

PLATELET FUNCTION DISORDERS

Second Edition

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Treatment of Hemophilia Monographs
Series Editor
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Platelet Function Disorders

Anjali A. Sharathkumar and Amy Shapiro

Summary

Platelets play a crucial role in hemostasis. Platelet dysfunction due to congenital and acquired etiologies is one of the most common causes of bleeding encountered in clinical practice. Bleeding manifestations are characterized by mucocutaneous bleeding like bruising, nose bleeding, and menorrhagia, and bleeding after hemostatic stress, such as after tonsillectomy and adenoidectomy, dental extraction, and, rarely, post-partum. This monograph was written following an extensive literature search through PubMed and Ovid using the search terms: “platelets,” “platelet function disorders and congenital/acquired,” “MYH9 disorders” and names of individual platelet function disorders. Further references not initially identified in the search but referenced within these articles were also reviewed. Review articles and textbooks were used to inform our discussion of the physiology of platelets, and current understanding of the biochemical mechanisms involved in platelet activation. An attempt has been made to summarize the spectrum of clinical manifestations of these disorders and their management.

Introduction

Clinical bleeding results from a disturbance in hemostasis. The term hemostasis applies to a myriad of physiological processes that are involved in maintaining vascular integrity and keeping the blood in fluid form. Normal hemostasis involves interaction between three forces first described by German pathologist Rudolf Virchow in 1856: blood (with soluble and cellular components), the blood vessel, and blood flow.

Human platelets are multifunctional anucleated cells that play a vital role in hemostasis. This monograph will discuss the physiology of platelets in hemostasis and platelet function defects. Disorders associated with decreased

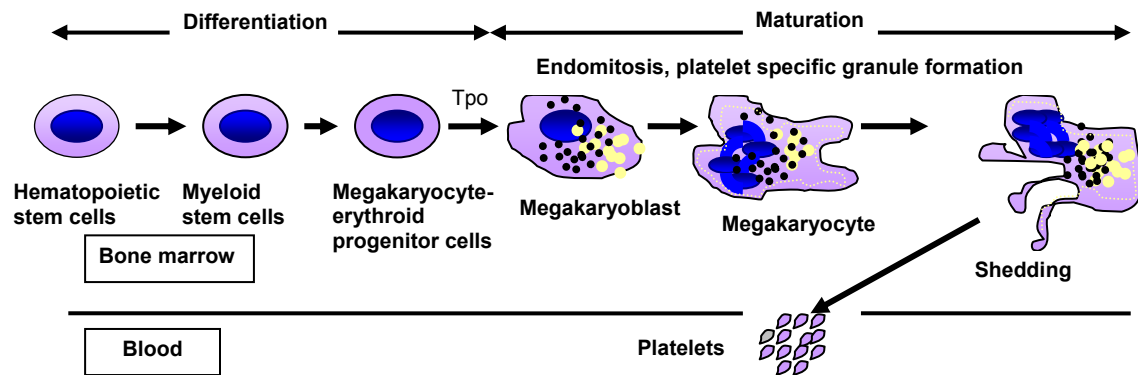
number of platelets (thrombocytopenia) are not covered in this monograph.

History of Platelet Discovery

German anatomist Max Schultze published the first accurate and convincing description of platelets in his newly-founded journal *Archiv für mikroskopische Anatomie* in 1865, as part of a study devoted mainly to white blood cells. He described “spherules” much smaller than red blood cells that occasionally clump and may participate in collections of fibrous material. He recognized them as a normal constituent of the blood and enthusiastically recommended further study by “those concerned with the in-depth study of the blood of humans.” In 1882, Italian pathologist Giulio Bizzozzero carried out a much more comprehensive study. He observed the “spherules” microscopically in the circulating blood of living animals and in blood removed from blood vessels. In well-planned experiments, he showed that they were the first component of blood to adhere to damaged blood vessel walls *in vivo*, and that *in vitro*, they were the first blood components to adhere to threads that subsequently became covered with fibrin. These initial discoveries are at the foundation of our current understanding of hemostasis.

Platelet Structure and Function

Platelets originate from the cytoplasm of bone marrow megakaryocytes (Figure 1). Platelets lack genomic DNA but contain megakaryocyte-derived messenger RNA (mRNA) and the translational machinery needed for protein synthesis. Circulating platelets are discoid in shape, with dimensions of approximately 2.0–4.0 by 0.5 μm , and a mean volume of 7–11 fl. Their shape and small size enables the platelets to be pushed to the edge of vessels, placing them in the optimum location to constantly survey the integrity of the vasculature. Platelets circulate in

Figure 1: Key steps in megakaryopoiesis

The diagram summarizes important steps of megakaryocyte development. Hematopoietic cells differentiate into megakaryocytes through exposure to the specific growth factor thrombopoietin (Tpo), with c-mpl as its receptor. Megakaryocyte maturation involves a process of endomitosis, nuclear duplication without cell division, resulting in DNA ploidy (8N-128N). Cytoplasmic organelles are organized into domains representing nascent platelets, demarcated by a network of invaginated plasma membranes. Within the marrow, megakaryocytes are localized near sinusoidal walls, facilitating shedding of large segments of cytoplasm into the circulation. The fragmentation of megakaryocyte cytoplasm into individual platelets results from shear force of circulating blood. Intracellular organelles are distributed into the platelet bud along microtubule tracks in shafts.

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a concentration of 150,000-450,000 cells/mL. Of the total body platelets, about 70% stay in circulation while the remaining 30% are continually but transiently sequestered in the spleen. Platelets remain in circulation for an average of 10 days. Most platelets are removed from the circulation by the spleen and liver after senescence, but a constant small fraction is continually removed through involvement in maintenance of vascular integrity.

On peripheral blood smears stained with Wright-Giemsa stain, platelets appear as small, granular staining cells with a rough membrane, and are normally present as 3-10 platelets per high-power oil-immersion field. Despite their simple appearance on the peripheral blood smear, platelets have a complex structure (Figure 2). Platelet internal structure has been divided into four zones:

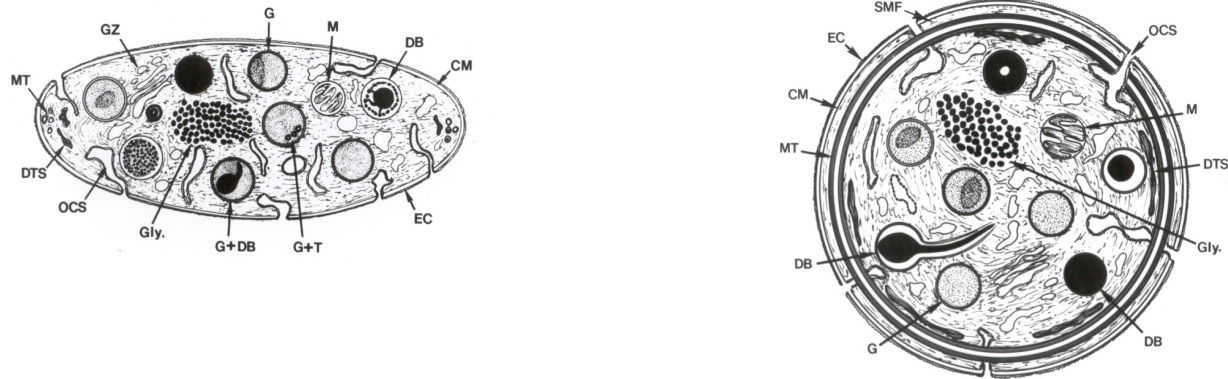
- Peripheral zone
- Sol-gel zone
- Organelle zone
- Membrane zone

The **peripheral zone** includes the outer membranes and closely associated structures. The platelet has a surface-connected system of channels called the open canicular system

(OCS). The walls of the OCS are included in this zone. The OCS provides access to the interior of the platelet to plasma substances, and an outlet channel for platelet products. The release of platelet products through the OCS after platelet activation is called "the release reaction."

The membranes of the platelet are rich in platelet receptors, which determine its specific cellular identity. These receptors are constitutively expressed on the platelets and require conformational change during platelet activation to express receptor function. The major classes of receptors and their ligands are shown in Table 1.

The peripheral zone also includes membrane phospholipids. Phospholipids are an important component of coagulation as they provide the surface upon which coagulation proteins react. Phospholipids also serve as the initial substrate for platelet enzymatic reactions to produce thromboxane A₂ (TXA₂), an important product of platelet activation and a powerful platelet agonist (substance that causes platelet aggregation). The platelet membrane also has the ability to translate signals from the surface into internal chemical signals.

Figure 2: Discoid platelets

The diagram summarizes ultrastructural features observed in thin sections of discoid platelets cut in cross-section. Components of the peripheral zone include the exterior coat (EC), trilaminar unit membrane (CM), and submembrane area containing specialized filaments (SMF) that form the wall of the platelet and line channels of the surface-connected open canalicular system (OCS). The matrix of the platelet interior is the sol-gel zone containing actin microfilaments, structural filaments, the circumferential band of microtubules (MT), and glycogen (Gly). Formed elements embedded in the sol-gel zone include mitochondria (M), granules (G), and dense bodies (DB). Collectively they constitute the organelle zone. The membrane systems include the surface-connected open canalicular system (OCS) and the dense tubular system (DTS), which serve as the platelet sarcoplasmic reticulum.

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The **sol-gel zone** is beneath the peripheral zone and consists of the framework of the platelet, the cytoskeleton. The cytoskeleton forms the support for the maintenance of the platelet's discoid shape as well as the contractile system that allows, upon activation, shape change, pseudopod extension, internal contraction, and release of granular constituents. The cytoskeleton comprises somewhere between 30-50% of the total platelet protein.

The **organelle zone** consists of the granules and cellular components such as lysosomes, mitochondria, etc. These organelles serve in the metabolic processes of the platelet and store enzymes and a large variety of other substances critical to platelet function. There are two compartments of adenine nucleotides: the storage or secretory pool in dense granules and the metabolic or cytoplasmic pool. Included in this zone are the alpha and dense granules.

The dense granules contain non-metabolic adenosine triphosphate (ATP) and adenosine diphosphate (ADP), serotonin, and calcium. The alpha granules contain adhesive proteins such as fibrinogen, fibronectin, von Willebrand factor

(VWF), thrombospondin, and vitronectin. The alpha granules also contain growth-promoting substances such as platelet-derived growth factor (PDGF), platelet factor 4, and transforming growth factor. Coagulation factors including

Table 1: Major platelet membrane receptors and their ligands

Glycoprotein (GP) Receptor	Structure	Function / Ligand
GP IIb/IIIa	Integrin $\alpha_{IIb}\beta_3$	Receptor for fibrinogen, VWF, fibronectin, vitronectin and thrombospondin
GP Ia/IIa	Integrin $\alpha_2\beta_1$	Receptor for collagen
GP Ib/IX/V	Leucine-rich repeats receptor	Receptor for insoluble VWF
GP VI	Non-integrin receptor, Immunoglobulin superfamily receptor	Receptor for collagen

factor V, high molecular weight kininogen, factor XI, and plasminogen activator inhibitor-1 are also present in the alpha granule.

The fourth zone is the **membrane zone**, which includes the dense tubular system. It is here that calcium, important for triggering contractile events, is concentrated. This zone also contains the enzymatic systems for prostaglandin synthesis.

Role of Platelets in Hemostasis

In a normal physiological state, platelets circulate without adhering to undisturbed vascular endothelium. Upon disruption of the integrity of the vascular endothelium or alteration in the shear stress of the blood flow, platelets are “activated.” Platelet activation plays a central role in both benign and pathological responses to vascular injury and thrombus formation. The process of transformation of inactivated platelets into a well-formed platelet plug occurs along a continuum, but may be divided into three steps: (1) adhesion; (2) aggregation; and (3) secretion.

The following section gives an overview of these events.

Platelet adhesion

Subendothelial components (e.g., collagen, VWF, fibronectin, and laminin) are exposed upon vessel wall damage. VWF facilitates the initial adhesion via binding to the glycoprotein (GP) Ib/IX/V complex, especially under high shear conditions. These interactions enable platelets to slow down sufficiently so that further binding interactions take place with other receptor-ligand pairs, resulting in static adhesion. In particular, the initial interaction between collagen and GPVI induces a conformational change (activation) in the platelet integrins GPIIb/IIIa and GPIa/IIa. VWF and collagen form strong bonds with GPIIb/IIIa and GPIa/IIa, respectively, anchoring the platelets in place. Recruitment of additional platelets occurs through platelet-platelet interaction that is mainly mediated through fibrinogen and its receptor, GPIIb/IIIa.

Patients with Bernard-Soulier syndrome and Glanzmann’s thrombasthenia have defective platelet adhesion due to decreased or absent expression of the glycoprotein receptors that are involved in platelet adhesion: the GPIb/IX/V and GPIIb/IIIa receptors, respectively.

Platelet aggregation and secretion

Platelets undergo morphological changes upon activation. Platelet shape changes from a disc to a spiny sphere with multiple pseudopodial extensions. The platelet membrane becomes rearranged, with exposure of negatively charged phospholipids that facilitate the interaction with coagulation proteins to form the tenase and prothrombinase complexes. The contents of platelet granules are secreted through the surface-connected canalicular system, with ADP, fibrinogen, and factor V appearing on the platelet surface and in the milieu immediately surrounding the platelet. PDGF is secreted and leads to smooth muscle proliferation. Repeated secretion of PDGF resulting from recurrent episodes of platelet activation increases smooth muscle proliferation and may initiate atherosclerosis. Platelet factor 3 is also expressed after platelet activation. Small pieces of the platelet are able to bud off to form circulating microparticles. Platelet-agonist interactions result in the production or release of a variety of intracellular messenger molecules that facilitate these reactions.

Biochemical processes involved in platelet aggregation and secretion

As platelets are recruited to the area of blood vessel damage, they become activated by a range of agonists including ADP, thrombin, and thromboxanes, which interact with transmembrane receptors. Receptor stimulation results in G protein interactions, which enable activation of enzymes involved in cellular metabolic pathways, in particular, phosphatidylinositol 3-kinase and phospholipase C. Metabolic pathway activation results in the elevation of cytoplasmic calcium and phosphorylation of substrate proteins, which bring about changes in the cytoskeleton, enabling platelet shape change and spreading, release of alpha- and dense-granular contents, stimulation of phospholipase A2 and liberation of TXA2, induction of a procoagulant surface,

and activation of GPIIb/IIIa receptors. The biochemical details of these reactions are illustrated in Figure 3.

A rare, diverse group of disorders of platelet signal transduction have been described, including defects in the agonist receptors for TXA₂, ADP and collagen; the membrane G proteins; and the prostaglandin pathway enzymes cyclooxygenase and TXA₂ synthetase (Figure 4). Disorders of the platelet storage granules are also well described and include dense granule deficiency, alpha granule deficiency, and combined dense and alpha granule deficiency (Figure 4).

Summary

The contribution of platelets to hemostasis lies in the formation of the primary hemostatic plug, the secretion of important substances for further recruitment of platelets, the provision of a surface for coagulation to proceed, the release of promoters of endothelial repair, and the restoration of normal vessel architecture. Disruption in any of the above described events and biochemical processes may lead to platelet dysfunction, which may be either inherited or acquired.

Clinical Features of Platelet Defects

The initial diagnosis of a platelet function disorder relies on careful evaluation of clinical findings and a detailed medical history, including family history. Reviewing the medical history can establish whether the disorder is hereditary or acquired. Bleeding manifestations typical of platelet function defects include:

- Unexplained or extensive bruising, particularly associated with soft tissue hematoma;
- Epistaxis, particularly lasting more than 30 minutes or causing anemia or admission to hospital;
- Menorrhagia, particularly if present since menarche;
- Gingival bleeding;
- Bleeding during childbirth;

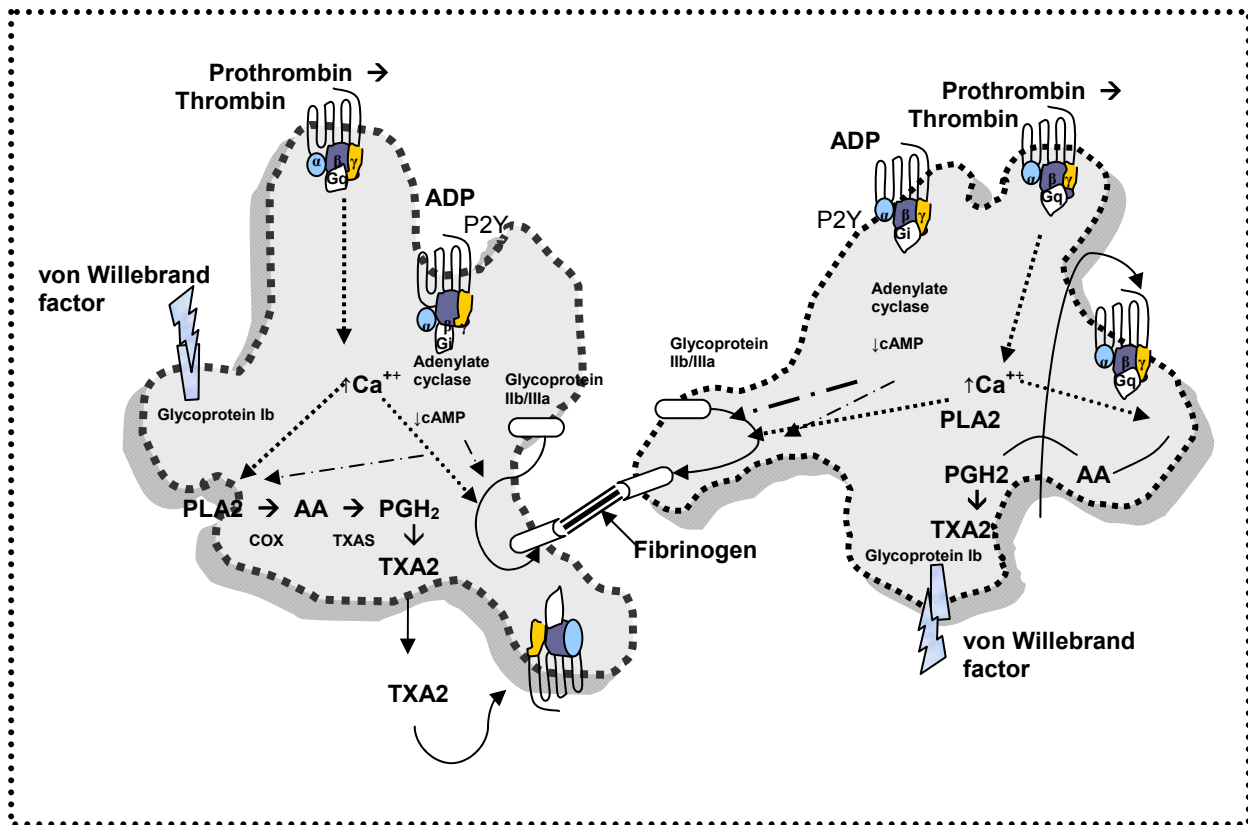
- Bleeding following invasive procedures (e.g., dental extraction, tonsillectomy, adenoidectomy).

Rarely, gastrointestinal bleeding, visceral hematoma, hemarthrosis, and intracerebral hemorrhage have also been reported, although these bleeding symptoms are more commonly observed in hereditary or acquired coagulation factor disorders.

In inherited platelet function disorders, bleeding is usually present since childhood but may be variable and exacerbated by conditions that stress hemostasis. In acquired hemorrhagic disorders, the clinical picture is dominated by the underlying disease; quite often these disorders are associated with multiple hemostatic defects such as thrombocytopenia or significant coagulation abnormalities. In most cases, a history and physical examination will reveal the etiology of the platelet dysfunction (e.g., a history of medication, diagnosis of myeloproliferative disorders, presence of cataracts or hearing defects in MYH-9 disorders, oculocutaneous albinism in patients with Hermansky-Pudlak syndrome, and hyperextensible joints in Ehlers-Danlos syndrome).

Laboratory evaluation

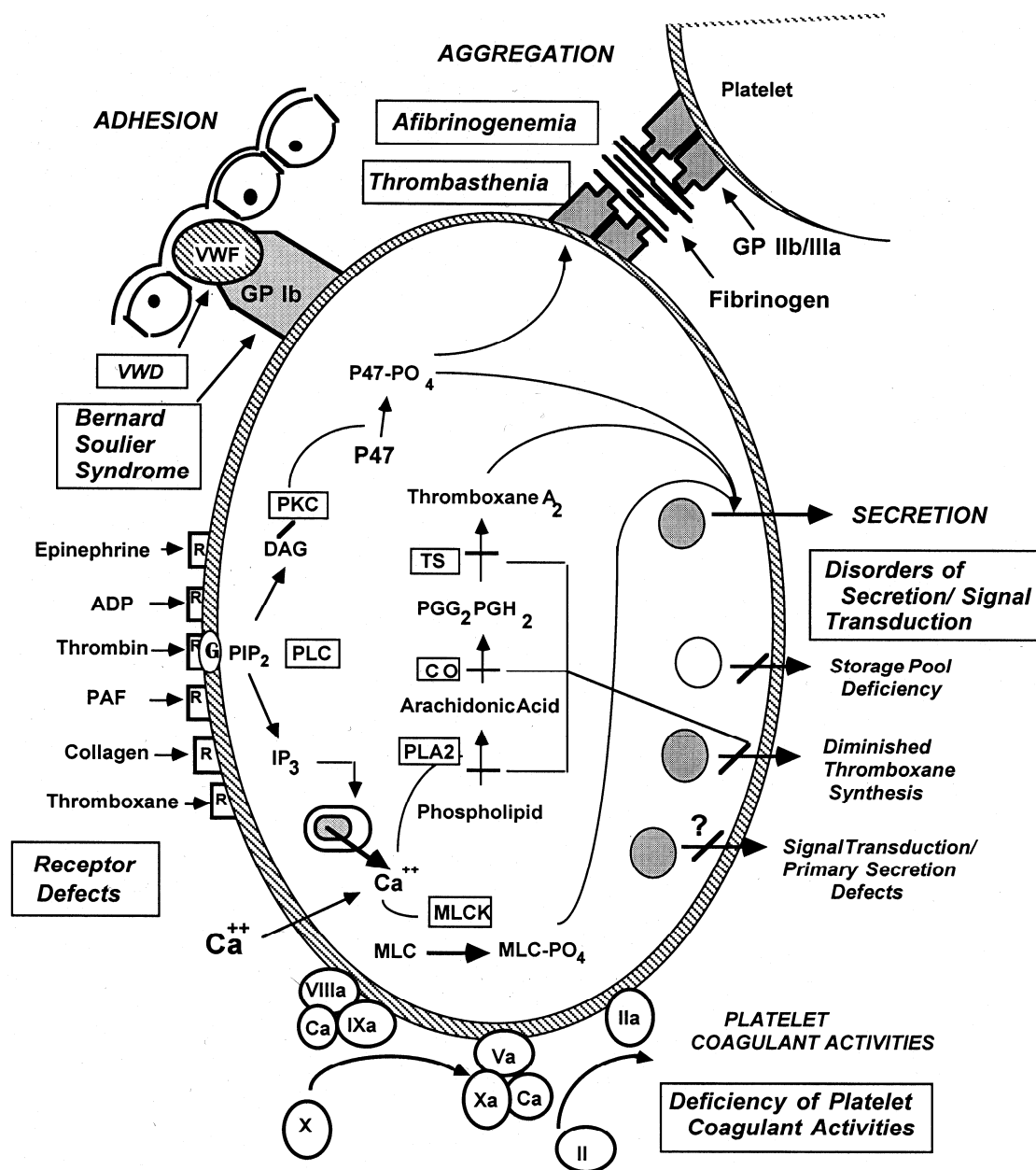
A reliably predictive “screening” test for platelet dysfunction does not exist. An approach for diagnostic testing to evaluate a clinically significant bleeding history without an identifiable underlying condition is shown in Figure 5. As platelets are easily activated, it is recommended that samples be drawn either in syringes or evacuated tubes. The anticoagulant of choice is 3.2% sodium citrate, which acts by chelating calcium ions (Ca⁺⁺). A blood/citrate ratio of 9:1 is recommended; higher concentration of citrate overchelates Ca⁺⁺ and subsequently interferes with later platelet function studies. Samples should be maintained at room temperature (20-25°C) during transport and storage. Tubes should be transported upright and care should be taken not to agitate or shake samples; manual transport is thus preferred to pneumatic tube systems.

Figure 3: Agonists, receptors, and effector systems in platelet activation

The diagram summarizes the molecular and biochemical mechanisms involved in platelet activation. The activation of platelets is induced by the interaction of several agonists, thromboxane A₂ (TXA₂), adenosine diphosphate (ADP), and thrombin with receptors expressed on the platelet membrane. TXA₂ is synthesized by activated platelets from arachidonic acid (AA) through the cyclooxygenase (COX) pathway. Once formed, TXA₂ can diffuse across the membrane and activate other platelets. In platelets, TXA₂ receptors couple to the G-proteins (G_q and G₁₂ or G₁₃), all of which activate phospholipase C (PLC). This enzyme degrades the membrane phosphoinositides (such as phosphatidylinositol 4,5-bisphosphate [PIP₂]), releasing the second messengers inositoltriphosphate (IP₃) and diacylglycerol (DAG). DAG activates intracellular protein kinase C (PKC), which causes protein phosphorylation. The release of IP₃ increases cytosolic levels of Ca⁺⁺, which is released from the endoplasmic reticulum. ADP is released from platelets and red cells. Platelets express at least two ADP receptors, P₂Y₁ and P₂Y₁₂, which couple to G_q and G_i, respectively. The activation of P₂Y₁₂ inhibits adenylate cyclase, causing a decrease in the cyclic AMP (cAMP) level, and the activation of P₂Y₁ causes an increase in the intracellular Ca⁺⁺ level. The P₂Y₁₂ receptor is the major receptor able to amplify and sustain platelet activation in response to ADP. Thrombin is rapidly generated at sites of vascular injury from circulating prothrombin and, besides mediating fibrin generation, represents the most potent platelet activator. Platelet responses to thrombin are largely mediated through G-protein-linked protease-activated receptors (PARs), which are activated after thrombin-mediated cleavage of their N-terminal exodomain. Human platelets express PAR1 and PAR4. The effects of agonists mediated by the decrease in cAMP levels and increase in intracellular Ca⁺⁺ levels lead to platelet aggregation through the change in the ligand-binding properties of the glycoprotein IIb/IIIa (αIIbβ₃), which acquires the ability to bind soluble adhesive proteins such as fibrinogen and von Willebrand factor. The release of ADP and TXA₂ induces further platelet activation and aggregation.

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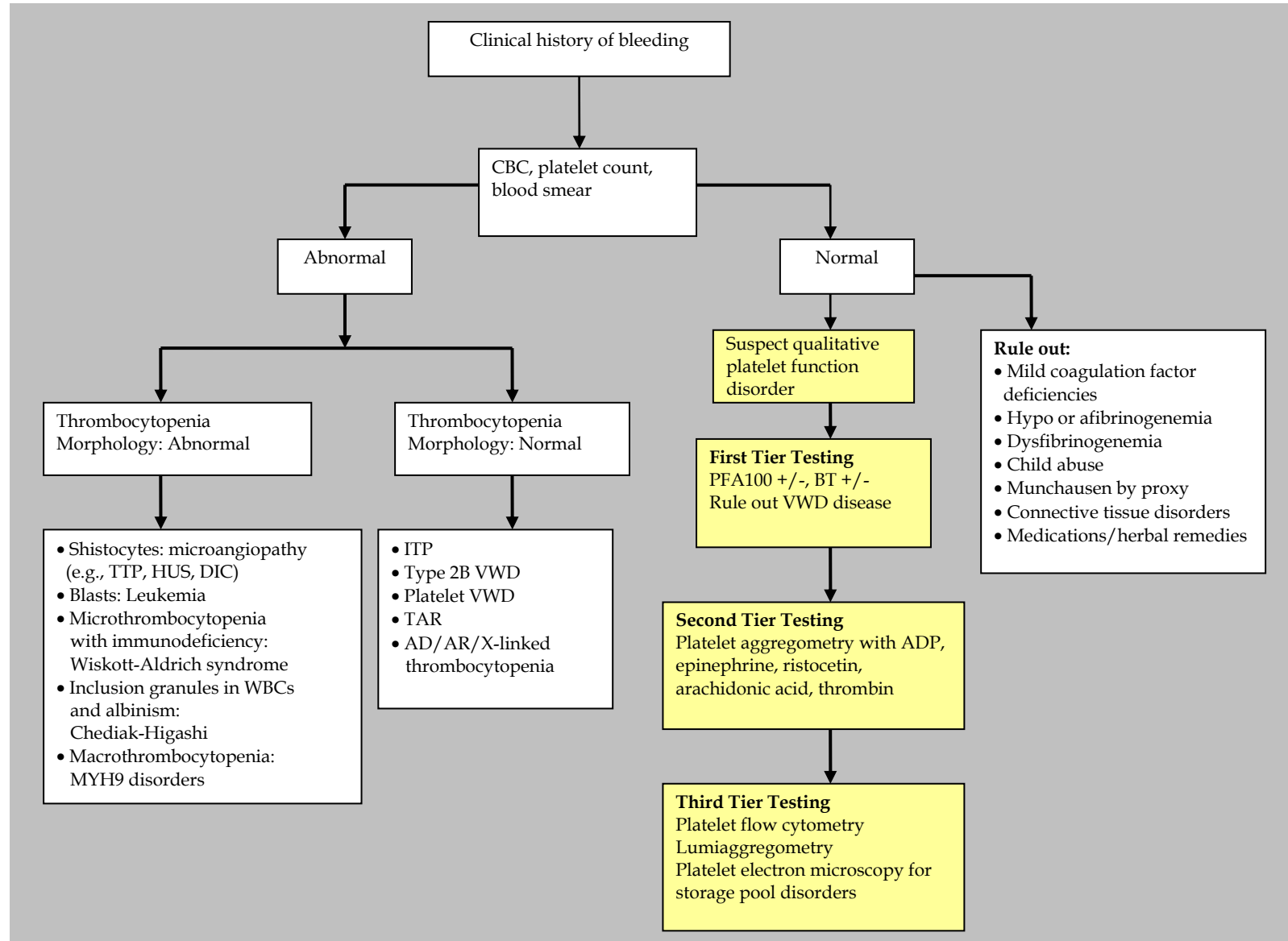
Figure 4: Schematic representation of normal platelet responses and the congenital disorders of platelet function



AKR/98

Abbreviations: CO = cyclooxygenase; DAG = diacylglycerol;
 IP3 = inositoltriphosphate; MLC = myosin light chain;
 MLCK = myosin light chain kinase;
 PIP2 = phosphatidylinositol bisphosphate;
 PKC = protein kinase C; PLC = phospholipase C;
 PLA2 = phospholipase A2;
 VWF = von Willebrand factor;
 VWD = von Willebrand disorder.

Reproduced with permission from: Rao, AK. Congenital disorders of platelet function: disorders of signal transduction and secretion. Am J Med Sci 1998; 316(2):69-76.

Figure 5: Algorithm for evaluation of a patient with suspected platelet disorders

Abbreviations: BT: bleeding time; PFA: Platelet function abnormalities; N: Normal; TTP: Thrombotic thrombocytopenic purpura; HUS: Hemolytic uremic syndrome; DIC: Disseminated intravascular coagulation; WBCs: White blood cell count; MYH 9: Myosine heavy chain gene disorders; ITP: Immune thrombocytopenic purpura; VWD: von Willebrand's disease; TAR: Thrombocytopenia and absent radi; AD: Autosomal dominant; AR: Autosomal recessive; ADP: Adenosine diphosphate.

Review of blood count

A complete blood count with review of peripheral blood smear should be the first step in the evaluation of a platelet function disorder. These basic tests will provide the investigator with important information, such as the platelet count and morphology of platelets/other cell lines, which is critical for the diagnosis of various inherited and acquired platelet disorders (Figures 5 and 6). The presence of schistocytes and helmet cells may lead to consideration of microangiopathic processes such as hemolytic uremic syndrome and thrombotic thrombocytopenic purpura. The presence of large but decreased platelet numbers with normal morphology of red and white blood cells may lead one to consider immune-mediated processes. Large inclusion granules in white cells are visible on smears in Chediak-Higashi syndrome. Platelets may be large in Bernard-Soulier syndrome and May-Hegglin anomaly. In Wiskott-Aldrich syndrome, platelets are small in size and decreased in number. Platelets may appear gray or washed out in Gray platelet syndrome. However, in the majority of platelet function defects such as release and storage pool defects and Glanzmann's thrombasthenia, platelet number and morphology are normal. Therefore, further assessment of platelet function is needed.

Bleeding time and PFA-100 closure time

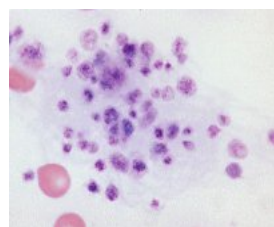
The laboratory tests that are conventionally used to assess platelet function include bleeding time (BT), and platelet function tests (which may be performed either on whole blood or platelet-rich

plasma). Neither the BT nor the platelet function analyzer (PFA) are good screening tools for platelet function disorders as each has limited sensitivity (~40%), even in symptomatic patients. The BT is operator-dependent and is affected by a subject's age and skin laxity. Both BT and PFA-100 closure time are prolonged in patients with low hematocrits and normal platelet function. Although technically sensitive when performed accurately, the BT is the only *in vivo* test to assess platelet function. Despite the limitations of BT and PFA-100 closure time, these tests can be useful for narrowing diagnostic considerations among patients with a history of mucocutaneous bleeding.

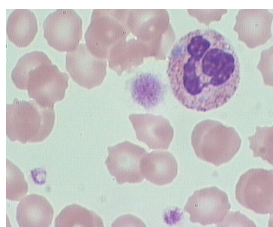
Platelet aggregation studies

Specific platelet function tests may be performed using assessment of aggregation to a panel of agonists with platelet-rich plasma or through whole blood aggregometers. The pattern obtained usually allows the investigator to diagnose and generally classify the defect. Common agonists employed in platelet function analysis include ADP, epinephrine, collagen, arachidonic acid, ristocetin, and thrombin. Less common agonists, such as low-dose ristocetin and cryoprecipitate, may be used in order to differentiate certain types of von Willebrand disease (VWD) from pseudo-von Willebrand disease (a defect of the platelet GPIIb). Platelet function analysis must be performed by experienced technicians on samples that are properly obtained and promptly transported to prevent activation of platelets before testing. Temperature, lipemia,

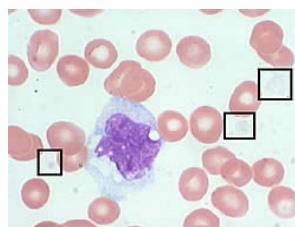
Figure 6: Morphological abnormalities seen on blood smear in patients with platelet function disorders



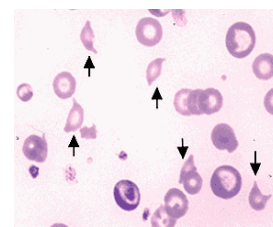
A: Platelet satellitism seen with EDTA



B: Giant platelets with eosinophilic and neutrophilic inclusions



C: Gray platelet syndrome



D: Schistocytes

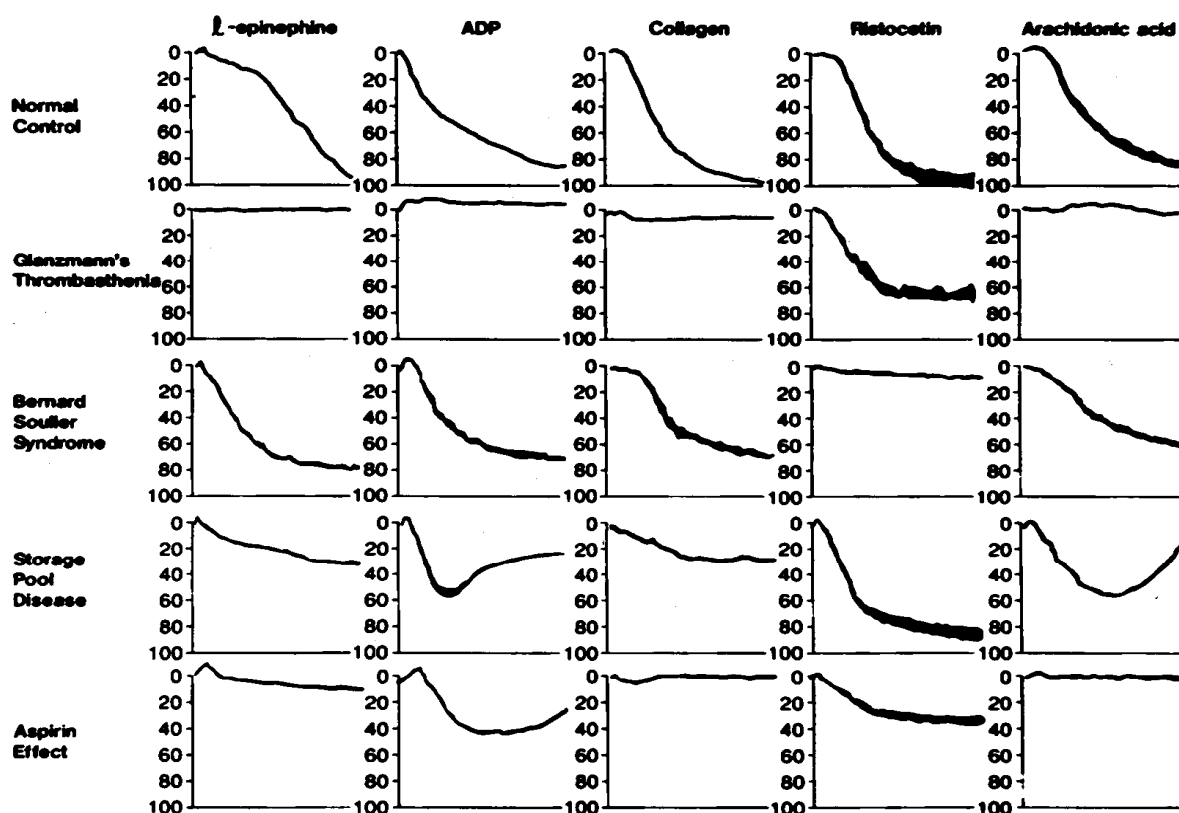
sample collection, interval from venipuncture, and preparation of platelet-rich plasma may all affect platelet aggregation profiles.

Platelet aggregation studies can confirm the effects of acetylsalicylic acid (Aspirin®), thienopyridines, β -lactam antibiotics, and paraproteins on platelet function. For example, VWD and Bernard-Soulier syndrome may be associated with defects in aggregation to ristocetin. Glanzmann's thrombasthenia has a flat aggregation profile to all agonists except ristocetin. Figure 7 and Table 2 describe abnormalities commonly encountered in platelet aggregation studies.

Platelet secretion assays

Platelet secretion is predominantly measured by two different assays. The first one, lumiaggregometry, simultaneously measures aggregation and luciferase luminescence. It measures the release or secretion of adenosine triphosphate (ATP) by the dense granules upon stimulation by agonists. Secretion can also be measured by allowing platelets to take up ^{14}C -labelled serotonin; its release is then measured in response to agonists. This test has a significant technical component, and is performed only in select laboratories. Abnormalities in these secretion assays may indicate release and storage pool defects.

Figure 7: Platelet aggregation studies in platelet-rich plasma from normal adult and patients with designated platelet function disorders



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Table 2: Platelet aggregation response to natural agonists in congenital and acquired platelet function defects

Disorder	Platelet aggregation					PFA-100 CT	
	ADP/epinephrine		Other platelet agonists				
	Primary wave	Secondary wave	Collagen	Arachidonic acid	Ristocetin	CADP	CEPI
Inherited							
von Willebrand disease Type 2B	N	N	N	N	I	P	P
Glanzmann's thrombasthenia	A	A	A	A	N	P	P
Bernard-Soulier syndrome	N	N	N	N	A	P	P
Storage pool disease	N/D	A	N/D	N/D	N	N/P	N/P
Acquired							
Aspirin	N/D	A	D	A	N	N	P
ADP receptor antagonists	D	D	NA	N	NA	N/P	N/P
GP IIb/IIIa inhibitors	D	D	D	A	NA	P	P

Abbreviations: PFA: platelet function analyzer; CT: closure time; ADP: adenosine diphosphate; CADP: collagen and ADP; CEPI: collagen and epinephrine; A: absent; D: decreased; I: increased (Type 2B VWD); N: normal; NA: not applicable; P: prolonged; GP: glycoprotein

Flow cytometry

Flow cytometry is the technique that measures protein expression on the cell using monoclonal antibodies. The most common clinical use for platelet flow cytometry is the diagnosis of inherited defects in platelet surface glycoproteins. Flow cytometry can detect decreased expression or absence of GPIb (Bernard-Soulier syndrome) and GPIIb/IIIa (Glanzmann's thrombasthenia). Other assays have been developed to test for storage pool disease and heparin-induced thrombocytopenia. Flow cytometry has also been used to assess a wide array of platelet characteristics in basic science and clinical studies. These include multiple techniques for assessing platelet activation and reactivity, measuring the effects of antiplatelet agents, and monitoring thrombopoiesis. These techniques have the advantage of using whole blood and are not

limited by thrombocytopenia, as are most other platelet function assays. In general, these techniques have yet to be used in routine clinical practice.

Electron microscopy

Electron microscopy (EM) will reveal structural platelet abnormalities, including a decreased number or abnormal morphology of platelet alpha and dense granules. EM studies are helpful in diagnosing platelet granule defects.

Molecular studies

Some families with severe platelet function disorders may benefit from identification of their molecular defect(s) to allow antenatal diagnosis to be offered. Molecular analysis of platelet GPs is available at a small number of specialist laboratories. These tests are not offered in routine clinical care.

More specialized tests

A wide variety of tests are available at specialist laboratories that may provide further diagnostic information, including analysis of receptor expression, protein phosphorylation, and formation of second messengers. In addition, several laboratories are at an early stage of developing genomic- and proteomic-based approaches for the analysis of individuals with platelet disorders, although the practical significance of these tests is presently unclear.

Acquired Disorders of Platelet Function

Disorders of platelet function are among the most common acquired hematologic abnormalities. The majority of these defects are detected incidentally due to abnormal laboratory tests including a prolonged BT or PFA-100 closure time. These defects as measured by laboratory tests do not correlate with bleeding risk. Hence, the clinical decision to evaluate acquired platelet function disorders depends solely on whether the observed derangement in platelet function poses a threat to the patient.

Acquired platelet disorders are classified broadly as those caused by defects that are intrinsic to the platelets and those caused by defects which are extrinsic to the platelets (Table 3). Drugs and many systemic diseases can lead to acquired platelet function defects. Acquired platelet function defects due to drugs are mild and ubiquitous. More than 100 drugs including Aspirin®, certain foods, spices, and vitamins have been reported to impair platelet function (Table 4). For almost all agents, the data are limited to descriptions of abnormal *in vitro* platelet aggregation tests or a prolonged bleeding time, which may have no clinical importance. Platelet dysfunction has also been reported in patients with uremia, liver dysfunction, cardiac bypass procedures, sepsis/infections, leukemia, and conditions including disseminated intravascular coagulation (DIC). It is important to underscore that Aspirin®, and presumably other causes of abnormal platelet function such as chronic renal failure and cardiac surgery, can profoundly

exacerbate bleeding in patients with an underlying inherited platelet disorder. The salient features of clinically significant conditions contributing to platelet dysfunction are summarized in Table 3.

In addition to platelet abnormalities resulting in decreased platelet function, defects leading to platelet gain-of-function are being recognized increasingly as a clinical entity. For instance, heparin-induced thrombocytopenia results in a transient, but highly thrombogenic condition that is due to “hyperfunction” of platelets. Hyperactive platelet syndrome (or sticky platelet syndrome) is an uncommon but well-described condition that results in arterial or venous thrombosis and complications during pregnancy. These gain-of-function abnormalities are beyond the scope of this monograph.

Management of Platelet Function Defects

Prevention and local care

There remain no good predictors of bleeding risk in patients who have platelet disorders. Most bleeding is a variation on normal anatomic and physiologic bleeding. Care should therefore be focused on prevention. Avoidance of medications such as Aspirin® and nonsteroidal analgesics is critical. Fastidious dental hygiene with regular teeth cleaning, hormonal control of menstrual bleeding, and presurgical treatment plans should be part of the comprehensive care. Most bleeding episodes can be controlled with local measures and platelet transfusions. Epistaxis and gingival bleeding are successfully controlled in most patients by nasal packing or the application of gel foam soaked in topical thrombin. Local bleeding during dental procedures can be treated by local measures, such as fibrin sealants. In addition, antifibrinolytic agents, such as aminocaproic acid, may be useful to control mild bleeding following scheduled dental procedures or other mucosal bleeding.

Table 3: Acquired disorders of platelet dysfunction presenting with bleeding symptoms

Site of platelet dysfunction	Systemic illness	Bleeding severity	Potential mechanism	Platelet aggregation abnormalities	Treatment
Intrinsic disorders of platelet function	Chronic myeloproliferative disorders	Mild to moderate	Defect at the level of committed megakaryocyte: 1) Abnormal lipid peroxidation and responses to thromboxane A ₂ 2) Subnormal serotonin uptake and storage 3) Abnormal expression of Fc receptors 4) Combined defect in membrane expression and activation of GPIIb/IIIa complexes 5) Acquired storage pool disorder 6) ↓ HMWM of plasma and platelet VWF	Inconsistent or defective aggregation	Treatment of underlying disorder
	Myelodysplastic syndrome/leukemias	Mild to moderate	Defective megakaryopoiesis: 1) Dilated canalicular system and abnormal microtubular formation 2) Reduced or giant granules may form by the fusion of several single granules 3) Acquired membrane defect with abnormal glycoprotein expression	Inconsistent or multiple aggregation defects	<ul style="list-style-type: none"> ▪ Treatment of underlying disorder ▪ Amicar®
Extrinsic disorders of platelet function	Uremia	Mild	1) platelet GPIIb/IX receptor number and function normal or ↓ 2) ↓ shear-induced platelet aggregation with high shear rates, possibly due to ↑ proteolysis by ADAMTS13 VWF metalloprotease 3) Defective activation-dependent receptor function GPIIb/IIIa for binding fibrinogen and VWF 4) Defective platelet secretion of ADP	↓ aggregation with collagen, ADP and epinephrine	<ul style="list-style-type: none"> ▪ Dialysis ▪ Correction of anemia ▪ DDAVP ▪ Conjugated estrogens ▪ Platelet transfusion ▪ rFVIIa ▪ Cryoprecipitate ▪ Humate-P®
	Liver dysfunction	Mild to severe	Altered platelet membrane palmitate and stearate metabolism	↓ aggregation to collagen, thrombin, ristocetin; absent secondary aggregation waves after aggregation with ADP and epinephrine	<ul style="list-style-type: none"> ▪ Correction of underlying disorder ▪ Platelet transfusion ▪ DDAVP
	Paraproteinemia	Mild to severe	Nonspecific binding of immunoglobulins to platelet surface +/- specific antigen/antibody interactions	Defective aggregation	<ul style="list-style-type: none"> ▪ Plasmapheresis ▪ Treatment of underlying disorder ▪ Platelet transfusions only during life-threatening bleeding

Table 3: Acquired disorders of platelet dysfunction presenting with bleeding symptoms (cont'd)

Site of platelet dysfunction	Systemic illness	Bleeding severity	Potential mechanism	Platelet aggregation abnormalities	Treatment
Extrinsic disorders of platelet function	Disseminated intravascular coagulation		Platelet activation by thrombin Acquired storage pool defect	↓ aggregation	<ul style="list-style-type: none"> ▪ Treatment of underlying disorder ▪ Platelet transfusion
	Cardiopulmonary bypass		1) Platelet activation and fragmentation due to hypothermia, contact with fibrinogen-coated synthetic surfaces, contact with blood/air interface, damage caused by blood suctioning, and exposure to traces of thrombin, plasmin, ADP, or complement 2) Drugs (e.g., heparin, protamine, and Aspirin®) and production of fibrin degradation products expected to impair platelet function	Abnormal <i>ex vivo</i> platelet aggregation in response to several agonists, ↓ platelet agglutination in response to ristocetin, and poor release reaction due to deficiency of alpha and dense granules	<ul style="list-style-type: none"> ▪ Platelet transfusion ▪ DDAVP ▪ Aprotinin ▪ Antifibrinolytics ▪ rFVIIa
	Hypothermia		1) ↓ plasma soluble P-selectin expression 2) ↓ levels of thromboxane B2	↓ platelet activation	Correction of hypothermia

Abbreviations: GP: Glycoprotein; HMWM: High molecular weight multimers; VWF: von Willebrand factor; ADP: Adenosine diphosphate; DDAVP: Desmopressin (1-deamino-8-D-arginine vasopressin); rFVIIa: recombinant factor VIIa,

Table 4: Acquired platelet dysfunction due to drugs and food substances

Agent	Potential Mechanism of Action
Drugs:	
Abciximab (ReoPro®), eptifibatide (Integrilin®), oral $\alpha_{IIb}\beta_3$ inhibitors, fibrinolytic agents	Inhibition of GPIIb/IIIa-fibrinogen interaction
Ticlopidine (Ticlid®), clopidogrel (Plavix®)	Inhibition of ADP receptors
Epoprostenol, iloprost, beraprost	Stimulation of adenylyl cyclase
Nitrites, nitroprusside	Stimulation of guanylyl cyclase
Methylxanthines (theophylline), dipyridamole, sildenafil and related drugs	Inhibition of phosphodiesterase
Aspirin, nonsteroidal anti-inflammatory drugs, moxalactam, losartan	Inhibition of thromboxane pathway
Verapamil, calcium channel blockers (nifedipine, diltiazem)	Inhibition of calcium influx
Tricyclic antidepressants, fluoxetine	Inhibition of serotonin uptake
Statins: lovastatin, pravastatin, simvastatin, fluvastatin, atorvastatin, cerivastatin	Interference with GTP signaling pathways
Antibiotics: β -lactam antibiotics, penicillins, nitrofurantoin	Mechanism unknown
Plasma expanders: dextran, hydroxyethyl starch	Impairment of platelet aggregation*
Anesthetics: dibucaine, procaine, cocaine, halothane	Impairment of platelet aggregation*
Phenothiazines: chlorpromazine, promethazine, trifluoperazine	Impairment of platelet aggregation*
Oncologic drugs: mithramycin, daunorubicin, BCNU	Impairment of platelet aggregation*
Radiologic contrast agents: Renographin-76, Renovist-II, Conray 60	Impairment of platelet aggregation*
Antihistamines: diphenhydramine, chlorpheniramine	Impairment of platelet aggregation*
Miscellaneous: clofibrate, hydroxychloroquine	↓ platelet response to ADP, epinephrine, and collagen
Foods and additives	
Omega-3 fatty acids, fish oil	Reduction in TXA ₂ synthesis
Vitamin E, onion	Inhibition of arachidonic acid metabolism
Onion, cumin, turmeric, clove	↓ platelet thromboxane production
Garlic	Inhibition of fibrinogen binding to platelets

*Indicates that the precise mechanism contributing to platelet dysfunction is not known.

Specific treatment options for patients with platelet dysfunction

Antifibrinolytic agents

Tranexamic acid (15-25 mg/kg orally every 6-8 hours, or 10 mg/kg intravenously every 8 hours) and epsilon aminocaproic acid (EACA, Amicar®; 50-100 mg/kg intravenously or orally every 4-6 hours) are helpful to stabilize the clot. These medications are useful for control of menorrhagia and other mild bleeding manifestations from mucous membranes, such as epistaxis. Tranexamic acid and EACA may be

used as a mouthwash (EACA in its liquid form or tranexamic acid, 10 mL of a 5% weight-to-volume solution daily, which is equivalent to a dose of 500 mg) for local oral bleeding such as from tonsillectomy site or from dental extractions.

Desmopressin

Desmopressin (1-deamino-8-D-arginine vasopressin, DDAVP) has been used with variable success in patients with platelet function disorders. The exact nature of the effect

of DDAVP is unclear as it has not been shown to induce a platelet release reaction.

Limited studies exist regarding treatment with DDAVP because of the rare nature of these disorders. One of the largest groups studied are those with Hermansky-Pudlak syndrome, with variable responses noted. Although most studies of DDAVP in Hermansky-Pudlak syndrome have used the bleeding time as the primary endpoint, the clinical relevance of bleeding time correction is not known, and evidence suggests that the bleeding time may not correlate with *in vivo* studies, such as platelet aggregation tests.

In a large study of patients with Bernard-Soulier syndrome, MHY9-related disorders, and Grey platelet syndrome, some responses to DDAVP were demonstrated, as indicated by reduced bleeding time and a 50% increase in ADP-induced platelet aggregation. Patients with Glanzmann's thrombasthenia failed to respond, supporting the notion that the effects of DDAVP on platelet function may depend on GPIIb/IIIa binding. Patients with storage pool disorders usually (but not always) respond. It is also not clear if laboratory correction (e.g., of the bleeding time) will correlate with clinical efficacy. The effects of DDAVP may involve an increase in the levels of circulating VWF.

DDAVP may cause flushing and hypotension. It should not be used in individuals with evidence of atherosclerosis. It causes fluid retention, and patients should be advised to restrict fluid intake in the subsequent 24 hours. Intravenous fluids should be given with caution due to the risk of water retention resulting in hyponatremia and potentially seizures. For this reason, it is not generally given to children under the age of two in whom the risk of hyponatremia may be higher. If given for more than a single dose, it is advisable to monitor daily weights and plasma electrolytes. DDAVP may be the agent of choice for mild bleeding problems where tranexamic acid or EACA alone are ineffective. There is no convincing evidence to support the practice of assessing DDAVP correction of the bleeding time. The effect must be assessed by clinical response.

DDAVP may be administered by:

- Intravenous infusion, at a dose of 0.3 µg/kg of a 4 µg/mL solution diluted to 30-50 mL in 0.9% saline, and infused over 30 minutes; there is some evidence that a smaller dose of 0.2 µg/kg is also effective when used in conjunction with antifibrinolytic agents such as tranexamic acid.
- Subcutaneous injection at a dose of 0.3 µg/kg once daily.
- Intranasal spray, available in a concentrated form (150 µg per dose) under the trade name Stimate®, to be administered at 300 µg for an adult (> 50 kg) and 150 µg for a child (20-50 kg) once daily.

The therapeutic efficacy of DDAVP decreases significantly after the first dose (tachyphylaxis). The initial response can be reproduced after a week. In procedures such as tonsillectomy, the dose of DDAVP can be repeated on day 7 in order to prevent bleeding from the separation eschar.

Platelet transfusions

Platelet transfusions are appropriate in severe disorders and when other agents have failed. However, these are blood products and carry risks of transfusion-transmitted infections and allergic reactions. It is likely that multiple-donor exposures increase the risk, so platelet transfusions should only be given if essential. Patients with platelet disorders may be subject to repeated episodes of transfusion, putting them at risk of developing alloantibodies either to human leukocyte antigens (HLAs) or missing GPs as in Bernard-Soulier syndrome (GPIb/IXa/Va) and Glanzmann's thrombasthenia (GPIIb/IIIa). Since sensitization leads to platelet refractoriness and failure to obtain hemostasis, it is advised to transfuse HLA-matched platelets unless the delay in obtaining them would compromise the clinical situation. However, these recommendations are based on expert opinion. Alternatively, single-donor platelet transfusions can be a practical option to avoid exposure to multiple alloantigens. In children the conventional dose of platelets is 10-15 mL/kg.

Recombinant factor VIIa (rFVIIa)

Experience using rFVIIa (NovoSeven®) in platelet disorders is limited, apart from managing bleeding in patients with

Glanzmann's thrombasthenia and storage pool disorders. rFVIIa has recently been approved in the European Union for use in Glanzmann's thrombasthenia patients refractory to platelet transfusion or those who have developed antibodies against GPIIb/IIIa. The dosing regimens used in patients with hemophilia with inhibitors, 90 µg/kg (80-120 µg/kg) every 3-4 hours, is felt to be sufficient to control bleeding in this population. The precise means by which rFVIIa exerts its efficacy in such patients has yet to be elucidated.

Splenectomy

Splenectomy has not been shown to have beneficial effects on any congenital or acquired platelet function disorders, with the exception of Wiskott-Aldrich syndrome. These patients can have a clear improvement in thrombocytopenia following splenectomy; however, these results have not been duplicated in other disorders. Splenectomy therefore should not be performed in hopes of improving platelet count or bleeding diathesis.

Other options

In a single unconfirmed study, prednisone at doses of 20-50 mg for 3-4 days was used to improve hemostasis in patients with inherited platelet disorders. However, it was ineffective in patients with Glanzmann's thrombasthenia and Aspirin®-induced defects. Bone marrow transplantation has been effective in reversing immunologic and hematologic manifestations in patients with Chediak-Higashi or Wiskott-Aldrich syndromes.

Specific Disorders of Platelet Function

The following section briefly describes the inherited platelet disorders (Table 5).

Defects in platelet receptors

Glanzmann's thrombasthenia

Glanzmann's thrombasthenia is a platelet disorder caused by an absence or decrease in the platelet receptor for fibrinogen IIb/IIIa. The platelet count, size, shape, and life span are normal in this disorder. It is inherited as an

autosomal recessive trait: therefore parental history of bleeding is negative. Males and females are equally affected. The bleeding time is invariably prolonged. Clot retraction is poor to absent. Platelet function studies reveal aggregation to ristocetin only (Figure 7 and Table 2). Adhesion to areas of damaged endothelium is normal but recruitment of further platelets into the primary hemostatic plug is defective. Assessment of GPIIb/IIIa receptors on the platelet membrane using flow cytometry is possible in reference laboratories. Therapy consists of platelet transfusions, as DDAVP is not helpful in these patients. Resulting platelet alloimmunization is a serious consequence of platelet transfusion. Therefore, platelet transfusions should be employed judiciously. rFVIIa has been shown to be effective for controlling bleeding in patients with Glanzmann's thrombasthenia at doses of 90 µg/kg. It has been licensed in Europe for the indication of treating bleeding in patients with Glanzmann's thrombasthenia. Because rFVIIa can reduce or eliminate the need for platelet transfusions, it can protect from the development of alloantibodies against the GPIIb/IIIa receptor.

Bernard-Soulier syndrome

Bernard-Soulier syndrome is a rare syndrome characterized by abnormally large platelets that may also be mildly decreased in number. The bleeding time is markedly prolonged. Platelet aggregation studies reveal that aggregation to ristocetin is abnormal (Figure 7 and Table 2). This abnormality is due to a decrease or absence of GPIb/IX, the VWF receptor. This disorder should be differentiated from VWD, which is due to a defect in the VWF rather than the platelet receptor. Bernard-Soulier syndrome is inherited as an autosomal recessive trait with males and females equally affected. Parental history of similar bleeding problems is absent. In contrast, VWD is inherited as an autosomal dominant trait; however, symptoms are variable and therefore parental history is an inadequate guide to excluding this diagnosis. In Bernard-Soulier syndrome, platelet transfusions are used therapeutically; however, as with Glanzmann's thrombasthenia, alloimmunization may occur. There are reports of successful control of bleeding

Table 5: Hereditary platelet function disorders

Disorder	Platelet count (K/mm ³)	Inheritance	Structural defect	Platelet characteristics	Defect in platelet function	Associations	Treatment options		
Disorders of adhesion and aggregation due to defects in receptors and defects in signal transduction							Platelet transfusion	DDAVP	rFVIIa
Bernard-Soulier syndrome	20-100	AR	GPIb/IX GPIb α GPIb β GPIX	Giant platelets	Abnormal adhesion	DiGeorge Velocardio facial syndrome	Y	Y	?
Glanzmann's thrombasthenia	Normal	AR	GPIIb/IIIa	None	Absent aggregation with physiological agonists, defective clot retraction	↑ bone thickening and ↓ fertility	Y	N	Y
Platelet type VWD	Normal or ↓	AD	GPIb α	Platelet size heterogeneity	Abnormal adhesion: ↑ sensitivity to ristocetin	Absence of HMWM	Y	N	?
$\alpha 2\beta 1$ collagen receptor	Normal	?	$\alpha 2$	Normal	Abnormal adhesion : ↓ response to collagen	Modifications in receptor density according to haplotype	Y	Y	?
GPVI collagen Receptor	Normal	?	GPVI absence can be secondary to proteolytic cleavage	Normal	Abnormal adhesion: ↓ response to collagen	Linked to FcR- γ ; receptor density depends upon haplotype	Y	Y	?
P2Y ₁₂ ADP receptor	Normal	AR	P2Y ₁₂ receptor	Normal	Abnormal aggregation to ADP	Not known	Y	Y	?
TP α , Thromboxane (TX) A ₂ receptor	Normal	AR	TP α	Normal	Absence of response to TXA ₂ analogues, ↓ response to collagen	Not known	Y	Y	?
Intracellular signaling	Normal or ↓	?	Phospholipase C- β_2 G α_q protein, among others	Normal	Variable aggregation and secretion defects on multiple agonists	Not reported	Y	Y	?
Cyclooxygenase deficiency	Normal or ↓	?AR	Cyclooxygenase enzyme	Not known	No aggregation with arachidonic acid, ↓ response to collagen and ADP	Not known	Y	Y	?
Scott syndrome	Normal	AR	ATP-binding cassette transporter A1	Normal	↓ procoagulant activity and microparticle release	Defects extend to other cell lines	Y	?	?
Wiskott-Aldrich syndrome	10-100	X-linked	WAS signaling defects	Small size, fewer granules	↓ aggregation and ↓ secretion	Eczema, immunodeficiency	Y	?	?
Disorders of secretion due to abnormalities of storage granules									
Dense granule deficiency with albinism: Hermanasky-Pudlak (HP); Chediak Higashi syndrome(CH)	Normal	AR	Proteins involved in vesicle formation and trafficking	↓ number of abnormal dense granules, giant granules (CH)	↓ aggregation and secretion with collagen	Oculocutaneous albinism, ceroid-lipofuscinosis (HP), infections (CH)	Y	Y	Y

Table 5: Hereditary platelet function disorders (cont'd)

Disorder	Platelet count (K/mm ³)	Inheritance	Structural defect	Platelet characteristics	Defect in platelet function	Associations	Treatment options		
Disorders of secretion due to abnormalities of storage granules							Platelet transfusion	DDAVP	rFVIIa
Gray platelet syndrome	30-100	AR or AD	Unknown, but prevents storage of proteins in α-granules	Empty α-granules	Abnormal but variable; can be decreased with thrombin, epinephrine and/or collagen	Myelofibrosis	Y	?	?
Quebec syndrome	~100	AD	↑ urokinase-type activator in α-granules, degraded proteins	Abnormal content of α-granules	Absent aggregation with epinephrine	None known	Y	?	?
Paris–Trousseau/Jacobsen syndrome (deletion of 11q23-24)	30-150	AD	Defective megakaryopoiesis	Giant megakaryocyte granules	Abnormal aggregation and secretion with thrombin, epinephrine, ADP and collagen	Psychomotor retardation, facial and cardiac abnormality	Y	Y	?
Dense granule deficiency without albinism	Normal	AD/X-R	Inability to package δ- granule contents	Quantitative deficiency of delta granules, ↓ serotonin content	Absent second wave of aggregation with ADP, epinephrine ATP:ADP ratio>3	Wiskott-Aldrich syndrome, TAR, Ehler-Danlos syndrome	Y	Y	Y
MYH9 disorders									
May-Hegglin	30-100	AD	MYH9; non-muscle myosin heavy chain IIA	Large size	No consistent defect	Neutrophil inclusions	Y	?	?
Fechtner syndrome	30-100	AD	MYH9	Large size	No consistent defect	Hereditary nephritis, hearing loss	Y	?	?
Epstein syndrome	5-100	AD	MYH9	Large size	Impaired response to collagen	Hereditary nephritis, hearing loss	Y	?	?
Montreal platelet syndrome	5-40	AD	Unknown	Large size	Spontaneous agglutination, ↓ response to thrombin	No known	Y	?	?
Dense granule deficiency with albinism: Hermanasky-Pudlak (HP) Chediak Higashi syndrome(CH)	Normal	AR	Proteins involved in vesicle formation and trafficking	↓ number of abnormal dense granules, giant granules (CH)	↓ aggregation and secretion with collagen	Oculocutaneous albinism, ceroid-lipofuscinosis (HP), infections (CH)	Y	Y	Y

Abbreviations: DDAVP: Desmopressin (1-deamino-8-D-arginine vasopressin); rFVIIa: recombinant factor VIIa; AD: Autosomal dominant; AR: Autosomal Recessive; ADP: Adenosine Diphosphate; ATP: Adenosine Triphosphate; HMWM: High molecular weight multimers; Y: Yes; N: No; FcR- γ : Fc Receptor γ ; TXA2: Thromboxane A2; GP: Glycoprotein; TAR: Thrombocytopenia and Absent Radii; ?: indicates no documented efficacy; MYH: Myosine Heavy Chain; WAS: Wiscott-Aldrich Syndrome

with rFVIIa in patients with Bernard-Soulier syndrome.

Defects in granule content / storage pool deficiencies

Storage pool disorders are a heterogeneous group of diseases in which there is an abnormality in the ability to store appropriate products within the platelet granules. The following represent a few of the recognized storage pool disorders not associated with a systemic disorder.

Grey platelet syndrome

Grey platelet syndrome is a disorder characterized by a protein deficiency (e.g., platelet factor 4, β -thromboglobulin, fibrinogen and PDGF) in the alpha granules, both in platelets and megakaryocytes. On the peripheral smear the platelets are gray in color and large. There is consistently impaired aggregation to thrombin in platelet function studies. A trial of DDAVP should be undertaken in these patients prior to the use of platelet transfusions. However, platelet transfusion may be required for severe bleeding or for those who fail to respond to DDAVP.

Quebec platelet disorder

Quebec platelet disorder is inherited as an autosomal dominant disorder that is associated with very abnormal aggregation with epinephrine. There is a defect in alpha granule proteolysis and a deficiency of alpha granule multimerin, a multimeric protein that binds factor V within the granule, thereby leading to a decreased content of platelet factor V and several other proteins (fibrinogen, VWF, etc).

The following storage pool disorders are reported to occur in association with other systemic inherited disorders.

Hermansky-Pudlak syndrome

Hermansky-Pudlak syndrome is inherited as an autosomal recessive disorder with associated oculocutaneous albinism. There is also a ceroid-like pigment in the bone marrow macrophages. Hermansky-Pudlak syndrome is characterized as a mild bleeding disorder with prolonged bleeding time and a marked absence of dense bodies. Platelet function studies show an absent secondary wave to ADP, epinephrine, and

ristocetin, and abnormal aggregation with collagen.

Chediak-Higashi syndrome

Chediak-Higashi syndrome is a rare autosomal recessive disorder with large abnormal granules that are apparent in melanocytes, leukocytes, and fibroblasts, but not in platelets. There is a partial oculocutaneous albinism and often-recurrent pyogenic infections. The platelet count is normal, with a prolonged bleeding time, decreased dense granules, and abnormal platelet aggregation associated with a bleeding tendency.

Wiskott-Aldrich syndrome

Wiskott-Aldrich syndrome is a rare X-linked recessive disorder caused by a defect in a protein now termed as Wiskott-Aldrich syndrome protein (WASp). The gene resides on Xp11.22-23, and its expression is limited to cells of hematopoietic lineage. This disease is characterized by thrombocytopenia, with small platelets and immunodeficiency. Patients with this disorder may have bleeding in association with the decreased number as well as abnormal function of the platelets. In some patients a storage pool deficiency has been described. Affected patients have a history of recurrent infections and eczema on physical examination. Laboratory abnormalities reveal absent isohemagglutinins. There are associated immunologic defects. Genetic testing has revealed abnormalities in many of these patients. Treatment of acute bleeding is through platelet transfusions. Splenectomy has shown to improve the thrombocytopenia. Bone marrow transplantation should be considered the definitive treatment for these patients.

Release defects

This group of patients most likely represents the largest group of platelet function disorders. Release defects may occur due to abnormalities in signal transduction from the membrane, abnormal internal metabolic pathways, and abnormal release mechanisms or structures involved in the release reactions (Figure 4). It is clear therefore that release defects are a heterogeneous group of disorders with a wide variety of underlying defects whose mechanisms are not yet fully elucidated. The final common abnormality within this group of

defects is the failure to successfully release granule contents upon platelet activation.

Release defects are associated with a prolonged bleeding time and an abnormal *in vitro* platelet aggregation profile characterized by abnormalities of aggregation in association with ADP, including an absent secondary wave, epinephrine and collagen, with a blunted or absent secondary wave. In more sophisticated studies, there is a measurable defect in ADP release. There are normal metabolic stores of ADP (those not associated with granule contents). The contents of granules are normal. Many patients with release defects may be treated with DDAVP.

Coagulation factor defects affecting platelet function

Abnormalities of plasma coagulation factors may lead to defects in platelet function, despite the presence of normal numbers of properly-functioning platelets. The most common abnormality in this category is VWD. Absence of plasma and platelet fibrinogen leads to a defect in platelet function, as fibrinogen is important in the platelet-platelet interaction within the primary hemostatic plug. Afibrinogenemia is a rare autosomal recessive defect. Both VWD and afibrinogenemia lead to adhesive defects in platelet function: VWD in the platelet vessel interaction, and afibrinogenemia in the platelet-platelet interaction.

von Willebrand disease

The defect in VWD resides within the VWF, which plays an important part in platelet function and whose platelet receptor is GPIIb/IX. Abnormalities in VWF may lead to mucocutaneous bleeding similar to that seen in platelet function defects. VWD is inherited as an autosomal dominant trait with males and females equally affected. The bleeding time may be prolonged. Coagulation factor studies may reveal abnormalities in factor VIII activity, quantitative VWF antigen, VWF activity (commonly measured in the ristocetin cofactor assay), and the structure of the protein itself (usually assessed through multimeric analysis by gel electrophoresis).

Afibrinogenemia

This is a rare autosomal recessive disorder in which there are extremely low or absent levels

of fibrinogen. The bleeding time may be prolonged. In some patients there may be an associated decrease in platelet counts as well as an abnormal platelet aggregation profile. The absence or severe deficiency of plasma fibrinogen leads to impaired platelet-platelet interaction.

Defects in platelet pro-coagulant activity

Scott syndrome

Scott syndrome is perhaps the best-described defect in platelet function in this category. In Scott syndrome, platelets have defective binding of factor Va-X and factor VIIIa-IXa complexes. Defective binding of these plasma coagulation factor complexes results in impaired activation of factor X and prothrombin, platelet-dependent fibrin formation, and an abnormality in platelet factor 3 activity. These defects have been attributed to an abnormality in the expression of phosphatidylserine in the plasma membrane.

Miscellaneous Congenital Disorders

There are disorders of platelet function that have been reported to occur in association with connective tissue disorders. These include but are not limited to such disorders as Ehlers-Danlos syndrome, Marfan's syndrome, osteogenesis imperfecta, and fragile X syndrome. May-Hegglin anomaly is an autosomal dominant disorder characterized by ineffective thrombopoiesis with normal platelet function studies, and abnormal inclusion granules in leukocytes. Platelet function defects have also been reported to occur in association with Down's syndrome.

Thrombocytopenia and absent radii syndrome (TAR) is characterized by thrombocytopenia and defects of the radial bone. Platelet function defects have been reported in TAR syndrome. Infants with this syndrome may suffer severe and even fatal bleeding in the first year of life, after which the thrombocytopenia gradually improves. Prophylactic platelet transfusions are recommended in this population of patients. Hereditary autosomal dominant thrombocytopenia resembling idiopathic thrombocytopenic purpura (ITP) has been reported and may be associated with a platelet function defect. Platelet function defects have

also been reported to occur in association with increased serum IgA, nephritis, deafness, and giant platelets.

Conclusion

Platelets are essential for primary hemostasis. Platelet function defects comprise a large and heterogeneous group of bleeding disorders that range in severity from mild to severe. Patients may be asymptomatic; however, the majority who are diagnosed present with easy bruising and mucocutaneous bleeding, or excessive hemorrhage following injury or surgery. As the complex internal biochemical and signal transduction pathways are further elucidated, and as structural analysis of platelets advances, more of the mechanisms leading to platelet function defects will be understood. Despite our advances in the understanding of the etiology of these defects in function, treatment remains fairly rudimentary. Adjunctive therapies (such as antifibrinolytics, microfibrillar collagen, fibrin glue, etc.), DDAVP, rFVIIa, and platelet transfusions remain the mainstay of therapy available at this time. For platelet function disorders associated with a defect in a plasma coagulation factor such as von Willebrand disease and afibrinogenemia, treatment consists of replacement of the deficient coagulation factor.

Resources

1. Brewer DB. Max Schultze (1865), G. Bizzozzero (1882) and the discovery of the platelet. *Br J Haematol*, 2006; 133: 251-8.
2. Michelson AD. *Platelets*, Second Edition, Academic Press.
3. George JN. Platelets. *Lancet*, 2000; 355: 1531-9.
4. Bolton-Maggs PH, Chalmers EA, Collins PW, Harrison P, Kitchen S, Liesner RJ, Minford A, Mumford AD, Parapia LA, Perry DJ, Watson SP, Wilde JT, Williams MD; UKHCDO. Review of inherited platelet disorders with guidelines for their management on behalf of the UKHCDO. *Br J Haematol*, 2006; 135(5):603-33.
5. Davi G, Patrono C. Platelet activation and atherothrombosis. *N Engl J Med*, 2007; 357: 2482-94.
6. Franchini M. The use of desmopressin as a hemostatic agent: a concise review. *Am J Hematol*, 2007 Aug; 82(8):731-5.
7. Poon MC, D'Oiron R, Von Depka M, Khair K, Nègrier C, Karafoulidou A, Huth-Kuehne A, Morfini M; International Data Collection on Recombinant Factor VIIa and Congenital Platelet Disorders Study Group. Prophylactic and therapeutic recombinant factor VIIa administration to patients with Glanzmann's thrombasthenia: results of an international survey. *J Thromb Haemos* 2004; 2: 1096-1103.



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