Detmar SB, Koopman HM, Grootenhuis MA. Health related quality of life of Dutch children: psychometric properties of the PedsQL in the Netherlands. *BMC Pediatr.* (2009) 9:68. doi: 10.1186/1471-2431-9-68

27. VanderZee KI, Sanderman R, Heyink JW, de Haes H. Psychometric qualities of the RAND 36-Item Health Survey 10: a multidimensional measure of general health status. *Int J Behav Med.* (1996) 3:104–22.

28. Zee KI. Het meten van de algemene gezondheidstoestand met de RAND-36, een handleiding. In: *Tweede Herziene Druk*. Groningen: UMCG/Rijksuniversiteit Groningen, Research Institute SHARE (2012).

29. Varni JW, Seid M, Rode CA. The PedsQL: measurement model for the pediatric quality of life inventory. *Med Care.* (1999) 37:126–39.

30. Varni JW. *The PedsQL™ Scoring Algorithm, Scoring the Pediatric Quality of Life Inventory™* Lyon: Mapi Research Trust (2015). Available online at: https://www.pedsql.org/contact.html

31. RAND Health Care. *36-Item Short Form Survey (SF-36), Version 1.0 Guidelines* Groningen: Noordelijk Centrum voor Gezondheidsvraagstukken (1993).

32. Frendl DM, Ware JE. Patient-reported functional health and well-being outcomes with drug therapy: a systematic review of randomized trials using the SF-36 health survey. *Med Care.* (2014) 52:439–45. doi: 10.1097/MLR.0000000000010311

33. Guyatt G, Walter S, Norman G. Measuring change over time: assessing the usefulness of evaluative instruments. *J Chronic Dis.* (1987) 40:171–8.

34. Morris C, Doll H, Davies N, Wainwright A, Theologis T, Willett K, et al. The Oxford ankle foot questionnaire for children: responsiveness and longitudinal validity. *Qual Life Res.* (2009) 18:1367–76. doi: 10.1007/s11136-009-9550-7

35. Gorelick MH, Scribano PV, Stevens MW, Schultz TR. Construct validity and responsiveness of the Child Health Questionnaire in children with acute asthma. *Ann Allerg Asthma Im.* (2003) 90:622–8. doi: 10.1016/S1081-1206(10)61866-2

36. Kazis LE, Anderson JJ, Meenan RF. Effect sizes for interpreting changes in health status. *Med Care.* (1989) 27:S178–89.

37. Lenhard WLA. Computation of effect sizes. *Psychometrica.* (2016) 4:15. doi: 10.13140/RG.2.2.17823.92329

38. Nguyen HL, Bonadurer GF, Tollefson MM. Vascular malformations and health-related quality of life: a systematic review and meta-analysis. *JAMA Dermatol.* (2018) 154:661–9. doi: 10.1001/jamadermatol.2018.0002

39. Stor MLE, Lokhorst MM, Horbach SER, Young-Afat DA, Kappen TM, van Hout NM, et al. Clinical characteristics associated with pain in patients with peripheral vascular malformations. *J Vasc Surg.* (2021) 75:1054–62. doi: 10.1016/j.jvs.2021.08.101

40. Pang C, Evans N, Jethwa P, Papadopoulou A, Khalifa M, Tsui J, et al. Single center experience of sirolimus therapy in head and neck low-flow vascular malformations. *Vasc Endovasc Surg.* (2021) 2021:15385744211010378. doi: 10.1016/j.ejvs.2021.10.020

41. Wang Y, Schork NJ. Power and design issues in crossover-based *N*-Of-1 clinical trials with fixed data collection periods. *Healthcare.* (2019) 7:84. doi: 10.3390/healthcare7030084

42. Lokhorst MM, Horbach SER, Waner M, van der Vleuten CJM, Mokkink LB. Responsiveness of quality-of-life measures in patients with peripheral vascular malformations: the OVAMA project. *Br J Dermatol.* (2020) 182:1395–403. doi: 10.1111/bjd.18619

43. Lokhorst MM, Horbach SER, Young-Afat DA, Stor MLE, Haverman L, Spuls PI, et al. Development of a condition-specific patient-reported outcome measure for measuring symptoms and appearance in vascular malformations: the OVAMA questionnaire. *Br J Dermatol.* (2021) 185:797–803. doi: 10.1111/bjd.20429

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