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REVIEW
Platelet Disorders in Children: A Diagnostic Approach

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The investigation of children with suspected inherited platelet disorders is challenging. The causes of mucocutaneous bleeding are many, and specialized testing for platelet disorders can be difficult to access or interpret. An algorithm developed for the investigation of suspected platelet disorders provides a sequential approach to evaluating both platelet function abnormalities and thrombocytopenia. Investigation begins with a clinical evaluation and laboratory testing that is generally available, including platelet counting, peripheral blood cell morphology, and aggregometry. Based on results of initial investigations, the algorithm recommends specialized testing for specific diagnoses, including flow cytometry, immunofluorescence microscopy, electron microscopy, and mutational analysis. Pediatr Blood Cancer 2011;56:975–983.

Key words: child; platelet disorders; platelet function tests; review; thrombocytopenia

INTRODUCTION

The primary physiological role of platelets is to support hemostasis at sites of vascular injury by forming platelet plugs that arrest blood loss (Fig. 1). Normally, disc-shaped platelets circulate in the bloodstream without adhering to the endothelium of the vessel wall. However, when the endothelium is damaged, platelets adhere to the exposed subendothelium, binding to subendothelial collagen via the integrin $\alpha2\beta1$ and GPVI membrane glycoprotein receptors and, at high shear, to collagen-immobilized von Willebrand factor (VWF) via the GPIb–IX–V complex. Platelet adhesion at the site of vessel wall damage initiates activation events via intracellular signaling pathways. Reorganization of the cytoskeleton in adherent platelets results in a shape change to irregular spheres with filopodia, spreading to increase surface contact. The contents of dense, or δ-granules (e.g., ADP and serotonin) and α-granules (e.g., adhesive proteins and growth factors) are secreted. Thromboxane A_2 (TxA_2), formed from arachidonic acid via the actions of cycooxygenase-1 (COX-1) and thromboxane synthase, is released from the cell. Scrambling of membrane phospholipids results in the exposure of phosphatidylserine on the platelet surface, providing a procoagulant surface for the assembly of coagulation factor complexes (tenase and prothrombinase) that accelerate the generation of thrombin. ADP, TxA_2, and thrombin bind to their specific membrane receptors, initiating signaling pathways that convert integrin $\alpha IIb\beta3$ (GPIIb-IIIa) from the low-affinity resting state to a high-affinity activated state for its ligands; these, divalent fibrinogen and multivalent VWF (the latter at high shear), function as bridges between $\alpha IIb\beta3$ on adjacent activated platelets, leading to aggregation. When platelet adhesion, activation, or aggregation processes fail, hemostasis is impaired.

Inherited platelet disorders encompass both function disorders and thrombocytopenia (Tables I and II) [1]. Most affected individuals present with symptoms and signs of mucocutaneous bleeding including bruising, epistaxis, bleeding from oropharynx or gastrointestinal tract, menorrhagia and surgical bleeding, particularly when mucous membranes are involved. These disorders may go undetected in young children unless a family history prompts early testing or until a hemostatic challenge results in bleeding. The investigation of a child with a suspected platelet disorder can be difficult because the possible causes of mucocutaneous bleeding are many, and specialized testing is often difficult to access or interpret. The purpose of this review is to present a diagnostic algorithm to aid in the investigation of suspected platelet disorders, and to provide some examples of how this algorithm can be used to identify specific inherited platelet disorders.

DIAGNOSTIC CHALLENGES

Testing for platelet disorders presents a number of challenges that are magnified in the pediatric setting. Practical considerations such as the need for relatively large volumes of blood for the most common platelet function assays, particularly light transmission aggregometry (LTA), preclude testing in younger children. Newer assays that require smaller amounts of blood are available in only a few centres, and await validation in younger age groups. Standardization of platelet function assays, only recently addressed by national and international interest groups [2], should improve the quality of testing for all patients, including children. Finally, access to specialized testing may present the most significant deterrent to pursuing the diagnosis of rare platelet disorders.

PREVALENCE

As there are no population-based data, the prevalence of inherited platelet disorders is unknown. A survey of pediatric centres in Germany, Austria, and Switzerland estimated two affected children per million population [3,4]. Ethnicity and consanguinity contribute to the variation in the frequency of specific

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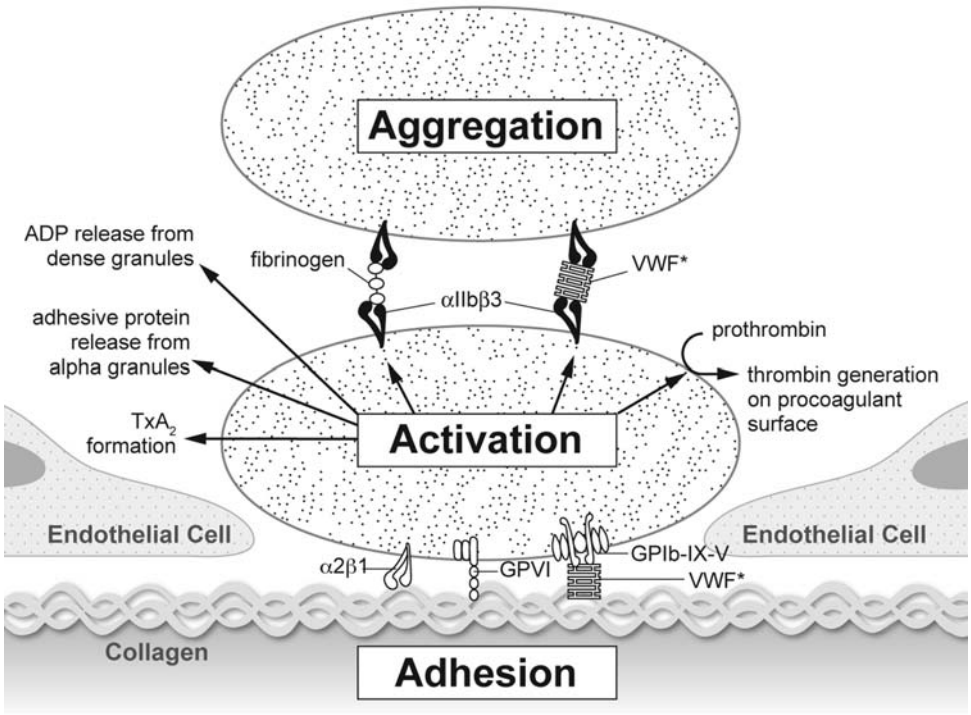

Fig. 1. Platelet plug formation at the site of vessel wall damage. A simplified diagram of adhesion, activation, and aggregation of platelets in response to exposed subendothelium in support of hemostasis. Note that alpha granules store and secrete >300 proteins involved in clot formation and wound healing, and that dense granules secrete serotonin and polyphosphate and other small molecules in addition to ADP. VWF, von Willebrand factor; TxA_2, thromboxane A_2. (*) interactions involving VWF that occur only at high shear.

TABLE I. Inherited Disorders of Platelet Function

Abnormalities of receptors for adhesive proteins
GPIb–IX–V complex (Bernard–Soulier syndrome[a], platelet-type von Willebrand disease[a])
GPIIb-IIIa (αIIbβ3; Glanzmann thrombasthenia)
GPIa-IIa (α2β1)
GPVI
GPIV
Abnormalities of receptors for soluble agonists
Thromboxane A_2 receptor
$P2Y_{12}$ receptor
α_2-adrenergic receptor
Abnormalities of platelet granules
δ-granules (δ-storage pool deficiency, Hermansky–Pudlak syndrome, Chediak–Higashi syndrome, thrombocytopenia with absent radii syndrome[a])
α-granules (Gray platelet syndrome[a], ARC syndrome[a], Quebec platelet disorder[a], Paris–Trousseau–Jacobsen syndrome[a])
α- and δ-granules (α, δ-storage pool deficiency)
Abnormalities of signal-transduction pathways
Primary secretion defects
Abnormalities of the arachidonic acid/thromboxane A_2 pathway
Gαq deficiency
Partial selective PLC-β_2 deficiency
Defects in pleckstrin phosphorylation
Defects in Ca^{2+} mobilization
Abnormalities of cytoskeleton
MYH9-related disorders (May–Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, Epstein syndrome)[a]
Wiskott–Aldrich syndrome[a]
X-linked thrombocytopenia[a]
Abnormalities of membrane phospholipids
Scott syndrome

[a]These disorders usually present with thrombocytopenia in addition to functional abnormalities.

TABLE II. Inherited Thrombocytopenias

Small platelets
Wiskott–Aldrich syndrome[a]
X-linked thrombocytopenia
Normal-sized platelets
Congenital amegakaryocytic thrombocytopenia
Amegakaryocytic thrombocytopenia with radio-ulnar synostosis
Thrombocytopenia with absent radii[a]
Familial platelet disorder and predisposition to acute myeloid leukemia[a]
Autosomal dominant thrombocytopenia
Large platelets
Bernard–Soulier syndrome[a]
DiGeorge/Velocardiofacial syndrome
Platelet-type von Willebrand disease[a]
Gray platelet syndrome[a]
ARC syndrome
MYH9-related disorders[a]
X-linked thrombocytopenia with thalassemia
Paris–Trousseau–Jacobsen syndrome
Benign Mediterranean macrothrombocytopenia
Dyserythropoietic anemia with thrombocytopenia

[a]These disorders may present with functional abnormalities in addition to thrombocytopenia.

disorders among populations. In clinical studies of patients presenting with mucocutaneous bleeding, platelet function abnormalities are at least as common as von Willebrand disease (VWD) [5–7]. A Canadian registry has collected 577 cases since 2004 (available at: http://www.fhs.mcmaster.ca/chr/data.html). Many of these patients have incompletely characterized platelet disorders, but the numbers suggest that platelet disorders are more common than previously appreciated.

APPROACH TO DIAGNOSIS

Development of a Diagnostic Algorithm

Precedents for the development of algorithms to address the diagnosis of children with suspected quantitative and qualitative platelet disorders include the algorithm for the diagnosis of inherited thrombocytopenias developed by the Italian *Gruppo di Studio delle Piastrine* in 2003 [8,9], and the schema for interpretation of laboratory assays in patients with suspected platelet disorders in the 2006 *United Kingdom Haemophilia Centre Doctors' Organisation* guideline for management of inherited platelet disorders [10]. The present algorithm was developed on behalf of the Rare Inherited Bleeding Disorders Subcommittee of the Association of Hemophilia Clinic Directors of Canada, to aid investigation (Fig. 2; available at: http://www.ahcdc.ca/index.php?option=com_content&view=article&id=27&Itemid=14, originally posted 2008, where it is accompanied by supporting descriptions of the platelet disorders). The algorithm begins with clinical evaluation and laboratory testing that is generally available, and proceeds to specialized testing for specific diagnoses. Similar approaches have been outlined by Nurden & Nurden [11] and Favaloro et al. [12].

Clinical History and Standardized Bleeding Questionnaires

The initial evaluation of a child with a suspected platelet disorder should begin with a detailed medical and bleeding history, including family history. Standardized, validated bleeding questionnaires may be useful in assessing the significance of bleeding symptoms in individual patients [13–15]. There is no specific questionnaire for platelet function disorders, with the exception of the one developed to assess bleeding in the Quebec platelet disorder (QPD) [16]. However, a standardized questionnaire for mucocutaneous bleeding in children with type 1 VWD has been validated [17,18]; available at: http://www.ahcdc.ca/index.php?option=com_content&view=article&id=27&Itemid=14). Based on adult questionnaires, the Pediatric Bleeding Questionnaire (PBQ) summates scores for 13 bleeding symptoms, graded according to severity from −1 or 0 to 4, and including a pediatric-specific category (umbilical stump bleeding, cephalohematoma, post-circumcision bleeding, venipuncture bleeding, conjunctival hemorrhage, macroscopic hematuria). Similar symptoms are observed in patients with platelet disorders, and recently, the PBQ has been shown to be potentially useful for assessing bleeding severity in children with these disorders [19].

Platelet Counts and Peripheral Blood Cell Morphology

The first decision point in the algorithm is the platelet count: normal platelet counts direct the investigations to the left side of the decision tree, while thrombocytopenia directs investigations to the right side of the decision tree. It should be noted, however, that automated cell counters underestimate platelet counts when platelet size is outside the established reference range. Similarly, the mean platelet volume (MPV) obtained from an automated cell counter may under or over estimate platelet size as the largest and smallest platelets are excluded from the analysis. Consequently, automated platelet counts and MPV can be less accurate in the presence of either macrothrombocytopenia or microthrombocytopenia [20], and should therefore be combined with evaluation of the Wright's or May–Grünwald–Giemsa-stained peripheral blood film to provide additional information regarding platelet number, size, clumping, and granularity (the platelet count can be estimated by the number of platelets per oil emersion field multiplied by 20×10^9/L; [21]). The absence of α-granules results in large pale platelets characteristic of Gray platelet syndrome [22]; leukocyte inclusions suggest *MYH9*-related disorders [23]; abnormal red blood cell morphology may indicate disorders related to mutations in the *GATA-1* gene [24]. Pseudothrombocytopenia resulting from clumping of platelets collected in EDTA anticoagulant can be identified by examination of the blood film, and confirmed by re-collecting a specimen in citrate-based anticoagulant [11].

Investigation of Functional Abnormalities

Although few studies have compared platelet function in healthy children with adult controls, available evidence suggests that, with the exception of neonates, platelet function is similar in healthy children and adults [25]. Bonduel et al. [26] demonstrated that platelet aggregation and secretion responses in children ages 1–18 years did not vary significantly with age and were not different than results in adults.

The Utility of Screening Tests

Considering the challenges of testing children for abnormalities of primary hemostasis, there is yet no ideal simple, inexpensive,

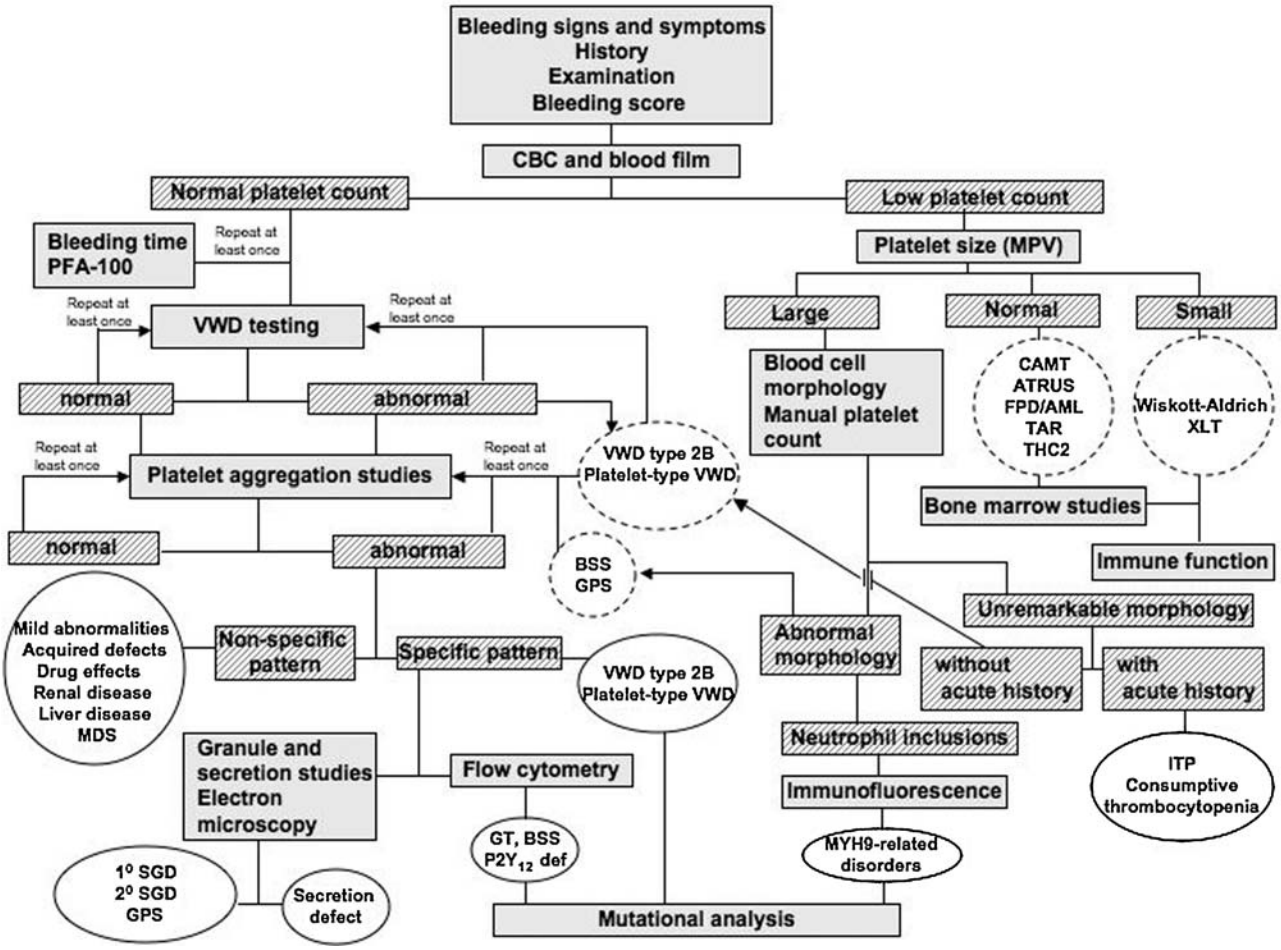

Fig. 2. Algorithm for evaluation of children with suspected platelet disorders. Suggested investigations are in gray boxes and potential results in hatched boxes. The circles and dotted circles contain diagnoses and suspected diagnoses, respectively. BSS, Bernard–Soulier syndrome; CAMT, congenital amegakaryocytic thrombocytopenia; ATRUS, amegakaryocytic thrombocytopenia with radio-ulnar synostosis; FPD/AML, familial platelet disorder and predisposition to acute myelogenous leukemia; GT, Glanzmann thrombasthenia; GPS, gray platelet syndrome; SGD, storage granule disorder; TAR, thrombocytopenia with absent radii; THC2, autosomal dominant thrombocytopenia; XLT, X-linked thrombocytopenia. Rare disorders not included in the algorithm: (i) Quebec platelet disorder—delayed-onset bleeding symptoms, absent aggregation with epinephrine, diagnosis by presence of platelet urokinase by immunoblotting or ELISA; (ii) Scott syndrome—mucocutaneous bleeding, normal aggregation with all agonists, diagnosis by absence of annexin A5 binding to activated platelets by flow cytometry; (iii) thromboxane A_2 receptor defect—mucocutaneous bleeding, decreased/absent aggregation with arachidonic acid and U46619, diagnosis by mutational analysis of *TBXA2R* gene.

sensitive screening test that reliably identifies patients requiring specialized testing of platelet function. Although both bleeding times and PFA-100® closure times have been used for this purpose, these tests are not adequately sensitive to rule out the need for further testing in patients with mucocutaneous bleeding [27,28], or in the pre-operative screening of unselected pediatric patients [29]. In most studies, the lack of specificity has limited their usefulness and, although they may have a role in the comprehensive evaluation of primary hemostatic abnormalities [30,31], these tests should be considered optional.

Testing for von Willebrand Disease

In children who present with mucocutaneous bleeding in the absence of a specific family diagnosis, differentiating VWD from platelet function abnormalities on the basis of clinical history is difficult [32]. Specific testing for VWD, either prior to or concurrently with platelet function testing, is recommended. Because both VWD and platelet function abnormalities are relatively common, some individuals will have combined disorders [5,33]; identifying VWD does not rule out the presence of a platelet function abnormality. Quiroga et al. [5] demonstrated that 11.5% of 113 individuals (age range 4–50 years) with mucocutaneous bleeding and laboratory evidence of abnormalities in primary hemostasis had both VWD and platelet dysfunction. VWD should also be considered in patients with thrombocytopenia: type 2B VWD may present with macrothrombocytopenia [[32]; see Montreal platelet syndrome (MPS), below].

Platelet Function Testing

The most common method of assessing platelet function is LTA, in which the optical density of a rapidly stirred sample of citrated platelet-rich plasma (PRP) at 37°C is measured by a photometer.

Upon addition of agonists (e.g., ADP, epinephrine, collagen, arachidonic acid, the stable TxA_2 mimetic U46619), the platelets change shape from discs to more rounded forms with extended filipodia, resulting in a transient, small decrease in light transmission that is followed by an increase as the platelets aggregate in a fibrinogen-dependent manner. Typically, the increase in light transmission (% aggregation) is measured. The secondary aggregation response observed with higher concentrations of ADP and epinephrine is due to TxA_2 formation and secretion of granule contents. Platelet agglutination stimulated by ristocetin, which changes the conformation of plasma VWF allowing it to bind to GPIb–IX–V, is also measured by LTA. Although many pre-analytical and analytical variables affect the results [34], and international surveys have shown that there is a wide variation in methods [35–38], LTA remains the gold standard platelet function test. Standardization of LTA has recently been addressed in an approved guideline by the Clinical and Laboratory Standards Institute [2], and the Platelet Physiology Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis is presently finalizing official guidelines.

LTA measurements with a complete agonist panel require at least 15 ml of blood to be drawn, and this is a major drawback in young children; use of a system that requires smaller PRP aliquots (250 μl) would minimize the amount of blood necessary for LTA testing. Whole blood aggregometry [2,39] that measures aggregation as the change in electrical impedance between electrodes, requires smaller blood volumes than LTA, but is not widely used. The more recently developed Multiplate® analyzer [40,41] that also works on the principle of electrical impedance, holds promise for pediatric use as it requires as little as 175 μl of anticoagulated whole blood per test sample.

Ideally, platelet aggregometry should be repeated once to ensure reproducibility of results. A detailed medication/supplement history is important to distinguish intrinsic platelet function disorders from acquired abnormalities due to ingestion of drugs or herbal supplements [2]. Characteristic patterns of aggregation tracings obtained using an agonist panel can be indicative of a specific diagnosis (Table III and Fig. 3 in Ref. [42]) [11,34,43].

An isolated increased agglutination response to a low concentration (0.5 mg/ml) of ristocetin is indicative of either type 2B VWD or platelet-type VWD (PT-VWD). This can be accompanied by the presence of thrombocytopenia and platelet clumping visible on the blood film. Type 2B VWD and PT-VWD can be differentiated by mixing studies using combinations of patient/normal control platelets and plasma [44], and by identification of the gain-of-function mutation in the *VWF* or *GPIBA* gene, respectively [1,45].

An absent aggregation response to all agonists with a normal response to ristocetin indicates Glanzmann thrombasthenia. This diagnosis can be confirmed by flow cytometric quantitation of the platelet membrane receptor αIIbβ3 [46]. Markedly decreased aggregation in response to all concentrations of ADP indicates a $P2Y_{12}$ ADP receptor defect. Mutational analysis can be used to identify the gene defect in these membrane receptor disorders: *ITGA2B*, *ATGB3* for Glanzmann thrombasthenia and *P2RY12* for a $P2Y_{12}$ ADP receptor defect [47,48].

Decreased secondary aggregation responses to ADP and epinephrine, and decreased aggregation in response to collagen can be indicative of abnormalities of platelet storage granules. Lumi-aggregometry studies, in which the secretion of ATP from the dense granules is measured by the use of a luciferin/luciferase reagent, are useful in the identification of dense granule disorders [11,34,39,43]. If secretion is abnormal, quantitation of platelet dense granules by a whole mount electron microscopy (EM) method [49] will distinguish a defect in the secretion process from a decrease in the number of dense granules. Although most of the gene defects associated with storage granule disorders are unknown, mutational analysis can confirm some dense granule disorders, for example, Hermansky–Pudlak syndrome and Chediak–Higashi syndrome [50].

Investigation of Thrombocytopenia

For patients with thrombocytopenia, further studies can be guided by platelet size (Fig. 2 and Table II; [8]). Classification into small, normal-sized, or large platelets on the basis of MPV should be confirmed by evaluation of the blood film, as discussed above. The clinical history may indicate whether the thrombocytopenia is acquired or inherited; acquired thrombocytopenias are significantly more common in children. Details regarding onset of bleeding symptoms, previous platelet counts and family history may aid in determining the duration of the thrombocytopenia. Evaluation of the

TABLE III. Aggregation Findings for Some Platelet Function Disorders

Disorder	LTA responses	Additional observations or testing
Bernard–Soulier syndrome	Absent response to ristocetin	Macrothrombocytopenia. Rule out VWD. Flow cytometry for GPIb quantitation
Type 2B VWD and platelet type-VWD	Increased agglutination with low concentrations of ristocetin	Thrombocytopenia and platelet clumping may be present. VWD testing
Glanzmann thrombasthenia	Absent response to all agonists except ristocetin	Flow cytometry for αIIbβ3 quantitation
Aspirin-like defect	Absent response to arachidonic acid with normal response to U46619; decreased response to low concentrations of collagen	Medication history for COX-1 inhibitors
Secretion defect, δ-granule defect	Decreased response to several agonists: ADP, collagen, and epinephrine	ATP release and/or electron microscopy for dense granule evaluation
ADP receptor defect	Decreased or absent response to ADP	Medication history for ADP receptor inhibitors. Flow cytometry for $P2Y_{12}$ quantitation
Gray platelet syndrome	Decreased response to thrombin and/or collagen	Macrothrombocytopenia with pale platelets on blood film

LTA, light transmission aggregometry; VWD, von Willebrand disease; COX, cyclooxygenase.

patient and family for evidence of clinical features in addition to the thrombocytopenia, such as skeletal anomalies [e.g., thrombocytopenia with absent radii (TAR)], immunodeficiency (e.g., Wiskott–Aldrich syndrome), or renal disease and hearing loss (e.g., *MYH9*-related disorders) may identify a syndromic cause for the thrombocytopenia. Although many of the inherited thrombocytopenias are associated with platelet dysfunction, in most cases these abnormalities are non-specific. LTA results on thrombocytopenic samples must be interpreted with caution, but they can be very useful, for example, in Bernard–Soulier syndrome—the specific abnormality of absent ristocetin-induced agglutination is diagnostic [34].

Small Platelets

Wiskott–Aldrich syndrome (WAS) and X-linked thrombocytopenia (XLT) are both caused by defects or deficiency of the WAS protein. Microthrombocytopenia (MPV <5 fl) in a male child should prompt investigation for other manifestations of WAS, including cellular and humoral immunodeficiency, eczema, and recurrent infection. Bone marrow studies may be required as part of the investigation, but megakaryocytes usually appear normal in number and morphology. Confirmatory studies for WAS/XLT include testing for the presence of the WAS protein by immunoblotting or by flow cytometry, and mutational analysis of the *WASP* gene [51].

Normal-Sized Platelets

Thrombocytopenia with platelets of normal size is often associated with inherited defects of megakaryopoiesis. Investigation should include an evaluation of the bone marrow: (i) to rule out acquired causes of thrombocytopenia including marrow infiltration, and (ii) to evaluate megakaryoctye number and morphology, and abnormalities of other cell lines [52]. Thrombocytopenia in neonates is relatively common, usually associated with acquired prenatal or perinatal factors. Rare inherited conditions, including congenital amegakaryocytic thrombocytopenia (CAMT), TAR and amegakaryocytic thrombocytopenia with radio-ulnar synostosis (ATRUS), present at birth with severe thrombocytopenia, which persists beyond the neonatal period. Few or no megakaryocytes in marrow aspirates [53] and elevated plasma thrombopoietin levels are characteristic of reduced platelet production [54]. Identification of typical skeletal abnormalities suggests the diagnosis of TAR and ATRUS. CAMT can be confirmed by mutational analysis of the thrombopoietin receptor gene *c-Mpl* [53].

Large Platelets

Acquired causes of macrothrombocytopenia in children are far more common than inherited defects. Primary immune thrombocytopenia (ITP) is the single most common cause of thrombocytopenia in children, and most of these patients will have a history of acute onset of bleeding that clearly suggests an acquired cause. Other immune- and non-immune destructive causes including drug effects and infections should be considered, particularly if evidence for congenital thrombocytopenia is absent. However, it is important to consider the possibility of inherited thrombocytopenias when children with a diagnosis of ITP have thrombocytopenia that is refractory to treatment and/or there is a family history of thrombocytopenia [55,56]. It has been suggested that the diagnosis of type 2B VWD should always be considered in patients with chronic thrombocytopenia and large platelets [57], especially if clumped platelets are observed on the blood film (this suggestion extends to PT-VWD). Testing for VWD and platelet function will confirm these diagnoses (see above). A reduced VWF:ristocetin cofactor/antigen ratio and an isolated increased agglutination response to a low concentration of ristocetin is indicative of type 2B VWD or PT-VWD.

The presence of Döhle-like inclusion bodies in the leukocytes in a May–Grünwald–Giemsa-stained blood film points to an *MYH9*-related disorder. This disorder encompasses the May–Hegglin anomaly, and Fechtner, Sebastian, and Epstein syndromes, now all known to be associated with molecular defects in a single gene, *MYH9*, coding for the heavy chain of non-muscle myosin IIA (NMMHC-IIA). Nephritis, sensorineural hearing loss, and/or cataracts occur but are not invariably present, particularly in children. Immunofluorescence analysis of NMMHC-IIA aggregates in neutrophils is proving to be very useful for the diagnosis of *MYH9*-related disorders, and mutational analysis can be used to identify the specific genetic defect [23,47,58,59].

Large pale platelets (due to the absence of α-granules) strongly suggest the diagnosis of Gray platelet syndrome, and can be confirmed by transmission EM [22,60]. Platelet function may be abnormal, with decreased aggregation response to collagen and/or thrombin. The genetic defect in Gray platelet syndrome is unknown, but recently the syndrome has been linked to chromosome 3p21.1-3p22.1 [61].

Giant granular platelets on the blood film suggest a diagnosis of Bernard–Soulier syndrome, and can be confirmed by an absent agglutination response to ristocetin, while the aggregation response to other agonists is preserved. Deficient or defective GPIb–IX–V can be confirmed by flow cytometry and/or mutational analysis of *GPIBA*, *GPIBB*, and *GP9* [47].

UNIQUE DISORDERS: QUEBEC PLATELET DISORDER AND MONTREAL PLATELET SYNDROME

Two Canadian inherited platelet function disorders, the QPD (originally Factor V Quebec) and the MPS, provide excellent examples of how insights into the molecular defects of platelet disorders have improved our understanding of platelet function and informed treatment choices for the affected patients.

Quebec Platelet Disorder

The story of this disorder began in 1984 with the description of a Quebec kindred with bleeding associated with oral surgery, and post-partum and intracranial hemorrhage. Unique to this disorder was the delayed onset of bleeding and the poor response to platelet transfusion; because of a marked deficiency of platelet factor V, it was named Factor V Quebec [62].

Subsequent studies showed that the inheritance pattern of the disorder was autosomal dominant, and that platelet factor V was proteolytically degraded, as were other platelet α-granule proteins, including VWF, fibrinogen, osteonectin, and thrombospondin [63,64]. Platelet counts were low to normal, and there was an absent aggregation response to epinephrine [63]. In contrast to platelets with inherited α-granule deficiencies, such as the Gray platelet and ARC (arthrogryposis, renal dysfunction, and

cholestasis) syndromes [22,65], the α-granule ultrastructure was intact. External membrane, lysosomal membrane, and plasma proteins were normal. In light of these observations, Factor V Quebec was renamed the Quebec platelet disorder (QPD; [66]).

The observed degradation of α-granule proteins suggested that QPD was associated with inappropriate protease activity. Further studies revealed that QPD platelets contain and secrete significant amounts of active urokinase plasminogen activator, abnormally synthesized by QPD megakaryocytes [67]. The secreted urokinase was active and in excess of the α-granule plasminogen activator inhibitor 1 (PAI-1) available to inhibit its activity [67]. Analysis of platelet proteins exposed to urokinase-mediated activation of plasminogen to plasmin confirmed that ectopic expression of urokinase by QPD platelets was the basis of the α-granule protein degradation [67,68]. Since plasma urokinase levels are close to normal in QPD patients, the cause of delayed-onset bleeding after trauma is due to abnormal urokinase secretion by platelets within the hemostatic plug, stimulating fibrinolysis and clot dissolution [67–69]. The observation that transgenic mice containing the urokinase gene (*PLAU*) selectively expressed in mouse platelets were resistant to occlusive carotid artery thrombosis and had rapid resolution of pulmonary emboli suggested that QPD patients may have similar protection against arterial and venous thromboembolism [70].

The abnormal expression of urokinase within platelets stems from abnormal expression in the developing megakaryocyte; expression occurs in temporal conjunction with other α-granule proteins [71], and urokinase co-localizes with these proteins in the α-granules. It has recently been shown that QPD is linked to the *PLAU* gene [72], whereby affected individuals have a tandem duplication of *PLAU* [73]. Although the tandem duplication of *PLAU* can explain mildly increased urokinase expression in cells, it does not explain the >100-fold increased expression in QPD megakaryocytes, suggesting that another as yet unknown mechanism is involved [72,73].

The appreciation that QPD is caused by abnormal expression of secreteable urokinase in patients' platelets has provided an explanation for the poor response to platelet transfusions, and for the effectiveness of fibrinolytic lysine analogue inhibitors (amino-caproic or tranexamic acid), which are now the treatment of choice for affected individuals even with severe bleeding symptoms.

Montreal Platelet Syndrome

In 1963, there was a description of a mother and three children from Montreal with a mucocutaneous bleeding disorder associated with macrothrombocytopenia and platelet clumping [74]. The name Montreal platelet syndrome (MPS) was assigned to this disorder by Milton and Frojmovic in 1979. They described a hypervolumetric shape change by MPS platelets upon activation with agonists or during PRP preparation suggesting a defect in the regulation of platelet size and shape change; this finding was proposed to explain the large platelets seen on blood film [75]. Spontaneous platelet aggregation was demonstrated in MPS whole blood; LTA with standard agonists (including high concentrations of ristocetin; low concentrations were not tested) was normal, with the exception of the response to thrombin [76]. MPS platelets suspended in normal plasma aggregated spontaneously but normal platelets suspended in MPS plasma did not, suggesting to the authors that MPS was an intrinsic platelet disorder [76]. It was also observed that MPS platelets were deficient in the Ca^{2+}-dependent protease calpain [77].

Members of the MPS kindred were recently re-evaluated, and it was noted that they had borderline low levels of VWF:antigen, discrepantly low VWF:ristocetin cofactor activity and absent plasma, but not platelet, high molecular weight VWF multimers [78]. Analysis of the *VWF* gene revealed a previously described heterozygous V1316M mutation in exon 28, confirming that the patients with MPS have type 2B VWD [78]. This study highlights the importance of recognizing that patients with macrothrombocytopenia may have defects in the interaction of VWF with platelets [57,79]. The recognition that MPS is really type 2B VWD changed the treatment recommendations for bleeding episodes in these patients to plasma-derived factor concentrates containing VWF activity.

CONCLUSIONS

Making a diagnosis of inherited platelet disorders in children can be hampered by a number of issues: blood sampling, access to specialized testing, and, in the face of more common diagnoses, delayed consideration of an alternative. Investigations of children with mucocutaneous bleeding are aided by a sequential approach that begins with a complete clinical evaluation of bleeding symptoms and signs, associated abnormalities and family history. Initial screening tests including platelet counting, MPV, and examination of the peripheral blood film can direct testing to the investigation of primary function defects or thrombocytopenia; VWD should be considered in the differential diagnosis of both. Generally available laboratory tests direct more specialized testing to specific confirmatory tests, including mutational analysis. Advances in our understanding of the molecular and biological basis of inherited platelet disorders have improved diagnostic proficiency and determination of appropriate therapy.

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