ABSTRACT
Platelet functional disorders are on the increase. The basic platelet functions are a continuous process starting when the vessel wall is injured. The three phases are 1 Initiation 2 Extension and 3 Consolidation. After these phases, a stable platelet plug is formed. Disorders of platelet function arise due to hereditary and acquired defects in the various steps of these functional phases. Acquired platelet dysfunctions are much more common than hereditary disorders. The usual causes are drugs especially anti platelet drugs, uremia, liver diseases, diabetes mellitus, cardiac bypass, hematological disorders like MDS, AML, paraproteinemias and myeloproliferative disorders. Common hereditary disorders are Bernard-Soulier syndrome, Glansmann thrombasthenia and storage pool disorders. Advanced investigations like platelet aggregation studies and flow cytometry are necessary for accurate diagnosis of hereditary platelet disorders. Treatment of these disorders is mainly symptomatic. Platelet transfusions are life saving in hereditary disorders but may induce antibodies if repeatedly used. For acquired disorders the offending drugs should be stopped. The diseases responsible for the platelet dysfunction should be effectively treated. Symptomatic treatment with red cell transfusion and platelet transfusion should be given. In future one may see a significant improvement in early diagnosis and medical management of inherited platelet disorders with improvement in platelet function measurements and proteomic (study of protein and their structure) approaches.

INTRODUCTION
Platelet disorders are very common in clinical practice. Quantitative platelet abnormalities (various causes of thrombocytopenia) are much more common than qualitative abnormalities (platelet functional abnormalities). Sometimes they can co exist. Basic understanding of the platelet functions is necessary to understand and evaluate the disorders of platelet dysfunction.

When there is a blood vessel injury the circulating platelets interact with a number of components of extra cellular matrix particularly collagen. This will lead to a series of receptor ligand interactions finally leading to the formation of a stable platelet plug or thrombosis. These interactions are a continuous process and consist of three phases.

1. Initiation
2. Extension
3. Consolidation

INITIATION PHASE
In this phase von Willibrand factor (vWF) binds to collagen. Then the vWF of this combination in turn binds to platelet membrane receptor glycoprotein GP Ib complex (Ib –IX – V complex). This is a very important step. At the same time more stable platelet monolayer is formed on the collagen mediated mainly by platelet receptor glycoprotein GP VI and platelet integrin $\alpha_2\beta_1$ (Fig 1).

EXTENSION PHASE
During this phase prothrombin is converted to thrombin on the activated platelet surface. Secretion
Platelet Dysfunction- What a Physician Should Know

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Fig. 1 : **Initiation of platelet adhesion by matrix components, particularly collagen.** Platelets employ a number of collagen receptors. These include von Willebrand factor (vWF)-mediated binding of collagen to the glycoprotein (GP) Ib complex (a heptamer composed of GPIbα, GPIbβ, GPV and GPIX), the direct engagement of collagen by the integrin αIIbβ3, and GPVI/FcRγ. Engagement and clustering of GPVI initiates tyrosine phosphorylation of FcRγ by a Src family kinase (SFK). The tyrosine kinase Syk then binds and is activated, in turn activating phospholipase Cγ, which then initiates phosphoinositide hydrolysis, secretion of adenosine diphosphate (ADP), and the production of thromboxane A2 (TXA2). ADP and TXA2 augment platelet activation by binding to their respective platelet receptors.

![Diagram showing GPVI and FcRγ interaction](image)

**Fig. 2 :** The extension phase of platelet plug formation accelerates and augments the activation of the platelet and is mediated largely by G-protein-coupled receptors, including the purinogenic receptors P2Y1 and P2Y12, which are bound by adenosine diphosphate (ADP); the α- and β-isoforms of the thromboxane A2 (TXA2) receptor TP; the protease-activated receptor (PAR) family members PAR1 and PAR4 that are recognized by thrombin; and the α2A-adrenergic receptor that is specific for epinephrine.

![Diagram showing P2Y1, P2Y12, TXA2, PAR1, PAR4, and α2A receptors](image)

of substances like ADP and thromboxane A2 occurs from α granules and δ granules. ADP plays an important role. It binds to its cognate platelet receptors to augment platelet activation.

**CONSORTIUM PHASE**

In this phase platelet aggregation (platelet-platelet cohesion) occurs. This is mediated by the binding of fibrinogen and or vWF to the activated platelet Integrin αIIbβ3 (GPIIb-IIIa). The aggregates of platelets with the fibrin network results in the generation of platelet plug or thrombi (Fig 3).

**CLASSIFICATION OF DISORDERS OF PLATELET DYSFUNCTION**

Qualitative disorders of platelet function could be classified as

1. Inherited causes
2. Acquired causes

With the increasing use of drugs like aspirin, other NSAIDS, antibiotics and other platelet inhibitors and increased incidence of diseases like chronic kidney disease, liver disease and diabetes mellitus acquired type of disorders of platelet dysfunction has become very common. These situations numerically outnumber the inherited disorders. A practicing physician encounters these acquired disorders in his or her daily practice and should be in a position to effectively evaluate and manage such patients.

Inherited disorders are classified according the phase of the platelet function which they affect i.e. initiation, extension or consolidation. Detailed classification is given in Table 1.

**APPROACH TO A PATIENT WITH PLATELET DYSFUNCTION**

A detailed patient and family history is the corner stone in
evaluation of these patients. A history of bleeding is usually subjective and sometimes very difficult to make out if it is significant. In a statistical analysis it was found that 25% of the persons who complain of serious bleeding do not have a bleeding disorder. On the other hand 1/3 of persons who have no bleeding may have von Willibrand disease. The international society on thrombosis and hemostasis (ISTH) suggests clinically significant bleeding should be considered if there are

1. Two or more distinct bleeding sites such as skin, nose, gums, vagina, GI tract or Genito- Urinary tract.
2. Bleeding involving single site severe enough to warrant blood transfusion.
3. Significant bleeding from single site which recurs on three or more unrelated and separate occasions.

A number of quantitative approaches to assess the bleeding have been proposed but is not practical. Sometimes it is very difficult to differentiate normal people from those with a bleeding disorder.

A very careful drug history is very important. Apart from the well known drugs, drugs like epoprostenol, statins, cilostazol, sildenafil, fluoxetine and cephalosporins, should be considered significant.

Bleeding manifestations include

a. Unexplained or extensive bruising
b. Epistaxis particularly lasting for more than 30 minutes causing anemia and admission to hospital.
c. Menorrhagia especially present from menarche
d. Oral cavity bleeding
e. Bleeding during childbirth
f. Bleeding following invasive procedure
g. Bleeding following dental extraction.

Severe inherited platelet disorders may present very early as

1. Intracranial/subdural hemorrhage after birth
2. Excessive bleeding from umbilical stump
3. After circumcision
4. Prolonged epistaxis during childhood
5. Easy bruisability

If the platelet dysfunction is mild it may manifest at any age usually following a hemostatic challenge such as surgery or dental extraction.

Consanguinity in the parents should be looked for in autosomal recessive disorders.

### Table 1: Disorders of platelet function

<table>
<thead>
<tr>
<th>Hereditary Platelet Dysfunction</th>
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<tbody>
<tr>
<td>Initiation Phase</td>
</tr>
<tr>
<td>✓ Bernard-Soulier syndrome</td>
</tr>
<tr>
<td>✓ Glycoprotein (GP) VI deficiency</td>
</tr>
<tr>
<td>Extension Phase</td>
</tr>
<tr>
<td>✓ Secretion disorders/granule deficiencies</td>
</tr>
<tr>
<td>• α-Granule abnormalities (gray platelet syndrome)</td>
</tr>
<tr>
<td>• δ-Granule (dense body) abnormalities</td>
</tr>
<tr>
<td>• Hermansky-Puttak syndrome</td>
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<tr>
<td>• Chediak-Higashi syndrome</td>
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<tr>
<td>• Wiskott-Aldrich syndrome</td>
</tr>
<tr>
<td>• α/δ-Granule deficiency</td>
</tr>
<tr>
<td>✓ Defects of signal transduction and secretion</td>
</tr>
<tr>
<td>• Impaired liberation of arachidonic acid</td>
</tr>
<tr>
<td>• Cyclooxygenase deficiency</td>
</tr>
<tr>
<td>• Thromboxane synthetase deficiency</td>
</tr>
<tr>
<td>• Thromboxane A&lt;sub&gt;2&lt;/sub&gt; receptor abnormalities</td>
</tr>
<tr>
<td>✓ Defects in calcium deficiency</td>
</tr>
<tr>
<td>✓ Defects of platelet procoagulant activity</td>
</tr>
<tr>
<td>Consolidation Phase</td>
</tr>
<tr>
<td>✓ Glanzmann thrombasthenia</td>
</tr>
<tr>
<td>Miscellaneous</td>
</tr>
<tr>
<td>✓ Hereditary macrothrombopathy/sensorineural hearing loss</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Acquired disorders of platelet functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug Induced Platelet Dysfunction</td>
</tr>
<tr>
<td>✓ Analgesics</td>
</tr>
<tr>
<td>✓ Antibiotics</td>
</tr>
<tr>
<td>✓ Cardiovascular drugs</td>
</tr>
<tr>
<td>✓ Psychotropic drugs</td>
</tr>
<tr>
<td>Secondary platelet dysfunction</td>
</tr>
<tr>
<td>✓ Uremia</td>
</tr>
<tr>
<td>✓ Paraproteinemia</td>
</tr>
<tr>
<td>✓ Liver Disease</td>
</tr>
<tr>
<td>✓ Cardio pulmonary bypass</td>
</tr>
<tr>
<td>✓ Diabetes Mellitus</td>
</tr>
<tr>
<td>✓ Myelodysplastic syndrome/Acute nonlymphocytic leukemia</td>
</tr>
<tr>
<td>✓ Myeloproliferative disorders</td>
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</tbody>
</table>

| Evaluation of these patients. A history of bleeding is usually subjective and sometimes very difficult to make out if it is significant. In a statistical analysis it was found that 25% of the persons who complain of serious bleeding do not have a bleeding disorder. On the other hand 1/3 of persons who have no bleeding may have von Willibrand disease. The international society on thrombosis and hemostasis (ISTH) suggests clinically significant bleeding should be considered if there are

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Sometimes features of vasculitis (systemic or cutaneous) like palpable purpura and superficial bleeding should be differentiated from the symptoms of platelet function disorders.

LABORATORY DIAGNOSIS

The following baseline investigations should be done in all patients suspected to have a platelet functional disorder.

1. CBC, ESR, CRP
2. Peripheral blood smear
3. Serum ferritin, (for an associated iron deficiency)
4. Prothrombin time (PT, INR), Partial thromboplastin time (PTT) and a Thrombin time (TT)
5. Blood urea, S. Creatinine, LFT, Blood sugar
6. Bone marrow studies (for secondary platelet dysfunction, myeloproliferative disorders, MDS, paraproteinemias, AML)

If the above investigations together with the history and physical examination suggest an acquired platelet functional disorder no more investigations are necessary. If on the other hand the patient is young, has a positive family history and acquired disorders are unlikely he/she should be referred to a good centre where platelet functional studies are carried out. The patient’s platelet count should be normal.

The following are advanced laboratory investigations for possible inherited platelet disorder

1. **Bleeding time**: Historically this was a test of platelet function. But it has fallen out of favor because it is poorly reproducible and it may be normal in mild platelet dysfunction. A prolonged bleeding time should be a good reason to perform additional tests of platelet function. In spite of all the drawbacks we use this test in India as a valuable test of platelet function if it is done by the same experienced technicians.

2. **Platelet function analyzer-100**: This test measures the rate of thrombus formation under high shear in citrated whole blood which passes through a membrane aperture coated with collagen/epinephrine or collagen/ADP. The closure time is increased in Glanzmann thrombasthenia and Bernard–Soulier syndrome.

3. **Platelet aggregation studies**: Platelet aggregation in platelet rich plasma is done by platelet aggregometer. The typical agonists used are ADP, epinephrine, collagen, arachidonic acid, ristocetin and thrombin.

4. **Adenine nucleotide content and release**: In storage pool disorders the measurement of platelet adenosine nucleotide (ADP and ATP) content and their release are very useful. Normal platelet aggregation study does not exclude the diagnosis of storage pool diseases. It is recommended that patients suspected of having platelet dysfunction should have both aggregation and adenine nucleotide release performed.

5. **Flowcytometry**: This investigation helps us to measure platelet surface receptor density, platelet activation, alpha granule release, procoagulant phospholipid expression and microvesicle production. One of the main applications of this investigation is that we can assess the GP 1b complex and GP IIb-IIIa in the diagnosis of Bernard–Soulier syndrome and Glanzmann thrombasthenia.

6. **Electron microscopy**: This is done only by specialized persons and is helpful in the assessment of platelet granule defects and changes in the platelet ultra structure.

7. **Additional assays**: There are several other tests done by very specialized centers for diagnosis of platelet disorders. These include analysis of receptor expression, specific molecular or genetic defects, protein phosphorylation, and formation of signal transduction intermediates or characterization of the platelet proteome.

Laboratory diagnosis of common and important inherited platelet disorders

**BERNARD-SOULIER SYNDROME**
1. Increased bleeding time
2. Giant platelets in the peripheral blood
3. A defective platelet aggregation with restocetin.
4. Low or absent levels of platelet GP 1b-V-IX (CD42a–d) by flow cytometry

**GLANZMANN THROMBASTHENIA**
1. Prolonged bleeding time
2. Deficient clot retraction
3. Decreased aggregation with ADP, collagen, epinephrine or thrombin.
4. Quantitative or qualitative abnormalities of GP IIb IIIa also known as integrin alpha IIb beta3

**STORAGE POOL DEFECTS**

This is a heterogeneous group of congenital disorders in which there is a deficiency of granules or their constituents. This results in a decreased ADP release from the activated platelets leading to a defect in secretion dependant platelet aggregation. This group consists of

i. **α granule storage pool disease**
ii. Isolated δ storage pool diseases
iii. Combined α δ storage pool diseases
OTHER RARE INHERITED PLATELET FUNCTIONAL DISORDERS

There are many other rare inherited platelet functional abnormalities. Hermansky-Pudlak and Chediak-Higashi syndromes are rare and have in common platelet dense granule deficiency, albinism and lysosomal granule defects.

The other rare platelet inherited disorders are inherited disorders of primary membrane receptors (congenital defects of P2Y12), signal transduction pathways (Scott syndrome) and additional hereditary defects of platelet function with thrombocytopenia (MYH-9 related thrombocytopenia syndrome and Wiskott-Aldrich syndrome – microthrombocytopenia, eczema and immunodeficiency.)

ACQUIRED DISORDERS OF PLATELET DYSFUNCTION

Normal platelet count, mucocutaneous bleeding and a negative family history (no bleeding in first degree relatives) in an adult is very suggestive of an acquired platelet dysfunction. Aspirin is the most common drug responsible for bleeding due to platelet dysfunction. It irreversibly acetylates COX-1 resulting in inhibition of TxA2 (Thromboxane A2). Aspirin produces 5-10% incidence of minor bleeding and 1-2% of major bleeding (requiring hospitalization or red cell transfusion). Other NSAIDS reversibly inhibit platelet COX-1. The bleeding usually stops within 48 hours when these drugs are stopped. Many patients are receiving a combination of aspirin and clopidogrel. The effect of both these drugs on platelets is irreversible. With clopidogrel the platelet function returns to 50% of normal levels by 72 hours after stopping this drug. Its effect is completely reversed only by 7 days. Large doses of beta lactam antibiotics can produce clinical bleeding. This is probably because of a non specific effect on ligand-receptor interactions. They also appear to modify the platelet membrane and decrease agonist binding. Their effect can be demonstrated after several days of treatment and will not resolve until 7-10 days after discontinuing. GP IIb IIIa blocking drugs like abciximab and tirofiban produce bleeding in 10% of the recipients. Intra cranial bleeding and death due to bleeding are rare.

Patients with uremia bleed due to a number of factors that include minor coagulation abnormalities, platelet dysfunction, thrombocytopenia and the wide spread use of a large number of medications that compromise hemostasis. Abnormalities in all phases of platelet functions including initiation, extension and consolidation have been noted in studies of platelet functions in uremic patients. Platelet dysfunctions are found in paraproteinemias, MDS, acute myeloid leukemia, and Ph chromosome negative myeloproliferative disorders. In many of these conditions there is an additional thrombocytopenia which complicates the clinical presentation of bleeding. Acquired platelet dysfunctions are also found in liver diseases, and during and after cardiac bypass surgery. In patients with diabetes mellitus dysregulation of several signaling pathways leads to increased platelet reactivity and platelet dysfunction. Unlike other acquired PFD (platelet functional defects) which produce bleeding, platelet dysfunction in diabetes mellitus produces thrombosis.

Clinical and laboratory records of 109 patients with acquired PFDs (Platelet Functional Defects) in All India Institute of Medical Sciences showed the following main causes in the order of commonness

i. Drug induced
ii. Idiopathic
iii. Hematological malignancies

64 patients had minor and 24 major bleeding episodes.

MANAGEMENT

Although many specific hereditary platelet disorders are described the treatment options are limited.

1. Topical anti fibrinolytic agents may be useful for minor bleeding in the oral and nasal cavities. Desmopressin may also be used in minor bleeding.
2. Epsilon amino caproic acid or tranexamic acid will reduce blood loss associated with epistaxis or menorrhagia.
3. In menorrhagia, hormonal suppression is very effective method to control bleeding. Endometrial ablation and hysterectomy are much more permanent methods to treat menorrhagia.
4. Transfusion of normally functioning platelets from a donor is a logical treatment for patients with hereditary platelet dysfunction when there is severe bleeding. But these patients are likely to develop iso and allo antibodies after a number of such transfusions. Isoantibodies develop in patients lacking the GP1b or GP IIb IIIa. These antibodies render the platelet transfusions ineffective later on. There is a recommendation to give HLA matched platelet transfusions. These are modern methods of successful removal of these antibodies in specialized centers. Hence it is recommended that platelet transfusions should be reserved only for severe life threatening bleeding. Leukocyte depletion of transfused platelets is recommended to decrease the frequency of sensitization.
5. Pregnancy and delivery represent a severe hemorrhagic risk. Platelet transfusion may be required prior to delivery and afterwards as well. We have been able to successfully manage a few patients with Glanzmann thrombasthenia during their pregnancy and delivery with symptomatic treatment including platelet transfusion.
6. Activated recombinant factor VII (rFVIIa) has recently been used to slow or arrest bleeding associated with platelet dysfunction. Though the outcome is variable it may be tried prior to platelet transfusion to avoid allo and iso immunization.

7. Bone marrow transplant or stem cell infusion following immune ablation has been successful in a few cases where the patient had recurrent life threatening bleeding.

ACQUIRED DISORDERS OF PLATELET FUNCTIONS

Drugs are the worst offenders. Platelet dysfunction by drugs in healthy individuals is not clinically significant. It becomes clinically significant in patients with coagulation disorders, uremia, liver disease or thrombocytopenia and patients receiving anticoagulant therapy. The offending drug should be immediately withdrawn. If the bleeding is serious enough to produce anemia red cell transfusion should be given. Platelet transfusions are also recommended to stop bleeding. The recommended analgesic for the group of patients with risk of bleeding is codeine and other opiates (tremedol) or synthetic analgesics such as paracetamol. Even paracetamol has been reported to have a modest inhibitory effect on platelets. There is no uniform consensus in the timing of restarting anti platelet drugs (aspirin and clopidogrel) after a bleeding episode. It may be safe to restart the drugs 7-14 days after the bleeding episode has stopped completely on a lower dose. Another debated issue is the question of stopping anti platelet drugs before minor and major surgeries for the fear of excessive bleeding. Now it is clear that these drugs need not be stopped before minor surgical procedures if their continuous usage is strongly indicated (e.g. during the first year after angioplasty and stent insertion). In case of a major surgery the decision of stopping anti platelet drugs is considered on an individual basis looking into the risks and benefits of doing so.

In a patient with uremia and bleeding, the most effective treatment is hemo dialysis or peritoneal dialysis though there may be a transient worsening after these procedures. Red cell transfusion and erythropoietin have been found to reduce bleeding. Conjugated estrogens have been found to be useful in uremic patients.

Secondary platelet dysfunction associated with hematological disorders like paraproteinemias, MDS, AML and Ph negative myeloproliferative disorders become well after the treatment of the primary conditions.

SUMMARY

In summary disorders of platelet dysfunction is not uncommon in clinical practice. A practicing physician is very likely to encounter such patients who present with bleeding almost every day. The acquired causes of platelet dysfunction are much more common than the inherited disorders. This is because of the increasing use of anti platelet and other drugs and an increase incidence of chronic renal and liver diseases. With a sound knowledge of the different aspects of a platelet functional disorder a physician will be able to manage many of these bleeding episodes. Only the inherited platelet disorders need referral to specialized centers. Once a definite diagnosis of these disorders is made they can be managed by their treating physicians. In diabetes mellitus platelet dysfunction leads to more platelet activity resulting in thrombosis. The treatment of platelet functional disorders is mainly symptomatic. Platelet transfusion which can be given in life threatening bleeding may lead to platelet antibody formation and refractoriness to further transfusion.

With improvement in platelet function measurement and proteomic approaches one can see significant improvement in early diagnosis and medical management of the inherited platelet disorders.

REFERENCES: