

ORIGINAL ARTICLE

Platelet function defects

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Summary. Inherited defects of platelet function are a heterogeneous group of disorders that can result in bleeding symptoms ranging from mild bruising to severe mucocutaneous haemorrhage. These defects may be classified according to their effect on the various steps of platelet microthrombi formation including initiation, extension and cohesion, or based on their particular structural or functional deficiency. Platelet membrane receptor deficiencies result in the rare, but well-characterized syndromes of defective clot initiation, such as Bernard–Soulier Syndrome. Platelet storage pool defects are the most common disorders affecting the extension phase of clot formation. Glanzmann thrombasthenia, with absent or dysfunctional

α IIb β 3 receptor is the prototypical defect of the cohesion/aggregation phase of microthrombi formation. Many of these disorders share common treatments although some therapies will have greater efficacy for one patient than another and should be individualized so as to provide optimal control of symptoms. Currently much effort is being put into methods to more rapidly and accurately diagnose patients with platelet disorders and to initiate appropriate therapy and prevent life threatening bleeding.

Keywords: bleeding disorders, clot formation, platelet function, platelet granules, platelet membrane receptors, platelets

Introduction: normal platelet function

The formation of a stable platelet plug hinges on the ability of the platelet to interact with the damaged vascular bed and recruitment of other cells in the process of haemostasis and repair. Any defect in this process can cause bleeding symptoms, ranging from clinically insignificant to severe. Platelet defects can be classified by their location in the three phases of clot formation: initiation, extension, and cohesion or aggregation or based on their particular structural or functional deficiency (Schema 1). Although it would be impractical to summarize all known platelet qualitative defects, some of the well-characterized and clinically significant syndromes will be discussed here with a focus on presentation, diagnosis and treatment. Additional excellent and more detailed articles on inherited platelet disorders, including congenital

thrombocytopenia, which are not covered here, have been published in the last few years and are highly recommended to the reader [1,2].

Adhesion

The first step in the initiation phase of thrombus formation involves plasma von Willebrand factor (VWF) binding to exposed collagen on the subendothelium via its VWF-A1 domain while simultaneously binding to the platelet membrane glycoprotein (GP) Ib-IX-V complex (Fig. 1a). Platelets also bind directly to collagen via the membrane GPVI receptor complex and the integrin α 2 β 1 collagen receptor [3,4]. This process results in a stable layer of platelets to facilitate the formation of the platelet plug. Bernard–Soulier syndrome (BSS) is characterized by the absence of the platelet membrane GPIb complex and thus is the prototypical mutation that affects initiation because this defect prevents normal adhesion to the VWF-A1 domain [5]. The normal engagement of these receptors and the formation of the platelet monolayer enhances platelet activation and signals the onset of the next stage of microthrombus formation.

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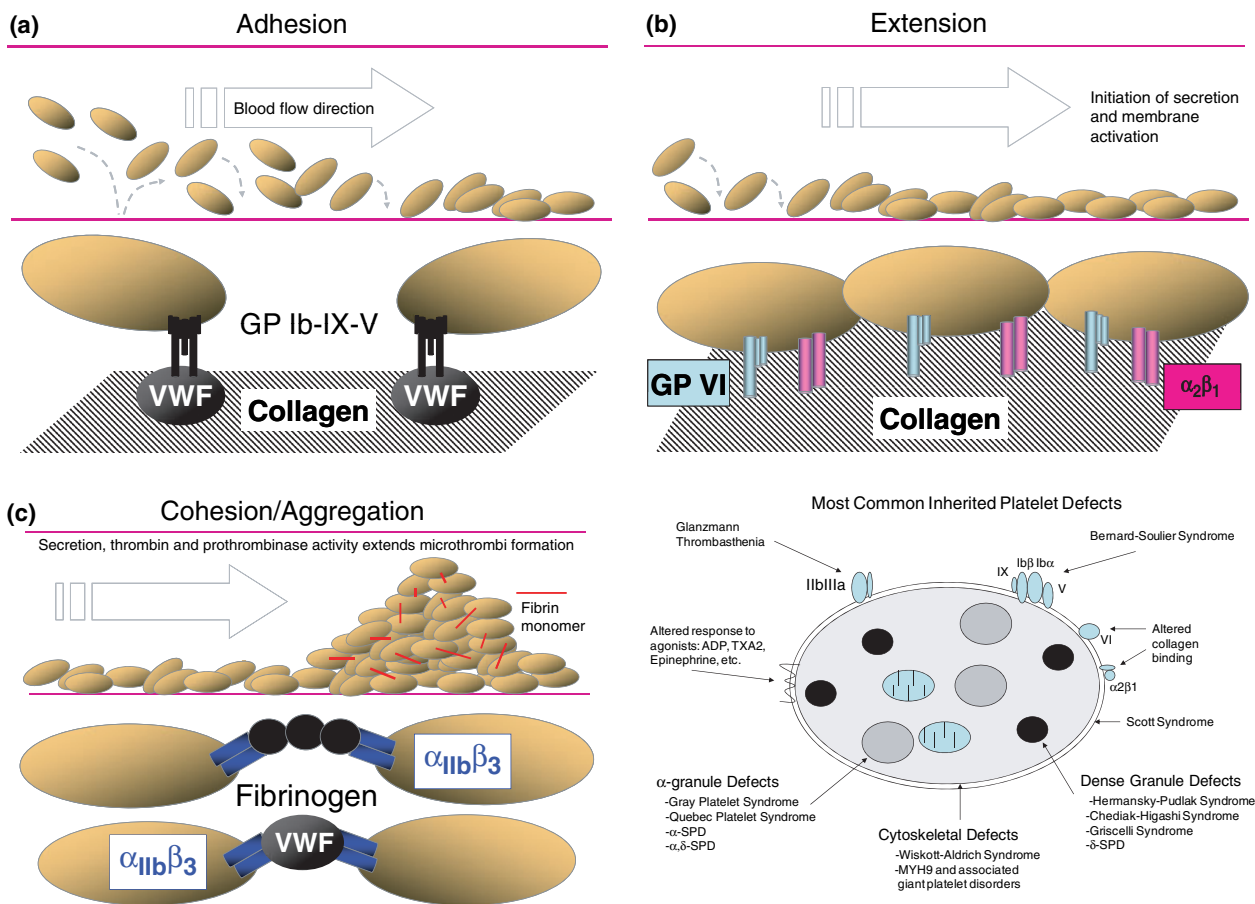


Fig. 1. Figs 1a, b and c outline the three phases of platelet plug formation including adhesion (1a), extension (1b) and aggregation/cohesion with clot formation. Each step requires a coordinated response to ligand and receptor interactions, signaling molecules, membrane expression of clotting protein components, secretion of granule contents, and cytoskeletal modifications. Abnormalities in any of these components will result in platelet dysfunction, although some maybe much more serious than others, based on the presence or absence of alternative pathways to complete the clot formation.

Extension

During this phase, adherent platelets become activated and secrete stored compounds from their α -granules and δ -granules (Fig. 1b). This stimulates circulating platelets to activate and release, which greatly enhances the propagation of the platelet plug. A key compound includes adenosine diphosphate (ADP), which helps to further activate platelets through binding to the respective ADP receptors on neighbouring platelets. During the course of this process, the activated platelet produces and/or releases additional agonists, including thromboxane A2 (TXA2). This is also the likely stage at which the activated platelet surface prothrombinase catalyzes the conversion of prothrombin providing a platform for fibrin clot propagation [6]. Numerous platelet defects that involve the extension phase of clot formation have been previously described [7]. Thus

far, storage pool defects appear to be the most common of these disorders, but membrane receptor and signal transduction pathway abnormalities are an active area of ongoing research.

Another defect related to the extension phase of clot formation is the rare autosomal recessive disorder Scott Syndrome [8]. Patients affected by Scott Syndrome ultimately have impaired platelet dependant fibrin formation leading to a potentially significant bleeding disorder. Scott syndrome is fundamentally a signal transduction pathway defect. Upon activation, Scott platelets are unable to transport phosphatidylserine from the inner to the outer phospholipid membrane. Consequently, the factor Va-Xa and VIIIa-IXa complexes are unable to bind to the membrane resulting in decreased thrombin generation and subsequently inadequate fibrin formation. Mutations of an ATP-binding cassette transporter A1 (ABCA1) are partly responsible for phosphatidylser-

ine translocation and have been implicated in the pathogenesis of Scott Syndrome [8,9].

Cohesion/aggregation

The final step in forming a platelet-rich thrombus is the cohesion/aggregation phase (Fig. 1c). Plasma VWF and fibrinogen bind activated platelets together via the platelet integrin α IIb β 3 (GPIIb–IIIa) complex. The classic disease state that results in a defective consolidation phase of clot formation is Glanzmann thrombasthenia (GT), which results in decreased levels or function of the GPIIb–IIIa complex, leading to absent or severely reduced platelet aggregation [10].

Thrombocytopenia whether inherited or acquired will impact all three phases to varying degrees based on the severity of the platelet deficiency. In a healthy individual, the platelet count must fall below 20 000–30 000 mm⁻³ before significant mucocutaneous bleeding will occur. In high turnover states, such as immune mediated thrombocytopenic purpura, the platelets are younger overall, slightly larger and more haemostatic, and so the platelet count may fall to <15 000 mm⁻³ before serious bleeding occurs. Other defects that warrant special consideration in the hereditary thrombocytopenias are the MYH-9 family of giant platelet disorders and Wiskott–Aldrich Syndrome with minute ‘dust-like’ platelets, both of which are associated with bleeding and striking morphology on the peripheral smear. These syndromes can be diagnosed with a simple CBC; for that reason, the importance of reviewing the smear in any evaluation of platelet disorders cannot be overstressed. Inherited thrombocytopenic syndromes are covered elsewhere in a recent review by Nurden *et al.* [11].

Incidence, racial/ethnic predilection

Fortunately, the severe forms of congenital platelet dysfunction are extremely rare. While it has been suggested that some of the disorders may be under diagnosed, some are known only to occur in a handful of families worldwide. Therefore, these defects are much more common in areas where consanguinity is more prevalent or in small, geographically or ethnically isolated communities. In the more common secretory defects, compound heterozygote mutations are more frequent. Rarely, the family history may suggest an autosomal dominant pattern with mucocutaneous bleeding present in multiple generations. As with all rare blood diseases, the age of onset and family history is very important

and may aid in the diagnostic work-up and choice of therapy.

General diagnostic evaluation

For the majority of haematologists, the diagnosis of platelet dysfunction is limited to a general category based on the combination of aggregation studies, evaluation of the platelet on smear, and in some centres, flow cytometry and electron microscopy. Reference centres can provide more specialized studies in the measurement of ATP/ADP levels, characterization of granules or signalling defects. This remained the state of the art until the most recent decade, which brought the promise of more exact diagnosis based on specific molecular and proteomic screening techniques, thus allowing haematologist to provide counselling for patients and families based on their own unique mutations.

Platelet Function Evaluation: In decades past, initial studies used the bleeding time as the mainstay of diagnostic manoeuvres to identify patients with platelet dysfunction [12]. Milder forms of platelet disorders could be elicited by administration of aspirin, which resulted in prolongation of the bleeding time, in these patients but not in individuals with normal platelet function. Although still very useful in diagnosis of platelet dysfunction, the bleeding time has fallen out of favour because of its unreliability for presurgical screening, particularly in patients with renal disease or with skin changes associated with medications or collagen defects. As a result, it is no longer widely available and very few clinical laboratories have technicians who can perform this assay well, especially on children.

Once von Willebrand disease, the most common diagnosis associated with mucosal bleeding has been excluded, the patients referred for evaluation of platelet dysfunction may have a number of studies performed either in series or with the initial visit. Importantly, no single assay or technology can diagnose all platelet disorders. Many facilities use the platelet function analyzer (PFA-100) or platelet thromboelastography in whole blood as an initial screen for platelet dysfunction prior to surgical procedures [13]. Further evaluation is necessary to actually define the platelet defect and may include; platelet aggregation with various agonists [14], calcium flux or secretion studies, protein biochemistry, electron microscopy and flow cytometry. Unlike congenital thrombocytopenia syndromes, such as MYH9 defects or Wiscott Aldrich, there is not yet a molecular screen that could bypass the initial

Table 1. Summary of platelet defects.

Diagnosis	Phase of clot formation	Key diagnostic findings	Thrombocytopenia?
Bernard–Soulier syndrome	Initiation	Lack of GPIb-IX-V Impaired aggregation to ristocetin Giant platelets	Mild
α -SPD (gray platelet syndrome)	Extension	Lack of α -granules on EM Large, gray, agranular platelets	Mild to moderate
δ -SPD	Extension	Lack of δ -granules on EM Reduced secondary wave of aggregation Low levels of platelet ADP, ATP	None
Chediak–Higashi syndrome	Extension	Oculocutaneous albinism δ -granule defects Progressive neurological deterioration Cytoplasmic inclusions	None
Hermansky–Pudlak syndrome	Extension	Oculocutaneous albinism δ -granule defects Pulmonary fibrosis	None
$\alpha\delta$ -SPD	Extension	Defects in primary and secondary aggregation Lack of α - and δ -granules on EM	None to mild
Glanzmann thrombasthenia	Consolidation/aggregation	Lack of GPIIb–IIIa Poor aggregation with ADP, collagen, Epi Normal ristocetin induced aggregation Absent clot retraction Morphologically normal platelets	None

ADP, adenosine diphosphate; ATP, adenosine triphosphate; EM, electron microscopy; GP, glycoprotein; SPD, storage pool disease.

functional or biochemical evaluation in platelet dysfunction.

Not all these assays are required for each diagnosis, however. Ongoing studies and working groups are validating, which are most useful for each diagnosis, to streamline the diagnostic workup, particularly in children [14,15]. A table summarizing pertinent findings for the syndromes described in this clinical review is listed in Table 1. In the future, platelet proteomics or molecular arrays may speed up the process of diagnosis by identifying upfront which protein or DNA sequence is defective or absent, but functional assays are still required today and will be for some time.

General approach to treatment

Because the treatment is similar for these syndromes, a brief review of the common therapeutic regimens used in platelet dysfunction will be discussed here and additional treatments unique to one particular disorder will be included under that specific heading. The first step in treatment and prevention of bleeding is always education of the patient and the family. All must be counselled that mucocutaneous bleeding will be common. Serious haemorrhage can occur in the event of trauma, surgery, in the gastrointestinal tract and frequently with menses or the postpartum period.

In any event, the patients should have a reliable way of contacting their haematologist, or providing their surgeons or ER physicians with a treatment plan in the event prophylaxis is needed or bleeding occurs. All patients with platelet defects must be counselled on avoiding certain medications that interfere with platelet function, such as aspirin and many non-steroidal anti-inflammatory agents or certain antidepressants [16].

Women with menorrhagia may require hormonal suppression to prevent menses altogether while others have undergone uterine ablation or hysterectomy to prevent life threatening bleeding in the most extreme cases. A comprehensive team approach is necessary for these patients and should include a gynaecologist with a special interest in bleeding disorders [17,18].

In general, certain non-specific agents have been used for years to minimize mucosal bleeding or as prophylaxis for minor surgical bleeding. Antifibrinolytic agents such as ϵ -aminocaproic acid (Amicar) or tranexamic acid (Cyklokapron) have been used with some success to decrease mucosal bleeding associated with epistaxis, menses or mucosal bleeding after dental work. In addition, desmopressin acetate (DDAVP) has been shown to be effective in preventing the bleeding in some of the platelet dysfunction syndromes [19]. A trial of DDAVP should always be performed to determine response before its use to

prevent bleeding prophylactically, as DDAVP has been known to trigger fibrinolysis in certain patients. Many articles have been published recently using activated recombinant factor VII (rFVIIa) to slow or arrest bleeding associated with platelet dysfunction [20,21]. Dosages have varied widely, but many patients have responded to this regimen when others have failed. Used in combination with anti-fibrinolytics, minor bleeding can be controlled in certain patients. This treatment is often used prior to platelet transfusion to avoid blood product exposure and isoimmunization.

Finally, in life-threatening bleeding, platelet transfusions will correct the bleeding defect in most cases, even if only one single-donor apheresis unit is given. Apheresis units (approximately equivalent to six pooled blood bank units) are strongly recommended and preferred to minimize multiple donor exposure, which can result in sensitization and a platelet refractory state. To minimize long-term sensitization to HLA class I proteins expressed on platelets, the blood products should always be leuko-poor or leuko-depleted. In syndromes with complete absence of a membrane GP such as GT or BSS, one should avoid excessive exposure to normal platelets because of the risk of developing an isoantibody to the missing proteins of the receptor complex. Isoimmunization appears to be rare in Glanzmann, but the risk of alloimmunization is still a major concern. When it does occur, an isoantibody may be directed against a functionally important region of the receptor complex, making subsequent transfusion of normal platelets ineffective. These isoantibodies are distinct from alloantibodies, which are formed to more subtle differences in the protein sequence, such as HPA-1 (PLA₁), where the membrane protein is still otherwise fully expressed and functional. These antibodies to normal platelets or HLA Class I have the potential of making the patient completely refractory to all future transfusions and, in the case of platelet directed antibodies, interfering with successful bone marrow transplantation for those patients even with an HLA matched donor.

Bernard–Soulier syndrome

Summary. Bernard–Soulier Syndrome (BSS) is a membrane receptor defect demonstrated to impact the initiation or adhesion phase of platelet plug formation and was initially described in 1948. The prominent member of the complex, GPIb, is a heterodimer composed of disulfide-bonded GPIb α and GPIb β subunits. GPIb then forms a non-covalent complex with two GPIX molecules. Two of these

trimers then associate non-covalently with one molecule of GPV to form the GPIb-IX-V complex. BSS is characterized by the inability of platelets to bind to VWF during the initial steps of platelet adhesion because these platelets either lack or have a qualitative defect in the platelet membrane glycoprotein GPIb-IX-V complex [22]. Mutations leading to absent expression or dysfunction have been uncovered in the genes for GPIb and GPIX [22]. BSS is nearly always inherited in an autosomal recessive pattern, with consanguinity a frequent finding. The platelets are decreased in number and very large on the peripheral smear. Of note, some patients with DiGeorge syndrome are missing the GPIb β chain, resulting in large platelets and mild thrombocytopenia, but there is minimal to no functional defect in these patients.

Clinical manifestations. Clinically, BSS typically presents in infancy with purpura, epistaxis or gingival bleeding. The age of onset is related to the severity of the disease. Later symptoms can include menorrhagia, gastrointestinal or genitourinary bleeding. Trauma or surgical procedures can also lead to excessive bleeding. The severity of bleeding symptoms can vary greatly among patients.

Diagnosis. One should start with a careful family history, including consanguinity and bleeding symptoms. Laboratory findings include mild thrombocytopenia but with much more severe bleeding than one would expect for the relatively small decrease in count. In type 1 BSS, the patients will also have giant platelets on peripheral blood smear [23,24]. A hallmark of the disease is the failure of platelets to agglutinate in the presence of ristocetin. This will differentiate BSS from other rare macrothrombocytopenic disorders, such as the MYH-9 family of platelet disorders. Lastly, flow cytometry should be performed with antibodies specific for each component of the complex (CD 42a-d) to characterize the decrease in the GPIb-IX-V membrane receptor. There is also a variant type BSS, in which the GPIb complex is dysfunctional with poor or absent binding of VWF, but there is still some complex present on the surface of the platelet. Patients with type 2 BSS may have normal platelet size and number [24]. These patients are identified by measuring the decreased amount of radiolabelled VWF binding in the presence of ristocetin cofactor compared with normal platelets.

Treatment. Significant bleeding or surgical procedures may require platelet transfusions. Given the absence of the GPIb-IX complex in BSS patients, the

risk of sensitization has to be considered. This can become a life-threatening complication because future platelet transfusions may be rendered useless by antibodies binding to the GPIb-IX-V complex on transfused platelets. In addition, leucodepleted platelets are required to decrease the exposure to HLA Class I antigens. Desmopressin and rFVIIa can be useful as well, but platelet transfusion remains the mainstay in treatment of severe bleeding. Because BSS has a wide clinical spectrum, the prognosis of BSS is related to the severity of an individual's disease and may change overtime based on hormonal alterations and the effects of aging.

Storage pool disease

Summary. Storage pool disease (SPD) is a heterogeneous group of congenital disorders that have in common a deficiency of granules, or their constituents, that results in a defect in ADP release from activated platelets and abnormal secretion-dependent platelet aggregation [25]. The *extension* phase of clot formation and platelet activation is mediated in large part by the release of stored compounds from platelet granules. The principal types of platelet granules are the α -granule and the δ -granule (dense body). The α -granule contains numerous proteins involved in platelet interaction, coagulation factors and proteins important in fibrinolysis [26]. The δ -granule contains primarily calcium, adenosine triphosphate, ADP and pyrophosphate. It is the presence of the high concentration of calcium in the δ -granule that gives its dense appearance on electron microscopy and allows it to be distinguished from the α -granule. Defects that affect the α -granule are termed α -SPD, those that affect δ -granules are δ -SPD and combined defects are termed $\alpha\delta$ -SPD [27].

α -Storage pool disease (α -SPD) or gray platelet syndrome

Summary. First described by Raccuglia in 1971, Gray platelet syndrome (GPS) is a deficiency in the number of α -granules and their contents in the platelet cytoplasm of affected patients. Absence of these granules results in a pale or 'grey' hue on the peripheral smear. GPS is thought to be extremely rare, with approximately 100 cases worldwide. The clinical manifestations of bleeding in GPS patients are because of a lack of these α -granule components resulting in a small and fragile platelet plug. GPS is inherited in an autosomal dominant or recessive pattern.

Pathophysiology. Megakaryocytes show defective α -granule production, with impaired uptake and storage of endogenously synthesized proteins, such as platelet factor 4, β -thromboglobulin or VWF, and defective storage of exogenous proteins, such as fibrinogen, albumin or factor V. These are key components in local platelet interaction and thrombin generation. It is most widely believed that the primary molecular defect in GPS occurs during the earliest stages of megakaryocyte maturation and involves a defect in the packaging of α -granule contents. Components of the α -granule contents and α -granule membrane, including P-selectin, have been found free in GPS platelet cytoplasm, indicating a failure of packaging [28].

Clinical manifestations. Bleeding symptoms may start from infancy, but the disease in GPS patients is usually less severe in general. Easy bruising, petechiae, mucosal membrane bleeding and postsurgical or traumatic bleeding may occur; life threatening spontaneous haemorrhage is rare.

Diagnosis. Peripheral blood Wright-Giemsa smear typically reveals mild to moderate thrombocytopenia and large gray agranular platelets. Platelet aggregation studies are variable with no classical response pattern to ADP, epinephrine, thrombin or collagen. In general, the secretion dependent aggregation studies are abnormal, but there are some patients who also show decreased response to thrombin or collagen [26]. Measurement of platelet adenosine nucleotide (ADP and ATP) content and release are useful in the diagnosis of storage pool and release defects [29]. When available, a primary tool used in diagnosis is electron microscopy, which reveals a near complete absence of α -granule in platelets and megakaryocytes [30].

δ -Storage pool disease (δ -SPD)

Summary. Dense granule storage pool defects were initially described in 1972 [31]. The platelets are morphologically normal on Wright-stained smears, but they are deficient in dense bodies by electron microscopy. These granules are storage sites for serotonin and the nucleotides ADP and ATP.

Pathophysiology. δ -SPD patients lack platelet dense granules resulting in a deficiency of ADP, ATP and serotonin, which when released, enhance platelet aggregation. The concentration of ADP correlates best with bleeding time and is likely mediated by its effect on VWF- and fibrinogen-dependent platelet

aggregation via the GPIIb–IIIa receptor. δ -SPD is likely inherited in an autosomal dominant pattern but neither the gene nor the molecular basis is known.

Clinical manifestations. δ -SPD patients have a bleeding spectrum and severity similar to those with α -SPD, including easy bruising, epistaxis and post-surgical bleeding.

Diagnosis. The diagnosis of δ -SPD is made using a combination of techniques. On peripheral blood smear, the platelet number is adequate and platelets appear normal in structure. A lack of dense granules can be better documented using transmission electron microscopy or fluorescent microscopy using special stains [32,33]. Recently, defects in adhesion under high shear and generation of the prothrombinase activity have also been reported in patients with δ -SPD, apparently as a result of decreased ADP secretion [6]. Platelet aggregation studies typically show a significantly impaired second wave of aggregation when stimulated by ADP, epinephrine or thrombin. The most consistent finding is that adenine nucleotides are reduced with an increased ratio of ATP to ADP and normal levels of lysosomal enzymes [34]. The combination of these findings with a clinical suspicion leads to the diagnosis of δ -SPD [6,32–34].

δ -SPD associated disorders

There are several disorders that have platelet dense granule deficiency in association with lysosome related organelles as part of their constellation of findings. Most notable is a melanosomal defect which results in a pattern of hypopigmentation. The best known are Hermansky–Pudlak syndrome (HPS) and related Griscelli syndromes, and Chediak–Higashi syndrome (CHS) [35].

HPS is an autosomal recessive disorder characterized by oculocutaneous albinism, lysosomal granule defects and platelet dense granule deficiency. These patients experience a variety of ocular manifestations and well-documented pulmonary fibrosis likely secondary to the lysosomal defects. From a haematological standpoint, these patients have a similar bleeding diathesis as patients with isolated δ -SPD. The results of their platelet aggregation studies will be abnormal in the secondary phase and secretion studies will be abnormal [6,32,33].

CHS is also characterized by oculocutaneous albinism and dense granule deficiency, but, in addition, features immune deficiency and a progressive neurological deterioration. CHS patients, moreover,

have cytoplasmic inclusions in other cell lines, which can be easily seen on the peripheral smear. These patients exhibit the same findings on platelet aggregation studies as in isolated δ -SPD, but in the accelerated phase of the disease, they develop thrombocytopenia distinguishing them from milder disorders. Bleeding symptoms are similar to isolated δ -SPD and can be managed accordingly, but only haematopoietic stem cell transplantation has been shown to improve the long-term outlook of this otherwise fatal syndrome [36].

$\alpha\delta$ -SPD

Briefly, combined α and δ platelet granule deficiencies are significantly less common than isolated defects. In these defects, δ -granules are always decreased, whereas the concentration of α -granules varies. These disorders also do not affect the entire platelet population uniformly because some platelets may have significantly more α and/or δ -granules than other platelets in the same patient. Unlike δ -SPD, laboratory testing reveals impaired primary aggregation in addition to secondary aggregation. Clinically, these patients behave much like α - or δ -SPD patients and respond to the same treatments in limited experience. Because of the lack of specificity of aggregation studies, the diagnosis also requires measurement of α - and δ -granule contents and/or electron microscopy to confirm the absence of platelet granules [37].

Management. The general treatment guidelines listed above are useful but vary from patient to patient in effectiveness. When bleeding occurs, treatment may include topical agents, DDAVP or Stimate nasal spray for responsive patients and antifibrinolytic agents. Initial treatment should include regular visits with healthcare team to be sure that proposed regimens provide hemostasis. In addition, the platelet transfusions may help for planned surgery, particularly for the ophthalmologic procedures in CHS, but this modality has not been studied systematically in SPD or GPS patients.

Membrane receptor disorders affecting the stages of clot formation

An evolving area of research is the field of inherited disorders of platelet membrane receptors affecting platelet extension and cohesion/aggregation activities. These disorders include the P2Y class of receptors, which respond to the adenine nucleotides secreted by platelet δ -granules, as

well as the TXA2 receptor and the epinephrine receptor.

The most studied of these receptors is the P2Y12 receptor which is responsible for ADP-induced platelet aggregation [38]. Defects in P2Y12 are inherited in an autosomal recessive pattern and can result from a number of different alterations in the gene coding for the receptor. Clinically, these patients exhibit a number of the same mild bleeding tendencies as SPD patients and, on laboratory workup, are found to have a very weak primary phase of aggregation when stimulated with ADP and other agonists. Only high concentrations of thrombin produce normal aggregation studies. These patients generally are treated with DDAVP for prophylaxis or bleeding episodes.

Glanzmann thrombasthenia (GT)

Summary. Recognized first in 1918, GT has been extensively studied and defines a defect in the consolidation phase of thrombus formation. GT patients have either a qualitative or quantitative disturbance in the platelet membrane GPIIb–IIIa complex. GT is a rare disorder and is inherited in an autosomal recessive fashion. Consanguinity has been identified in most cases.

Pathophysiology. The GPIIb–IIIa receptor is integral to platelet aggregation because in its activated state, it preferentially binds to fibrinogen and VWF. Fibrinogen and VWF then cross-link platelets by binding to the activated GPIIb–IIIa molecule on adjacent platelets. GT platelets are able to adhere with other receptors, such as GPIb–IX–V complex, to exposed subendothelium in damaged capillaries, but are unable to spread effectively without GPIIb–IIIa and cannot form platelet microthrombi because of their impaired aggregation.

Diagnosis. GT patients have normal numbers of platelets that appear morphologically normal on peripheral blood smear. Screening coagulation studies i.e. Protime and aPTT are unaffected, but bleeding time is prolonged. The typical pattern of platelet aggregation studies shows poor aggregation in response to ADP, collagen, epinephrine and thrombin, but normal aggregation in the presence of ristocetin. If GT is suspected, flow cytometry can be performed to confirm the diagnosis by determining the quantity of GPIIb–IIIa expressed on the platelet membrane using specific antibodies binding to either GPIIIa (CD61) or GPIIb (CD 41).

Table 2. Classification of Glanzmann thrombasthenia.

	Glycoprotein IIb–IIIa	Fibrinogen binding	Clot retraction
Type 1	<5%	Absent or severely deficient	Absent
Type 2	10–20%	Present	Normal or moderately deficient
Variant	>50%	Variable	Variable

Molecular basis. GT results from either a qualitative or quantitative defect of platelet GPIIb–IIIa complex. The genes coding for GPIIb and GPIIIa are located nearby on the long arm of chromosome 17. More than 100 mutations have been identified that either inhibit synthesis of the receptor altogether or interfere with its ability to be processed normally [39,40]. Genetic testing can be performed in special academic laboratories to determine the exact location of the mutation, but on a research basis only at this point. (<http://sinaicentral.mssm.edu/intranet/research/glanzmann>).

Clinical manifestations. Overall, three types of GT have been recognized based on the level of expression of GPIIb–IIIa (see Table 2).

Patients with type 1 express <5% of the normal amount, whereas patients with type 2 express 5% to 20%. Heterozygotes express 50% of normal and are typically asymptomatic, whereas patients with type 2 may have a severe phenotype. On the basis of these data, it seems that some level between 20% and 50% is needed to prevent bleeding. Unlike many of the other platelet defects, the bleeding from GT can be severe and may rarely result in death if not treated appropriately. Bleeding symptoms usually start in early infancy and include epistaxis, oral bleeding and purpura. The onset of menarche is associated with severe menorrhagia, often requiring transfusions. Bleeding symptoms in GT patients are usually worse in childhood, particularly epistaxis, but improve with age [40]. Antibodies against GPIIb–IIIa and HLA Class I have been detected in multiply transfused patients and cause serious complications if the patient becomes refractory to platelet transfusions.

Management. Localized bleeding can usually be treated with topical thrombin or antifibrinolytic agents, but invasive procedures usually require prophylactic platelet transfusion. Childbirth also requires aggressive management with platelet transfusion both during and after delivery [14]. HLA-matched platelets should be used if possible.

Recombinant factor VIIa has been used with success in some patients with alloantibodies or in an effort to avoid platelet exposure in the first place. Allogeneic bone marrow transplantation has been used successfully in the most severe of cases [41].

Prognosis. Generally with aggressive supportive care, GT patients have a good prognosis. Like many other platelet disorders, poor outcomes have most often been attributed to posttraumatic or unanticipated postsurgical bleeding.

Summary

The most severe forms of platelet dysfunction are associated with deficiencies in the platelet membrane receptors, but defects in the number and distribution of secretory granules, signalling or prothrombinase function can also cause significant bleeding. Whereas proper diagnosis of a particular platelet dysfunction is important to the patient and family for genetic counselling and prognosis, the current treatment options are fairly limited and generic, aimed at treating the symptoms rather than eliminating the primary defect. Registries and international mutational analysis projects will aid in further characterization of these syndromes and identify the specific genes involved. With the further expansion of gene therapy and novel therapeutic agents, newly diagnosed patients in the future may look forward to a normal life span free of major hemorrhagic events.

Disclosures

The authors stated that they had no interests which might be perceived as posing a conflict or bias.

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Links to organizations with interest in these areas

1. National Hemophilia Foundation: <http://www.hemophilia.org>
2. International Society on Thrombosis and Haemostasis Scientific and Standardization Committee (ISTH/SSC): http://www.med.unc.edu/isth/ssc_home.htm
3. National Organization for Rare Diseases, Inc.: <http://www.rarediseases.org>