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Guest Editors' Editorial

Haemostatic disorders in children pose a unique and ever-evolving clinical challenge. Unlike in adults, paediatric coagulation is shaped by developmental physiology and age-dependent laboratory parameters. As a result, diagnostic and therapeutic strategies should not simply be adopted from adult practice. Moreover, the awareness of paediatric thrombosis has risen and thrombotic complications, once considered rare in children, are increasingly recognized as significant causes of morbidity and, in severe cases, even mortality.

Over the past decade, the field has undergone remarkable transformation. In inherited bleeding disorders, advances in factor replacement therapies and the advent of non-factor therapies such as emicizumab have redefined prophylactic strategies and improved quality of life for many patients. At the same time, therapeutic options for thrombosis have also expanded. The introduction of direct oral anticoagulants into paediatric care represents a paradigm shift, offering practical alternatives to traditional anticoagulation.

This special issue aims to provide a comprehensive overview of current diagnostic strategies, emerging therapies, and unresolved challenges in paediatric thrombosis and haemostasis. We hope it will serve as a practical resource for paediatricians and general practitioners, supporting them in recognizing and evaluating bleeding and thrombotic disorders in children and in understanding modern therapeutic options. Increased awareness and knowledge in everyday clinical practice are key to improving patient care.

Phu Quoc Le and Veerle Labarque

How to Recognize a Child with a Bleeding Disorder?

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Abstract
Children often exhibit bleeding symptoms as a result of their active lifestyle, or due to trauma or surgery. In growing children, mild bleeding symptoms are frequently observed. However, this does not necessarily indicate an underlying bleeding problem. As a paediatrician, it can be challenging to determine whether these symptoms are normal or indicative of an underlying bleeding disorder. This article provides a comprehensive overview of how to evaluate bleeding symptoms in children. It covers clinical presentations, diagnostic tools and laboratory assessments, and aims to help paediatricians identify children who may have a bleeding disorder and determine which patients require referral to a paediatric haematologist.

Physiology of haemostasis and coagulation

The process of blood clotting is initiated in response to injury to a blood vessel, with the objective of minimising blood loss (Figure 1). In the event of a wound, vasoconstriction will predominate, with platelets adhering to the exposed subendothelium due to von Willebrand factor (VWF). This process will result in the aggregation of additional platelets and the subsequent formation of a platelet clot. This phenomenon has been referred to as primary haemostasis. Subsequent to this, secondary haemostasis is initiated, which is characterised by a multifaceted interaction between coagulation factors and cofactors. This interaction occurs through the intrinsic and extrinsic coagulation cascade, resulting in the formation of fibrin. The fibrin formed will stabilise the platelet clot into a solid blood clot. In order to prevent excessive clot growth, the inhibitors of the coagulation system are activated and regulate clot formation and fibrinolysis (1-3).

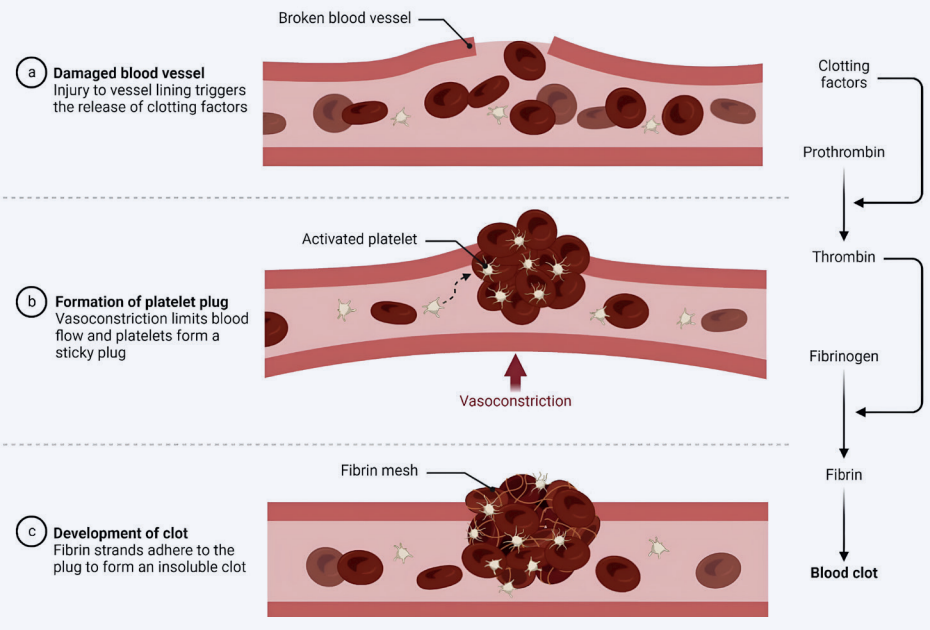
The process of clot formation is dependent on the interplay between three fundamental components: the blood vessel wall, platelets, and clotting factors. Impaired blood clotting resulting in increased bleeding tendency may result from a disorder in any of these components (Table 1) (1-6).

Evaluation of a child with bleeding tendency

In children, typical manifestations of normal bleeding are occasional nosebleeds or small, superficial bruises (mainly on the lower extremities), often resulting from minor trauma. However, pathological bleeding may manifest as spontaneous, recurrent, or excessive bleeding from minor injuries, mucocutaneous bleeding (e.g., frequent gum bleeding or nosebleeds, haematuria, excessive menstrual blood loss), or unexplained bruising in unusual locations (e.g., buttocks, back, thorax) (1-5,7-9).

A survey of 228 otherwise healthy children who had undergone a straightforward tonsillectomy and/or adenoidectomy revealed that a considerable number of them reported bruising easily (24%), bruising at least once a week (36%) and suffering from nosebleeds (39%). The questionnaires were compared with those of 31 patients with bleeding disorders (von Willebrand disease (VWD) and/or platelet function disorders). Respondents with a bleeding disorder more frequently displayed bruising on more than one body part

FIGURE 1: Blood clot formation in broken vessel. BioRender. (2019). https://app.biorender.com/biorender-templates/details/t-5de84c1d615c7a0081eb975a-blood-clot-formation-in-broken-vessel



(38.5%). Furthermore, a higher prevalence of larger bruises (29.6%) and haematomas (21.7%) was observed. These findings were in contrast to the control group without bleeding disorders, where such complications occurred in only 4.9%, 3.5% and 2.7% of cases, respectively(10).

History

A detailed medical history of the child is crucial. This comprises the presence of underlying diseases (e.g., liver, kidney, heart diseases) and the onset, frequency, and severity of bleeding episodes. For instance, the presence of intracranial haemorrhage, umbilical cord haemorrhage, bleeding following heel prick, or intramuscular vaccination in a neonate may be indicative of a bleeding pathology. The number of nosebleeds experienced by the child in the preceding 12 months should be ascertained, as should the duration of each episode and whether medical intervention was necessary, which could include cauterisation or transfusion. Furthermore, clinicians should inquire about bleeding following surgical or dental procedures, minor injuries and vaccinations. Adenoidectomy, tonsillectomy, as well as circumcision, are procedures that may reveal a blood clotting disorder due to the pronounced bleeding tendency during these procedures. For adolescent girls, a menstrual history is imperative, encompassing inquiries into the duration and volume of menstrual bleeding, the frequency of changing pads or tampons, and the presence of substantial blood clots (1-11).

The medical history should include information on the use of any medication that inhibits platelet aggregation (e.g., aspirin or non-steroidal anti-inflammatory drugs (NSAIDs), some anti-epileptics and herbal medication) or inhibits coagulation (e.g., vitamin K antagonists or direct oral anticoagulants (DOACs)) (3,6,8).

An extensive family history of bleeding disorders should also be explored, both for female and male family members. In X-linked diseases, such as haemophilia, the presence of a bleeding tendency in brothers, (grand)fathers or uncles of the mother may serve as an indication of potential genetic susceptibility. In addition, consideration should always be given to the possibility of rare coagulation factor deficiencies in cases of consanguinity (1,5,7).

Physical Examination

A detailed physical examination should assess for signs of mucocutaneous bleeding, joint or muscle haemorrhages, and other abnormal bleeding patterns (1-11).

When inspecting the skin, particular attention should be paid to the presence of petechiae, as well as to the size and distribution of bruising. Haematomas measuring more than 5cm in diameter, or located in an atypical location, such as the trunk or back, may be indicative of underlying pathology. In addition to the skin, examination of the mucous membranes (e.g., the oral cavity and nasal mucosa) should be conducted to look for the presence of petechiae or bleeding foci. Redness, warmth, swelling, restricted movement or asymmetry in a joint or muscle may be signs of a hemarthrosis or muscle haemorrhage. As illustrated in Table 2, the clinical presentation may assist in differentiating between a platelet abnormality and a clotting factor defect.

Moreover, congenital thrombocytopenia and thrombocytopathy can be associated with a wide range of congenital abnormalities (e.g., cardiac, facial, parathyroid, renal, radius and thymus anomalies, psychomotor impairment, eczema, immunodeficiency, albinism,...). The presence of hyperlaxity of the joints and skin may be indicative of a connective tissue disorder.

Furthermore, an abnormal distribution of bruising (e.g., on the cheeks, ears, neck, upper arms, torso, genital region, or in the form of slap marks) or abnormal bleeding (including vaginal bleeding in prepubescent girls) should always be considered as possible indications of non-accidental trauma or child abuse (8,9).

TABLE 1: Bleeding disorders (non-exhaustive) based on the three components of coagulation (1-5, 9).

Platelet disorders	
Thrombocytopenia	Decreased production (e.g., congenital platelet deficiencies, bone marrow failure or infiltration, medication) Increased breakdown (e.g. immune thrombocytopenic purpura (ITP), haemolytic uremic syndrome) Pooling (splenomegaly) Dilution
Thrombopathies	Glanzmann thrombasthenia Bernard-Soulier syndrome Other platelet function defects Storage pool diseases Aspirin, anti-aggregants Chronic kidney insufficiency Cardiopulmonary bypass, etc.
Coagulation factor disorders	
Congenital procoagulant deficiencies	Haemophilia A (factor VIII) and B (factor IX) von Willebrand disease (VWD) rare coagulation factor deficiencies (factor II, V, VII, X, XI, XIII) a-, hypo- or dysfibrinogenemia
Acquired deficiencies	Vitamin K deficiency Liver disease Vitamin K antagonists (± overdose) Direct oral anticoagulants (± overdose) Diffuse intravascular coagulation Sepsis Inhibitors
Hyperfibrinolysis	α2-antiplasmin deficiency Plasminogen activator inhibitor-1 deficiency
Vascular	
Ehlers-Danlos syndrome (type IV), other connective tissue disorders	
Osteogenesis imperfecta	
Trauma	
Vitamin C deficiency	affecting collagen formation

Bleeding Assessment Tools

Standardized bleeding assessment tools have been developed to assist clinicians in a systematic evaluation of bleeding symptoms and the determination of the likelihood of an underlying bleeding disorder. The most widely used and validated bleeding assessment tools in paediatrics include the ISTH-BAT (International Society on Thrombosis and Haemostasis Bleeding Assessment Tool) and the Paediatric Bleeding Questionnaire (PBQ) (11-16).

The ISTH-BAT is a widely utilised instrument for both adults and children that assigns a numerical score based on the presence and severity of bleeding symptoms across multiple domains, namely epistaxis, cutaneous bruising, minor wounds, oral cavity, gastrointestinal tract bleeding, bleeding following surgery or dental procedures, haematuria, menorrhagia, post-partum haemorrhage, central nervous system bleeding, muscle haematomas, hemarthrosis and other bleedings. In children, a score of three or higher is considered abnormal and should prompt further evaluation by a paediatric haematologist. However, it is imperative to acknowledge the limitations of this instrument, as it may underestimate bleeding

in younger children who have not yet encountered the common haemostatic challenges that are typically experienced by older children and adults. Furthermore it may exhibit reduced sensitivity for acquired disorders such as immune thrombocytopenia (ITP) (13).

The PBQ is another tool that has been validated for the purpose of screening for VWD in children. The PBQ is a questionnaire that, beside the abovementioned domains, also assigns points for paediatric-specific symptoms, such as cephalohematoma, umbilical cord bleeding, post-venipuncture bleeding, post-circumcision bleeding and macroscopic haematuria. A score of two or more is considered as a 'positive' bleeding score. The PBQ has a high negative predictive value to exclude VWD in children (14-16).

These tools facilitate the standardisation of the evaluation of bleeding symptoms and can assist in determining which children require further laboratory testing or referral to a specialist. The sensitivity and specificity of these tests for predicting bleeding disorders in children are moderate to high depending of the pretest probability, and their interpretation should be informed by clinical judgment and in combination with laboratory findings (11-16) The American Academy of Paediatrics emphasises that while a detailed bleeding history is essential, family and patient history alone are insufficient to rule out bleeding disorders, and abnormal bleeding scores should prompt further evaluation (8).

Laboratory tests

The general strategy for laboratory elaboration of patients with a suspected bleeding disorder is outlined in Figure 2 and Table 3 (1-9,11).

Indications derived from the patient's or family history and physical examination may assist in the selection of preliminary screening tests for increased bleeding tendency. For instance, in a male patient with bleeding symptoms where haemophilia A is present in the family, a Factor VIII (FVIII) level will be determined as early as the first step.

It is also important to interpret laboratory results within the context of the clinical picture, as mild abnormalities may be observed in healthy children or may be influenced by factors such as medication, stress, infection, inflammation or blood type (where blood type O is associated with lower VWF levels). However, pre-analytical errors can also lead to incorrect test results. Examples include incorrectly or insufficiently filled tubes, inadequate mixing, a long period between collection and analysis, and excessive pinching. Furthermore, it should be noted that reference values of aPTT are higher and all coagulation factors (with the exception of FVIII and VWF) are lower in the initial six months following birth in both pre-term and full-term infants when compared with adult values. Consequently, it is strongly recommended that these children undergo re-evaluations at an older age to ascertain the presence of any abnormal values (7,17).

FIGURE 2: Algorithm for a practical approach of children with bleeding symptoms. aPTT: Activated partial thromboplastin time, PT: Prothrombin time, TT: Thrombine time, F: factor, VWF: von Willebrand Factor, FVIII: factor VIII, PFT: platelet function tests, FXIII: factor XIII, PAI-1: plasminogen activator inhibitor-1. Created in biorender.com

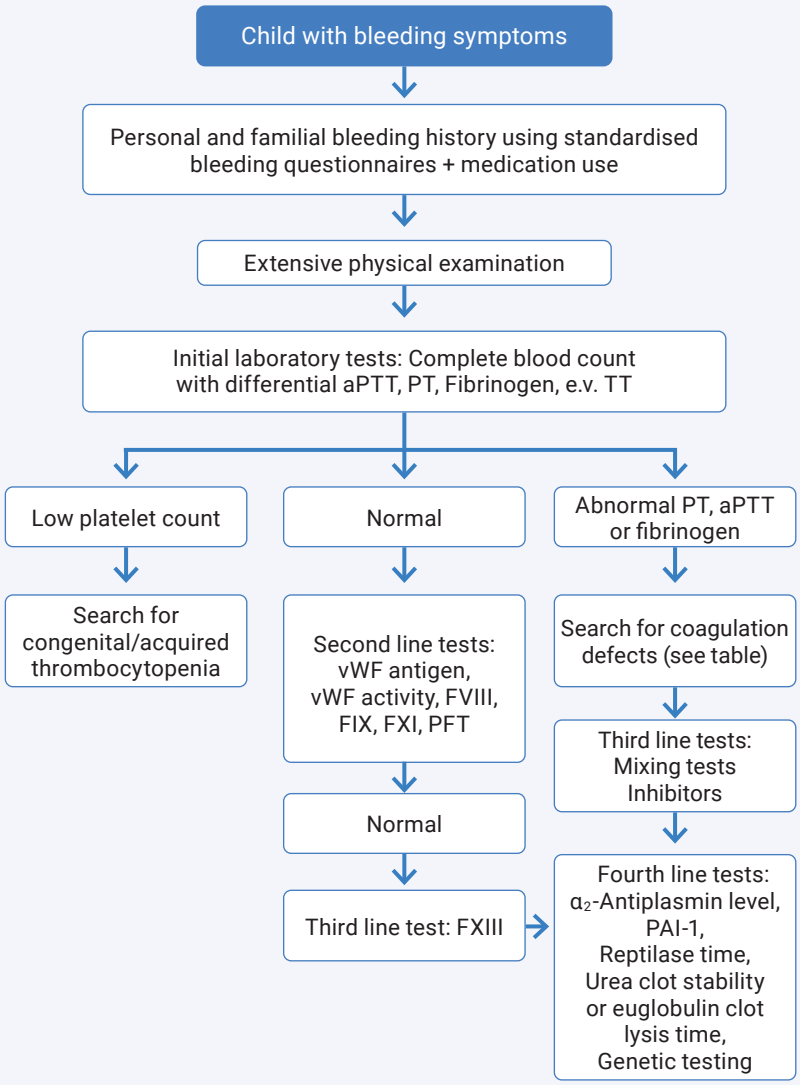


TABLE 2: Differences in presentation of bleeding symptoms in platelet disorders and coagulation factor deficiencies.

	Platelet disorder	Coagulation factor deficiency
Localisation bleeding	Skin Mucosa (epistaxis, gums, vaginal, gastro-intestinal tract)	Deep in soft tissues
Petechiae	Yes	No
Ecchymoses	Small, superficial	Large, deep
Haemarthrosis/muscle haemorrhage	Rare	Frequent
Bleeding after cut or scratch	Yes	No
Bleeding after surgery or trauma	Immediate or delayed (48h), mostly mild	Delayed (48h), mostly extensive

TABLE 3: Perinatal infection Risk (PIR) tool.

aPTT	PT	TT	Fibrinogen	Disorder
Normal	Normal	Normal	Normal	Normal profile, mild factor deficiency (incl. mild VWD), FXIII deficiency, Platelet function disorder, α2-antiplasmin deficiency, PAI-1 deficiency, connective tissue disorder
↑	Normal	Normal	Normal	Deficiency of FVIII, VWF, FIX, FXI, FXII or lupus anticoagulant
Normal	↑	Normal	Normal	Deficiency of FVII, early liver failure or DIC, warfarin therapy, mild vitamin K deficiency
↑	↑	Normal	Normal	Deficiency of FII, FV, FX, vitamin K deficiency, supratherapeutic vitamin K antagonists, combination of factor deficiencies
↑	Normal or ↑	↑	Normal	Heparin (normal reptilase time)
Normal	Normal	↑	↓ (1-1.5g/L)	Hypofibrinogenemia, mild dysfibrinogenemia
↑	↑	↑	↓ (<1g/L)	Dysfibrinogenemia, afibrinogenemia, late DIC, liver failure

Of note, some patients may have a clinically significant bleeding phenotype but normal tests using the approach described here. However, they may have a bleeding disorder. In these patients genetic testing may help to get to the diagnosis (discussed in another paper of this special issue).

Referral to a paediatric haematologist

It is essential that children with suspected or confirmed bleeding disorders are referred to a paediatric haematologist. The urgency of referral depends on the severity of the symptoms. Immediate referral is indicated for children with life-threatening bleeding, such as intracranial, gastrointestinal or retroperitoneal, joint or muscle bleeding. It is also indicated for children with active bleeding and severe thrombocytopenia. A semi- or non-urgent referral should be considered for children with abnormal laboratory results, such as prolonged aPTT and/or PT on multiple occasions, low factor levels, persistent menorrhagia or a family history of bleeding disorders accompanied by suspicious symptoms. Even when child abuse is suspected, a comprehensive medical history, physical examination and laboratory tests are necessary, and a referral to a paediatric haematologist may be required (8,9).

Referral is also indicated for pre-surgical evaluation in all children with an unexplained history of bleeding, a positive bleeding score, and/or abnormal laboratory results, in order to ensure appropriate perioperative management. Also, children with a bleeding phenotype of unknown origin should stay in follow-up in a paediatric haematology centre (7,8).

Summary

Easy bruising or bleeding symptoms are commonly observed in children, and it may be challenging for the paediatrician to distinguish between a normal situation, bleeding disorders or non-accidental traumatism and child abuse. The American Academy of Paediatrics stresses the importance of a meticulous evaluation of bleeding symptoms, a comprehensive medical history and physical examination, and a systematic initial laboratory workup. The initial diagnostic approach should be tailored based on clinical presentation and initial test results, with further specialised testing if necessary. Referral to a paediatric haematologist affiliated with a haemophilia centre is crucial for a definitive diagnosis and adequate management.

Statement

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REFERENCES

1. van Ommen CH, Peters M. The Bleeding Child. Part I: Primary Hemostatic Disorders. Eur J Pediatr. 2012;171(1):1-10. doi:10.1007/s00431-011-1532-4.
2. van Herrewegen F, Meijers JC, Peters M, van Ommen CH. Clinical practice: the bleeding child. Part II: disorders of secondary hemostasis and fibrinolysis. Eur J Pediatr. 2012;171(2):207-14. doi: 10.1007/s00431-011-1571-x.
3. Sarnaik A, Kamat D, Kannikeswaran N. Diagnosis and management of bleeding disorder in a child. Clin Pediatr (Phila). 2010;49(5):422-31. doi: 10.1177/0009922809351090.
4. Revel-Vilk S. Clinical and laboratory assessment of the bleeding pediatric patient. Semin Thromb Hemost. 2011;37(7):756-62. doi: 10.1055/s-0031-1297166.
5. Khair K, Liesner R. Bruising and Bleeding in Infants and Children--a Practical Approach. Br J Haematol. 2006;133(3):221-31. doi:10.1111/j.1365-2141.2006.06016.x.
6. Vora A, Makris M. Personal practice: An approach to investigation of easy bruising. Arch Dis Child. 2001;84(6):488-91. doi: 10.1136/adc.84.6.488. PMID: 11369565; PMCID: PMC1718790.
7. Acharya SS. Rare bleeding disorders in children: identification and primary care management. Pediatrics. 2013;132(5):882-92. doi: 10.1542/peds.2012-3662. Epub 2013 Oct 14. PMID: 24127475.
8. Anderst J, Carpenter SL, Abshire TC, Killough E. Evaluation for Bleeding Disorders in Suspected Child Abuse. Pediatrics. 2022;150(4):e2022059276. doi:10.1542/peds.2022-059276.
9. Carpenter SL, Abshire TC, Killough E, Anderst JD. AAP Section on Hematology/oncology, the American Society of Pediatric Hematology and Oncology, and the AAP Council on Child Abuse and Neglect. Evaluating for Suspected Child Abuse: Conditions That Predispose to Bleeding. Pediatrics. 2022;150(4):e2022059277. doi:10.1542/peds.2022-059277.
10. Nosek-Cenkowska B, Cheang MS, Pizzi NJ, Israels ED, Gerrard JM. Bleeding/bruising symptomatology in children with and without bleeding disorders. Thromb Haemost. 1991;65(3):237-41.
11. Chaigneau M, James PD. Approach to the Patient With Bleeding. Hematol Oncol Clin North Am.. 2021;35(6):1039-49. doi:10.1016/j.hoc.2021.07.001.
12. O'Brien SH, Zia A. Hemostatic and thrombotic disorders in the pediatric patient. Blood. 2022;140(6):533-41. doi: 10.1182/blood.2020006477. PMID: 34724564.
13. Rodeghiero F, Tosetto A, Abshire T, Arnold DM, Coller B, James P, et al. ISTH/SSC joint VWF and Perinatal/Pediatric Hemostasis Subcommittees Working Group. ISTH/SSC bleeding assessment tool: a standardized questionnaire and a proposal for a new bleeding score for inherited bleeding disorders. J Thromb Haemost. 2010;8:2063-65.
14. Mittal N, Naridze R, James P, Shott S, Valentino LA. Utility of a Paediatric Bleeding Questionnaire as a screening tool for von Willebrand disease in apparently healthy children. Haemophilia. 2015;21(6):806-11. doi:10.1111/hae.12689
15. Laoaroon N, Empremsilapa S, Sirachainan N. Pediatric standardized bleeding assessment tool for screening bleeding disorder in school-age children. Hematol Transfus Cell Ther. 2022;44(3):453-5. doi:10.1016/j.htct.2021.11.020.
16. Kalot MA, Husainat N, Tayiem S, El Alayli A, Dimassi AB, Diab O, et al. Bleeding assessment tools in the diagnosis of VWD in adults and children: a systematic review and meta-analysis of test accuracy. Blood Adv. 2021;5(23):5023-5031. doi:10.1182/bloodadvances.2021004368.
17. Monagle P, Barnes C, Ignjatovic V, Furmedge J, Newall F, Chan A, et al. Developmental haemostasis. Impact for clinical haemostasis laboratories. Thromb Haemost. 2006;95(2):362-72. doi: 10.1160/TH05-01-0047. PMID: 16493500.

Von Willebrand Disease

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Abstract
Von Willebrand disease (VWD) is the most common inherited bleeding disorder, caused by quantitative or qualitative defects of von Willebrand factor (VWF). This multimeric glycoprotein plays a central role in haemostasis by binding to extracellular matrix proteins such as collagen and to platelets via the glycoprotein Ib receptor. In addition, VWF acts as a carrier for factor VIII, preventing its premature clearance and degradation. Defective VWF function results in a bleeding tendency, predominantly manifesting as mucocutaneous haemorrhages (epistaxis, easy bruising, gum bleeding, and menorrhagia). The severity of symptoms varies not only between patients but also over time in the same individual, leading to a significant impact on quality of life. Diagnosis relies on the evaluation of bleeding history, family history, and specialized laboratory assays, yet it often remains challenging despite recent advances.

Introduction

Von Willebrand disease (VWD) affects approximately 1% of the general population, though clinically significant bleeding occurs in about 1 in 1000 individuals. Its prevalence is consistent across different races and ethnicities. Unlike haemophilia, VWD is transmitted in an autosomal manner, affecting both men and women. However, due to menstruation, pregnancy, and childbirth, women experience a disproportionate clinical burden (1-4).

The disorder is caused by either quantitative or qualitative deficiencies in VWF and is classified into three main types: type 1, 2 (with subtypes 2A, 2B, 2M, and 2N), and 3. Type 1 is the most frequent, involving partial deficiency; type 3 represents complete absence of VWF; and type 2 includes functional abnormalities. While type 1 predominates, diagnostic thresholds - particularly in cases with borderline VWF levels (30–50 IU/dL, historically termed "low VWF") - remain debated. Diagnosis should integrate clinical phenotype and family history alongside laboratory results (4,5).

Classification

Most cases of VWD are inherited in an autosomal dominant manner, while type 3 and type 2N follow autosomal recessive inheritance. Type 1 accounts for 70–80% of cases, type 2 for about 20%, and type 3 for less than 5% (6,7).

- Type 1: Partial quantitative deficiency of VWF. A subtype, type 1C, involves increased VWF clearance.
- Type 3: Complete absence of VWF, leading to severe bleeding.
- Type 2: Qualitative abnormalities, subdivided as follows:
  - 2A: Defective VWF multimerization.
  - 2B: Increased affinity for GPIb.
  - 2M: Defective binding despite normal multimer structure.
  - 2N: Impaired FVIII binding, mimicking haemophilia A.

Historically, patients with VWF levels between 30–50 IU/dL were categorized as "low VWF". However, many experience clinically significant bleeding, warranting individualized management. According to 2021 guidelines, a diagnosis of type 1 VWD is supported when VWF levels are ≤30 IU/dL, regardless of bleeding history, or ≤50 IU/dL in the presence of abnormal bleeding (5). Table 1 summarizes the different subtypes of VWD and their characteristics.

Clinical Characteristics

VWD typically presents with mucocutaneous bleeding, including recurrent epistaxis, oral cavity bleeding, easy bruising, and menorrhagia. Gastrointestinal bleeding - often linked to angiodysplasia - is a difficult-to-treat complication, especially in elderly patients and those with type 2A or type 3 disease (8-9).

Bleeding may also occur after surgery, trauma, or childbirth. Hemarthroses and deep muscle bleeds are rare, usually confined to type 3 VWD. Spontaneous bleeding is uncommon even in severe deficiency. Women are more symptomatic due to menstrual and obstetric challenges. The phenotype often fluctuates over a patient's lifetime, requiring individualized diagnosis and management (4,10).

Diagnosis

Diagnosis relies on three elements: a personal bleeding history often obtained through the application of a bleeding assessment tools (BATs), specialized laboratory findings consistent with reduced levels and/or dysfunction of VWF, and family history.

Assessment of bleeding phenotype

BATs are increasingly used to quantify bleeding severity. The 2021 guidelines recommend validated BATs for screening, especially in women, before proceeding to laboratory testing. However, some limitations appear when using these tests with young people without any bleeding challenges to overcome (11).

Laboratory Evaluation

Complete blood count, activated partial thromboplastin time (aPTT) and prothrombin time are usually normal, though aPTT may be prolonged in severe cases and platelets may be variably lower in type 2B disease. Initial diagnostic tests include (12-14):

- VWF antigen (VWF:Ag)
- VWF platelet-dependent activity (VWF:Act)
- Factor VIII activity (FVIII:C)

Both quantitative and functional assays are required for accurate classification. The VWF activity-to-antigen ratio is used to differentiate between quantitative vs qualitative deficiency of VWF (VWD type 1 vs type 2), with type 2 characterized by an activity/antigen ratio <0.7. Multiple assessments are often necessary, as VWF levels fluctuate with age, stress, inflammation, blood group (lower in type O), pregnancy, and hormonal factors (15).

Furthermore, VWF multimer analysis helps distinguish the patient's subtype of VWD. In types 1, 2M, and 2N VWD, all sizes of multimers are seen, while preferential loss of high-molecular-weight multimers is seen in type 2A and type 2B. Type 3 VWD is characterized by almost complete absence of VWF multimers (5).

Genetic Testing

Molecular testing can confirm difficult cases, differentiate subtypes, and guide family planning. It is particularly valuable in distinguishing type 2N VWD from mild haemophilia A, type 2B from platelet-type VWD, and congenital from acquired forms (16).

Treatment

Therapeutic strategies are generally safe and effective when guided by specialist care. Management involves antifibrinolytics, desmopressin, and VWF concentrates, tailored to the type and severity of disease (14,16,17).

- Tranexamic acid: Antifibrinolytic used for mild bleeding and minor procedures. Can be administered topically, orally, intravenously, or subcutaneously.

TABLE 1: Characteristics of the subtypes of VWD

VWB subtype	Inheritance	Pathobiology and Lab testing
Type 1	Autosomal dominant but incomplete penetrance	- Partial quantitative VWF deficiency. - Concordant reductions in VWF:Ag and VWF functional assays. - Includes VWF mutations causing rapid clearance
Type 2A	Mostly autosomal dominant	- Decreased VWF-dependent platelet adhesion. - Discordant reduction in VWF functional assays compared to VWF:Ag levels (ratio VWF:Act/VWF:Ag < 0.7). - Reduction in high-molecular weight (HMW) multimers.
Type 2B	Autosomal dominant	- Increased VWF affinity for platelet Gp1b. - Discordant reduction in VWF functional assays compared to VWF:Ag levels (ratio VWF:Act/VWF:Ag < 0.7). - May be associated with loss of HMW multimers ± thrombocytopenia.
Type 2M	Autosomal dominant	- Decreased VWF-dependent platelet adhesion. - Discordant reduction in VWF functional assays compared to VWF:Ag levels (ratio VWF:Act/VWF:Ag < 0.7). - Normal multimers. - Diagnosis by exclusion.
Type 2N	Autosomal recessive	- Decreased VWF binding affinity for FVIII. - Reduced FVIII:C levels (ratio FVIII:C/VWF:Ag < 0.7). - Plasma VWF:Ag often normal or slightly reduced.
Type 3	Autosomal recessive	- Severe quantitative VWF deficiency. - Plasma VWF:Ag < 5 IU/dL.

- Desmopressin (DDAVP): Stimulates release of endogenous VWF and FVIII. Effective in many type 1 and select type 2 cases, but contraindicated in type 2B and ineffective in type 3. However, this can only be used after a challenge test and not before the age of 2-3 years.
- VWF concentrates: Indicated for patients unresponsive to DDAVP or with severe disease (type 2B, type 3). May also be indicated in patients responsive to DDAVP undergoing major surgery.
- Hormonal and iron therapy: Used for menorrhagia and to correct anaemia.

Perioperative Management

Careful risk stratification and perioperative planning are essential. Factors include type of surgery, baseline VWF levels, prior haemostatic response, and bleeding history. Tailored dosing of VWF/FVIII substitution is needed and should be prescribed in consultation with the paediatric haematologist.

Emerging Therapies

Recombinant VWF (rVWF) has shown efficacy in clinical trials, though it was not superior to tranexamic acid for heavy menstrual bleeding. The off-label use of Emicizumab, a bispecific monoclonal antibody that mimics FVIII function, in type 3 VWD has shown promising results and is currently being investigated in a trial to confirm its efficacy and safety (18,19).

Conclusion

VWD is associated with significant morbidity due to recurrent mucocutaneous bleeding. Early recognition and individualized treatment are key to minimizing complications and burden. Advances in understanding VWF biology have refined diagnostic and therapeutic strategies, yet challenges persist in ensuring accurate diagnosis and equitable access to effective treatment. Ongoing research into novel therapies remains essential to address unmet needs in this patient population.

Statement

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REFERENCES

1. Bowman M, Hopman W.M, Rapson D, Lillicrap D and James P. The prevalence of symp- tomatic von Willebrand disease in primary care practice. J Thromb Haemost. 2010;8(1):213-216.
2. Rodeghiero F, Castaman G, Dini E. Epidemiological investigation of the prevalence of von Willebrand's disease. Blood 1987; 69(2): 454–459. pmid:3492222
3. Sadler JE, Mannucci PM, Berntorp E, Bochkov N, Boulyjenkov V, Ginsburg D, et al. Impact, diagnosis and treatment of von Willebrand disease. Thromb Haemost. 2000,84:160–174.
4. Brignardello-Petersen R, El Alayli A, Husainat N, Kalot MA, Shahid S, Aljabirii Y et al. Gynecologic and obstetric management of women with von Willebrand disease: summary of systematic reviews of the literature. Blood Adv 2022; 6(1):228–237.
5. James PD, Connell NT, Ameer B, Di Paola J, Eikenboom J, Giraud N, et al. ASH ISTH NHF WFH 2021 guidelines on the diagnosis of von Willebrand disease. Blood Adv. 2021;5:280–300.
6. Leebeek FW, Eikenboom JC. Von Willebrand's Disease. N Engl J Med. 2016 Nov 24;375(21):2067–2080
7. Sadler JE, Budde U, Eikenboom JCJ, Favaloro EJ, Hill FGH, Holmberg L, et al. Update on the pathophysiology and classification of von Willebrand disease: a report of the Subcommittee on von Willebrand Factor. J Thromb Haemost. 2006;4:2103-2114.
8. de Wee EM, Sanders YV, Mauser-Bunschoten EP, van der Bom JG, Degenaar-Dujardin ME, Eikenboom J, et al. Determinants of bleeding phenotype in adult patients with moderate or severe von Willebrand disease. Thromb Haemost, 2012 ;108, 683–692.
9. Makris M, Federici AB, Mannucci PM, Bolton-Maggs PHB, Yee TT, Abshire T et al. The natural history of occult or angiodysplastic gastrointestinal bleeding in von Willebrand disease. Haemophilia 2015; 21(3):338–342.
10. van Galen KP, Mauser-Bunschoten EP, Leebeek FW. Hemophilic arthropathy in patients with von Willebrand disease. Blood Rev 2012; 26(6):261–266.
11. Kalot MA, Husainat N, Tayiem S, El Alayli A, Dimassi AB, Diab O, et al. Bleeding assessment tools in the diagnosis of VWD in adults and children: a systematic review and meta-analysis of test accuracy. Blood Adv. 2021;5:5023–31.
12. Favaloro EJ, Pasalic L, Curnow J. Laboratory tests used to help diagnose von Willebrand disease: an update. Pathology. 2016;48:303-318.
13. Sharma R, Haberichter SL. New advances in the diagnosis of von Willebrand disease. Hematology Am Soc Hematol Educ Program. 2019;2019:596–600.
14. Laffan MA, Lester W, O'Donnell JS, Will A, Campbell Tait R, Goodeve A, et al. The diagnosis and management of von Willebrand disease: a United Kingdom Haemophilia Centre Doctors Organization guideline approved by the British Committee for Standards in Haematology. Br J Haematol. 2014;167:453-465
15. Abou-Ismail MY, James PD, Flood VH, Connell NT. Beyond the guidelines: how we approach challenging scenarios in the diagnosis and management of von Willebrand disease. J Thromb Haemost. 2023;21(2):204- 214.
16. James P, Leebeek F, Casari C, Lillicrap D. Diagnosis and treatment of von Willebrand disease in 2024 and beyond. Haemophilia. 2024;30(Suppl. 3):103–111.
17. Connell NT, Flood VH, Brignardello-Petersen R, Abdul-Kadir R, Arapshian A, Couper S, et al. ASH ISTH NHF WFH 2021 guidelines on the management of von Willebrand disease. Blood Adv. 2021;5(1):301-325.
18. Ragni MV, Rothenberger SD, Feldman R, Nance D, Leavitt AD, Malec L,et al. Recombinant von Willebrand factor and tranexamic acid for heavy menstrual bleeding in patients with mild and moderate von Willebrand disease in the USA (VWDMin): a phase 3, open-label, randomised, crossover trial. Lancet Haematol. 2023;3026(23):1-12.
19. Shanmukhaiah C, Jijina F, Kannan S, Pai NG, Kulkarni B, Khuba SV, et al. Efficacy of emicizumab in von Willebrand disease (VWD) patients with and without alloantibodies to von Willebrand factor (VWF): report of two cases and review of literature. Haemophilia. 2022;28(2):286-291.

Molecular Testing in the Diagnosis of Inherited Bleeding Disorders – Insights and Advances

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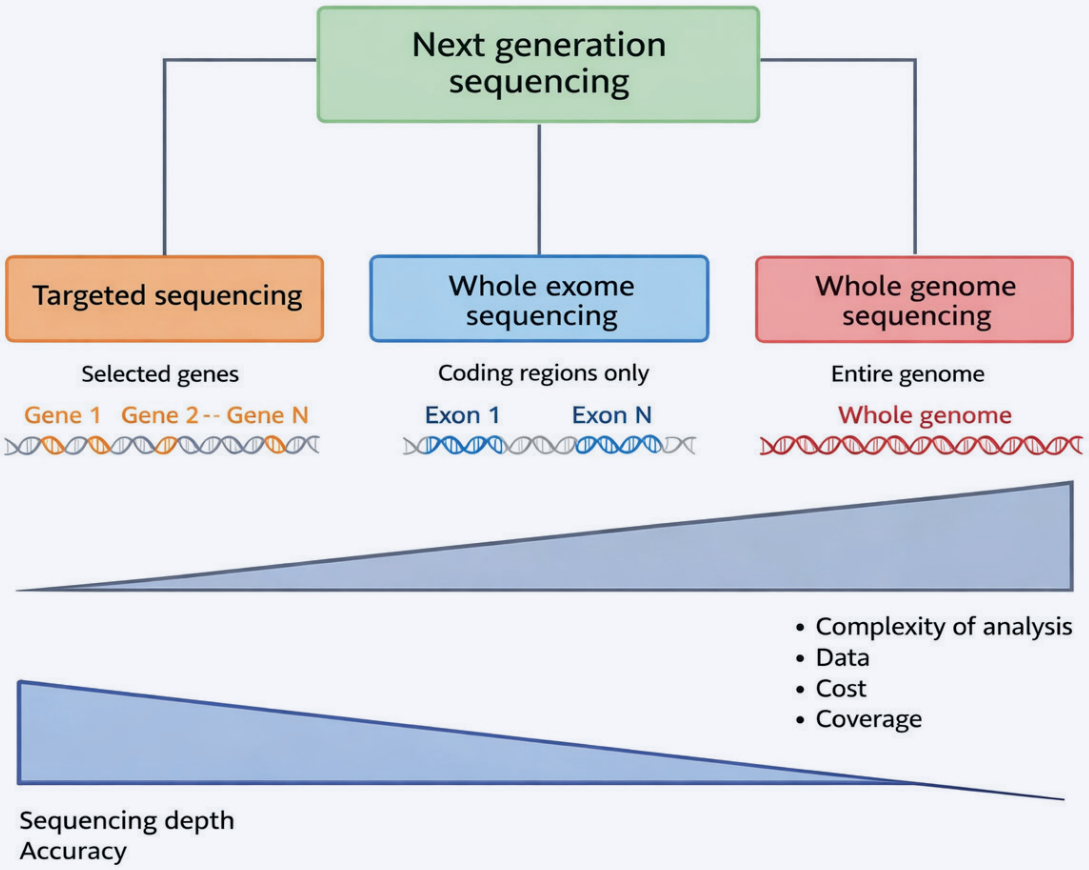
Abstract
Inherited bleeding disorders represent a diverse group of conditions characterized by an increased bleeding tendency that is most often caused by defects in platelets or coagulation factors. The clinical and genetic heterogeneity of these disorders entails significant diagnostic challenges, as traditional approaches based on clinical evaluation, family history and specialized laboratory assays often lack both sensitivity and specificity, especially for rare or atypical presentations. Recent advances in molecular genetics, particularly high throughput sequencing (HTS), have transformed the diagnostic landscape of inherited bleeding disorders. HTS enables the simultaneous analysis of multiple genes, facilitating the identification of causative DNA variants in an increasing number of patients. Targeted gene panels, whole exome sequencing, and whole genome sequencing each offer unique advantages in terms of coverage, depth, and the ability to detect a broad spectrum of genetic alterations. The implementation of multigene panels has led to a significantly increased diagnostic yield and reduced diagnostic delays. Despite these advances, important challenges remain, particularly regarding the interpretation of variants of uncertain significance, detection of complex rearrangements, and the management of incidental findings. In this review, we describe recent insights and advances in the application of HTS for the diagnosis of inherited bleeding disorders and discuss the implications for clinical practice.

Introduction

Inherited bleeding disorders represent a highly heterogeneous group of conditions, characterized by a lifelong increased bleeding diathesis. These disorders are broadly categorized into inherited platelet disorders (IPDs) and inherited coagulation disorders. IPDs include quantitative defects (thrombocytopenia) and qualitative defects known as thrombocytopathies (impaired platelet function). Inherited coagulation disorders involve deficiencies or dysfunction of specific clotting factors, with von Willebrand disease (VWD) and haemophilia A being the most common inherited coagulation disorders (1,2). Traditionally, the diagnosis of inherited bleeding disorders has relied on a combination of clinical evaluation, family history, and specialized laboratory investigations, with functional assays - such as platelet function tests and coagulation factor activity measurements - serving as the cornerstone of the initial diagnostic process (3). Despite their central role, these functional assays also present notable limitations, and therefore the diagnostic journey can be complicated. Clinical symptoms often overlap among different disorders, and functional assays may be unable to distinguish between them. Additionally, functional assays may lack sensitivity and specificity, particularly in mild or atypical forms of inherited conditions. They can fail to distinguish between inherited and acquired causes of bleeding, and results can be influenced by preanalytical factors such as the time between sample collection and processing, as well as external factors like stress, fever, and pregnancy. For example, acute stress can significantly increase plasma levels of von Willebrand factor (VWF) and factor VIII (FVIII) due to endothelial cell release triggered by catecholamine surges. Similarly, during pregnancy, VWF and FVIII levels can rise threefold as part of the body's physiological preparation for delivery (4). These elevations may transiently normalize abnormal values, thereby masking an underlying inherited bleeding disorder and producing false-negative results. Currently, there are no functional assays available to directly assess the integrity or function of the vascular wall; as a result, endothelial dysfunction cannot be evaluated using standard laboratory testing. In clinical practice, some patients present with a clear bleeding phenotype despite entirely normal results on comprehensive functional haemostatic assays - referred to as having a bleeding disorder of unknown cause (BDUC) (5). This underscores a key limitation of functional testing to detect bleeding defects: while valuable, our current assays may not detect all defects, particularly when the underlying pathophysiology lies outside the scope of what these tests can measure.

All these factors can result in diagnostic delays and uncertainty. In recent years, the advent of molecular diagnostic testing has revolutionized the approach to inherited bleeding disorders by enhancing diagnostic capabilities and improving the accuracy of identifying underlying genetic causes (6,7). This review explores the evolving role of molecular testing in the diagnosis of inherited bleeding disorders. We describe the advances in HTS technologies, the application of gene panels, and the implementation of molecular testing into clinical and research settings, with the overarching goal of improving diagnostic precision and patient outcomes.

FIGURE 1: Overview of next-generation sequencing technologies: targeted panel sequencing, whole exome sequencing (WES), and whole genome sequencing (WGS). The figure illustrates differences in genomic coverage, sequencing depth, cost, and suitability for detecting various types of genetic variants. Targeted panels focus on selected genes and offer high depth for specific clinical questions. WES captures all coding regions (exons), providing a balance between coverage and cost. WGS covers the entire genome and is most comprehensive, enabling detection of structural variants and non-coding mutations. CNV: copy number variation



Advancements in sequencing methods

Prior to the advent of high throughput sequencing (HTS), Sanger sequencing, a method that allows for the targeted analysis of a specific gene or gene region, was used to confirm a functional diagnosis. Sanger sequencing remains the gold standard for variant validation and is still used for the analysis of single genes or for familial segregation studies (8). By genetically validating a functional diagnosis, it is possible to offer appropriate genetic counselling, establish a prenatal diagnosis, or identify carriers (e.g., women who are carriers of pathogenic variants in the coagulation factor 8 (F8) or 9 (F9) but who have normal factor levels). However, the labour-intensive nature and substantial cost, coupled with its low throughput, make it impractical to simultaneously investigate multiple candidate genes, which is often required due to the genetic heterogeneity of bleeding disorders. HTS platforms, which can sequence millions of DNA fragments in parallel, facilitate the simultaneous analysis of multiple genes, exomes, or even the entire genome, offering a faster and more cost-effective alternative to Sanger sequencing. HTS can be used both to provide molecular confirmation of an established clinical or functional diagnosis, and to identify a genetic diagnosis in cases where a clear functional diagnosis has not yet been established. HTS comprises several techniques, each with its own applications, which we will provide a brief overview of here (Figure 1) (9).

Targeted gene panels are designed to capture and sequence a predefined set of genes that are known to be associated with specific conditions (10). The use of targeted panels offers several advantages, including high sequencing depth, rapid turnaround, and cost-effectiveness compared to broader approaches. This approach is particularly valuable in the diagnosis of disorders where pathogenic variants may occur in different genes, such as for congenital thrombocytopenia. Panels can in theory also detect copy number variations (CNVs), including larger deletions and duplications, within the targeted gene set. However, the ability of panels to detect CNVs is dependent on the design of the panel and bioinformatics pipelines (11). Whole exome sequencing (WES) analyses the entire exome, which is the protein-coding portion of the genome. The exome accounts for about 1-2% of the total genome and encompasses approximately 23 000 genes (12). WES is especially useful where targeted panel testing fails to identify a causative variant, as it allows for the discovery of novel disease genes or the confirmation of atypical presentations of known disorders (13). However, it also presents challenges, including the interpretation of incidental findings unrelated to the phenotype and the increased burden of data analysis. Whole genome sequencing (WGS) provides the most extensive analysis, where the entire genome (coding and non-coding regions) is examined. The complexity and cost of WGS remain significant, and its use is currently limited to research settings or particularly challenging diagnostic cases.

Each of these techniques has its own advantages and disadvantages (14). Firstly, the major difference between the three methods is the volume of data that are generated. WGS produces substantially more data than targeted sequencing,

and the complexity of analysis is inherently linked to this data volume, making WGS analysis far more complex compared to targeted sequencing. Secondly, as the volume of data analysed increases, so do the associated costs. Lastly, targeted sequencing demonstrates the highest accuracy, while WGS has the lowest accuracy, with a higher likelihood of errors. Consequently, findings from WES or WGS are routinely confirmed using Sanger sequencing. Although traditional short-read next generation sequencing (NGS) was previously limited in detecting certain CNVs, current WES and WGS methods, equipped with advanced bioinformatics pipelines, are now sufficiently sensitive to detect most deletions and duplications. Despite advances in short-read NGS, challenges remain in identifying complex structural variants, such as large inversions and repeat expansions. These limitations can lead to certain complex insertions being missed when relying solely on standard targeted HTS panels (15). To overcome these limitations, complementary techniques such as multiplex ligation-dependent probe amplification (MLPA) and array comparative genomic hybridization (aCGH) arrays are widely employed. MLPA offers targeted, sensitive detection of exon-level deletions and duplications in genes like VWF, F8, and F9 (16,17). These confirmatory methods remain essential for the validation of certain complex genomic alterations.

A major recent advancement in sequencing technology is the development of long-read sequencing methods (18). Most current high-throughput sequencing in clinical diagnostics rely on short-read sequencing, where DNA is fragmented into short stretches of approximately 100–300 base pairs. While highly effective for detecting single nucleotide variants and small indels, short-read methods are inherently limited in their ability to map large structural variants, repetitive elements, and complex rearrangements. Long-read sequencing platforms can sequence much longer DNA fragments (thousands to tens of thousands of base pairs), greatly increasing the ability to resolve complex structural variants, large inversions, repeat expansions, and other changes in challenging genomic regions. Several recent studies illustrate the growing clinical utility of long-read sequencing in inherited bleeding disorders. For example, Chatron et al. used nanopore long-read sequencing to identify an unbalanced chromosomal rearrangement responsible for severe haemophilia A (19). This complex structural variant had eluded detection and characterization by conventional genetics sequencing. Another study applied targeted long-read sequencing in cases of inherited platelet disorders, successfully identifying and precisely characterizing structural variants - including deletions, insertions, and complex rearrangement - that were missed or incompletely resolved by short-read approaches and conventional diagnostic tests (20). These initial studies show that long-read sequencing can improve the detection and precise characterization of genetic variants in certain bleeding disorders, leading to more accurate diagnoses.

Variant interpretation and clinical significance

Variants identified through molecular testing are systematically categorized to guide clinical interpretation and patient management. Each variant is assessed based on available evidence and classified as benign (class 1), likely benign (class 2), variant of uncertain significance (VUS; class 3), likely pathogenic (class 4), or pathogenic (class 5) (21). This standardized framework supports consistent reporting and facilitates communication between laboratories and clinicians, guiding genetic counselling and clinical decision-making. In accordance with the American College of Medical Genetics and Genomics (ACMG) guidelines, certain laboratories implement additional stratification within the VUS category (21,22). Specifically, they further distinguish between standard VUS and VUS+ classifications. The VUS+ designation is applied to variants for which there is preliminary—yet insufficient—supporting evidence for pathogenicity, but not enough to justify reclassification as likely pathogenic.

Application of molecular testing in inherited bleeding disorders

The International Society on Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee Subcommittee (SSC) has curated a list of disease-causing (TIER1) genes specifically associated with bleeding and thrombotic disorders, which is updated annually (23). To date, 109 TIER1 genes have been recognized as causative for (anti)coagulation and platelet defects (Table 1). These genes form the basis of multigene panels used in clinical molecular testing of bleeding and thrombotic disorders. Our UZ Leuven multigene panel has included an additional third subpanel with genes that are not part of the ISTH TIER1 list (Table 1). These genes are related to vascular disorders such as Hereditary Haemorrhagic Telangiectasia and Ehlers-Danlos syndrome. This subpanel is used for screening patients with BDUC.

To evaluate the effectiveness of implementing a multigene panel in clinical practice, it is important to consider the diagnostic yield. Several studies have reported their diagnostic rates; however, direct comparison is challenging, as the composition of multigene panels can differ between centres. Additionally, differences in patient selection criteria, sequencing methods, and variant interpretation guidelines may further complicate meaningful comparisons. The ThromboGenomics study represents the largest international evaluation to date of multigene panel sequencing for patients with suspected inherited coagulation, thrombotic and platelet disorders (25). In this cohort of 2,396 patients, the panel achieved an overall diagnostic rate of 49.2% among those with thrombotic, coagulation, platelet count, or platelet function disorders, but only 3.2% in BDUC patients. Building on this international framework, UZ Leuven implemented a similar HTS panel for inherited bleeding disorders in clinical diagnostics from early 2019 onward. Over a three-year period, the clinical application of this multigene panel testing was evaluated (24). A total of 487 patients from 27 hospitals were tested, with at least one genetic variant detected in 58% of cases, indicating a substantial and similar diagnostic yield. These figures refer to bleeding and thrombosis. Of these genetic variants, half were classified as likely pathogenic or pathogenic, while the remainder were VUS.

The clinical advantages of molecular testing, particularly NGS, are considerable and extensive. This technology has advanced diagnostic capabilities in haematology and beyond, enabling more precise, timely, and informative assessments of hereditary conditions. Firstly, genetic testing offers the ability to establish a definitive molecular diagnosis. In many cases, clinical features alone may be insufficient to distinguish between phenotypically similar disorders. NGS enables the precise identification of the causative genetic variant, thereby providing diagnostic certainty. This allows clinicians to transition from a presumptive or phenotypic diagnosis to a definitive molecular diagnosis. This certainty is not only important for patient management, but also for communication with patients and families, as it provides a clear explanation for symptoms and test results that may have previously remained inconclusive (26). Secondly, a molecular diagnosis enables more accurate risk stratification and prognostic assessment. Different genetic variants, even within the same gene, can be associated with variable disease severity, progression, and response to treatment. Identifying the precise genetic cause enables clinicians to anticipate potential complications, monitor for disease-associated risks, and make well-informed decisions regarding surveillance and intervention strategies. For example, in Glanzmann Thrombasthenia, specific genetic variants within the *ITGA2B* or *ITGB3* genes are associated with differences in bleeding

severity and the risk of alloimmunization, which significantly influence clinical management, including the need for platelet transfusion support or the use of alternative therapies such as recombinant factor VIIa (27,28). Thirdly, genetic testing can reveal a broader and clinically significant underlying pathology that may not be apparent based on clinical features alone. Certain genes, such as *RUNX1*, *ETV6*, *ERG* and *ANKRD26*, are known not only for their role in inherited thrombocytopenia with increased bleeding tendencies, but also for their association with an increased risk of haematological malignancies, including myelodysplastic syndrome and acute leukaemia (29–32). In these cases, the identification of a pathogenic variant has implications beyond the immediate presentation, prompting the need for proper counselling, long-term monitoring, and early detection of malignant transformation. Fourthly, the identification of a pathogenic variant has profound implications for genetic counselling and family planning. Molecular testing remains essential not only in cases where the diagnosis cannot be established through functional assays, but also when functional testing provides a clear diagnosis, as it is crucial for genetic counselling, targeted carrier screening, and predictive testing. Once a causative variant is known, at-risk relatives can be offered predictive testing, and carriers can be identified with accuracy. This enables tailored counselling regarding inheritance patterns, recurrence risks, and reproductive options, including prenatal and preimplantation genetic diagnosis (33). Fifthly, a molecular diagnosis can prevent inappropriate or unnecessary treatments. For instance, distinguishing a case of inherited thrombocytopenia from immune thrombocytopenia, based on genetic findings may prevent the initiation of immunosuppressive therapies, which would be ineffective and potentially harmful in the inherited context (26). Finally, NGS is considered cost-effective, particularly given the complexity and genetic heterogeneity of inherited bleeding disorders which often require the simultaneous analysis of multiple candidate genes.

Despite the significant advances in molecular testing, several challenges remain. One of the most prominent issues is the interpretation of VUS, which complicates the process of determining their clinical relevance. To further elucidate a VUS, further investigation is critical to clarify its clinical impact (34). Segregation studies within affected and non-affected family members help determine whether the variant co-segregates with the disease phenotype, providing important clues about its potential pathogenicity. It is important to keep incomplete penetrance in mind, which can complicate the interpretation of segregation analyses. Furthermore, the variant frequency in large population databases, such as gnomAD, is evaluated; a variant that is rare or absent in healthy populations but more common in affected individuals may suggest a pathogenic role. In parallel, curated databases like ClinVar offer clinical interpretations of variants based on submitted evidence, aiding the assessment of their significance in a diagnostic context (21). Other supportive strategies include literature reviews for new case reports, the use of computational prediction tools to evaluate the variant's likely effect, and periodic reanalysis of genomic data as scientific knowledge evolves. Lastly, sometimes functional testing, which may include in vitro or in vivo assays, is conducted to assess the effect of the variant on protein function or cellular processes. All these mechanisms may be conducted to reclassify a VUS. However, despite these supplementary investigations, it is by far not always feasible to definitively reclassify a VUS. This uncertainty can pose substantial difficulties in clinical decision-making, as it leaves both clinicians and patients uncertain about the potential impact of these variants on disease manifestation or prognosis. In addition, incomplete coverage of certain genes can lead to missed diagnoses, as not all regions of the genome may be fully captured or sequenced. NGS platforms may have limitations in detecting large-scale genomic alterations, such as large deletions or duplications. Furthermore, the association of certain genes related to inherited bleeding disorders with an increased risk of malignancies offers a clear clinical benefit, as it enables early detection, appropriate counselling, and timely surveillance. However, this advantage is accompanied by important ethical considerations, particularly regarding the disclosure of incidental findings and the psychological burden such information may place on patients and their families.

NGS-based approaches in diagnostically challenging bleeding disorders

It is acknowledged that NGS has significantly transformed the diagnostic landscape of inherited bleeding disorders. However, despite the implementation of multigene panels, in some cases no definitive functional or genetic diagnosis can be established. This encompasses clinical scenarios in which the underlying pathobiology remains insufficiently understood or the relevant genes have yet to be identified (as observed in patients with BDUC), as well as individuals diagnosed with a known bleeding disorder—even when functionally characterized—who lack an identifiable pathogenic variant (e.g., certain patients with haemophilia). This section will outline the research-based approaches that may be pursued in such cases to further investigate the underlying genetic causes.

RNA sequencing (RNAseq) has become an essential tool for uncovering the molecular consequences of genetic variants, especially those outside coding regions, by enabling the detection of aberrant splicing events, altered gene expression, and the functional impact of non-coding variants. It should be noted, however, that this approach depends on the gene being sufficiently expressed in the analysed tissue, often blood cells, which may not always apply to the specific genes that are being studied. For example, in haemophilia A, systematic analysis combining NGS of the entire F8 locus and mRNA from patients without detectable coding variants, confirmed that deep intronic variants could create new splice sites, leading to the aberrant inclusion of intronic DNA into the mature mRNA and premature stop codons, thereby establishing their pathogenicity. It is important to note that the cDNA analysed in this study was derived from RNA extracted from blood cells, which are not primary sites of factor VIII synthesis. Therefore, tissue-specific alternative splicing events occurring in factor VIII-producing cells may not be represented (35). Similarly, in von Willebrand disease, transcriptomics of platelets and leukocytes has revealed that deep intronic and synonymous VWF mutations can cause aberrant splicing, with RNAseq data providing direct evidence of their functional impact (36). These findings underscore the essential role of RNAseq in uncovering the pathogenic consequences of non-coding and intronic mutations that are missed by conventional genomic approaches, thereby improving the molecular diagnosis and understanding of inherited bleeding disorders.

In addition to facilitating variant detection, NGS has significantly advanced the understanding of the underlying pathobiology of rare bleeding disorders. *SLFN14*-related thrombocytopenia serves as a notable example (37). Pathogenic missense variants in *SLFN14*, which encodes for an endoribonuclease involved in ribosome degradation, have been identified as the cause of an autosomal dominant form of thrombocytopenia with increased bleeding tendency through WES and RNAseq. Transcriptomic analyses of patients carrying the *SLFN14 K219N* variant revealed extensive dysregulation of gene expression, including marked upregulation of ribosomal protein genes and activation of mitochondrial translation and transcription pathways. This transcriptomic signature indicates ribosomal stress and a compensatory response mediated by mTORC1 signalling, which appears central to disease pathogenesis. These insights illustrate how NGS technologies not

only enable genetic diagnosis but also contribute to the elucidation of molecular mechanisms underlying rare platelet disorders.

Genetic manipulation of immortalized megakaryocyte progenitor cell lines (imMKCLs) could be a powerful research tool to further unravel the underlying pathophysiology and validate the functional consequences of genetic variants identified in patients with inherited platelet disorders (38). These imMKCLs are derived from human induced pluripotent stem cells and can be expanded in culture for several months and differentiate into functional platelets. By using CRISPR-Cas9, researchers can introduce patient-specific mutations into imMKCLs and study their impact on megakaryocyte differentiation, platelet production, gene expression, and signalling pathways. This approach not only helps to confirm or deny the pathogenicity of a VUS but also provides mechanistic insights into disease development. For example, Lo et al. investigated the molecular mechanisms underlying Gray Platelet Syndrome, a rare inherited bleeding disorder caused by pathogenic variants in the *NBEAL2* gene, leading to the absence of α-granules in platelets and resulting in bleeding symptoms (39,40). Using imMKCLs, they demonstrated that the endoplasmic reticulum protein SEC22B physically interacts with *NBEAL2* and is essential for proper α-granule biogenesis. Importantly, disease-associated variants in *NBEAL2* disrupt this interaction, and genetic knockout of SEC22B in imMKCLs results in a failure to produce α-granules, recapitulating key features of Gray Platelet Syndrome. These findings provide new insights into the pathogenesis of Gray Platelet Syndrome using the imMKCL-based model.

Conclusion

The diagnosis of inherited bleeding disorders has been fundamentally transformed by advances in genetic testing. The transition from phenotype-driven algorithms to molecular diagnostics has enabled the identification of causative variants in an increasing proportion of patients, improving risk stratification, guiding management, and facilitating genetic counselling. While targeted gene panels remain the first-line approach in clinical practice, WES and WGS, along with emerging research tools such as RNAseq, are essential for resolving undiagnosed cases and for expanding our understanding of the genetic architecture of these disorders. Despite these advances, it remains essential that diagnostic requests continue to be targeted and guided by clinical expertise to ensure appropriate test selection, efficient resource use, and meaningful interpretation of results.

Challenges remain, including the interpretation of variants of uncertain significance, the need for equitable access to testing, and the ethical considerations associated with incidental findings. The future of diagnostics in inherited bleeding disorders will be shaped by the integration of multi-omics data and the continued collaboration between clinicians, geneticists, and researchers.

Statements

There are no competing interests to disclose.

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TABLE 1: Overview of the multigene Thrombosis and Haemostasis panel of UZ Leuven, divided in the three subpanels.

Subpanel	Gene	Related Disorder(s)
Coagulation*	ADAMTS13	Thrombotic thrombocytopenic purpura
Coagulation	F2	Prothrombin deficiency Prothrombin G20210A mutation-associated thrombophilia
	F5	F5 deficiency Factor V Leiden thrombophilia
	F7	Factor VII deficiency
	F8	Haemophilia A
	F9	Haemophilia B
	F10	FX deficiency
	F11	FXI deficiency
Coagulation ***Angioedema***	F12	FXII deficiency
Coagulation	FGA	Fibrinogen deficiency
	FGB	Fibrinogen deficiency
	FGG	Fibrinogen deficiency
	GGCX	Vitamin K-dependent coagulation factor deficiency
	HRG	Histidine-rich glycoprotein deficiency
	KLKB1	Hereditary prekallikrein deficiency (Fletcher factor deficiency)
	KNG1	Kininogen deficiency
	LMAN1	Combined FV and FVIII deficiency
	MCFD2	Combined FV and FVIII deficiency
	PIGA	Paroxysmal nocturnal haemoglobinuria
	PLG	Plasminogen deficiency
	PROC	Protein C deficiency
	PROS1	Protein S deficiency
	SERPINC1	Antithrombin deficiency
	SERPIND1	Heparin cofactor 2 deficiency
	SERPINE1	Plasminogen activator inhibitor 1 deficiency
	SERPINF2	Alpha 2 antiplasmin deficiency
	THBD	Thrombomodulin deficiency
	VKORC1	Vitamin K-dependent coagulation factor deficiency
	VWF	Von Willebrand Disease

Subpanel	Gene	Related Disorder(s)
Unexplained bleeding	ACVRL1	Hereditary Haemorrhagic Telangiectasia
	CHST14	Ehlers-Danlos syndrome
	COL1A1	Ehlers-Danlos syndrome, arthrochalasia type, 1
	COL3A1	Ehlers-Danlos syndrome, vascular type
	COL4A1	Brain small vessel disease with or without ocular anomalies
	COL4A2	Brain Small Vessel Disease 2
	COL5A1	Ehlers-Danlos syndrome, classic type, 1
	COL5A2	Ehlers-Danlos syndrome, classic type, 2
	ENG	Hereditary Haemorrhagic Telangiectasia
	GDF2	Hereditary Haemorrhagic Telangiectasia
	SMAD4	Hereditary Haemorrhagic Telangiectasia

* The coagulation subpanel includes genes related to bleeding and thrombosis.

Subpanel	Gene	Related Disorder(s)
Platelet	ABCC4	Reduced ADP-induced platelet aggregation
	ABCG5	Sitosterolemia with macrothrombocytopenia
	ABCG8	Sitosterolemia with macrothrombocytopenia
	ACTB	Baraitser-Winter syndrome with macrothrombocytopenia
	ACTN1	Macrothrombocytopenia
	ANKRD26	Thrombocytopenia and increased risk for cancer
	ANO6	Scott syndrome (platelet function disorder)
	AP3B1	Hermansky Pudlak syndrome (platelet function disorder)
	AP3D1	Hermansky Pudlak syndrome (platelet function disorder)
	ARPC1B	Platelet abnormalities and immune-mediated inflammatory disease predisposition
	BLOC1S3	Hermansky Pudlak syndrome (platelet function disorder)
	BLOC1S5	Hermansky Pudlak syndrome (platelet function disorder)
	BLOC1S6	Hermansky Pudlak syndrome (platelet function disorder)
	CDC42	Takenouchi-Kosaki syndrome with thrombocytopenia
	CYCS	Thrombocytopenia
	DIAPH1	Macrothrombocytopenia and sensorineural hearing loss
	DTNBP1	Hermansky Pudlak syndrome (platelet function disorder)
	ERG	Thrombocytopenia and susceptibility to cancer
	ETV6	Thrombocytopenia and increased risk for cancer
	FERMT3	Leukocyte Adhesion deficiency type III (platelet function disorder)
	FLI1	Paris-Trousseau syndrome and Jacobsen syndrome (TP)
	FLNA	Syndrome with macrothrombocytopenia
	FYB1	Thrombocytopenia
	GATA1	Thrombocytopenia with dyserythropoiesis
	GFI1B	Macrothrombocytopenia
	GNE	Myopathy associated with thrombocytopenia
	GP1B1	Bernard-Soulier syndrome Macrothrombocytopenia (mild) Platelet-type VWD
	GP1BB	Bernard-Soulier syndrome Macrothrombocytopenia (mild)
	GP6	Glycoprotein VI deficiency
	GP9	Bernard-Soulier syndrome
	HOXA11	Amegakaryocytic thrombocytopenia with radio-ulnar synonstosis syndrome
	HPS1	Hermansky Pudlak syndrome (platelet function disorder)
Platelet	HPS3	Hermansky Pudlak syndrome (platelet function disorder)
	HPS4	Hermansky Pudlak syndrome (platelet function disorder)
	HPS5	Hermansky Pudlak syndrome (platelet function disorder)
	HPS6	Hermansky Pudlak syndrome (platelet function disorder)
	IKZF5	Thrombocytopenia, Pegasus syndrome
	ITGA2B	Glanzmann Thrombasthenia
	IGAB3	Glanzmann Thrombasthenia
	KDSR	Thrombocytopenia and erythrokeratoderma
	LYST	Chediak-Higashi syndrome
	MECOM	Amegakaryocytic thrombocytopenia with radio-ulnar synostosis syndrome
	MPIG6B	Thrombocytopenia, anaemia and myelofibrosis
	MPL	Amegakaryocytic thrombocytopenia
	MYH9	Macrothrombocytopenia, sensorineural hearing loss, kidney disease, cataracts
	NBEA	Autism with platelet dense granule defect
	NBEAL2	Gray platelet syndrome
	P2RY12	ADP receptor defect
	PLA2G4A	Phospholipase A2 deficiency, group IV A
	PLAU	Quebec platelet disorder
	PTGS1	Platelet function disorder
	RAP1B	Syndromic thrombocytopenia
	RASGRP2	Platelet function disorder
	RBM8A	Thrombocytopenia-absent radius (TAR) syndrome
	RUNX1	Thrombocytopenia and increased risk for haematological cancer
	SLFN14	Platelet function disorder
	SRC	Thrombocytopenia
	STIM1	Stormorken syndrome = York platelet syndrome (thrombocytopenia)
	STXBP2	Familial hemophagocytic lymphohistiocytosis type 5
	TBXA2R	Thromboxane A2 receptor defect
	THPO	Thrombocytopenia progressing to bone marrow failure
	TPM4	Macrothrombocytopenia
	TUBB1	Macrothrombocytopenia
	VIPAS39	Arthrogryposis, renal dysfunction, and cholestasis
	VPS33B	Arthrogryposis, renal dysfunction, and cholestasis
	VWF	Von Willebrand disease
	WAS	Wiskott-Aldrich syndrome

REFERENCES

1. Soucie JM, Evatt B, Jackson D. Occurrence of hemophilia in the United States. Am J Hematol. 1998 Dec;59(4):288–94.
2. Werner EJ, Broxson EH, Tucker EL, Giroux DS, Shults J, Abshire TC. Prevalence of von Willebrand disease in children: A multiethnic study. J Pediatr. 1993 Dec;123(6):893–8.
3. Gresele P, Harrison P, Gachet C, Hayward C, Kenny D, Mezzano D, et al. Diagnosis of inherited platelet function disorders: Guidance from the SSC of the ISTH. Journal of Thrombosis and Haemostasis. 2015 Feb;13(2):314–22.
4. Soleimani Samarkhazan H, khaksari MN, Rahmati A, Esfahani ML, Solouki A, Aghaei M. Von Willebrand disease (VWD) and pregnancy: a comprehensive overview. Thromb J. 2025 Dec;23(1):41.
5. Baker RI, Choi P, Curry N, Gebhart J, Gomez K, Henskens Y, et al. Standardization of definition and management for bleeding disorder of unknown cause: communication from the SSC of the ISTH. Journal of Thrombosis and Haemostasis. 2024 Jul;22(7):2059–70.
6. Heremans J, Freson K. High-throughput sequencing for diagnosing platelet disorders: lessons learned from exploring the causes of bleeding disorders. Int J Lab Hematol. 2018 May;40:89–96.
7. Freson K, Turro E. High-throughput sequencing approaches for diagnosing hereditary bleeding and platelet disorders. Journal of Thrombosis and Haemostasis. 2017 Jul;15(7):1262–72.
8. Al-Shuhaib MBS, Hashim HO. Mastering DNA chromatogram analysis in Sanger sequencing for reliable clinical analysis. Journal of Genetic Engineering and Biotechnology. 2023 Nov;21(1):115.
9. Xue Y, Ankala A, Wilcox WR, Hegde MR. Solving the molecular diagnostic testing conundrum for Mendelian disorders in the era of next-generation sequencing: Single-gene, gene panel, or exome/genome sequencing. Genetics in Medicine. 2015 Jun;17(6):444–51.
10. Hu T, Chitnis N, Monos D, Dinh A. Next-generation sequencing technologies: An overview. Hum Immunol. 2021 Nov;82(11):801–11.
11. Moreno-Cabrera JM, del Valle J, Castellanos E, Feliubadaló L, Pineda M, Brunet J, et al. Evaluation of CNV detection tools for NGS panel data in genetic diagnostics. European Journal of Human Genetics. 2020 Dec;28(12):1645–55.
12. Marian AJ. Sequencing your genome: what does it mean? Methodist Debakey Cardiovasc J. 2014 Jan;10(1):3–6.
13. Platt CD, Zaman F, Bainter W, Stafstrom K, Almutairi A, Reigle M, et al. Efficacy and economics of targeted panel versus whole-exome sequencing in 878 patients with suspected primary immunodeficiency. Journal of Allergy and Clinical Immunology. 2021 Feb;147(2):723–6.
14. Ver Donck F, Downes K, Freson K. Strengths and limitations of high-throughput sequencing for the diagnosis of inherited bleeding and platelet disorders. Journal of Thrombosis and Haemostasis. 2020 Aug;18(8):1839–45.
15. Dillon OJ, Lunke S, Stark Z, Yeung A, Thorne N, Gaff C, et al. Exome sequencing has higher diagnostic yield compared to simulated disease-specific panels in children with suspected monogenic disorders. European Journal of Human Genetics. 2018 May;26(5):644–51.
16. Yadegari H, Driesen J, Hass M, Budde U, Pavlova A, Oldenburg J. Large deletions identified in patients with von Willebrand disease using multiple ligation-dependent probe amplification. Journal of Thrombosis and Haemostasis. 2011 May;9(5):1083–6.
17. Rafati M, Ravanbod S, Hoseini A, Rassoulzadegan M, Jazebi M, Enayat MS, et al. Identification of ten large deletions and one duplication in the F8 gene of eleven unrelated Iranian severe haemophilia A families using the multiplex ligation-dependent probe amplification technique. Haemophilia. 2011 Jul;17(4):705–7.
18. Di Marco F, Spitaleri A, Battaglia S, Batignani V, Cabibbe AM, Cirillo DM. Advantages of long- and short-reads sequencing for the hybrid investigation of the Mycobacterium tuberculosis genome. Front Microbiol. 2023 Feb;14:1104456.
19. Chatron N, Schluth-Bolard C, Frétigny M, Labalme A, Vilchez G, Castet SM, et al. Severe hemophilia A caused by an unbalanced chromosomal rearrangement identified using nanopore sequencing. Journal of Thrombosis and Haemostasis. 2019 Jul;17(7):1097–103.
20. Zamora-Cánovas A, de la Morena-Barrio B, Marín-Quilez A, Sierra-Aisa C, Male C, Fernández-Mosteirin N, et al. Targeted long-read sequencing identifies and characterizes structural variants in cases of inherited platelet disorders. Journal of Thrombosis and Haemostasis. 2024 Mar;22(3):851–9.
21. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in Medicine. 2015 May;17(5):405–24.
22. Houge G, Laner A, Cirak S, de Leeuw N, Scheffer H, den Dunnen JT. Stepwise ABC system for classification of any type of genetic variant. European Journal of Human Genetics. 2022 Feb;30(2):150–9.
23. Megy K, Downes K, Simeoni I, Bury L, Morales J, Mapeta R, et al. Curated disease-causing genes for bleeding, thrombotic, and platelet disorders: Communication from the SSC of the ISTH. Journal of Thrombosis and Haemostasis. 2019 Aug;17(8):1253–60.
24. Van Laer C, Jacquemin M, Baert S, Labarque V, Thys C, Vanassche T, et al. Clinical application of multigene panel testing for bleeding, thrombotic, and platelet disorders: a 3-year Belgian experience. Journal of Thrombosis and Haemostasis. 2023 Apr;21(4):887–95.
25. Downes K, Megy K, Duarte D, Vries M, Gebhart J, Hofer S, et al. Diagnostic high-throughput sequencing of 2396 patients with bleeding, thrombotic, and platelet disorders. Blood. 2019 Dec;134(23):2082–91.
26. Kim B. Diagnostic workup of inherited platelet disorders. Blood Res. 2022 Apr;57(S1):11–9.
27. Botero JP, Lee K, Branchford BR, Bray PF, Freson K, Lambert MP, et al. Glanzmann thrombasthenia: Genetic basis and clinical correlates. Haematologica. 2020 Apr;105(4):888–94.
28. Nurden AT, Fiore M, Nurden P, Pillois X. Glanzmann thrombasthenia: A review of ITGA2B and ITGB3 defects with emphasis on variants, phenotypic variability, and mouse models. Blood. 2011 Dec;118(23):5996–6005.
29. Homan CC, Scott HS, Brown AL. Hereditary platelet disorders associated with germ line variants in RUNX1, ETV6, and ANKRD26. Blood. 2023 Mar;141(13):1533–43.
30. Zhang MY, Churpek JE, Keel SB, Walsh T, Lee MK, Loeb KR, et al. Germline ETV6 mutations in familial thrombocytopenia and hematologic malignancy. Nat Genet. 2015 Feb;47(2):180–5.
31. Noris P, Perrotta S, Seri M, Pecci A, Gnan C, Loffredo G, et al. Mutations in ANKRD26 are responsible for a frequent form of inherited thrombocytopenia: Analysis of 78 patients from 21 families. Blood. 2011 Jun;117(24):6673–80.
32. Song WJ, Sullivan MG, Legare RD, Hutchings S, Tan X, Kufrin D, et al. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. Nat Genet. 1999 Oct;23(2):166–75.
33. Bastida JM, del Rey M, Lozano ML, Sarasquete ME, Benito R, Fontecha ME, et al. Design and application of a 23-gene panel by next-generation sequencing for inherited coagulation bleeding disorders. Haemophilia. 2016 Jul;22(4):590–7.
34. Walsh N, Cooper A, Dockery A, O'Byrne JJ. Variant reclassification and clinical implications. J Med Genet. 2024 Feb;61(3):207–11.
35. Pezeshkpoor B, Zimmer N, Marquardt N, Nanda I, Haaf T, Budde U, et al. Deep intronic "mutations" cause hemophilia A: Application of next generation sequencing in patients without detectable mutation in F8 cDNA. Journal of Thrombosis and Haemostasis. 2013 Sep;11(9):1679–87.
36. Borràs N, Orriols G, Batlle J, Pérez-Rodríguez A, Fidalgo T, Martinho P, et al. Unraveling the effect of silent, intronic and missense mutations on VWF splicing: Contribution of next generation sequencing in the study of mRNA. Haematologica. 2019 Mar;104(3):587–98.
37. Ver Donck F, Ramaekers K, Thys C, Van Laer C, Peerlinck K, Van Geet C, et al. Ribosome dysfunction underlies SLFN14-related thrombocytopenia. Blood. 2023 May;141(18):2261–74.
38. Nakamura S, Takayama N, Hirata S, Seo H, Endo H, Ochi K, et al. Expandable megakaryocyte cell lines enable clinically applicable generation of platelets from human induced pluripotent stem cells. Cell Stem Cell. 2014 Apr;14(4):535–48.
39. Lo RW, Li L, Pluthero FG, Leung R, Eto K, Kahr WHA. The endoplasmic reticulum protein SEC22B interacts with NBEAL2 and is required for megakaryocyte a-granule biogenesis. Blood. 2020 Aug;136(6):715–25.
40. Gunay-Aygun M, Falik-Zaccai TC, Vilboux T, Zivony-Elboum Y, Gumruk F, Cetin M, et al. NBEAL2 is mutated in gray platelet syndrome and is required for biogenesis of platelet γ-granules. Nat Genet. 2011 Jul;43(8):732–4.

Haemophilia Treatment in 2025

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Abstract
Haemophilia is a rare disease with high morbidity and mortality in its severe form. The mainstay of haemophilia management has been regular prophylactic infusions of the missing coagulation factor (FVIII/FIX) to reduce morbidity and mortality associated with chronic, crippling arthropathy. This approach is burdensome and costly for patients due to the need for frequent intravenous infusions, high costs, limited availability, and the development of inhibitors. Advances in the engineering and manufacturing of clotting concentrates have led to the creation of novel molecules that address many of these limitations. These include the development of extended half-life factors, which require less frequent infusions, and new non-factor replacement treatments that can be administered subcutaneously and infrequently, such as FVIII-mimetic antibodies (e.g., emicizumab) and downregulators of natural anticoagulants (e.g., antithrombin, tissue factor pathway inhibitor, or activated protein C). Finally, gene therapy is set to offer patients the possibility of a cure by transferring a functional copy of the gene required to express the missing or dysfunctional clotting factor. This therapy was approved for the treatment of haemophilia A and B in 2022 in Europe and the United States and is currently in late-phase clinical investigation.

Introduction
The most prevalent X-linked inherited bleeding disorders are haemophilia A and B, characterized by a deficiency or absence of clotting factors VIII (haemophilia A - HA) and IX (haemophilia B - HB), respectively. The severity of the disease is determined by the residual amount of FVIII or FIX, which can be classified as severe (<1%), moderate (1–5%) or mild (6-49%) (1).

Haemophilia A and B share similar symptoms, including bleeding into large joints such as the elbows, knees, and ankles (referred to as index joints). This bleeding can lead to painful and disabling haemophilic arthropathy. Regardless of the severity, life-threatening bleeding, such as intracranial haemorrhages or internal organ bleeding may occur, albeit more frequently in severe cases. While spontaneous bleeding is common in severe haemophilia, those with moderate or mild forms may also experience significant bleeding due to trauma or surgery. Inadequate management can result in chronic disease and lifelong disability (2,3). Fortunately, therapeutic advances and comprehensive care have significantly improved morbidity and mortality in the 21st century.

Treatment
Haemophilia management includes on-demand treatment for bleeding episodes and perioperative management, as well as regular prophylactic treatment designed to reduce bleeding frequency, morbidity, and mortality, thereby enhancing quality of life. Treatment options encompass factor replacement therapy, non-replacement therapies, inhibition of natural anticoagulant pathways that promote thrombin generation, and gene therapies that enable endogenous production of clotting factors. These therapies differ in their approach to prophylaxis and on-demand treatment, method and frequency of administration, duration of effect, level of haemostatic protection, and side effect profiles. Annual bleeding rates and joint damage are crucial markers for evaluating prophylactic treatment efficacy. Personalized management strategies aligned with individual goals, such as participating in competitive sports, should begin at diagnosis and continue throughout life. A multidisciplinary team is essential to support these strategies and provide education to both clinicians and patients (2-4).

Over the past three decades, prophylaxis has become the evidence-based standard of care, proven to better preserve joint integrity than on-demand therapy, especially in patients with severe haemophilia (5,6). The wide availability of recombinant clotting factor concentrates - manufactured using ultrafiltration and nanofiltration viral inactivation techniques and free from animal or human proteins - has facilitated widespread implementation of prophylaxis, at least in high-income countries. However, despite the significant benefits of primary prophylaxis, frequent intravenous injections due to the short half-life of standard half-life (SHL) factor VIII (10–12 hours) and IX (18–20 hours) pose adherence challenges, especially for younger patients. This often necessitates the use of ports or other central venous access devices (3,4,7).

To address these limitations, extended half-life (EHL) recombinant FVIII/FIX concentrates were developed between 2010 and 2020. Techniques include fusing clotting factors with proteins like the Fc domain of IgG1 or albumin, or pegylation using polyethylene glycol (PEG). While FIX modifications yielded a fivefold increase in half-life, results for FVIII were more modest (1.5–1.7x increase). EHL-FIX can allow weekly or every two weeks dosing while maintaining trough levels above 5%, effectively converting a severe phenotype to a mild one. EHL-FVIII's impact is less obvious, typically enables twice-weekly dosing, instead of three times weekly, maintaining trough levels of 2% - 3% thereby transforming severe bleeding into a moderately severe bleeding phenotype. Thus, by reducing the number of venipunctures, prophylaxis became more feasible as the standard, particularly for patients with HB. The EHL factors are satisfactory in terms of safety. Their safety profiles in clinical trials have been consistent with SHL products. No concerns were raised about the high degree of engineering of the molecules, resulting in neoantigenicity and more inhibitors (3,7,8).

Despite these advancements, development of inhibitory antibodies against FVIII/FIX remains a major challenge. Around 30% of individuals with severe haemophilia A and 10% with severe haemophilia B develop inhibitors that neutralize replacement

therapy, limiting prophylaxis options and increasing morbidity and mortality. Bypassing agents (BPAs), such as recombinant activated factor VII (rFVIIa, NovoSeven) and activated prothrombin complex concentrate (FEIBA), were game-changers in the 1990s, but unmet needs, especially regarding injection frequency and practicality, persisted (3-5).

Innovative non-factor therapies (NFTs) emerged between 2010 and 2020. These drugs enhance thrombin generation (e.g., emicizumab, MIM8) or inhibit natural anticoagulant pathways (e.g., fitusiran, concizumab, and marstacimab). Fitusiran, a monthly subcutaneous siRNA therapy, reduces antithrombin levels to restore haemostasis in both HA and HB, with or without inhibitors. Concizumab, an anti-tissue factor pathway inhibitor (TFPI) monoclonal antibody, and marstacimab, a TFPI neutralizing antibody approved in 2024–2025, are administered subcutaneously and aim to reduce bleeding frequency by counteracting TFPI. SerpinPC, another agent under development, targets activated protein C to support thrombin generation in the absence of FVIII or FIX (3-8).

Currently, emicizumab is the only widely used NFT for prophylaxis in HA. This bispecific monoclonal antibody mimics FVIII by bridging FIXa and FX and is administered subcutaneously every 7, 14, or 28 days. Data from the HAVEN 1–4 and HAVEN 7 trials support its use across all age groups, with or without inhibitors. However, emicizumab does not normalize haemostasis, so FVIII or BPAs remain necessary for bleeding or surgery. Breakthrough bleeds still occur, though less frequently (9,10). Emicizumab is not effective for HB.

According to the 2020 WFH guidelines, clinicians aimed for FVIII trough levels >3% - 5% (2). Evidence increasingly supports targeting levels >5%, or even 15%–50%, to achieve near-zero joint bleeding (11). Efanesoctocog alfa, a next-generation FVIII therapy, surpasses the VWF half-life ceiling and maintains FVIII levels well above traditional products. The unique structure of efanesoctocog alfa allows for independent operation of endogenous VWF, resulting in a significantly longer half-life compared to standard and EHL FVIII products. When given prophylactically at 50 IU/kg intravenously weekly, FVIII activity levels increased to 100 – 120% and remained above 40% to day 4 and then were 13–15% at day 7 post infusion in adolescents and adults. Following infusion in children <6 years of age, the time to 40 IU/dL was 59.2 hours and for the 6 to <12 -year-old, 72.2 hours compared to 81.7 hours and 97 hours for the 12 to < 18 and ≥18 -year-old, respectively. In other words, efanesoctocog alfa is a new type of FVIII replacement with an extended half-life also called ultra-extended product, which allows for once weekly dosing to achieve haemostasis and FVIII trough levels of 13-15 IU/dL. This provides a highly effective option for prevention of bleeding in haemophilia A. It also provides a better option for treatment of bleeding and coverage for surgery with fewer infusions (12.13).

Despite these improvements, regular injections and breakthrough bleeds still limit quality of life. Gene therapy offers a potential cure. In 2022, the EMA conditionally approved valoctocogene roxaparvovec (AAV5-hFVIII-SQ) for HA, while the FDA approved etranacogene dezaparvovec-drlb (AAV5-FIX Padua) for HB. However, variability of factor expression level and durability of response are concerns. Median FVIII activity after valoctocogene roxaparvovec declined from 22.9 IU/dL in year 1 to 8.3 IU/dL in year 3. In contrast, FIX levels remained stable at around 37% for three years post-etranacogene dezaparvovec (14-17).

Conclusion

Maintaining FVIII/FIX levels within the normal range is ideal but still difficult. SHL and EHL therapies have long used trough levels as markers of efficacy. However, new findings suggest that time spent above protective thresholds may be more relevant than simply reaching a minimum trough. Non-factor therapies and gene therapy present opportunities to elevate and maintain coagulation activity more effectively and with fewer injections.

Thanks to EHL products and non-replacement therapies, we are shifting from managing severe disease to aiming for a mild—or even non-haemophilia—phenotype. The therapeutic goal is now evolving toward achieving zero bleeds. Gene therapy may be the ultimate step in this evolution. However, due to variability in response and durability, close clinical monitoring remains essential to avoid unrecognized disease activity.

There is no doubt that gene therapy and other innovative treatments will continue to evolve. The future of haemophilia care is bright, with real potential for patients to become haemophilia-free.

Statement

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REFERENCES

1. Blanchette VS, Key NS, Ljung LR, Manco-Johnson MJ, van den Berg HM, Srivastava_A, Subcommittee on Factor VIII, Factor IX and Rare Coagulation Disorders of the Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis. Definitions in hemophilia: communication from the SSC of the ISTH. J Thrombosis Haemostasis 2014;12(11):1935-9.
2. Srivastava A, Santagostino E, Dougall A, Kitchen S, Sutherland M, Pipe SW, et al. WFH Guidelines for the management of hemophilia, 3rd ed. Haemophilia. 2020; 26:1–158.
3. Mancuso ME, Mahlangu JN, Pipe SW. The changing treatment land- scape in haemophilia: from standard half-life clotting factor concentrates to gene editing. Lancet. 2021;397(10274):630-640.
4. Mancuso ME, Croteau SE, Klamroth R. Benefits and risks of non-factor therapies: Redefining haemophilia treatment goals in the era of new technologies. Haemophilia. 2024;30(Suppl. 3):39–44.
5. Manco-Johnson MJ, Abshire TC, Shapiro AD, Riske B, Hacker MR, Kilcoyne R, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. N Engl J Med 2007; 357: 535–544.
6. Gringeri A, Lundin B, von Mackensen S, Mantovani L, Mannucci PM. A randomized clinical trial of prophylaxis in children with hemophilia A (the ESPRIT study). J Thromb Haemost 2011; 9: 700–710.
7. Hart DP, Kessler CM, Aledort L. Re-personalization and stratification of hemophilia care in an evolving treatment landscape, Hematology 2019, 24:1, 737-741
8. Okaygoun D, Oliveira DD, Soman D, Williams R. Advances in the management of haemophilia: emerging treatments and their mechanisms, J Biomed Sci (2021) 28:64
9. Callaghan MU, Negrier C, Paz-¬Priel I, Chang T, Chebon S, Lehle M, et al. Long-¬term outcomes with emi¬cizumab prophylaxis for hemophilia A with or without FVIII inhibitors from the HAVEN 1-4 studies. Blood. 2021;137(16):2231-2242.
10. Pipe S, Collins P, Dhalluin C, Kenet G, Schmitt C, Buri M, et al. Emicizumab prophylaxis in infants with severe hemophilia A (HAVEN 7): primary analysis of a phase 3b open-label trial. Blood. 2024;143(14):1355-1364
11. Malec L, Matino D. Targeting higher factor VIII levels for prophylaxis in haemophilia A: a narrative review. Haemophilia. 2023;29(6):1419-1429.
12. von Drygalski A, Chowdary P, Kulkarni R, Susen S, Konkle BA, Oldenburg J, et al. Efanesoctocog alfa prophylaxis for patients with severe hemophilia A. N Engl J Med. 2023;388:310–318.
13. Malec L, Peyvandi F, Chan AKC, Königs C, Zulfikar B, Yuan H, et al. Efanesoctocog alfa prophylaxis for children with severe hemophilia A. N Engl J Med. 2024;391:235-246.
14. VandenDriessche T, Pipe SW, Pierce GF, Kaczmarek R. First conditional marketing authorization approval in the European Union for hemophilia "A" gene therapy. Mol Ther. 2022;30:3335–3336.
15. Herzog RW, VandenDriessche T, Ozelo MC. First hemophilia B gene therapy approved: more than two decades in the making. Mol Ther. 2023;31:1–2.
16. Madan B, Ozelo MC, Raheja P Symington E, Quon DV, Leavitt AD et al. Three-year outcomes of valoctocogene roxaparvovec gene therapy for hemophilia A. J Thromb Haemost. 2024 Jul;22(7):1880-1893
17. von Drygalski A, Gomez E, Giermasz A, Castaman G, Key NS, Lattimore SU et al. Stable and durable factor IX levels in patients with hemophilia B over 3 years after etranacogene dezaparvovec gene therapy. Blood Adv. 2023 Oct 10;7(19):5671-5679.

The Initial Approach to Paediatric Thrombosis

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Abstract

Venous thromboembolism (VTE) in children presents unique challenges distinct from adult thrombosis, primarily due to developmental differences in the haemostatic system and the rarity of traditional adult risk factors. Paediatric VTE is increasingly recognized, especially in neonates and adolescents, and is predominantly associated with underlying comorbidities such as central venous catheters (CVCs), cancer, or congenital heart disease. The concept of "developmental haemostasis" underscores the age-dependent evolution of coagulation, influencing both risk and management strategies.

Diagnosis relies on clinical signs and is confirmed via Doppler ultrasonography, MRI, or CT, depending on the site. Pulmonary embolism, though rare, requires prompt evaluation in cases of respiratory distress or collapse. Laboratory tests, including D-dimer, are less specific in children due to overlapping inflammatory conditions.

Anticoagulation therapy, typically initiated with heparinoids, must account for age-related pharmacokinetic differences and the risk of bleeding. Low-molecular-weight heparin (LMWH) and direct oral anticoagulants (DOACs) are increasingly used, though evidence-based guidelines remain limited. Treatment duration and intensity are tailored to clinical context, with special considerations for asymptomatic CVC-related thrombosis and high-risk scenarios like neonatal renal vein thrombosis or purpura fulminans.

Thrombophilia testing is reserved for recurrent or unprovoked VTE, with genetic confirmation advised for severe deficiencies. While paediatric VTE management has advanced, ongoing research is essential to address unresolved questions and optimize evidence-based care, ensuring therapies align with the unique physiology of children.

Introduction

The risk and context of thrombo-embolism (TE) in children are substantially different compared to adults. Diseases that damage the vascular endothelium and that are typically associated with thrombosis risk in adults, occur less frequently in children and they are less frequently exposed to acquired prothrombotic risk factors such as smoking, the use of hormonal therapies, etc. Moreover, the haemostatic system of children differs substantially from adults. The systematic description of the evolution and development of the haemostatic system of children of all ages by Maureen Andrew has led to the concept of 'developmental haemostasis' (1-3). This concept provides the framework and background for the approach to paediatric bleeding and thrombosis and describes the evolution of the haemostatic system from foetal life through adulthood. Paediatric TE has historically been considered of little significance, but is being increasingly recognized, especially in infancy and adolescence.

Contrary to the typical risk factors considered in adults, TE is a problem most frequently occurring in "the sick child", paralleling the risk factors for venous thrombosis from Virchow's triad: endothelial injury, impaired blood flow (stasis) and hypercoagulability. Over 90% of paediatric thromboses are related to underlying medical or surgical risk factors and/or, most commonly, the presence of central venous catheters (CVC) (4). Other risk factors include inherited hypercoagulable states, cardiovascular diseases, infection, trauma, malignancy, immobility and chronic inflammation. Idiopathic primary TE is exceedingly rare in children. It is important to note, however, that adult risk factors, such as obesity and the use of oral contraceptives, are on the rise in the adolescent age group (5). Inherited thrombophilia is a coagulation disorder associated with an increased genetic predisposition to develop thrombosis. Most other acquired risk factors mentioned above are associated with the development of acquired hypercoagulable states.

This review concerns exclusively venous thrombo-embolism (VTE) of various types. Arterial thrombosis is not discussed here.

Diagnostic approach to venous thrombosis

Clinical manifestations of VTE depend on the affected blood vessel and the degree of occlusion caused by the thrombosis. Initial assessment of paediatric TE should start with careful personal and family history in order to identify potential underlying conditions associated with increased risk for thrombosis.

Venous thrombosis in the upper or lower limbs causes acute onset of pain and swelling of the limb and represents the most frequent clinical manifestation of VTE in children. Measurement of limb circumference and clinical pictures are helpful in the diagnosis of limb VTE in children. Several clinical investigations may provide additional clues in the clinical suspicion of thrombosis, such as Homan's sign (calf pain upon dorsiflexion of the foot), May's sign (calf pain upon compression) or Payr's sign (sole pain upon sole compression).

VTE of vessels of internal organs causes specific symptoms, usually related to organ swelling and subsequent loss of function. For example, renal vein thrombosis (RVT) causes abdominal pain, palpable mass, haematuria and renal failure, while cerebral sinus thrombosis (CSVT) is related to signs and symptoms of intracranial hypertension. Portal vein thrombosis usually remains asymptomatic until symptoms of chronic portal hypertension occur, or may present acutely with symptoms of acute abdomen (6).

Pulmonary embolism (PE) is rare in children, but has to be considered in cases of chest pain, acute respiratory distress or sudden collapse in case of massive PE. Clinical signs of deep vein thrombosis may be present, but this is not always the case. Later onset clinical manifestations may include prominent collateral circulation, stroke and chylothorax.

Clinical suspicion of TE is most commonly confirmed by Doppler ultrasonography (US), echocardiography, computed tomography (CT) or magnetic resonance (MRI), depending on the location. Compression Doppler US is most frequently used for diagnosing limb TE in children. Vessels with thrombosis are identified by absent Doppler signals and non-compressibility of the affected vein, with or without visible intraluminal thrombotic material. As with all US-based imaging, diagnostic yield depends on the experience of the operator and patient compliance. Specific anatomic regions such as the upper intrathoracic venous system, are more difficult to access, influencing the sensitivity of Doppler US. MRI (MR venography) or CT scan (CT venography) should be considered in cases of high suspicion when Doppler US fails to reveal thrombosis.

Conventional venography is still considered the gold standard for diagnosing VTE, although in practice, this technique is rarely used in children. However, injection of contrast through the line followed by X-ray is commonly used for diagnosis of CVC-related thrombosis or dysfunction.

CT pulmonary angiography (spiral CT) is the diagnostic modality of choice for suspected PE in children, in cases without contra-indications to contrast injection (6, 7). Ventilation perfusion scans are associated with lower irradiation but they can only be used in cooperative children with normal chest X-ray and no concurrent cardiopulmonary disease. They are more time-consuming and often require sedation, making them less widely applicable. In case of suspected PE, echocardiography is indicated to rule out signs of right ventricular dysfunction. Echocardiography may also be preferred for diagnosing PE in neonates.

There are no specific laboratory tests that can accurately confirm the diagnosis of thrombosis in children. In neonates, thrombosis is often associated with sudden onset unexplained thrombocytopenia, which should raise suspicion for CVC thrombosis. For example, thrombocytopenia associated with haematuria in a newborn is suggestive of RVT. Routine coagulation analyses such as aPTT and PT/INR, as well as fibrinogen levels, may indicate low or high coagulation factor levels. D-dimer testing has limited usefulness because it lacks specificity as high D-Dimer levels can also occur in pro-inflammatory states such as sepsis or malignancy.

Anticoagulation therapy

Anticoagulation therapy in children is usually initiated with heparinoids, either unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH), for at least 5 days. Treatment can then continue with either ongoing LMWH therapy, vitamin K antagonists (VKAs) or a direct oral anticoagulant (DOAC). There are relatively few clinical trials evaluating the use of these treatments in children across all age groups and various underlying pathologies, which limits evidence-based care. Additionally, there is a lack of general and global consensus regarding the best therapeutic and monitoring recommendations for the different treatment options. It is important to keep in mind that the use of antithrombotic drugs in children differs from adults, due to age-dependent differences in the haemostatic system and drug metabolism (8).

Children generally require higher doses of UFH and LMWH, with weight-specific doses varying depending on age (8). Heparin anticoagulant activity is related to its binding to antithrombin (AT), thereby activating AT. The response to heparinoids may be impaired by low AT levels, which can be related to AT deficiency, young age or iatrogenic factors such as asparaginase use. Treatment with UFH or LMWH in children therefore needs to be monitored through regular anti-Xa activity measurements, which assess the degree of inhibition of activated factor X by heparinoids, and according dose adaptations (Table 1). AT replacement therapy should be reserved for children who fail to respond clinically to heparinoid treatment and have low AT levels, as well as for children with inherited or iatrogenic AT deficiency, nephrotic syndrome, liver failure or disseminated intravascular coagulation (9).

VKAs are generally used for children who require long-term anticoagulant treatment, especially in indications where the safety and efficacy of DOACs are so far not well established or insufficient, such as thromboprophylaxis for mechanical valves or antiphospholipid syndrome. VKA treatment requires regular INR measurements that can be performed by home point-of-care systems, and regular dose adaptations. Control of adequate

TABLE 1: Proposed dose adaptations of LMWH in children, based on anti-Xa level measurements.

anti-Xa level (U/ml)	delay next injection	dose change
<0.35	no	increase by 25%
0.35-0.49	no	increase by 10%
0.5-1.0	no	no change
1.1-1.5	no	decrease by 20%
1.6-2.0	after 3 hours	decrease by 30%
>2.0	discontinue until anti-Xa ≤ 0.5	decrease by 40%

if dose is changed, recheck next day

INR ranges is challenging due to the myriad of factors influencing VKA induced anticoagulation, such as dietary factors, many medications and intercurrent viral infections.

In recent years, DOACs have more frequently been used in children, thanks to the paediatric development programs that have been performed. Their use is discussed in more detail elsewhere in this issue.

Thrombosis in specific situations and age groups

CVC-related thrombosis

CVC-related thrombosis represents the most frequent cause of secondary thrombosis in neonates, infants and children and is caused by endothelial damage, disruption of blood flow and infusion of potentially prothrombotic agents. Factors influencing catheter-related thrombosis risk extend beyond host-related elements such as congenital heart disease and low birth weight, which is associated with increased catheter-to-vessel size ratio. They also include catheter-related risk factors like the type of CVC (e.g., PICC lines), insertion technique and the nature of infused therapy (e.g., total parenteral nutrition) (10).

CVC-related thrombosis should be suspected whenever regular symptoms of limb thrombosis occur in the presence of a CVC, but equally in cases of repeated loss of CVC patency, CVC sepsis or the appearance of collateral circulation in the skin.

CVC-related thrombosis can be asymptomatic. The true incidence, clinical significance and complication risk of asymptomatic CVC-related thrombosis are unknown. Jones et al. reviewed the literature to determine the incidence of asymptomatic VTE after screening in different clinical settings, and found widely variable incidences, ranging from 5-15.2% in neonates to 5.9-65% in paediatric cancer patients, with critically ill children, children with congenital heart disease or other cohorts, displaying intermediate incidences ranging from 7.1 up to 46% (11).

Decisions about treatment of these asymptomatic VTEs depend on recurrence risk, residual thrombosis risk, post thrombotic syndrome occurrence and risk of embolism or mortality. Decisions about anticoagulation in unwell children should be informed by the risk of not treating balanced by the risk of acute or long-term morbidity.

Symptomatic CVC-related thrombosis, however, requires anticoagulant treatment, especially if extension of the thrombus occurs after initial observation in patients who were deemed too unwell to receive anticoagulant treatment. Given the risk of development of paradoxical emboli after CVC removal, it has been suggested that the CVC be removed after 3-5 days of treatment, especially in neonates (8). However, based on two multicentre observational studies that addressed this question and could not corroborate this finding, the most recent guidelines do not advise against immediate removal of a non-functioning catheter (12-14). Duration of treatment is generally shorter than in non-CVC related thrombosis, and can be limited to 6 weeks, especially after CVC removal and complete thrombus resolution. Prophylactic anticoagulation should be considered, however, if the CVC is still in place at the end of the therapeutic treatment period.

Neonates

Neonates represent the paediatric age group associated with the highest thrombosis risk, which is associated with gestational diabetes, the use of intravascular catheters in small vessels, sepsis, inflammation and hypoxia.

Treatment decisions for neonatal thrombosis should be based on careful consideration of the haemorrhagic risk versus anticoagulation benefit. In cases with increased haemorrhagic risk, such as low gestational age and/or weight, necrotizing enterocolitis, intraventricular haemorrhage or other co-morbidities, initial observation may be preferred before initiation of anticoagulation therapy. If thrombosis extension is identified after initial radiologic observation of the thrombus, anticoagulation is recommended.

Renal vein thrombosis

RVT is the most prevalent form of thrombosis in neonates, apart from CVC-related thrombosis, and should be suspected in cases of abdominal pain, abdominal mass, haematuria and/or thrombocytopenia.

Anticoagulation therapy is recommended because of the potential benefit for reducing hypertension and kidney function damage, although 75% of affected kidneys become atrophic regardless of whether anticoagulation treatment is initiated. In cases of bilateral RVT, thrombolytic treatment followed by anticoagulation is suggested (14).

Anticoagulation therapy in the setting of neonatal RVT requires strict therapeutic monitoring (anti-Xa), especially if LMWH are used, because of potential drug accumulation in the presence of renal failure. Continuous infusion of UFH may be the preferred treatment option. Evidence regarding the use of DOACs in this setting is limited (15).

Purpura fulminans

Purpura fulminans is a rare, life-threatening condition caused by congenital severe (homozygous or compound heterozygous) protein C and/or protein S deficiency and presents in the first days of life with progressive haemorrhagic skin necrosis. It may be associated with concurrent large vessel thrombosis (16).

Later-onset purpura fulminans may be due to consumption or decreased synthesis of protein C and S due to sepsis or hepatic failure or acquired antibodies to protein S in the setting of chickenpox.

Treatment should consist of the prompt initiation of anticoagulation therapy and urgent analysis of protein C and S levels. Fresh frozen plasma infusions and/or administration of protein C concentrate should be added after confirmation of the deficiency. Genetic confirmation of *PROC* and *PROS1* genes and genetic consultation should be offered.

Cancer

Paediatric cancer patients are particularly prone to the development of VTE and its aetiology is multifactorial. Prevalence is variable, from 16% of symptomatic to 40% of asymptomatic TE in children with cancer (17). Occurrence of TE in children with cancer is related to the interaction of cancer with prothrombotic factors in the patient, and factors related to cancer treatment.

For example, children with acute lymphoblastic leukaemia (ALL) are shown to have activated coagulation at diagnosis (18). Cancer in general can lead to pro-inflammatory states. Chemotherapeutic agents such as Asparaginase influence the production of anticoagulant and procoagulant factors and cause endothelial activation. The presence of a CVC and surgical procedures further lead to endothelial damage and activation.

The decision to treat VTE in cancer patients is particularly challenging because of the delicate risk/benefit equilibrium and the haemorrhagic risk in these patients, and should be made on an individual basis. Most experts recommend maintaining a platelet count of greater than 50 x 10^9/L for anticoagulated patients. Special attention is required for anticoagulated patients undergoing invasive procedures.

For patients who develop thrombo-embolic complications after asparaginase therapy, it is generally recommended to restart asparaginase treatment provided the thrombosis is stabilized after treatment, and to continue thromboprophylaxis until at least 4 weeks after the last dose of PEGylated asparaginase.

Thrombosis in paediatric cancer patients can be treated by either LMWH or DOACs such as rivaroxaban (19).

Congenital heart disease

Paediatric cardiac disease represents an important risk factor for the development of thrombo-embolic complications. VTE in the setting of paediatric cardiac surgery occurs in 19-40% of children. Prevalence is influenced by the type of surgery, the severity of the heart defect and the degree of cardiac insufficiency. This patient group is also particularly at risk for arterial ischemic stroke. Management of these thrombotic complications does not differ substantially from TE in other children. Long-term thromboprophylaxis is required for patients with mechanical valves or other risk factors that predispose to a high risk of thrombosis, such as coronary dilatation after Kawasaki disease, or uncorrected states of cardiac insufficiency (16).

Cerebral sinovenous thrombosis

Thrombosis occurring in the venous sinuses of the brain results in venous outflow obstruction, causing a specific set of symptoms and complications. Subsequent increase in capillary hydrostatic pressure may lead to infarction, which is often haemorrhagic.

The occurrence of CSVT is multifactorial, and a comorbid condition can usually be identified (20). Around 40% of cases occur in the neonatal period. Symptoms can be non-specific, especially in neonates, and consist of progressive headache, altered consciousness, seizures and focal neurological deficits. Diagnosis is confirmed by contrast-enhanced venous imaging by MRI or CT. Mortality may be as high as 10% in neonates and older children, whereas up to 40-50% of patients suffer long-term neurological sequelae (21).

Anticoagulant treatment with LMWH or DOACs for a minimum of 3 months is recommended, and may be prolonged if imaging studies reveal persistent thrombosis, especially in the presence of ongoing symptoms (8, 14). Specific consideration is required for neonates, patients with associated haemorrhage or infection. However, haemorrhagic transformation of an infarction is not a contraindication for anticoagulation in the setting of CSVT. Treatment duration may be safely reduced to 6 weeks for patients with low-risk CSVT such as partially occlusive thrombosis and the absence of ongoing risk factors (22).

Thrombophilia

Thrombophilia refers to the propensity to form blood clots and may be inherited or acquired. Thrombophilia contributes to the risk of VTE, but given the fact that TE most commonly occurs in the setting of certain clinical conditions as described above, it constitutes rather a risk factor than an isolated cause of most occurrences of TE in children. The inherited thrombophilias for which a pathogenic link is most clearly established include the Factor V Leiden mutation, the prothrombin G20210A mutation, antithrombin deficiency, protein C deficiency and protein S deficiency (5, 23). Acquired risk factors include the presence of antiphospholipid antibodies (lupus anticoagulant, anti-cardiolipid antibodies, anti-b2-glycoprotein antibodies). Other factors, with less clear association to thrombotic risk include elevated factor VIII, elevated lipoprotein(a) and hyperhomocystinaemia. Some children may present a combination of thrombophilic factors, further increasing their thrombotic risk.

Indications for thrombophilia testing remain based on expert opinion, given the small patient numbers and heterogeneity of patient groups, as well as the multifactorial origin of paediatric thrombosis. Most importantly, the usefulness of diagnostic testing should be discussed on an individual basis. These discussions should include detailed information on the pros and cons of testing, as well as on adequate information regarding consequences of positive or negative results.

Thrombophilia testing is indicated, however, in cases of unprovoked or recurrent VTE. It is generally not indicated for patients with CVC-related VTE.

For patients with other provoked VTE, the utility for testing should be discussed with patients and parents.

Thrombophilia test results rarely influence acute treatment decisions in children with VTE, with the exception of neonates presenting with purpura fulminans, for whom AT, protein C and S analysis is recommended. For all other cases, test results do currently not provide a risk-based therapeutic approach.

Testing of asymptomatic children based on positive family history of VTE or thrombophilia remains controversial. It may be considered in situations where the child will be exposed to other thrombotic risk factors such as oral contraceptives, or cases of severe thrombo-embolic family history.

In all other cases, it is reasonable to postpone testing in asymptomatic children until they reach an age at which they can decide for themselves whether or not they want to know that they may be at increased risk for thrombosis.

In the case that the patient and the family decide against thrombophilia testing for a first VTE or positive family history, strict thromboprophylactic measures are indicated in situations of increased risk, such as prolonged immobilisation, complex surgery, CVC, etc.

Thrombophilia testing in the acute thrombotic phase may be difficult and should in most cases be deferred for 3-6 months and until after stopping the anticoagulant treatment. As for most haemostasis laboratory analysis, abnormal test results should be confirmed on a separate blood sample. Inherited thrombophilia should additionally be confirmed by testing of both parents whenever possible.

Conclusion

Paediatric thrombosis, while historically considered of little significance, is increasingly recognized, especially in neonates and adolescents, and is most frequently associated with underlying co-morbidities such as cancer, the presence of a CVC or heart disease. The diagnostic and therapeutic approach must take into consideration the unique developmental aspects of the haemostatic system in children, which means that adult guidelines must not be extrapolated. Continued research and dedicated clinical trials are essential to addressing the many remaining unresolved questions, to establish evidence-based guidelines and improve the management of paediatric thrombosis.

References

1. Andrew M, Paes B, Milner R, Johnston M, Mitchell L, Tollefsen DM, et al. Development of the human coagulation system in the full-term infant. Blood. 1987;70(1):165-72.
2. Andrew M, Paes B, Milner R, Johnston M, Mitchell L, Tollefsen DM, et al. Development of the human coagulation system in the healthy premature infant. Blood. 1988;72(5):1651-7.
3. Andrew M, Paes B, Johnston M. Development of the hemostatic system in the neonate and young infant. Am J Pediatr Hematol Oncol. 1990;12(1):95-104.

4. van Ommen CH, Peters M. Venous thromboembolic disease in childhood. Semin Thromb Hemost. 2003;29(4):391-404.
5. Young G, Albisetti M, Bonduel M, Brandao L, Chan A, Friedrichs F, et al. Impact of inherited thrombophilia on venous thromboembolism in children: a systematic review and meta-analysis of observational studies. Circulation. 2008;118(13):1373-82.
6. Lassandro G, Palmieri VV, Palladino V, Amoruso A, Faienza MF, Giordano P. Venous Thromboembolism in Children: From Diagnosis to Management. Int J Environ Res Public Health. 2020;17(14).
7. Chalmers E, Ganesen V, Liesner R, Maroo S, Nokes T, Saunders D, et al. Guideline on the investigation, management and prevention of venous thrombosis in children. Br J Haematol. 2011;154(2):196-207.
8. Monagle P, Chan AKC, Goldenberg NA, Ichord RN, Journeycake JM, Nowak-Gottl U, et al. Antithrombotic therapy in neonates and children: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141(2 Suppl):e737S-e801S.
9. Monagle P, Cuello CA, Augustine C, Bonduel M, Brandao LR, Capman T, et al. American Society of Hematology 2018 Guidelines for management of venous thromboembolism: treatment of pediatric venous thromboembolism. Blood Adv. 2018;2(22):3292-316.
10. Pelland-Marcotte MC, Avila L, Temple M, Williams S. Venous Thrombosis. In: Blanchette V, Brandao L, Carcao M, Kumar R, Rand M, Revel-Vilk S, editors. SickKids Handbook of Pediatric Thrombosis and Hemostasis. 3rd. rev. an ext. ed. Basel, Switzerland: Karger AG; 2024. p. 256-74.
11. Jones S, Monagle P, Newall F. Do asymptomatic clots in children matter? Thromb Res. 2020;189:24-34.
12. van Ommen CH, Bergman KA, Boerma M, Bouma HA, Donker AE, Gouvernante M, et al. NEOnatal Central-venous Line Observational study on Thrombosis (NEOCLOT): evaluation of a national guideline on management of neonatal catheter-related venous thrombosis. J Thromb Haemost. 2023;21(4):963-74.
13. Jaffray J, Baumann Kreuziger L, Branchford B, Wee CP, Faustino EVS, Zakai NA, et al. Symptomatic pulmonary embolus after catheter removal in children with catheter related thrombosis: A report from the CHAT Consortium. J Thromb Haemost. 2022;20(1):133-7.
14. Monagle P, Azzam M, Bercovitz R, Betensky M, Bhat R, Biss T, et al. American Society of Hematology/International Society on Thrombosis and Haemostasis 2024 updated guidelines for treatment of venous thromboembolism in pediatric patients. Blood Adv. 2025;9(10):2587-636.
15. Labarque V, Mandel-Shorer N, Robertson J, Williams S. Thromboembolic Events at Specific Organ Sites. In: Blanchette V, Brandao L, Carcao M, Kumar R, Rand M, Revel-Vilk S, editors. SickKids Handbook of Pediatric Thrombosis. 3rd. rev. and ext. ed. Basel Switzerland: Karger AG; 2024. p. 288-306.
16. Sabapathy C, Amid A, Kumar R, Avila L, van Ommen CH. Diagnostic Approach to Thrombosis and Thrombophilia. In: Blanchette V, Brandao L, Carcao M, Kumar R, Rand M, Revel-Vilk S, editors. SickKids Handbook of Pediatric Thrombosis. 3rd. rev. and ext. ed. Basel Switzerland: Karger AG; 2024. p. 233-55.
17. Athale U. Thrombosis in pediatric cancer: identifying the risk factors to improve care. Expert Rev Hematol. 2013;6(5):599-609.
18. Athale UH, Chan AK. Thromboembolic complications in pediatric hematologic malignancies. Semin Thromb Hemost. 2007;33(4):416-26.
19. Palumbo JS, Lensing AWA, Brandao LR, Hooimeijer HL, Kenet G, van Ommen H, et al. Anticoagulation in pediatric cancer-associated venous thromboembolism: a subgroup analysis of EINSTEIN-Jr. Blood Adv. 2022;6(22):5821-8.
20. Pulcine E, Andrade A, Vargas Nino AC, Williams S, Ichord RN, Dlamini N. Stroke. In: Blanchette V, Brandao L, Carcao M, Kumar R, Rand M, Revel-Vilk S, editors. SickKids Handbook of Pediatric Thrombosis. 3rd. rev. and ext. ed. Basel Switzerland: Karger AG; 2024. p. 307-35.
21. Moharir MD, Shroff M, Pontigon AM, Askalan R, Yau I, Macgregor D, et al. A prospective outcome study of neonatal cerebral sinovenous thrombosis. J Child Neurol. 2011;26(9):1137-44.
22. Goldenberg NA, Kittelson JM, Abshire TC, Bonaca M, Casella JF, Dale RA, et al. Effect of Anticoagulant Therapy for 6 Weeks vs 3 Months on Recurrence and Bleeding Events in Patients Younger Than 21 Years of Age With Provoked Venous Thromboembolism: The Kids-DOTT Randomized Clinical Trial. JAMA. 2022;327(2):129-37.
23. van Ommen CH, Nowak-Gottl U. Inherited Thrombophilia in Pediatric Venous Thromboembolic Disease: Why and Who to Test. Front Pediatr. 2017;5:50.

Direct Oral Anticoagulants in the Field of Paediatric Thrombosis

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Abstract
Direct oral anticoagulants (DOACs) have largely replaced traditional anticoagulants in adults for many years. However, their use in children has only emerged more recently. In this narrative review, we provide an overview on the current knowledge regarding DOACs in paediatric thrombosis.

Rivaroxaban and dabigatran have been shown to be safe and effective options for the treatment of venous thromboembolic events in children. In addition, apixaban, edoxaban and rivaroxaban can be used safely for thromboprophylaxis in children with congenital heart disease who are at high risk for thrombosis. In contrast, the benefits of DOACs in the treatment of arterial thrombosis, stroke, or in the prevention of thromboembolic events in children with leukaemia remain less well established. Owing to their oral administration, predictable pharmacokinetics, fixed dosing, and minimal monitoring requirements, DOACs represent an important advancement in paediatric anticoagulation and offer effective, practical alternatives to traditional therapies. Ongoing research and real-world data will be essential to further define their role across the full spectrum of paediatric thromboembolic disease.

Introduction

Thrombo-embolic events (TEs) are an increasingly recognized clinical problem in children, due to improvements in diagnostic imaging, increased clinical awareness, and better survival of children with complex medical conditions. The treatment and prevention of TEs have long relied on traditional anticoagulants, including unfractionated heparin (UFH), low molecular weight heparin (LMWH), and vitamin K antagonists (VKAs). However, most practices for use of these drugs in children were extrapolated from adult data or based on observational studies and small-scale trials. Moreover, these anticoagulants require injections and/or regular monitoring and dose adjustments, which can be burdensome for both patients and providers.

In recent years, direct oral anticoagulants (DOACs) have emerged as a promising alternative and are increasingly used in paediatric patients (1). These drugs offer oral administration, predictable pharmacokinetics, and fixed dosing regimens, potentially simplifying care and improving quality of life for the patients. Additionally, clinical trials to establish the safety, efficacy, and appropriate use of DOACs in children are available. Thus, DOACs are expected to replace traditional anticoagulants for many indications, making it important for clinicians to become well-acquainted with their characteristics and use.

This review aims to provide an overview of the available evidence on the use of DOACs in the treatment and prevention of thrombosis in children.

Classification, pharmacology, bleeding complications and reversal of DOACs

DOACs fall into two classes based on their mechanism of action: direct thrombin inhibitors and factor Xa inhibitors. Dabigatran (Pradaxa®) is currently the only direct thrombin inhibitor, while factor Xa inhibitors include apixaban (Eliquis®), rivaroxaban (Xarelto®), and edoxaban (Lixiana®). These drugs differ in pharmacokinetics and renal clearance with apixaban being less dependent on renal excretion, potentially making it safer for patients with impaired renal function. Although serum drug levels can be measured for DOACs, their clinical significance is unclear. For example, only a weak correlation between apixaban levels and clinical effect was observed in paediatric cardiac patients (2). Nevertheless, measuring levels could be considered in special situations, such as renal dysfunction, post-pyloric feeding, possible drug–drug interactions.

Overall, clinical trials have demonstrated that DOACs are safe for use in paediatric populations. The most common bleeding symptoms in children treated with DOACs include epistaxis (8.4%), subcutaneous hematoma (6.4%), and wound haemorrhage (3.7%) but major and clinically relevant non-major (CRNM) bleeding episodes are rare (2.4%) (3). In a comparative analysis, 9.4% of the 9,470 patients on rivaroxaban, apixaban or edoxaban reported one or more relevant bleeding events at least 7 days after their first prescription, while higher rates were observed among patients treated with LMWH (14.1%; n=51,330) and warfarin (16.1%; n=6,330)

(4). Notably, neonates, thrombocytopenic cancer patients or those at high risk for bleeding, and patients with renal or liver failure were underrepresented or excluded from trials, limiting information on the use of DOACs in these patient groups. Moreover, reversing DOACs remains challenging. Idarucizumab is under investigation in children as a reversal agent for dabigatran, while andexanet alfa, approved in adults for factor Xa inhibitors, is expensive, not widely available, and lacks paediatric data. Prothrombin complex concentrates could serve as an alternative option, though they are not specifically approved for this purpose. In case elective surgery is scheduled, DOACs should be discontinued 24-48 hours before, depending on the renal function and type of surgery (risk of bleeding).

The use of DOACs in the treatment of venous thrombo-embolic events

Rivaroxaban and dabigatran have emerged as the most studied DOACs in the treatment of paediatric venous thrombo-embolic events (VTEs) (5). Overall, a meta-analysis of 3 randomized clinical trials including 790 children with a VTE, demonstrated that DOACs were associated with a reduced risk of VTE recurrence compared to standard anticoagulation (risk difference = -3%, p = 0.04), while there was no significant difference in mortality or bleeding events (6). Based on this evidence, both rivaroxaban and dabigatran received EMA approval for treatment of paediatric VTE in 2020. To date, available data on the efficacy and safety of apixaban in the treatment of VTE in children are limited to adolescents, whereas results of a randomized trial with edoxaban are still awaiting publication. It should also be noted that the use of DOACs is contra-indicated in patients with confirmed antiphospholipid syndrome, due to the increased risk of TEs shown in adults (7). Table 1 provides an overview of studies of DOACs in children.

Rivaroxaban (EINSTEIN-Jr)

Thrombus resolution occurred in a similar frequency in patients treated with rivaroxaban (47%) compared to those receiving standard-of-care (SOC) (42%) and recurrence rates were similarly low (1% and 3% respectively). Major and CRNM bleeding events occurred in 0% and 3%, respectively, in the rivaroxaban group compared to 1% and less than 1% in the SOC group (8). After completing the initial 3-month treatment phase, patients could continue with secondary prophylaxis for up to 1 year. During the extended-phase treatment, recurrent VTE occurred in three of the 214 children (1%; cumulative incidence 3%; 95% CI 0.9-9.8). No major bleeding events were reported and CRNM bleeding episodes occurred in 3 children on rivaroxaban (2%). No deaths were observed during the study (9).

Dabigatran (DIVERSITY)

This study demonstrated that dabigatran was non-inferior to SOC for thrombus resolution (46% for dabigatran; 42% for SOC) and recurrent VTE (4% for dabigatran; 8% for SOC). No deaths were reported in either group. The incidence of bleeding symptoms were the same for both groups (major and CRNM bleeding in 2% and 1% respectively, in each group) (10). In a subgroup analysis, Brandão et al. demonstrated that among patients with inherited thrombophilia, thrombus resolution and recurrence-free interval was better in those who received dabigatran (36%) than in those treated with SOC (22%) (11). For patients requiring secondary prophylaxis (n=203), dabigatran was administered for up to 12 months and was associated with 1.0% recurrence rate, 1.5% major bleeding events, and 1.0% CRNM bleeding events. There were no deaths reported (12).

Apixaban

In this open-label phase 2 study, 63.6% (14 of 22) of adolescents with a VTE achieved a complete response after 3 months of treatment, while the others demonstrated a partial response. No patients experienced a new VTE and no major or CRNM bleeding events occurred (13). In a separate case report, Manis et al. documented the successful use of apixaban in combination with thrombectomy in a 17-year-old boy with Paget-Schroetter syndrome complicated by VTE (14).

Edoxaban (Hokusai VTE PEDIATRICS Study (NCT02798471))

Preliminary data available on ClinicalTrials.gov show that recurrent VTE occurred in 3.4% and 4.8% of patients treated with edoxaban for 3 months and 12 months respectively, compared to 1.4% and 1.4% in the SOC group at both time points. The difference was not significant at 3 months but the analysis is not yet available for the 12 months' time point. Major and CRNM bleeding were reported in 2.1% for the edoxaban group versus 3.5% in the SOC group.

Real-world data

A single-centre retrospective review provided real-world evidence on the use of DOACs in 65 children with VTE (rivaroxaban in 61.5%, apixaban in 37%, and dabigatran in 1.5%). The median duration of follow-up was 11.5 months (ranging from 12-120 months). Among these patients, six (9.2%) experienced recurrent VTE (one on apixaban and five on rivaroxaban). A major bleeding episode occurred in one surgical patient (2%), while six patients (9%) had CRNM bleeding. These findings indicate a higher risk of recurrent VTE compared to randomized trials, likely due to ongoing risk factors (including anti-phospholipid syndrome and systemic lupus erythematosus in 1 patient each) and longer follow-up. Nevertheless, bleeding rates were consistent with findings from previous trials (15).

The use of DOACs in the treatment of arterial thrombosis and stroke

The use of DOACs in the treatment of paediatric arterial thrombosis and/or stroke is limited to a few cases, hence lacking evidence. Gupta et al. described a 6-year old boy with inherited thrombophilia who was treated with rivaroxaban for a posterior a. basilaris stroke (16). He experienced no recurrence during the first 6 months of treatment. In addition, apixaban was used for the treatment of an intracardiac thrombus in 3 patients (2-6 years old) with congenital heart disease (17). Complete resolution was obtained in 2 of them, a partial resolution in the other patient. No clinical bleeding events occurred.

DOACs as thromboprophylaxis in acute lymphoblastic leukaemia and lymphoblastic lymphoma

During induction therapy for acute lymphoblastic leukaemia or lymphoblastic lymphoma, children face an increased risk of TEs. The Phase 3 PREVAPIX-ALL trial tested apixaban in this high-risk population, in a randomization to no anticoagulation. VTE occurred in 12% (31/256) of the apixaban group versus 18% (45/256) in the control group (p = 0.080), a non-significant difference. One death in the SOC group was attributed to a haemorrhagic cerebral sinus vein thrombosis. Major bleeding was equal (2 patients per group),

TABLE 1: Overview of studies investigating the use of DOACs in children.

DOAC	Phase/design/ comparator	Age, number (n)	Efficacy	Safety
Treatment of VTE				
Rivaroxaban (8) (EINSTEIN-Jr)	Phase 3, randomized, SOC	> 37 wks. GA; n = 500	CR: 47% vs 42% Improvement: 29% vs 33% Recurrence: 1% vs 3%	Major bleed: 0% vs 1% CRNMB: 3% vs 1%
Dabigatran (10,11) (DIVERSITY)	Phase 2b/3, randomized, SOC	3 mo. – 18 y; n = 267	Resolution: 46% vs 42% 36% vs 22% (inherited thrombophilia) Recurrence: 4% vs 8%	Major bleed: 2% in each group CRNMB: 1% in each group
Apixaban (13)	Phase 2, open-label	Adolescents > 40 kg; n = 26	CR: 64% PR: 36% Recurrence: 0%	No bleeding events
Edoxaban (Hokusai VTE PEDIATRICS Study)	Phase 3, randomized, SOC	n = 290	Recurrence: 3% vs 1% at 3 mo. 5% vs 1% at 12 mo.	Major and CRNMB: 2.1% vs 3.5%
Rivaroxaban, apixaban or dabigatran (15)	Real-world	n = 65	Recurrence: 9%	Major bleed: 2% CRNMB: 9%
Thromboprophylaxis in ALL and LBL				
Apixaban (18) (PREVAPIX-ALL)	Phase 3, randomized, no anticoagulation	1-18 y; n = 265	VTE occurrence: 12% vs 18%	Major bleed: 0.8% CRNMB: 4% vs 1%
Thromboprophylaxis in congenital heart disease				
Rivaroxaban (19)* (UNIVERSE)	Phase 3, randomized, aspirin	2-8 y; n = 100	TE occurrence: 2% vs 9%	Bleeding: 36% vs 41%
Apixaban (23)*	Real-world	≤ 18 y n = 62	TE occurrence: 0.07 per 1000 person-days	Major or CRNMB: 0.22 per 1000 person-days
Apixaban (22) (SAXOPHONE)	Phase 2, randomized, SOC	28 d – 18 y; n = 192	TE occurrence: 0%	Major or CRNMB: 0.8% vs 4.8%
Apixaban (2)	Real-world	< 19 y; n = 172	TE occurrence: 0%	Major bleed: 0.5% CRNMB: 1.7%
Edoxaban (27) (ENNOBLE-ATE)	Phase 3, randomized, SOC	< 18 y, n = 167	TE occurrence: 0% vs 1.7%	Major or CRNMB: 0.9% vs 1.7%
Edoxaban (28)	Real-world	< 18 y, n = 1651	Event-free survival 88% at 10 y, 70% at 12 y	none

* included only Fontan patients; ALL: acute lymphoblastic leukemia; CR: complete resolution; CRNMB: clinically relevant non-major bleed; DOAC: direct oral anticoagulant; GA: gestational age; LBL: lymphoblastic lymphoma; PR: partial resolution; SOC: standard-of-care; (V)TE: (venous) thrombo-embolic event; y: years

but CRNM bleeding was higher with apixaban (4% vs. 1%, p = 0.030). Overall, these findings demonstrate that apixaban is safe but did not significantly reduce VTE in these children, limiting its routine use for thromboprophylaxis (18).

DOACs as thromboprophylaxis in congenital heart disease

Beyond malignancies, children with congenital heart disease are also at increased risk for thrombosis. This is especially true after a Fontan procedure in single-ventricle patients, where thrombosis remains a common complication. Other high-risk conditions include heart failure, giant coronary artery aneurysms due to Kawasaki disease, ventricular assist devices, and mechanical heart valves. Standard prophylaxis for these patients includes LMWH, VKA, and/or platelet antagonists, but their limitations have led to growing interest in DOACs as a more convenient and potentially effective alternative. Several clinical trials and real-world studies on rivaroxaban or apixaban have indeed highlighted their potential for the prevention of thrombosis after a Fontan procedure. Furthermore, evidence for the use of DOACs in children with other cardiac conditions is also growing. Particularly, apixaban and edoxaban have been studied in these patients.

Rivaroxaban

In the phase 3 UNIVERSE study (19), patients within 4 months of a Fontan procedure had similar TE rates with rivaroxaban (2%; 1/66) compared to aspirin (9%; 3/34) during a 12 month treatment. Moreover, the proportion of bleeding events was similar in both groups (36% vs 41%).

The use of rivaroxaban in non-Fontan paediatric cardiac patients is limited. Montanez et al. described an 8-year-old boy with a dilated coronary artery aneurysm secondary to Kawasaki disease in whom rivaroxaban appeared to be a safe and effective option for thromboprophylaxis (20). Additionally, a small real-world case series reported no new thrombus formation and few complications in 15 children on rivaroxaban (including those with Fontan circulation, high-risk surgical shunts and high-risk stent angioplasty) (21).

Apixaban

The phase 2 SAXOPHONE study evaluated apixaban for 1 year in children with single ventricle physiology (74%), Kawasaki disease (14%), and other congenital or acquired heart conditions (22). Major or CRNM bleeding occurred in 0.8% (1/129) with apixaban compared to 4.8% (3/63) with SOC. However, overall bleeding events were more frequent with apixaban (100 vs. 58.2 events per 100 patient-years), primarily due to 12 participants with ≥4 minor bleeding events. Importantly, no TEs or deaths occurred in either group. Real-world evidence further supports the efficacy and safety of apixaban in 219 cardiac patients ≤19 years (median age of 6.8 years). This retrospective single-centre analysis demonstrated no clinical signs or imaging evidence of new thrombus formation in any of the 172 patients requiring thromboprophylaxis post-cardiac surgery (42%), for failing Fontan physiology (13%), Kawasaki disease (8%), cardiomyopathy and heart failure (9%), and other cardiac reasons (21%) (2). There were 1 major and 3 CRNM bleeding events. The efficacy and safety of apixaban in patients with Fontan is also supported by another real-world study that reported only 1 TE (0.07 per 1000 person-days) and 3 serious or CRNM bleeding events (0.22 per 1000 person-days) (23).

Furthermore, apixaban was administered prophylactically to 18 children awaiting a heart transplant. Of these, 3 patients had a ventricular assist device. No increased perioperative bleeding, no major or CRNM bleeding episodes nor TEs were reported (24). Similarly, Kobayashi et al. as well as Van Edom et al. demonstrated in small case series that apixaban can be used in children and adolescents supported with the HeartMate 3 ventricular assist device (25,26).

Edoxaban

The Phase 3 ENNOBLE-ATE trial demonstrated the efficacy and safety of oral edoxaban once daily for 3 months with an open-label extension up to 1 year in patients with Fontan circulation (44%), Kawasaki disease (22%), heart failure (4%) and other cardiac disorders (30%). No TEs were reported with edoxaban versus 1.7% with SOC (27). Bleeding rates were similarly low in both groups. Real-world data on edoxaban use in children and adolescents with a history of Kawasaki disease and giant coronary artery aneurysms demonstrated event-free survival rates of 88% at 10 years and 70% at 12 years from diagnosis (28). These outcomes are comparable to the expected risk for thrombosis of 18 +/- 2% per 10 patient-years, as calculated from the International Kawasaki Disease Registry (29).

Mechanical and bioprosthetic valves

To date, only 1 single-centre study in adults with bioprosthetic aortic or mitral valves (ENAVLE) is available, demonstrating non-inferiority of edoxaban compared to warfarin for the prevention of TEs (30). On the contrary, a higher incidence of both thrombotic and bleeding events compared to warfarin has been reported in adults with mechanical valves receiving dabigatran (RE-ALIGN study) (31). Although the use of DOACs in patients with either mechanical or bioprosthetic valves is increasing (32), evidence is limited and the use of DOACs in these patients therefore remains relatively contra-indicated.

Anticoagulation using DOACs in congenital vascular malformations

DOACs are increasingly adopted to treat painful venous malformations (VMs) in adults. However, limited data exist regarding their use in paediatric patients. In 2017, Yasumoto et al. reported the first successful use of dabigatran to treat consumptive coagulopathy in a 17-year-old adolescent (33). More recently, a case series involving seven children with painful VMs and chronic intravascular consumptive coagulopathy, demonstrated that treatment with DOACs (dabigatran in 2 patients, rivaroxaban in 5 patients) resulted in effective pain relief and improved coagulation profiles, with no reported adverse events. These findings suggest that DOACs may represent a viable treatment option for children with VMs complicated by intravascular coagulopathy (34).

Conclusion

DOACs are increasingly being studied in the paediatric population and have been shown to be effective and safe in treating VTE as well as preventing TEs in children with congenital heart disease who have a high risk of thrombosis. However, the benefit of DOACs in treating arterial thrombosis or stroke has not yet been fully established. Further research and real-world data are urgently needed to better define the role of DOACs for all possible indications in children.

Statement

The author has no conflicts of interest to disclose relating to the topic discussed in this manuscript.

REFERENCES

1. Mack, M.; Xavier, F.; McCormick, M. Patterns of Direct Oral Anticoagulant Use in Pediatric Patients: Results From a Multicenter National Database. Pediatric Blood & Cancer 2025, 72, e31816, doi:10.1002/pbc.31816.
2. VanderPluym, C.; Esteso, P.; Ankola, A.; Hellinger, A.; Ventresco, C.; Hawkins, B.; et al. Real-World Use of Apixaban for the Treatment and Prevention of Thrombosis in Children with Cardiac Disease. Journal of Thrombosis and Haemostasis 2023, 21, 1601–1609, doi:10.1016/j.jtha.2023.03.005.
3. Chong, S.; Sun, L.; Mu, G.; Hua, M.; Xiang, Q.; Cui, Y. Adverse Events of Factor Xa Inhibitors in Pediatric Patients: A Meta-Analysis and Pharmacovigilance Study. Pediatr Drugs 2025, 27, 351–366, doi:10.1007/s40272-024-00665-3.
4. D'Abrantes, S.; Male, C.; Brown, N.; Bjursell, M.; Ambery, P.; Berggren, A.; et al. Anticoagulant Prescribing Trends, Bleeding Events, and Reversal Agent Use in Pediatric Patients: A Retrospective, Real-World Study. PLoS One 2025, 20, e0323137, doi:10.1371/journal.pone.0323137.
5. Al-Ghafry, M.; Sharathkumar, A. Direct Oral Anticoagulants in Pediatric Venous Thromboembolism: Review of Approved Products Rivaroxaban and Dabigatran. Front. Pediatr. 2022, 10, 1005098, doi:10.3389/fped.2022.1005098.
6. Yu, X.; Zhu, W.; Liu, C.; Lu, R. Direct Oral Anticoagulants for Treating Acute Venous Thromboembolism in Children: Meta-Analysis of Randomized Controlled Trials. Clin Appl Thromb Hemost 2024, 30, 10760296241271386, doi:10.1177/10760296241271386.
7. Ortel, T.L.; Neumann, I.; Ageno, W.; Beyth, R.; Clark, N.P.; Cuker, A.; Hutten, B.A.; Jaff, M.R.; Manja, V.; Schulman, S.; 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, 4693–4738, doi:10.1182/bloodadvances.2020001830.
8. Thom, K.; Lensing, A.W.A.; Nurmeev, I.; Bajolle, F.; Bonnet, D.; Kenet, G.; Massicotte, M.P.; Karakas, Z.; Palumbo, J.S.; Saracco, P.; et al. Safety and Efficacy of Anticoagulant Therapy in Pediatric Catheter-Related Venous Thrombosis (EINSTEIN-Jr CVC-VTE). Blood Advances 2020, 4, 4632–4639, doi:10.1182/bloodadvances.2020002637.
9. Male, C.; Lensing, A.W.A.; Chan, A.K.C.; Kenet, G.; Young, G.; Bhat, R.; Pap, A.F.; Kubitza, D.; Prins, M.H.; Monagle, P.; et al. Extended-Phase Anticoagulant Treatment of Acute Venous Thromboembolism in Children: A Cohort Study from the EINSTEIN-Jr Phase 3 Trial. The Lancet Haematology 2025, 12, e357–e364, doi:10.1016/S2352-3026(25)00067-5.
10. Halton, J.; Brandão, L.R.; Luciani, M.; Bomgaars, L.; Chalmers, E.; Mitchell, L.G.; Nurmeev, I.; Sharathkumar, A.; Svirin, P.; Gorbatikov, K.; et al. Dabigatran Etexilate for the Treatment of Acute Venous Thromboembolism in Children (DIVERSITY): A Randomised, Controlled, Open-Label, Phase 2b/3, Non-Inferiority Trial. The Lancet Haematology 2021, 8, e22–e33, doi:10.1016/S2352-3026(20)30368-9.
11. Brandão, L.R.; Tartakovsky, I.; Albisetti, M.; Halton, J.; Bomgaars, L.; Chalmers, E.; Luciani, M.; Saracco, P.; Felgenhauer, J.; Lvova, O.; et al. Dabigatran in the Treatment and Secondary Prophylaxis of Venous Thromboembolism in Children with Thrombophilia. Blood Advances 2022, 6, 5908–5923, doi:10.1182/bloodadvances.2021005681.
12. Brandão, L.R.; Albisetti, M.; Halton, J.; Bomgaars, L.; Chalmers, E.; Mitchell, L.G.; Nurmeev, I.; Svirin, P.; Kuhn, T.; Zapletal, O.; et al. Safety of Dabigatran Etexilate for the Secondary Prevention of Venous Thromboembolism in Children. Blood 2020, 135, 491–504, doi:10.1182/blood.2019000998.
13. Pinchinat, A.; Levendoglu-Tugal, O.; Perez, Y.; Thatcher, E.; Otero, N.; Budhram, A.; Mahanti, H.; Morris, E.; Klejmont, L.; Schaefer, E.; et al. A Pilot Safety, Feasibility and Efficacy Study of an Oral Anticoagulant, Apixaban, in Secondary Prophylaxis of a Primary Venous Thromboembolism in Children and Adolescents. Br J Haematol 2023, 201, doi:10.1111/bjh.18804.
14. Manis, M.M.; Cummins, L.E.; Kyle, J.A.; Taylor, S.M. Successful Use of Apixaban for Paget-Schroetter Syndrome in a Pediatric Patient. The Journal of Pediatric Pharmacology and Therapeutics 2021, 26, 508–511, doi:10.5863/1551-6776-26.5.508.
15. Valenti, G.G.; Sabo, C.; Hyde, M.; Rajpurkar, M. Real-world Experience of Direct Oral Anticoagulant Use in a Single Pediatric Center. Pediatric Blood & Cancer 2024, 71, e30926, doi:10.1002/pbc.30926.
16. Gupta, J.; Garhwal, P.; Sharma, V.; Choudhary, R.; Sehra, R.N.; Devpura, K. Rivaroxaban in a Child with Inherited Thrombophilia and Stroke — New Kid on the Block. Indian J Pediatr 2024, 91, 1107–1107, doi:10.1007/s12098-024-05191-1.
17. Esch, J.J.; Hellinger, A.; Friedman, K.G.; VanderPluym, C.J. Apixaban for Treatment of Intracardiac Thrombosis in Children with Congenital Heart Disease. Interactive CardioVascular and Thoracic Surgery 2020, 30, 950–951, doi:10.1093/icvts/ivaa041.
18. O'Brien, S.H.; Rodriguez, V.; Lew, G.; Newburger, J.W.; Schultz, C.L.; Orgel, E.; Derr, K.; Ranalli, M.A.; Esbenshade, A.J.; Hochberg, J.; et al. Apixaban versus No Anticoagulation for the Prevention of Venous Thromboembolism in Children with Newly Diagnosed Acute Lymphoblastic Leukaemia or Lymphoma (PREVAPIX-ALL): A Phase 3, Open-Label, Randomised, Controlled Trial. The Lancet Haematology 2024, 11, e27–e37, doi:10.1016/S2352-3026(23)00314-9.
19. McCrindle, B.W.; Michelson, A.D.; Van Bergen, A.H.; Suzana Horowitz, E.; Pablo Sandoval, J.; Justino, H.; Harris, K.C.; Jefferies, J.L.; Miriam Pina, L.; Peluso, C.; et al. Thromboprophylaxis for Children Post-Fontan Procedure: Insights From the UNIVERSE Study. JAHA 2021, 10, e021765, doi:10.1161/JAHA.120.021765.
20. Montanez, N.; Sexson Tejtel, S.K.; Menon, N.M. Pediatric Thromboprophylaxis of Large Coronary Artery Aneurysm Using Rivaroxaban. Journal of Pediatric Hematology/Oncology 2023, 45, 356–359, doi:10.1097/MPH.0000000000002690.
21. Duran, S.; Gerber, C.; Bilsky, S.; Plummer, S.; Bocks, M. Use of Rivaroxaban in Children with Congenital Heart Disease: A Single-Center Case Series. Pediatr Cardiol 2024, 45, 1295–1300, doi:10.1007/s00246-022-03094-6.
22. Payne, R.M.; Burns, K.M.; Glatz, A.C.; Male, C.; Donti, A.; Brandão, L.R.; Balling, G.; VanderPluym, C.J.; Bu'Lock, F.; Kochilas, L.K.; et al. Apixaban for Prevention of Thromboembolism in Pediatric Heart Disease. Journal of the American College of Cardiology 2023, 82, 2296–2309, doi:10.1016/j.jacc.2023.10.010.
23. Adkins, K.; Williams, R.J.; Esteso, P.; Kobayashi, R.; Gauvreau, K.; VanderPluym, C.; Hellinger, A. Real-World Use and Outcomes of Apixaban for Early Post-Surgical Fontan Thromboprophylaxis. Pediatr Cardiol 2024, doi:10.1007/s00246-024-03641-3.
24. Benvenuto, V.; Hartje-Dunn, C.; Vo, L.; Hellinger, A.; Esteso, P.; Fynn-Thompson, F.; VanderPluym, C. Use of Apixaban in Children Awaiting Heart Transplantation. Pediatric Transplantation 2024, 28, e14632, doi:10.1111/petr.14632.
25. Kobayashi, R.L.; Cetatoiu, M.A.; Esteso, P.; Ventresco, C.; Hawkins, B.; Daly, K.P.; Blume, E.D.; Fynn-Thompson, F.; VanderPluym, C. Apixaban Anticoagulation in Children and Young Adults Supported With the HeartMate 3 Ventricular Assist Device. ASAIO Journal 2023, 69, e267–e269, doi:10.1097/MAT.0000000000001889.
26. Van Edom, C.J.; Cools, B.; Droogné, W.; Jacobs, S.; Van Puyvelde, J.; Vlasselaers, D.; Vanassche, T.; Meyns, B. Apixaban Plasma Levels in Patients with HeartMate 3 Support. The Journal of Heart and Lung Transplantation 2025, 44, 550–557, doi:10.1016/j.healun.2024.11.003.
27. Portman, M.A.; Jacobs, J.P.; Newburger, J.W.; Berger, F.; Grosso, M.A.; Duggal, A.; Tao, B.; Goldenberg, N.A.; Brothers, M.; Marino, B.; et al. Edoxaban for Thromboembolism Prevention in Pediatric Patients With Cardiac Disease. Journal of the American College of Cardiology 2022, 80, 2301–2310, doi:10.1016/j.jacc.2022.09.031.
28. Sagiv, E.; Newland, D.M.; Slee, A.; Olson, A.K.; Portman, M.A. Real-World Experience with Edoxaban for Anticoagulation in Children at Risk for Coronary Artery Thrombosis. Cardiol Young 2024, 34, 870–875, doi:10.1017/S1047951123003761.
29. McCrindle, B.W.; Manlhiot, C.; Newburger, J.W.; Harahsheh, A.S.; Giglia, T.M.; Dallaire, F.; Friedman, K.; Low, T.; Runeckles, K.; Mathew, M.; et al. Medium-Term Complications Associated With Coronary Artery Aneurysms After Kawasaki Disease: A Study From the International Kawasaki Disease Registry. JAHA 2020, 9, e016440, doi:10.1161/JAHA.119.016440.
30. Shim, C.Y.; Seo, J.; Kim, Y.J.; Lee, S.H.; De Caterina, R.; Lee, S.; Hong, G.-R.; Explore the Efficacy and Safety of Edoxaban in Patients after Heart Valve Repair or Bioprosthetic Valve Replacement (ENAVLE) study group Efficacy and Safety of Edoxaban in Patients Early after Surgical Bioprosthetic Valve Implantation or Valve Repair: A Randomized Clinical Trial. J Thorac Cardiovasc Surg 2023, 165, 58-67.e4, doi:10.1016/j.jtcvs.2021.01.127.
31. Eikelboom, J.W.; Connolly, S.J.; Brueckmann, M.; Granger, C.B.; Kappetein, A.P.; Mack, M.J.; Blatchford, J.; Devenny, K.; Friedman, J.; Guiver, K.; et al. Dabigatran versus Warfarin in Patients with Mechanical Heart Valves. N Engl J Med 2013, 369, 1206–1214, doi:10.1056/NEJMoa1300615.
32. Ryu, R.; Tran, R. DOACs in Mechanical and Bioprosthetic Heart Valves: A Narrative Review of Emerging Data and Future Directions. Clin Appl Thromb Hemost 2022, 28, 10760296221103578, doi:10.1177/10760296221103578.
33. Yasumoto, A.; Ishiura, R.; Narushima, M.; Yatomi, Y. Successful Treatment with Dabigatran for Consumptive Coagulopathy Associated with Extensive Vascular Malformations. Blood Coagulation & Fibrinolysis 2017, 28, 670–674, doi:10.1097/MBC.0000000000000666.
34. Boccara, O.; Bonigen, J.; Soupre, V.; Puzenat, E.; Bisdorff, A.; Drouet, L. Efficacy and Safety of Direct Oral Anti-Coagulants in Painful Venous Malformations in Children. Pediatric Dermatology 2025, pde.15930, doi:10.1111/pde.15930.

Post-Thrombotic Syndrome in Children: A Comprehensive Narrative Review

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Keywords
Anticoagulants ; Administration, Oral ; Thrombosis ; Child

Abstract
Background and Objective
Post-thrombotic syndrome (PTS) is a chronic, often debilitating consequence of deep vein thrombosis (DVT), characterized by pain, swelling, and venous insufficiency. With paediatric venous thromboembolism (VTE) increasingly being recognized due to improved diagnostics and rising hospital interventions, understanding PTS in this population is critical. This narrative review aims to consolidate current knowledge on the incidence, risk factors, diagnosis, and management of PTS in children, while identifying clinical and research gaps unique to this age group.

Methods
A narrative literature review was conducted from January 15– April 30, 2025, incorporating data from systematic reviews, cohort studies, and guideline papers. Key sources included peer-reviewed studies focused on paediatric thrombosis and PTS, particularly drawing from databases such as PubMed and EMBASE. Articles in English were prioritized, and both retrospective and prospective data were considered.

Key Content and Findings
This review highlights that the incidence of paediatric VTE is increasing, particularly in hospitalized and high-risk groups, with PTS occurring in up to 40% of children and 20% of neonates.

Major risk factors include involvement of multiple vessels, incomplete thrombus resolution, and recurrent DVT. Protective factors identified were regular physical activity, catheter-related DVT, and provoked thrombosis. Diagnostic challenges persist due to limited validation of assessment tools, with the Manco-Johnson instrument being the only validated paediatric-specific tool. Current management emphasizes anticoagulation, physical therapy, and selective use of thrombolysis or stenting in adolescents, though evidence remains limited.

Conclusions
PTS represents a significant, underrecognized burden in paediatric patients with prior DVT. Improved risk stratification, diagnostic criteria, and individualized management protocols are urgently needed. This review calls for multicentre prospective studies to better guide clinical decisions and inform policy for PTS in children.

Introduction
Venous thromboembolism (VTE), which includes deep vein thrombosis (DVT) and pulmonary embolism (PE), is a rising concern in hospitalized children.

Historically viewed as rare in children, the reported incidence of paediatric VTE has steadily increased, particularly in tertiary care and intensive care settings. This trend is largely attributed to heightened clinical awareness and improved diagnostic capabilities.

Among the most concerning long-term complications of DVT is post-thrombotic syndrome (PTS), a condition characterized by venous hypertension that leads to symptoms such as limb pain, chronic swelling, and skin changes. While adult literature on PTS is robust, paediatric data remain sparse. The paediatric population has unique physiological characteristics and risk profiles, necessitating a tailored approach to understanding and managing PTS.

Methods
The literature search strategy is summarized in Table 1.

Epidemiology and Incidence of Paediatric DVT and PTS
Recent studies estimate the annual incidence of VTE in children at 0.07 to 0.49 per 10,000 children, rising to as high as 106 per 10,000 among hospitalized paediatric patients (1). In the Netherlands, 94% of neonatal DVT cases were found to be related to central venous catheters (CVCs), whereas older children exhibited more heterogeneous risk factors (2).

Van Ommen (1) notes that paediatric VTE has been increasing by nearly 10% annually, driven by heightened awareness, more frequent use of central venous lines, and complex comorbid conditions such as cancer and inflammatory bowel disease. Despite being rare compared to adults, the absolute number of paediatric thrombosis cases is growing, making this a critical area for intervention. PTS incidence in children is reported to range widely from 9.5% to 70%, with a mean incidence of 26% based on a systematic review (3). A recent cohort study revealed a 20% incidence in neonates and 40% in older children, underscoring the need for age-specific assessment (2).

Pathophysiology of Post-Thrombotic Syndrome
PTS results from sustained venous hypertension following a thrombotic event. Damage to venous valves caused by thrombosis, either through obstruction or inflammation-induced fibrosis, disrupts normal venous return. This chronic dysfunction results in venous reflux and tissue oedema. In paediatric patients, physiological differences in vascular and coagulation systems

TABLE 1: . Literature search strategy.

Specification	Details
Date of search	January 15– April 30, 2025
Databases and other sources searched	PubMed, EMBASE, Cochrane Library, and reference lists of relevant articles
Search terms used	"post-thrombotic syndrome" OR "PTS" OR "postphlebitic syndrome" AND "children" OR "pediatrics" OR "adolescents" OR "neonates" OR "infants"; MeSH terms: "Postthrombotic Syndrome", "Venous Thrombosis", "Pediatrics"
Timeframe	From January 2000 to April 2025
Inclusion and exclusion criteria	Inclusion: Studies on PTS in children (≤18 years), English language, observational studies, cohort studies, systematic reviews, and relevant clinical guidelines. Exclusion: Case reports, editorials, conference abstracts without full data, adult-only studies
Selection process	Articles were screened by an independent reviewer (pediatric hematology fellow); disagreements were resolved by discussion and consensus with a senior hematologist
Any additional considerations	Preference was given to studies using validated pediatric outcome measures (e.g., Manco-Johnson instrument); data from both high-income and low-to-middle-income countries were included to ensure global relevance

complicate the understanding of PTS development, and age-specific studies are needed to clarify these mechanisms.

Risk Factors and Protective Factors

Several studies identify distinct risk factors for the development of PTS in paediatric patients:

1. Extent of Thrombosis: Involvement of three or more vessels and incomplete thrombus resolution were associated with a significantly increased risk of PTS in both neonates and older children (2).
2. Recurrent DVT: Children with a history of recurrent thrombosis had a higher likelihood of developing PTS.

But also some protective factors were identified such as (2):

1. Exercise (at least three times per week),
2. Catheter-associated DVT, and
3. Provoked thrombosis (e.g., from surgery or infection) .

Clinical Manifestations and Diagnostic Challenges

PTS manifests with limb discomfort, swelling, heaviness, and occasionally skin discoloration or ulceration. In paediatric populations, functional limitations can interfere with play, school attendance, and physical development.

Diagnosis in children remains challenging due to a lack of consensus on assessment tools. Adaptations of adult scales such as the Villalta score have been used as well as the CAPTSure score, but the Manco-Johnson instrument is the only one with validated paediatric data (Figure 1) (3, 4).

Regular clinical evaluations and patient-reported symptom diaries may assist in long-term monitoring.

Impact on Quality of Life

PTS can profoundly affect quality of life, especially in active children and adolescents. Chronic pain, reduced mobility, and cosmetic concerns (such as skin pigmentation or limb asymmetry) can contribute to emotional distress, reduced physical activity, and social withdrawal. Studies show that children with moderate to severe PTS often require long-term follow-up and rehabilitative services, further highlighting the burden on families and healthcare systems (2, 5, 6).

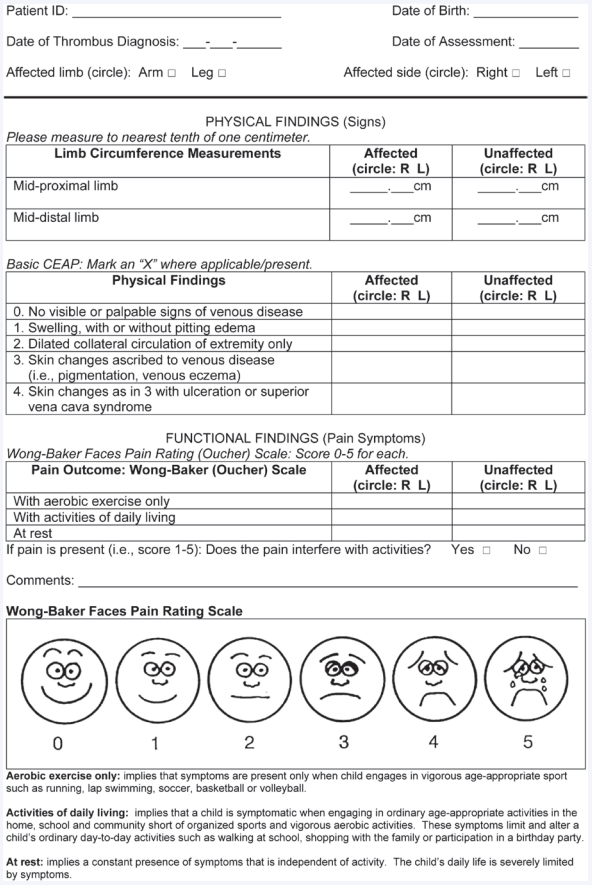
Management Strategies

Current treatment strategies focus on anticoagulation during the acute phase of DVT, aiming to prevent progression and recurrence. However, optimal strategies for preventing or managing PTS remain undefined in paediatrics.

Anticoagulation

Low-molecular-weight heparin (LMWH) remains the cornerstone of anticoagulant therapy and plays an important role in the prevention of PTS by promoting thrombus resolution and reducing DVT recurrence, which may in turn limit venous damage that contributes to PTS. Newer direct oral anticoagulants are being investigated for their safety and efficacy in children. Van Ommen notes that evidence supporting pharmacologic thromboprophylaxis in

FIGURE 1: The Manco-Johnson instrument for paediatric PTS outcome measurement.



Patient ID: _____ Date of Birth: _____

Date of Thrombus Diagnosis: ___-___-_____ Date of Assessment: _____

Affected limb (circle): Arm □ Leg □ Affected side (circle): Right □ Left □

PHYSICAL FINDINGS (Signs)

Please measure to nearest tenth of one centimeter.

Limb Circumference Measurements	Affected (circle: R L)	Unaffected (circle: R L)
Mid-proximal limb	_____.___cm	_____.___cm
Mid-distal limb	_____.___cm	_____.___cm

Basic CEAP: Mark an "X" where applicable/present.

Physical Findings	Affected (circle: R L)	Unaffected (circle: R L)
0. No visible or palpable signs of venous disease		
1. Swelling, with or without pitting edema		
2. Dilated collateral circulation of extremity only		
3. Skin changes ascribed to venous disease (i.e., pigmentation, venous eczema)		
4. Skin changes as in 3 with ulceration or superior vena cava syndrome		

FUNCTIONAL FINDINGS (Pain Symptoms)

Wong-Baker Faces Pain Rating (Oucher) Scale: Score 0-5 for each.

Pain Outcome: Wong-Baker (Oucher) Scale	Affected (circle: R L)	Unaffected (circle: R L)
With aerobic exercise only		
With activities of daily living		
At rest		

If pain is present (i.e., score 1-5): Does the pain interfere with activities? Yes □ No □

Comments: _____

Wong-Baker Faces Pain Rating Scale

Aerobic exercise only: implies that symptoms are present only when child engages in vigorous age-appropriate sport such as running, lap swimming, soccer, basketball or volleyball.

Activities of daily living: implies that a child is symptomatic when engaging in ordinary age-appropriate activities in the home, school and community short of organized sports and vigorous aerobic activities. These symptoms limit and alter a child's ordinary day-to-day activities such as walking at school, shopping with the family or participation in a birthday party.

At rest: implies a constant presence of symptoms that is independent of activity. The child's daily life is severely limited by symptoms.

paediatrics remains limited and emphasizes careful balancing of bleeding risk against potential benefit. Importantly, while effective treatment of DVT may reduce the risk of subsequent PTS, prophylaxis aimed solely at preventing DVT does not appear to alter the incidence of PTS once a DVT has occurred (1).

Thrombolysis and Intervention

In selected adolescents with iliofemoral DVT, pharmaco-mechanical thrombectomy and stenting may reduce PTS incidence, though evidence is limited (7).

Compression Therapy

Avila et al. demonstrated that compression gear (CG) was associated with a decrease in post-traumatic stress (PTS) scores in children at follow-up, provided it was worn for at least 8–12 hours per day for three or more days per week, and provided the pressure applied by the CG was relatively low (5).

Physical Therapy and Exercise

Regular physical activity has been shown to reduce PTS risk and improve limb function. Exercise regimens should be age-appropriate and individualized (6, 8).

Multidisciplinary Approaches and Long-Term Monitoring

Effective management of paediatric PTS requires coordinated care involving haematology, vascular medicine, rehabilitation/physiotherapy, nursing, and psychosocial support to address both venous pathology and functional impairment. This multidisciplinary care model should be paired with structured, long-term surveillance, as PTS may evolve slowly over years.

It is recommended that an initial standardised PTS assessment is carried out six months after DVT, followed by annual evaluations (± six months) using validated paediatric PTS tools. For non-neonates, monitoring should continue for at least 5 years, while neonates and children with CVC-associated DVT require extended follow-up for at least 10 years, reflecting the delayed and progressive nature of PTS in younger patients. This longitudinal approach enables early recognition of symptom progression, timely referral to supportive services (e.g., physiotherapy, pain management), and adjustment of interventions to preserve function and quality of life (5).

Research Gaps and Future Directions

There is an urgent need for multicentre trials, standardized outcome definitions, and paediatric-specific interventions.

Significant challenges persist in paediatric PTS research. The lack of standardized diagnostic criteria and outcome measures hampers data comparison across studies. Few randomized controlled trials exist, and many interventions are extrapolated from adult data. Ethical constraints and low incidence rates further complicate trial design. Investment in paediatric-specific studies and long-term cohort tracking is essential.

Further prospective studies and clinical trials are needed to validate diagnostic tools, determine the most effective prevention strategies, and evaluate the long-term outcomes of therapeutic interventions. International registries and multicentre studies will be vital to enhance understanding and optimize care for paediatric PTS. Collaborative networks and standardized treatment pathways may help streamline patient care and improve long-term outcomes.

Conclusion

Postthrombotic syndrome is a significant but often underdiagnosed complication of paediatric DVT. The rise in paediatric thrombosis cases mandates a systematic approach to diagnosis, management, and prevention of PTS. Greater awareness, standardized protocols, age-specific diagnostic tools, and interdisciplinary care models are essential to reduce long-term morbidity and improve quality of life. A multidisciplinary approach is key to effective care.

Statement

The author has no conflicts of interest to disclose relating to the topic discussed in this manuscript.

REFERENCES

1. van Ommen CH. Thromboprophylaxis in Children: Navigating Uncharted Waters. Hamostaseologie. 2025;45(4):302-11.
2. Klaassen I, Sari S, van Ommen H, Rettenbacher E, Fijnvandraat K, Suijker M, et al. Incidence and risk factors for postthrombotic syndrome in neonates and children in a single-center cohort study. Journal of Thrombosis and Haemostasis. 2025;23(1):181-9.
3. Goldenberg NA, Donadini MP, Kahn SR, Crowther M, Kenet G, Nowak-Gottl U, et al. Post-thrombotic syndrome in children: a systematic review of frequency of occurrence, validity of outcome measures, and prognostic factors. Haematologica. 2010;95(11):1952-9.
4. Avila ML, Brandao LR, Williams S, Montoya MI, Stinson J, Kiss A, et al. Development of CAPTSure(TM) - a new index for the assessment of pediatric postthrombotic syndrome. J Thromb Haemost. 2016;14(12):2376-85.
5. Avila L, Amiri N, De R, Vincelli J, Pullenayegum E, Brandao LR. Compression garments for the management of pediatric post-thrombotic syndrome: A prospective longitudinal study. J Thromb Haemost. 2021;19(12):3073-9.
6. Goldenberg NA, Brandao L, Journeycake J, Kahn S, Monagle P, Revel-vilk S, et al. Definition of post-thrombotic syndrome following lower extremity deep venous thrombosis and standardization of outcome measurement in pediatric clinical investigations. J Thromb Haemost. 2012;10(3):477-80.
7. Lombardi M, Glass J, Wasan S, Marston W. Acute iliofemoral deep venous thrombosis in adolescents and young adults is associated with hypercoagulable states and a significant incidence of post-thrombotic syndrome. Journal of Vascular Surgery: Venous and Lymphatic Disorders. 2025;13(4):102211.
8. Avila L, Betensky M, Cohen C, Ahuja S, Goldenberg N, Zia A. Clinical care of pediatric patients with or at risk of postthrombotic syndrome: guidance from the ISTH SSC Subcommittee on pediatric and neonatal thrombosis and hemostasis. J Thromb Haemost. 2024;22(2):365-78.

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