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REVIEW

Clinical practice

The bleeding child. Part I: primary hemostatic disorders

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Abstract Mucocutaneous bleeding is common in childhood and may be the result of primary hemostatic disorders such as vascular abnormalities, von Willebrand disease, thrombocytopenia, and platelet dysfunction. A detailed bleeding history and physical examination are essential to distinguish between normal and abnormal bleeding and to decide whether it is necessary to perform further laboratory evaluation. Initial laboratory tests include complete blood count, peripheral blood smear, mean platelet volume, von Willebrand factor (VWF) antigen assay, VWF ristocetin cofactor activity, and factor VIII activity. Once thrombocytopenia and von Willebrand disease have been excluded, platelet function should be tested by platelet aggregation. Additional specific diagnostic tests, such as platelet secretion tests and flow cytometry for the detection of platelet surface glycoprotein expression, are needed to confirm the raised hypothesis.

Keywords Primary hemostasis · Thrombocytopenia · Von Willebrand disease · Platelet function disorders · The bleeding child · Diagnostics

Introduction

Children either with overt bleeding or with a history of easy bruising and/or recurrent bleeding episodes are frequently seen in daily practice. These signs and symptoms may be caused by an underlying bleeding disorder, and the challenge for pediatricians is to decide whether these children have an "abnormal" bleeding pattern and need further laboratory investigations. Knowledge of the hemostatic physiology and pathology is essential to order and interpret proper laboratory tests. This review focuses on the clinical and laboratory diagnosis of primary hemostatic disorders in children.

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Physiology of primary hemostasis

Hemostasis is a complex process that leads to the formation of a blood clot at the site of vessel injury and three phases can be distinguished: primary hemostasis or formation of a platelet plug, secondary hemostasis, or coagulation and fibrinolysis [21]. Primary hemostasis starts immediately after damage of the vessel wall with vasoconstriction as a result of local contraction of vascular smooth muscle cells (Fig. 1). Next, von Willebrand factor (VWF) binds to the exposed subendothelial collagens. Platelets are tethered to the site of endothelial cell injury through the binding of VWF to the glycoprotein Ib receptor of the platelet. Platelets roll over VWF in the direction of flow and get slightly activated. Platelets finally attach to the subendothelium by participation of other receptors, such as GPIIbIIIa, and the collagen receptors GPVI and $\alpha 2\beta 1$. After adhesion, spreading of the platelets occurs, which is important to endure the shear forces of the blood flow. The platelets are subsequently activated by strong agonists present at the site of injury, mainly collagen and thrombin. Upon activation, the GPIIbIIIa receptor changes conformation, thereby facilitating aggregation. The GPIIbIIIa receptor binds fibrinogen or VWF, which cross-links platelets together by binding GPIIbIIIa receptors on neighboring platelets. At the same time, the contents of the platelet α-

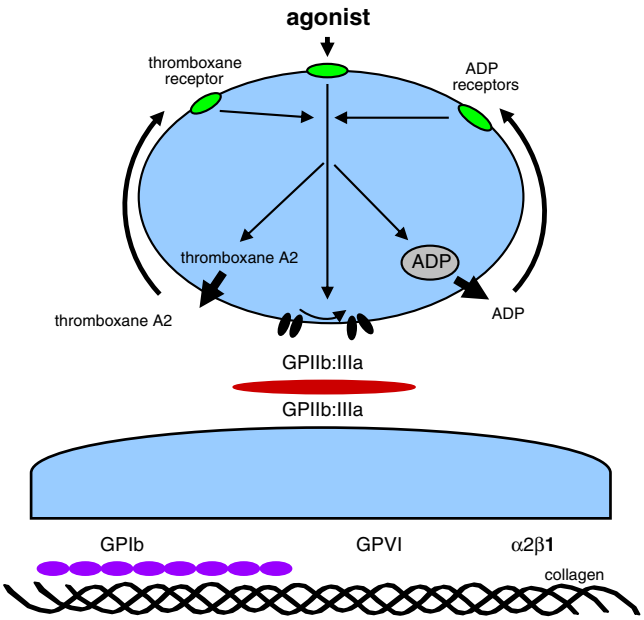

Fig. 1 Primary hemostasis. Von Willebrand factor ( ) binds to the exposed collagen. Platelets are tethered to the site of the injured endothelium through the binding of VWF to the glycoprotein Ib (*GPIb*). They attach to the collagen by participation of other receptors including GPVI and α2β1. After activation, the GPIIb:IIIa changes conformation and binds fibrinogen ( ) or VWF, initiating platelet aggregation. Adenosine 5′-diphospate (*ADP*) and thromboxane A2 are released, supporting aggregation

granules and dense granules, including the soluble agonist adenosine 5′-diphosphate (ADP), are released and thromboxane A2 (TXA2) is synthesized from arachidonic acid released from platelet membrane phospholipids. Both ADP and TXA2 bind to their platelet receptors to support platelet aggregation. Concomitantly, platelets undergo a change in morphology and expose negatively charged phospholipids on their surface membrane. These phospholipids are essential in the assembly of several coagulation factors for the secondary hemostasis, the coagulation process.

In summary, to form a firm platelet plug, it is necessary to have healthy blood vessels, VWF, and sufficient and well-functional platelets. Diseases of these three players cause primary hemostatic disorders including vascular anomalies, von Willebrand disease (VWD), thrombocytopenia, and platelet function disorders.

Primary hemostatic disorders

Vascular anomalies

Vascular anomalies causing bleeding complications in children are various forms of structural anomalies, such as hereditary hemorrhagic telangiectasia, disorders of the connective tissue (including Ehlers–Danlos disease and osteogenesis imperfecta), and small vessel vasculitis [24]. The Ehlers–Danlos disease includes a clinically and genetically heterogeneous group of connective tissue diseases of which the key features are skin hyperextensibility, delayed wound healing with atrophic scarring, joint hypermobility, easy bruising, and generalized connective tissue fragility.

In children, excessive bruising is frequently the presenting symptom, especially in the vascular type of Ehlers–Danlos disease. Other common signs are bleeding from the gums following brushing of the teeth and after tooth extraction. Ehlers–Danlos syndrome is often associated with platelet or coagulation abnormalities such as platelet function disorders and deficiencies of factors XIII, IX and XI. However, generalized vascular fragility dominates the clinical picture. It sometimes leads to severe varicosities and arterial rupture, which may cause sudden death, usually in the third or fourth decade of life.

Von Willebrand disease

VWD was first described by Erik von Willebrand in 1926. Several decades later, it was recognized that it is caused by deficient or defective VWF, a very large multimeric glycoprotein, which (1) promotes the adhesion of platelets to the injured vessel and to each other and (2) binds and stabilizes factor VIII (FVIII). It is the most frequent inherited bleeding disorder with an incidence around 1% [6]. VWD is classified into six different types: type 1 is due to partial quantitative deficiency of VWF, types 2A, 2B, 2M, and 2N are due to qualitative defects of VWF, and type 3 to a total absence of VWF. Type 1 accounts for about 70% of all VWD cases and is inherited in an autosomal dominant manner. Type 2 accounts for 25% of all cases and the inheritance is either autosomal dominant or autosomal recessive. Type 3 accounts for less than 5% and is transmitted as an autosomal dominant trait. The clinical picture varies but usually comprises mild to moderate mucocutaneous bleeding including bruising without trauma, epistaxis, prolonged bleeding after dental extractions, menorrhagia, and prolonged or excessive bleeding after childbirth. Clinical manifestations in type 2N resemble those of mild hemophilia A. Type 3 is the most severe form of VWD because of the combination of the absence of VWF and profound deficiency of FVIII. Bleeding manifestations are mucocutaneous hemorrhages but most of all prolonged bleeding after surgery. Desmopressin is generally an effective preventative or curative treatment for bleeding in type 1. Desmopressin is a synthetic analog of the pituitary antidiuretic hormone vasopressin. It induces an increase in plasma levels of VWF and FVIII, and it improves platelet adhesion and aggregation. The exact mechanism of action, however, is

not fully understood [16]. In patients with type 2 VWD, the response to desmopressin is variable and therapy with purified human VWF is often required. Patients with type 3 VWD need therapy with purified human VWF in combination with FVIII. These children should be seen by an experienced hematologist.

Thrombocytopenia

The normal platelet count for neonates and children ranges from 150 to 450×10^9/L. Thrombocytopenia is defined as a platelet count of less than 150×10^9/L. In general, the risk of bleeding is relatively small unless the platelet count drops to less than 50×10^9/L. Spontaneous bleeding usually does not occur until the platelet count is less than 20×10^9/L and is also dependent on the cause of thrombocytopenia. For example, the risk of intracranial hemorrhage is higher in neonates with a platelet count of less than 30×10^9/L caused by alloimmune thrombocytopenia than by autoimmune thrombocytopenia. When thrombocytopenia is detected, therapy is not always necessary. It is essential to implement reasonable precautions to minimize bleeding complications such as avoiding aspirin and nonsteroidal anti-inflammatory drugs, intramuscular injections, and contact sports in school children and adolescents. However, in some patients, prompt treatment is required to prevent long-term disability.

Neonatal thrombocytopenia

Neonatal thrombocytopenia usually develops within 72 h of birth (early neonatal thrombocytopenia) or after 72 h of birth (late neonatal thrombocytopenia) [29] (Table 1). The most frequent causes of early neonatal thrombocytopenia are placental insufficiency, perinatal asphyxia, and perinatal infections. Thrombocytopenia in these neonates is usually self-limiting and resolves within 10 days. Late neonatal thrombocytopenia is the result of sepsis and necrotizing enterocolitis in more than 80% of the neonates. Thrombocytopenia develops very quickly, can be severe, and lasts for more than 1 week.

The most important cause of early neonatal thrombocytopenia is neonatal alloimmune thrombocytopenia (NAITP), although it only accounts for a small portion of all cases of early neonatal thrombocytopenia. The incidence is estimated to be 1:1,000–1,500 pregnancies. It occurs when fetal platelets contain an antigen inherited from the father and absent in the mother. The mother forms IgG class antiplatelet antibodies against the foreign antigen. The

Table 1 Classification of thrombocytopenia in the neonate and child

Neonatal thrombocytopenia	Childhood thrombocytopenia
Early onset (<72 h)	Destructive thrombocytopenia
Placental insufficiency	Immune thrombocytopenia
Perinatal asphyxia	Medication
Perinatal infection	Infection
Disseminated intravascular coagulation	Hemolytic uremic syndrome
Alloimmune	Thrombotic thrombocytopenic purpura
Autoimmune	Catheter-related thrombosis
Congenital infection	Heart disease
Thrombosis	Disseminated intravascular coagulation
Bone marrow infiltration replacement	Von Willebrand disease type 2B
Kasabach–Merritt syndrome	Kasabach–Merritt syndrome
Metabolic disease	Hypersplenism
Congenital/inherited	Hypothermia
	Massive bleeding
Late onset (>72 h)	
Sepsis	Decreased production
Necrotizing enterocolitis	Inherited (large/normal/small platelets)
Congenital infection	Congenital aplastic anemia
Autoimmune	Congenital amegakaryopoeiseis
Catheter-related thrombosis	Bone marrow infiltration
Kasabach–Merritt syndrome	Vitamin B_{12} deficiency
Metabolic disease	Folic acid deficiency
Medication	Acidosis
Congenital	Infection
Inherited (large/normal/small platelets)	Medication

antibodies cross the placenta and destroy fetal platelets. About 75% of the cases are caused by fetomaternal incompatibility for human platelet antigen-1a (HPA-1a). Other antibodies, such as HPA-5b (16%) and HPA-15b (4%), are often involved as well [10]. Neonatal thrombocytopenia is often severe (less than 20×10^9/L) and results in intracranial hemorrhage in 10–20% of the untreated pregnancies. The diagnosis is made by demonstrating platelet antigen incompatibility between the mother and the father. To prevent intracranial hemorrhages, it is extremely important to consider NAITP as a possible diagnosis in every healthy neonate with thrombocytopenia at birth, especially term neonates. Neonates with NAITP should have a cranial ultrasound to look for intracranial hemorrhages. As a result of the high risk of intracranial hemorrhages, neonates with a platelet count of less than 30×10^9/L and sick or preterm neonates with platelets less than 50×10^9/L should be treated with platelet transfusions [28]. The treatment of choice is HPA-compatible platelets (HPA-1a/5b-negative platelets). Alternatives are random platelets, immunoglobulin, steroids, and washed maternal platelets.

Less common thrombocytopenia in newborns is caused by maternal platelet autoantibodies as result of maternal immune thrombocytopenia (ITP) or other immune-mediated thrombocytopenia. The incidence is 1–5 in 10,000 pregnancies. Thrombocytopenia is usually mild and intracranial hemorrhages do not often occur (<1%). Thrombocytopenia may worsen over the first few days, making close monitoring of platelets for at least 5 days after birth mandatory. The platelet count returns to normal within 3 months. In neonates with a platelet count less than 30×10^9/L, treatment consists of immunoglobulin 1 g/kg for 2 days.

Inherited thrombocytopenias are very rare. The classification is usually based on the size of the platelets [13].

Thrombocytopenia in children

In children, the causes of thrombocytopenia can be classified according to the pathologic mechanism involved: destruction of platelets (including sequestration and pooling) or decreased platelet production (Table 1). The most common cause of thrombocytopenia as a result of platelet destruction is ITP. ITP is defined as a peripheral blood platelet count of less than 100×10^9/L and the absence of any obvious initiating and/or underlying cause of thrombocytopenia. Only 3% of children with ITP have clinically significant manifestations. Severe bleeding occurs more often in children with platelets of $<10\times10^9$/L. The incidence of intracranial bleeding is about 0.1% to 0.5%. As result of the lack of significant bleeding, most children can be managed without therapy. Initial treatment of children with moderate to severe bleeding or at increased risk of bleeding consists of intravenous immunoglobulin (0.8–1 g/kg), corticosteroids (1–2 mg/kg/day for a maximum of 14 days, or 4 mg/kg/day for 3–4 days), or intravenous anti-D immunoglobulin (50–75 ug/kg). In life-threatening situations, a double dose of platelets should be given together with intravenous high-dose corticosteroids and immunoglobulin or anti-D. In children with ITP and no improvement after 3 to 6 months, it is recommended to test for antinuclear and antiphospholipid antibodies and immunoglobulins (IgG, IgA, and IgM) to exclude chronic diseases associated with thrombocytopenia. Bone marrow evaluation is recommended when abnormalities of other cell lines in the bone marrow are present or systemic features such as bone pain or enlarged spleen. All children with chronic ITP (≥12-month duration) should be seen by an experienced hematologist. In these children, treatment options include dexamethasone, high-dose methylprednisolone, and rituximab. Recently, an international consensus with the abovementioned recommendations has been published [27].

Platelet function disorders

Platelet function disorders in children can be classified into acquired or inherited disorders. Most platelet function disorders are acquired. The most frequent cause of acquired platelet dysfunction is medication including nonsteroidal anti-inflammatory drugs, aspirin, valproic acid, beta-lactam antibiotics, and selective serotonin reuptake inhibitors [23]. Acquired platelet dysfunction can also be a complication of an underlying disease such as renal failure, liver disease, and malignant disorders [4]. Inherited disorders of platelet function are a rare and heterogeneous group of diseases, which are characterized by easy bruising, epistaxis, menorrhagia, and mucocutaneous and perioperative bleeding. Classification of these disorders is usually based on alterations in function or structure [13]. In the German-speaking countries, the incidence has been estimated to be 1.3 to 2.2 affected children per one million children [18]. Only in half of the children, defects were well classified: 32% presented with Glanzmann thrombasthenia, 21% with aspirin-like defects, 17% with platelet receptor defects, 15% with storage pool disorders, 8% with Bernard–Soulier syndrome, and 7% with other defects.

Glanzmann thrombasthenia is a rare autosomal recessive defect in the platelet membrane receptor GPIIbIIIa, the main fibrinogen receptor on the platelet surface, resulting in ineffective platelet aggregation. Platelet aggregation responses to all agonists except ristocetin.

Bernard–Soulier syndrome results from a defect in one of the components of the GP Ib-IX-V complex on the platelet, causing a defective binding of platelets to VWF. It

is characterized by giant platelets and thrombocytopenia. It is an autosomal recessive disorder.

The term *storage pool deficiency* (SPD) is used for platelet disorders associated with deficiencies in platelet secretion granules, changing the contents of dense granules (δ-SPD), alpha-granules (α-SPD or gray platelet syndrome), or both (αδ-SPD). These deficiencies can be idiopathic or part of a more complex disorder such as Hermansky–Pudlak syndrome. The bleeding tendency is usually mild.

Treatment of platelet function disorders consists of desmopressin, antifibrinolytic agents, or transfusion of platelets. Many disorders respond well to desmopressin [18, 33]. It is advisable to test the therapeutic efficacy of desmopressin. Platelet transfusions should be used in patients with severe bleeding complications, which do not respond on medical therapy and platelet defects that cannot be managed by desmopressin therapy. Alloantibodies either to human leucocyte antigens or missing GPs may easily occur. An alternative in patients who no longer respond to platelet transfusions is recombinant factor VIIa [14].

Medical history and physical examination

To decide whether a bleeding child needs further evaluation for one of the above discussed primary hemostatic disorders, the medical history and clinical findings are important tools (Table 2). Bleeding can be called "abnormal" if the duration or the quantity of the bleeding is longer and more severe than one would expect. Small bruises can be seen on forehead, knees, and shins in all children from the time they begin to crawl. Children with underlying bleeding disorders usually have bruises on parts of the body that are involved in falls or trauma. If these bruises are larger or more than one would expect, a bleeding disorder must be ruled out. Before crawling, bruising is unusual and one should keep the possibility of nonaccidental trauma in mind. Uncommon sites of bruising such as the back, buttocks, arm, and abdomen should also trigger suspicion for child abuse [32].

The type and pattern of bleeding may be important indications for primary or secondary bleeding disorders. Petechiae, bruising, and mucosal bleeding, such as gingival hemorrhage, epistaxis, and menorrhagia, are suggestive of disorders of platelets and blood vessels or VWD. Bleeding into soft tissues, muscles, and joints implies the presence of a coagulation factor deficiency such as hemophilia. A persistent bleeding, for example after surgery, is indicative for a primary hemostasis problem, whereas a delayed bleeding is more suggestive of a secondary bleeding disorder.

Table 2 Evaluation of a bleeding child: medical history

Type, localization, severity, and course of bleedings
Pregnancy: medication of mother, immune thrombocytopenia of mother, HELPP syndrome, placental insufficiency
Birth: asphyxia, cephalohematoma, and type of delivery
Heel prick
Circumcision
Umbilical stump
Immunizations
Time when child starts to become mobile
Dental extractions
Surgery, such as adenotonsillectomy
Menstruation
Trauma
Concomitant illnesses and medication
Family history

HELLP hemolysis, elevated liver enzymes, low platelet count

It is important to explore the time of onset of the bleeding symptoms. The symptoms of acquired disorders, including ITP, usually present over days, whereas symptoms of a longer duration are suggestive of a congenital disorder such as congenital platelet disorders or VWD. Parents tend to forget events from the past, so it is worthwhile to ask precisely about any bleeding symptoms directly after birth, the shedding of the umbilical stump, heel prick, immunizations, minor surgical interventions, including circumcision and adenotomy, and the time that their child started to crawl. The hemostatic system needs to be adequately challenged before the bleeding disorder becomes evident. Severe bleeding disorders may manifest in infancy or early childhood. Mild bleeding disorders, however, may become apparent later in childhood or even in adulthood after more significant challenges to the hemostatic system such as surgery, dental extractions, or menstruation.

A comprehensive family history is extremely valuable as most children have not come across a lot of challenges to the hemostatic system. All family members of both sexes should be recorded with regard to abnormal bleeding tendencies. For X-linked disorders, such as X-linked thrombocytopenia, one should particularly take a detailed history of maternal grandfathers, uncles, and cousins. For autosomal recessive disorders, such as type 3 VWD, history of consanguinity is of significance. It is essential to ask about surgery, dental extractions, menstruations, transfusions, fluxus postpartum, and unexpected deaths of all family members. The questions should be specific. Do not ask whether blood loss after dental extractions was normal, but ask precisely how many days it took before bleeding stopped.

The presence of concomitant illnesses can be an indication for the cause of bleeding. Chronic renal failure is associated with platelet function disorders [4]. An enlarged spleen can be the cause of thrombocytopenia. Wilms' tumor is known to cause acquired VWD, which can be the cause of bleeding. Children with systemic lupus erythomatosus may bleed because of ITP or systemic vasculitis. Furthermore, many platelet function disorders are associated with inherited syndromes [12]. For example, thrombocytopathy as result of platelet dense granule deficiency occurs in children with oculocutaneous albinism in Hermansky–Pudlak syndrome or Chediak–Higashi syndrome. On the other hand, most congenital bleeding disorders occur in otherwise healthy-looking children.

Medication history is essential as well. For example, aspirin and nonsteroidal anti-inflammatory drugs cause irreversible as well as reversible platelet function disorder. Valproate therapy may cause thrombocytopenia or acquired VWD [20].

Collection of the bleeding history and its interpretation is very subjective. For this reason, questionnaires on bleeding history have been developed [34]. The Bleeding Score was originally developed as a research tool to evaluate bleeding severity in adults with known VWD [30]. A pediatric adaptation of this adult score has been developed by Bowman et al., termed the Pediatric Bleeding Questionnaire, and a prospective study has shown that a score of ≥2 was predictive of a diagnosis of VWD in a primary care setting [7]. In a pediatric tertiary care setting, however, this score appeared to have limited predictive value for identifying patient with common mild bleeding disorders such as type 1 VWD or platelet function disorders [25]. So, until now, this score cannot be used as a diagnostic tool to discriminate between patients with or without bleeding disorder.

Physical examination is another important tool for diagnosis of a bleeding disorder. Petechiae are usually present in primary hemostatic disorders, especially thrombocytopenia or thrombocytopathy. Other signs of primary hemostatic disorders are small and/or many scattered ecchymoses as well as mucosal hemorrhages. Ecchymoses in unusual places, such as the thorax or back, may be suggestive for both primary and secondary bleeding disorders. The same is true for large bruises, too large for the degree of trauma, or for palpable hematomas. In case of epistaxis, the nose should be examined to exclude excoriations and damaged vessels as results of trauma. Joint and intramuscular bleeding with or without trauma is always abnormal in children and suggestive for especially secondary bleeding disorders. Physical examination is also important for the evidence of associated abnormalities of syndromes with platelet function disorders such as albinism, bilateral hypoplastic or absent radii in thrombocytopenia with absent radii syndrome, and eczema in Wiskott–Aldrich syndrome [12]. The possibility of child abuse should always be kept in mind while evaluating a bleeding child.

Laboratory evaluation

Children with a positive individual and/or familial history of bleeding should undergo coagulation testing. Laboratory evaluation of bleeding disorders includes testing for defects of the secondary hemostasis (prothrombin time and activated partial thromboplastin time), which will not be discussed in the present article. Initial coagulation studies for primary hemostatic disorders include complete blood count, peripheral blood smear, mean platelet volume (MPV), bleeding time or platelet function analyzer-100 (PFA-100), VWF antigen assay (VWF:Ag), VWF ristocetin cofactor activity (VWF:RCo), and FVIII activity. If thrombocytopenia and VWD have been excluded, platelet function tests should be performed including light transmission aggregometry and platelet secretion tests (Fig. 2).

Complete blood count

It is essential to perform a complete blood count to study the number of platelets. The result of the blood count will rapidly eliminate or diagnose thrombocytopenia as the cause of bleeding. It is important to realize that a low number of platelets affects potential platelet function testing. In the past, platelets were counted manually with phase-contrast microscopy. The introduction of automated full blood counters has improved the precision and velocity of counting. Additionally, these counters made it possible to record various platelet indices including MPV. Normal ranges for platelet counts and MPV do not differ from adult normal ranges [2, 9]. In giant platelet disorders such as Bernard–Soulier, MPV is high, whereas in Wiskott–Aldrich syndrome, MPV is low, reflecting the small size of the platelets in this syndrome. MPV may not always reflect the actual platelet size, especially in thrombocytopenia and in giant-platelet disorders. Furthermore, the presence of anemia may give an indication for the severity of acute or chronic blood loss as a result of bleeding. Chronic blood loss is usually represented by a hypochomic, microcytic anemia, while acute blood loss is characterized by a normocytic anemia. Both anemias are accompanied by a low number of reticulocytes. If thrombocytopenia is caused by bone marrow failure, it is usually accompanied by another suppressed blood cell line, i.e., normocytic anemia with reticulopenia and/or leucopenia.

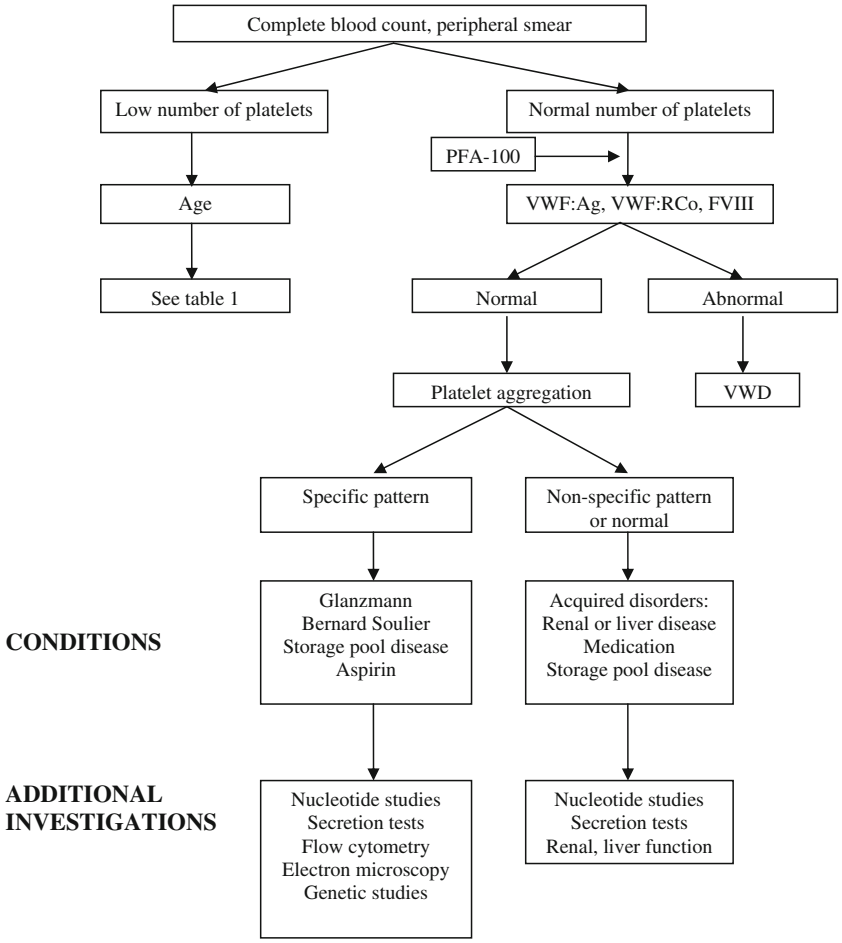

Fig. 2 Algorithm for children with symptoms and signs of primary hemostatic disorders

Peripheral blood smear

A peripheral blood smear is indispensable in any patient with a potential platelet disorder to rule out thrombocytopenia which is the result of clumping of the platelets, to identify abnormal cells including blasts, and to examine platelet morphology. Electron microscopy can, for example, identify the absence of α-granules in gray platelet syndrome.

Bleeding time and platelet function analyzer-100

The bleeding time, developed by Duke in 1910, was the first test to assess primary hemostasis in vivo [11]. In this test, which was improved by Ivy, the patient was stabbed by a lancet on the ventral side of the forearm while a blood pressure cuff was placed on the upper arm and inflated to 40 mmHg. Blood was drawn off every 30 s by a filter paper, and the bleeding time was documented as the time from when the wound was made until bleeding stopped. Andrew et al. used a modified device that makes a smaller skin incision and showed that bleeding times in neonates are shorter than those in adults [1]. The shorter bleeding time or enhanced platelet–vessel interaction in healthy neonates compared to older children or adults is caused by the higher concentration and activity of circulating VWF and higher hematocrit [15]. In the recent past, this test has become less popular due to its invasiveness, low sensitivity and specificity, and its inability to predict bleeding in unselected patients. Especially in neonates and infants, this test is time-consuming and difficult to perform and standardize [8]. In most hospitals, the in vivo bleeding time is, therefore, replaced by the in vitro bleeding time devices in particular the PFA-100. This test needs about 1 ml of citrated whole blood, is easy to perform, and produces results very rapidly. In the PFA-100, citrated whole blood is applied to a cartridge with a membrane that is coated with collagen/epinephrine or collagen/ADP. This membrane has a very small aperture, which occludes after contact with the applied blood. The time necessary to occlude the aperture is the closure time. Term neonates have shorter closure times than older children or adults [5]. Infants and older children have closure times in the same range as those in adults [22]. Although the PFA-100 is more

Table 3 Classification of von Willebrand disease based on specific von Willebrand factor tests

VWD type	VWF:Ag	VWF:RCo	RCo/Ag	FVIII	Multimer pattern	Low-dose RIPA	Platelets
1	↓	↓	=	N or ↓	N	Absent	N
2A	↓	↓	RCo<Ag	N or ↓	Abnormal	Absent	N
2B	↓	↓	RCo<Ag	N or ↓	Abnormal	↑	N or ↓
2M	↓	↓	RCo<Ag	N or ↓	N	Absent	N
2N	N or ↓	N or ↓	=	<30 IU/dL	N	Absent	N
3	Absent	Absent	NA	<10 IU/dL	Absent	Absent	N

RIPA ristocetin-induced platelet aggregation, *VWF* von Willebrand factor, *VWD* von Willebrand disease, *NA* not applicable, *N* normal, *VWF:Ag* VWF antigen assay, *VWF:RCo* VWF ristocetin cofactor activity, *FVIII* factor VIII

sensitive and reliable than the bleeding time, it is not the perfect screening tool for primary hemostatic disorders [31]. It is highly sensitive for severe VWD and platelet defects, including Bernard–Soulier syndrome, but it is insensitive for mild disorders such as mild type 1 VWD, storage pool disease, and Hermansky–Pudlak syndrome. False-positive results may easily occur, especially in anemic patients. As a consequence, PFA-100 may help to exclude severe forms of VWD and platelet defects in children, especially when it is difficult to obtain blood. However, if clinical suspicion of a bleeding disorder is high and PFA is normal, other tests are still required including VWF and platelet function tests. Additionally, because abnormal PFA-100 results are not specific, further investigation is required in patients with abnormal PFA-100 results.

Diagnosis of von Willebrand disease

Besides mediating the initial adhesion of platelets at the sides of vascular injury, an additional function of VWF is binding and stabilizing FVIII in the circulation. Therefore, initial tests to detect VWD or low VWF include VWF:Ag, VWF:RCo, and FVIII activity [26]. The normal values of these tests range from 50 to 150 IU/dL. VWF:Ag is a qualitative immunoassay that measures the concentration of VWF protein in the plasma. VWF:RCo is a functional assay of VWF that measures its ability to interact with normal platelets. In this assay, the antibiotic ristocetin causes VWF to bind to platelets resulting in platelet clumps. Several methods can be used to detect platelet clumping.

FVIII coagulant assay is a measure of the function of the clotting factor FVIII in plasma. In VWD, the VWF:Ag and VWF:RCo are both decreased. A low VWF may result in a low level of FVIII leading to a prolonged activated partial thromboplastin time.

The coefficient of variation of all three assays is high, especially that of the VWF:RCo assay. It has been measured in laboratory surveys at 30% or greater, and it is still higher when the VWF:RCo is lower than 12–15 IU/dL. Nevertheless, it is still the most broadly accepted laboratory test of VWF function. The coefficient of variation of the VWF:Ag and FVIII assay is also relatively high (about 10–20% or greater) [17]. The quality of laboratory testing varies among laboratories as well. Repeated testing for VWD is sometimes necessary to detect low levels of VWF. Plasma levels of VWF increase due to systemic inflammation, administration of oral contraceptives, and stress including surgery, exercise, anxiety, and crying in an anxious child. The phlebotomy should be performed in a quiet setting and as atraumatic as possible to limit exposure of tissue factor from the site and the activation of clotting factors, minimizing falsely high or low values. Furthermore, individuals who have blood type O have concentrations approximately 25% lower compared to persons

Table 4 Characteristic findings on light transmission aggregometry

		Platelet aggregation				
Disorder	Platelet morphology	Epinephrine	ADP	Collagen	Ristocetin	AA
Glanzmann	Normal	−	−	−	+	−
Bernard Soulier	Giant platelets	+	+	+	−	+
Aspirin	Normal	+	+	±	+	−
Storage pool disease						
Gray platelet syndrome	Large with decreased α-granules	+	−	−	+	+
δ-Granules deficiency	Decreased δ-granules	−	±	−	±	±

AA arachidonic acid, *ADP* adenosine 5′-diphosphate

who have other ABO blood types. In conclusion, the high variability of the three diagnostic assays as well as the variability of plasma levels of VWF and FVIII as result of conditions of the patient or blood sample make it difficult to diagnose VWD or classify the VWD subtype.

The VWF:RCo-to-VWF:Ag ratio can help to discriminate between type 1 and type 2 VWD. A ratio of <0.5–0.7 is suggestive of type 2A, 2B, and 2M VWD (Table 3). Most tests to classify the subtypes of VWD, such as multimer analysis, can only be performed in specialized coagulation laboratories. Classification of subtypes of VWD is mandatory, as desmopressin therapy causes thrombocytopenia in type 2B and is therefore contraindicated. Genetic testing may help to find the correct diagnosis and select affected family members.

Diagnosis of platelet function disorders

Platelet aggregation tests remain the "gold standard" for the evaluation of platelet function. In the second step, specific diagnostic tests should be used to confirm the hypothesis such as platelet secretion tests, flow cytometry, and measurement of platelet nucleotides. All these specialized tests are only performed in specialized coagulation laboratories. These tests are time-consuming, technically challenging, and require large volumes of freshly drawn citrated blood, which is an important drawback in studying young children. Investigating neonates without bleeding symptoms but with a positive family history should therefore be deferred to a later date when the child is older. In healthy children older than 1 year, platelet aggregation and dense granule release of adenosine triphosphate (ATP) do not vary significantly with age and are not different than results in adults, which suggest that established adult normal ranges may be used in children beyond 1 year [19].

The most common method of assessing platelet function is light transmission aggregometry. In this test, agonists such as ADP, epinephrine, collagen, or arachidonic acid are added to platelet-rich plasma in a cuvette at 37°C, which is stirred between a light source and a photocell. When an agonist is added, the platelets aggregate and absorb less light, which is detected by the photocell, producing a specific aggregation trace. Characteristic patterns of aggregation tracings obtained using an agonist panel can be indicative of a specific diagnosis, especially abnormalities of the platelet receptors (Table 4). For example, an absent aggregation to all agonists with a normal response to ristocetin indicates Glanzmann thrombasthenia. The diagnosis can be confirmed by flow cytometry that determines the lack of GPIIbIIIa on the platelet surface. In patients with abnormalities of the platelet granules causing SPD, there is a large variability in platelet aggregation. In a large study of 106 patients with δ-SPD, only 33% had typical aggregation traces, whereas about 25% had normal aggregation. To detect patients with SPD, measurement of platelet nucleotides or lumi-aggregometry may be more sensitive than light transmission aggregometry. Lumi-aggregometry is a modification of light transmission aggreggometry, which measures ATP release from the dense granules. Detailed algorithms for diagnostic approach to children with suspected inherited disorders of platelet function have been published [3, 13].

Summary

Children with bruising and bleeding are a major challenge for pediatricians. A comprehensive medical history and physical examination should help the pediatrician to decide which children need further diagnostic laboratory evaluation. Initial laboratory testing of primary hemostatic disorders includes the number of platelets, examination of the blood smear, and VWD testing. If thrombocytopenia and VWD have been excluded, platelet function tests should be performed in a specialized coagulation laboratory. And finally, keep vascular abnormalities in mind!

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