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CLINICAL LABORATORY ASSESSMENT OF CONGENITAL AND ACQUIRED DISORDERS OF PLATELET FUNCTION

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ABSTRACT
Platelet function disorders are known to cause abnormality in primary haemostasis and produce signs and symptoms different from coagulation factor deficiencies which on the other hand cause disorders of secondary haemostasis. Under normal circumstances, the resistance of the endothelial cell lining to interactions with platelets and coagulation factors prevents thrombosis. When there is vascular damage and the underlying matrix of the blood vessels are exposed, a coordinated series of events are set in motion to seal the defect. The disorders of platelet function can be classified into two (2) types: congenital (inherited) which occurs relatively rare or acquired which on the other hand is common. Over the years, various methods of assessing platelet function in the clinical laboratory have been derived; however with the advent of automation, more research is still on to further unveil specific defects in the structure and functions of platelets. Methods are however being specific for investigating certain stages of haemostasis process. Certain substances are however known to affect platelet function causing various clinical conditions, e.g. therapeutic anti-platelet agents (e.g. prostanoids synthesis antagonists), agents that bind to platelet receptors and membranes, antibiotics, agents that increase cyclic adenosine monophosphate (C-AMP) as well as some other chemical substances. Research however, has helped medical personnel to design different therapeutic options for platelet disorders and more research is still on to further improve on medical laboratory investigations and the management of these physiological disorders.

INTRODUCTION

Although platelets are classified as cells, they are actually cytoplasmatic fragments derived from megakaryocytes in the bone marrow. Platelet formation and release probably occur through the sinus endothelial cells.However, others postulate that megakaryocytes undergo cytoplasmatic fragmentation in the pulmonary capillary bed. Their life span in the peripheral blood is approximately 9 days[1]. The average platelet count in peripheral blood ranges from 150,000 to 400,000 per microliter. The diameter of normal platelets is 1–4 µm. At a given point, 70% of the platelets are in the circulation and 30% are in the spleen (splenic pool). The daily platelet production of 40,000/µl can be increased eightfold[1].

The function of platelets in the blood stream is mainly to assist in the coagulation process by creating a platelet plug and stimulating the formation of a solid fibrin clot that helps to trap blood components as well as cover the surface of wounds. It

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helps to stop bleeding.However, platelet function disorders include several rare congenital disorders alongside a wide range of commonly acquired disorders e.g. aspirin use, effects of other drugs, liver diseases, uremia, etc. The Hematologist is often asked to evaluate patients with a bleeding disorder with clinical characteristics suggesting the presence of a qualitative and/or quantitative platelet disorder.

This review will briefly summarize normal platelet function as well as available Medical Laboratory investigations of platelet function. The major emphasis will be on the aetiology, medical laboratory evaluation, and some available therapeutic options in patients with disorders of platelet function.

NORMAL PLATELET FUNCTION

A review of normal platelet function is required in order to understand functional platelet disorders, especially with the increasing number of therapeutic agents available that specifically target various stages of platelet function. There are four sequential steps that describe this function:

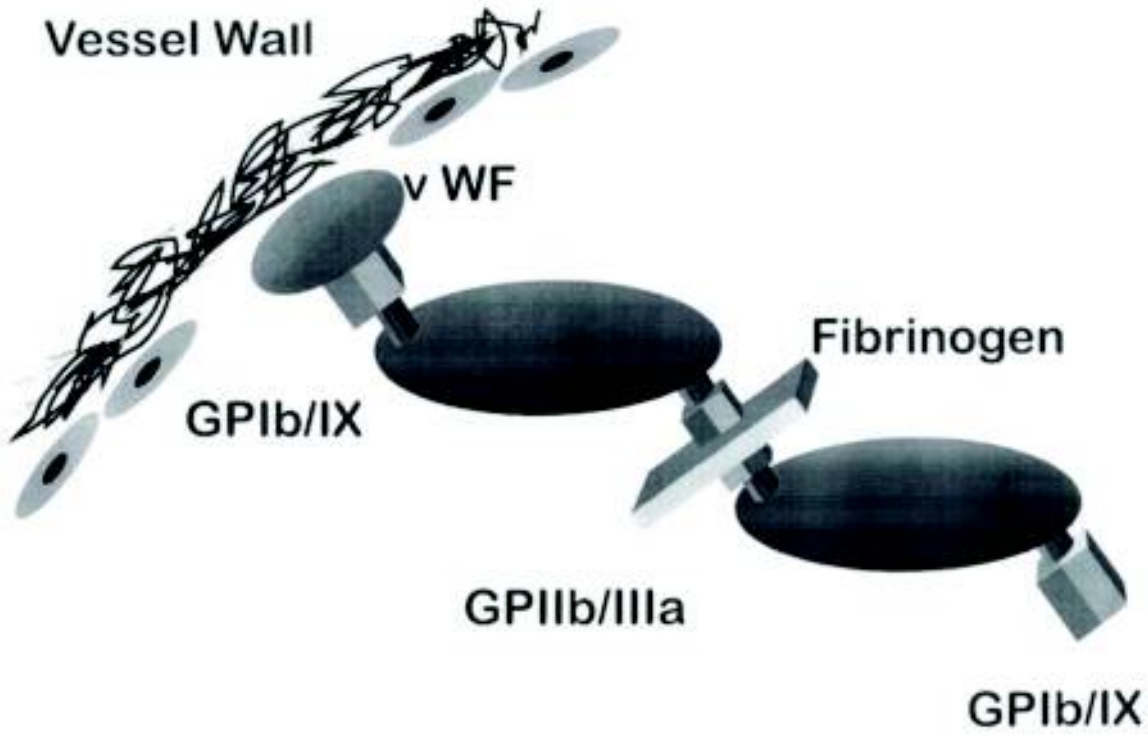
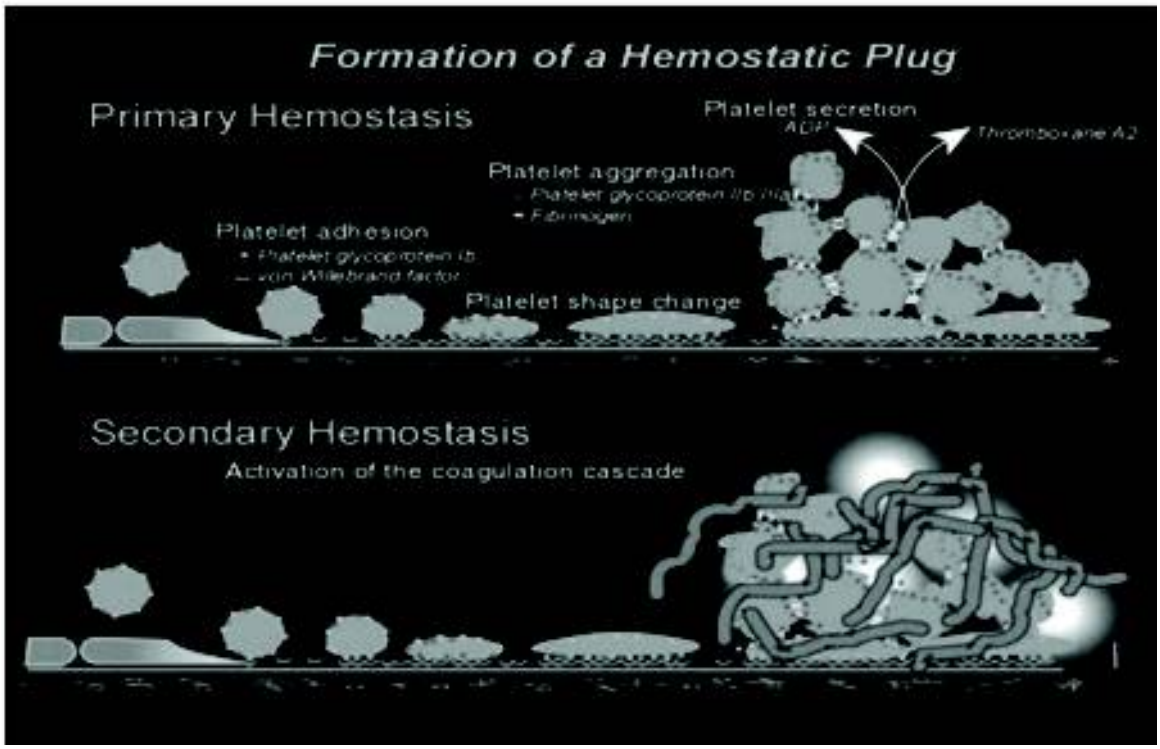


Figure 1: Scheme of platelet adhesion and aggregation.
Source: Modern Haematology, 2nd edition [2007] [2]



Platelet Adherence

Platelet-mediated haemostasis is initiated by exposure of the vascular sub-endothelium following injury to the endothelial surface. Circulating platelets are recruited to the site of injury and rapidly bind to exposed components of the sub-endothelium, including collagen, fibronectin, von Willebrand factor (vWF), fibrinogen, and thrombospondin via glycoprotein (GP) receptors on the platelet surface e.g., GPIb/IX, GPIa/IIa, GPIIb/IIIa[3].

Platelet Activation

Receptor-ligand binding leads to platelet activation, resulting in change in the shape of the structure, mediated through calcium-dependent cytoskeletal changes in the platelet. This outside in-signaling is followed by the release of substances from two sources (release reaction) which makes the content of the alpha and dense granules available at the platelet surface where they facilitate further local platelet adhesion and aggregation, thrombin generation and wound healing[1].

Platelet Aggregation

Platelet aggregation is the clustering together of thrombocytes. The platelet GP IIb/IIIa complex mediates platelet-to-platelet interactions. On resting platelets, GP IIb/IIIa is unable to bind fibrinogen or vWF. Platelet activation allows binding of these proteins, which bridges adjacent platelets. Morphologically, the platelets change dramatically from discs to spheres in a process called shape change[4].

Interaction With Other Cell Components

Cellular interactions between platelets and vascular endothelium or other blood cellular components regulate the haemostatic process. Platelets can even interact and play a role in the activity of pathologic elements such as tumour cells or infectious agents. Moreover, it has been described that platelet interactions can interfere with the activity of anti-platelet drugs.

Vascular endothelium: The endothelium does not form a passive barrier for blood circulation. Endothelial cells and several of

their active metabolites, including eicosanoids such as prostacyclin (PGI2) and endothelial-derived relaxing factor (nitric oxide) are known to directly influence platelet reactivity.

Leukocytes: The activating and inhibitory mechanisms triggered by the interactions between platelets and leukocytes are widely recognized. Lipoxygenase products such as HETEs and several surface glycoproteins play a role in platelet-leukocyte interaction, which is favoured by pathophysiologic events at sites of inflammation, thrombosis and vascular injury.

Erythrocytes: Red blood cells modulate platelet reactivity with sub endothelial structures, mainly through rheological mechanisms (i.e., erythrocyte deformability and aggregation)

Infectious agents: Platelet binding by bacterial pathogens is thought to facilitate the establishment of certain infections. Furthermore, association of viruses with platelets may represent a viral transfer and a passive vehicle for viral dissemination[6].

DISORDERS OF PLATELET FUNCTION

Most platelet disorders are due to an insufficient number of platelets, a condition known as thrombocytopenia[7].There are a wide variety of causes of platelet disorders, and they can occur at any age. Some are hereditary disorders that may become obvious as early as infancy; for example, there may be excessive bleeding following circumcision. In older people, platelet disorders may manifest after a dental extraction or surgical procedure.

Outcome varies widely, depending on the cause and severity of the disorder.Some forms are mild and lifelong, with only minor bruising or limited periods of excessive bleeding; others arise suddenly and may be fatal. In general, older patients seem to be at higher risk of serious complications, such as intracranial hemorrhage[7].

Symptoms of platelet function disorders may include the following:

- ? Minor bleeding just under the surface of the skin or in the mouth—usually appears as multiple tiny, pinpoint-size specks (petechiae) on the lower legs or inner cheeks
- ? Bleeding of the gums or blood blisters in the mouth
- ? Bleeding from the gums when baby teeth fall out or after tooth extractions
- ? Nosebleeds
- ? Easy bruising
- ? Prolonged or heavy menstrual periods.
- ? Prolonged bleeding after a minor cut or after dental or surgical procedures.
- ? Bleeding into the stomach or intestine
- ? Purpura: a purple discoloration of the skin after blood has leaked under it forming a bruise--often from no known trauma.
- ? Emergency symptoms: sudden onset of severe headache, nausea, vomiting, vision loss, or confusion (signs of a brain hemorrhage); black or tarry stools may indicate bleeding from the gastrointestinal tract[7].

However, the classification of these disorders can be divided into two: Congenital and Acquired disorders[8].

CONGENITAL DISORDERS

Several hemorrhagic disorders can be attributed to abnormalities in platelet function occurring in the presence of adequate numbers of circulating platelets.

The abnormalities may result from processes involving adhesion, activation, aggregation or a deficient release reaction that could be genetically inherited[1]. People with these disorders usually have a family history of a bleeding disorder that causes prolonged bleeding after minor cuts or surgery, or easy bruising. Examples of these congenital disorders include:

Von Willebrand Disease

Von Willebrand disease (vWD) is the most common inherited bleeding disorder. It is autosomal dominant, and its prevalence is estimated to be as high as 1 case per 1000 population. The hallmark of von Willebrand disease is defective platelet adhesion to sub-endothelial components caused by a deficiency of the plasma protein vWf. This factor is a large, multimeric protein that is synthesized, processed, and stored in the Weibel-Palade bodies of the endothelial cells, and secreted constitutively following stimulation[9].

vWf has a major role in primary haemostasis as mediator of the initial shear-stress–induced interaction of the platelet to the sub-endothelium via the GP Ib complex. In addition, von Willebrand protein acts as a carrier and stabilizer of coagulation factor VIII by forming a complex in the circulation. Von Willebrand disease is a relatively mild bleeding disorder, except in the occasional patient who is homozygous for the defect and who has severe bleeding often indistinguishable from classic haemophilia. The bleeding manifestations are predominantly skin-related and muco-cutaneous (i.e., easy bruising, epistaxis, Gastro-Intestinal haemorrhage)[9].

Most bleeding episodes occur following trauma or surgery. In women, menorrhagia is common, often exacerbated by the concurrent administration of non-steroidal anti-inflammatory drugs. Pregnant patients with this disease usually do not have problems. Bleeding time is prolonged in persons with von Willebrand disease, and because the Von Willebrand protein is phase-reactant (i.e., increased synthesis in the presence of inflammation, infection, tissue injury and pregnancy), a mild prolonged bleeding time may be normalized, resulting in difficulty in diagnosis[10].

In addition to the prolonged bleeding time, characteristic abnormalities in platelet aggregation tests occur. In patients with von Willebrand disease, platelets aggregate normally to all agonists except the antibiotic Ristocetin, which induces binding of the von Willebrand protein to platelets, similar to what happens with platelets following vessel wall injury in vivo. Ristocetin-induced platelet aggregation correlates with the platelet-aggregating activity of the von Willebrand protein. Levels of coagulation factor VIII are also low, resulting from a decrease in vWF[9].Although the common form of von Willebrand disease (type I) results from a quantitative deficiency of vWf, the variants result from abnormalities in the von Willebrand protein[10].

A common variant (type IIA) of von Willebrand disease results from functionally defective vWf that is unable to form multimers or be more susceptible to cleavage by ADAMTS13. Larger multimers are more active in mediating platelet vessel-wall interaction. In these variants, the factor VIII level may be normal[10]. In the type IIB variant, the von Willebrand protein has heightened interaction with platelets, even in the absence of stimulation. Platelets internalize these multimers, leading to a deficiency of von Willebrand protein in the plasma[9].

A disorder of platelet GP Ib has also been described. In this condition, increased affinity for von Willebrand protein in the resting stage leads to the deletion of plasma von Willebrand protein. This disease is called pseudo von Willebrand disease or platelet-type von Willebrand disease[10].

Type III von Willebrand disease is a severe form of von Willebrand disease that is characterized by very low levels of vWf and clinical features similar to haemophilia A, but with autosomal recessive inheritance. This condition results from a homozygous state or double heterozygosity[9].

Bernard-Soulier Syndrome (BSS)
BSS is a rare congenital bleeding disorder transmitted as an autosomal recessive trait. The platelets are usually moderately decreased in number and on review of the blood smear they are large and variable in their morphology. Bleeding from cutaneous and mucous membranes is quite common. The major functional abnormality in this disease is an impaired adhesion of the platelets to the sub-endothelial matrix causing a markedly prolonged bleeding time. The adhesion defect is due to a deficiency or absence of the GP Ib/IX complex, which results in impaired interaction of platelets with VWF at the vessel wall[9].

Thus, BSS should be suspected when giant platelets are found on the blood smear and when ristocetin is unable to aggregate platelets in the presence of normal VWF. GP Ib/IX is the binding site for VWF when platelets are exposed to ristocetin. The diagnosis is confirmed by quantization of the GP Ib/IX complex by platelet flow cytometry. Patients with BSS may need platelet transfusions to prevent or treat haemorrhage. However, multiple transfusions will lead to the development of alloantibodies that destroy donor platelets and limit the efficacy of subsequent transfusions[10].

Thrombasthenia (Glanzmann'S Disease)
Glanzmannthrombasthenia is an autosomal recessive disorder characterized by moderate to severe muco-cutaneous bleeding, including menorrhagia, and postoperative bleeding. In most patients, the bleeding diathesis is severe, although there are exceptions[1].

The hallmarks of the disease are a prolonged bleeding time and an absent of aggregation response to all aggregation agonists such as ADP, collagen, epinephrine, thrombin, and arachidonate[10]. The typical form of the disease is marked by a deficiency or absence of the platelet membrane GP IIb/IIIa complex. As a result, the platelets cannot bind fibrinogen following platelet activation and cannot aggregate to form the platelet plug[9]. Thrombasthenic platelets show normal secretory responses and can adhere to the vessel wall. The diagnosis is confirmed by demonstrating the absence of agonist-induced platelet aggregation and the absence or partial deficiency of the GP IIb/IIIa complex by platelet flow cytometry[9].
Glanzmannthrombasthenia is classified into:

I. Type I patients, who completely lack GP IIb/IIIa and fibrinogen on the surface of their platelets, and
II. Type II patients, who have a partial deficiency of GP IIb/IIIa (5– 20%) and fibrinogen on the surface of their platelets[10].

Abnormalities of Platelet Secretion
Platelet cytoplasm contains four types of granules:

I. dense granules (containing ADP, ATP, calcium, and serotonin),
II. α-granules (containing a variety of proteins),

III. lysosomes (containing acid hydrolases), and
IV. microperoxymes (containing peroxidase activity)[10].

Following platelet activation, the contents of these granules are secreted in a process called platelet secretion. Inherited disorders of platelet secretion result from the deficiency of one or more of these types of platelet granules or from abnormalities in the mechanism of platelet secretion with a quantitatively normal granule population[1]. These disorders usually cause mild to moderate bleeding manifesting itself by easy bruising, menorrhagia, and excessive postoperative or postpartum blood loss. Patients with a disorder of platelet secretion usually have a moderately prolonged bleeding time, absence of the second wave of platelet aggregation after stimulation with the agonists ADP and epinephrine (δ-granule defect), and decreased aggregation after stimulation with collagen[9].

Similar laboratory patterns may be observed in acquired abnormalities of platelet secretion induced by drugs such as aspirin, or in systemic diseases such as uraemia and dysproteinemias.

α-Granule Deficiency(Gray Platelet Syndrome, **α**-Storage Pool Disease)
The Gray Platelet Syndrome is a rare bleeding disorder resulting from the absence of morphologically recognizable α-granules in the platelets of affected patients. The name derives from the large gray platelets that appear on the blood smear. The disorder presumably results from a defect in granule formation or packing within the megakaryocytes. Alpha granules (α-granules) contain a variety of proteins such as platelet factor (PF)-4, β-thromboglobulin (TG), platelet-derived growth factor (PDGF), thrombospondin, fibrinogen, VWF, and others. The lack of these proteins can cause a life-long history of mild muco-cutaneous bleeding and a prolonged bleeding time. The number of platelets is usually decreased (60,000–100,000/μL). Aggregation studies give conflicting results, but usually there is a decrease or absence of collagen-induced aggregation[10].

Dense Granule Deficiency (**δ**-Storage Pool Disease)
The diagnosis of δ-granule deficiency is usually suspected upon finding a reduction in the amount of δ-granule substances (e.g., ADP, serotonin) in the platelets. A defect in the platelet release reaction results in a single reversible aggregation wave, because there are no dense body components to recruit platelets for the second wave.A hereditary deficiency in dense granules is also a feature of Hermansky- Pudlak syndrome, which is associated with oculo-cutaneous albinism and a steroid-likepigment in marrow macrophages. Another dense granule deficiency disorderis Chediak-Higashi syndrome(oculocutaneous albinism, chronic infections, andhaemorrhage)[10].

Platelet granule abnormalities have also been reported in Wiskott-Aldrich syndrome (thrombocytopenia, infections, eczema). Platelets that havenormal dense granule components but are unable to release them have alsobeen classified as having an “aspirin-like” defect because the clinical symptomsresemble those following the ingestion of aspirin.

Patients with δ-storagepool disease present with a mild to moderate bleeding diathesis characteristic ofplatelet secretion defects. The number of platelets and their morphology areusually normal, whereas the bleeding time is often, but not always, prolonged[1].

ACQUIRED DISORDERS OF PLATELET FUNCTION

Several systemic diseases are complicated by qualitative abnormalities in platelet function. In these systemic diseases, usually external or un-physiological components of the plasma result in partial or complete interference with platelet functions. Examples include:

Uraemia: Many patients with uraemia have a bleeding diathesis characterized by a prolonged bleeding time and abnormal platelet adhesion, aggregation, secretion, and platelet pro-coagulant activity. The pathogenesis of the platelet defect is not clear. Abnormalities in plasma vWf, reduction in GP Ib, and a decreased adhesion via GP IIb/IIIa have been reported[1].Uremic platelets however exhibit, when stimulated, a reduced release of arachidonic acid from membrane phospholipids. Platelet function disorder is mainly because of the significant accumulation of urinary toxins. Both dialyzable and nondialyzable substances found in uremic plasma can inhibit platelets in vitro at high concentrations[1].

The bleeding diathesis and the prolonged bleeding time in uraemia often improve with dialysis. Infusion of Desmopressin (DDAVP) a vasopressin analogue that releases vWf from endothelial cells will improve haemorrhage and bleeding times in more than 50% of uremic patients[10].

Myeloproliferative Disorders: Platelet dysfunctions are observed in patients with chronic myelo-proliferative disorders (essential thrombocythemia, polycythemia vera), but also in some patients with acute leukemia and myelodysplastic syndromes. The patterns of abnormality vary from patient to patient, and none of the functional defects reliably predicts a potential clinical haemorrhage.

In most of these disorders, thrombocytopenia is usually a more significant cause of bleeding than platelet dysfunction. However, in essential thrombocythemia, bleeding may occur with elevated platelet counts, even if the bleeding time is normal. These same patients may also experience vascular ischemic syndromes due to micro-vascular platelet occlusion[10].

Dysproteinemias: The dysproteinemias (multiple myeloma, Waldenström macro-globulinemia) are another group of systemic disorders in which the hemorrhagic diathesis has multiple causes. One major cause of bleeding complications is due to the blockage of surface-connected platelet functions.

Paraproteins adsorbed on platelets and vessel surfaces result in the inhibition of GP IIb/IIIa, platelet adhesion, and clot retraction. Platelet survival is shortened. Additional defects include thrombocytopenia, inhibition of coagulation factors (factor VIII), defective fibrin polymerization, and hyper-viscosity[1].

Liver Disease: Disorders of haemostasis in liver disease are multi-factorial. Clotting factor deficiencies, thrombocytopenia, enhanced fibrinolysis, a mild persistent disseminated intravascular coagulation (DIC), and dysfibrinogenemia all contribute to bleeding and measurable coagulation defects. Excess fibrinogen/fibrin degradation products that are present in liver disease adsorb to platelets and may interfere with platelet functions[1].

Bleeding time is frequently prolonged. Desmopressin (DDAVP) is effective in some patients, but in the case of severe bleeding and platelet counts less than 50,000/µL, platelet transfusions will be necessary[10].

Cardiopulmonary Bypass: During extra-corporeal perfusion for cardiac surgery, a variable degree of thrombocytopenia almost always occur. The mechanism of the thrombocytopenia is multi-factorial. The drop in platelet number is primarily due to haemo-dilution[1]. In addition, there is evidence of platelet activation and loss in the plastic tubing. The drop in platelet count generally persists for several days following bypass probably due to increased clearance of damaged platelets. The bypass procedure can also lead to abnormalities in platelet function[11].

LABORATORY ASSESSMENT OF PLATELET FUNCTION

Full Blood Countand Peripheral Smear Examination: These simple procedures can be used to investigate platelet function[12]. Careful examination of the peripheral smear is essential in a patient with thrombocytopenia. Spurious thrombocytopenia due to platelet clumping or platelets adhering to neutrophils (platelet satellitism) can be seen on a smear. Peripheral smear of a patient reported to have platelet counts of 10,000-150,000/µL on various occasion shows clumping of the platelets and satellitism involving neutrophils and platelets[10].

Examination of the peripheral smears in immune thrombocytopenic purpura often showgiant platelets. These platelets reflect the increased megakaryocytic mass in the marrow[13]. Rare disorders, such as Bernard-Soulier syndrome, can be diagnosed based on the result from the peripheral smear. Examination of the smear is essential to exclude thrombotic thrombocytopenic purpura and rare instances of acute leukemia. In thrombotic thrombocytopenic purpura, a striking degree of red blood cell fragmentation is seen in addition to thrombocytopenia. Examination of the peripheral smear shows red blood cell fragments, basophilic cells, in addition to thrombocytopenia in thrombotic thrombocytopenic purpura[9].

However, the minimum criteria for the diagnosis of thrombotic thrombocytopenic purpura are thrombocytopenia and micro-angiopathic haemolytic anaemia without an apparent etiology. Examination of the smear shows thrombocytopenia and a micro angiopathic picture (characteristic helmet cells/schistocytes and basophilic red blood cells). In addition, the lactic dehydrogenase(LDH) level is high, with brisk reticulocytosis. Signs of intravascular coagulation are characteristically absent in patients with thrombotic thrombocytopenic purpura[9].

Bleeding Time: The bleeding timeis defined as the time between the infliction of a small standard cut and the moment the bleeding stops[1].

This is a valuable test for disorders of primary haemostasis; however, this test is highly operator-dependent and is not recommended as a routine screening test. Primary haemostasis bleeding time is performed by measuring the duration required for bleeding to stop from a fresh superficial cut (1 mm deep, 1 cm long) made on the volar surface of the forearm using a template under standard conditions[14].

Under these conditions, the cessation of bleeding results from the formation of a primary haemostatic plug. A fairly linear correlation exists between bleeding time and platelet counts of 10,000-100,000/µL[10]. Bleeding time is prolonged with platelet counts below 75,000/µL, although that finding provides no insight into reason the count is low[10].

Primary haemostasis bleeding time should not however be performed on patients with thrombocytopenia. A prolonged bleeding time with a normal platelet count is very significant and indicates a qualitative platelet disorder[14].

Platelet Aggregation Assays: Platelet aggregation is measured by turbidimetric methods. When platelets aggregate, the opalescent suspension of platelet-rich plasma (PRP) becomes clearer and allows more light transmission. The extent of aggregation is determined by measuring the increase in light transmission[15].

The light absorbance of platelet-rich-plasma decreases as platelets aggregate. The amount and the rate of fall are dependent on platelet reactivity to the added agonist, provided that other variables such as temperature, platelet count, and mixing speed, are controlled. The absorbance changes are monitored on a chart recorder[15].

Small doses of ADP (< 1 µmol) induce a reversible form of platelet aggregation (primary wave), unaccompanied by thromboxane synthesis or release of intra-platelet ADP. However, with increasing doses of ADP, sufficient stimulation of platelets occur and leads to the release of intra-platelet ADP and the synthesis of thromboxane A2 from arachidonic acid, thus resulting in more pronounced irreversible aggregation (secondary wave)[10].

Ristocetin induces platelet aggregation by inducing von Willebrand protein binding to the platelet GP Ib complex[1].Platelet aggregation tests are useful in distinguishing different disorders of platelet function. They are also particularly useful in the diagnosis of von Willebrand disease, in which ristocetin-induced platelet aggregation is defective[15].

Automated Platelet Function Screening Test: The platelet function analyzer 100 (PFA-100) is an example of an automated screening equipment for platelet function. It is a bench-top automated instrument that assesses primary haemostasis under shear stress.

The PFA-100 uses a disposable test cartridge that contains a membrane impregnated with collagen plus ADP (Col/ADP membrane) or epinephrine (Col/Epi membrane). A blood sample of 0.8 mL of citrated blood is placed in a cup and is aspirated through the aperture. The shear stress and the agonists in the membrane activate platelets, leading to platelet aggregation[16].

The end point, expressed as closure time, is when blood flow stops because of occlusion of the aperture by platelet aggregates[16].

The platelet aggregate formation depends on:

(1) von Willebrand factor [vWf] binding to collagen-coated nitrocellulose membranes,
(2) Platelet adhesion to vWf via platelet GP Ib platelet activation, and
(3) Platelet aggregation mediated by the interaction of GP IIb/IIIa with vWf and fibrinogen[10].

Normal closure times range from 77 to 133 seconds for the Collagen/ADP membrane and 98-185 seconds for the CollagenEpinephrine membrane. The PFA-100 has been tested in persons with bleeding disorders. The closure time using the Col/Epi cartridge is abnormal in patients with congenital platelet function defects, von Willebrand disease, or aspirin ingestion, whereas the closure time with

the Col/ADP cartridge is abnormal mainly in patients with von Willebrand disease or congenital disorders[10].

Aspirin prolongs the closure time 94% of the time with the Col/Epi cartridge and only 27% of the time with the Col/ADP cartridge[17]. Glanzmannthrombasthenia, Bernard-Soulier syndrome, and most mild von Willebrand diseases are associated with a prolonged closure time with both cartridges, whereas a storage pool defect and giant platelet thrombopathy have a prolonged closure time only with the Col/Epi cartridge[17].

The advantages of this instrument include simplicity and reproducibility. The PFA-100 has been reported to have a coefficient of variation of less than 10%. It may be useful for determining global platelet function and for assessing the efficacy of anti-platelet therapy[17].

THERAPEUTIC ANTI-PLATELET AGENTS

Many drugs affect platelet function in vitro, these substances can cause platelet function abnormalities although relatively few of them prolong the bleeding time. These can however lead to acquired disorders of platelet function.

Aspirin ingestion results in abnormal platelet function with a moderately prolonged bleeding time and a defective platelet aggregation due to the inhibition of the cyclooxygenase and the thromboxane synthetase[10].

Large doses of Carbenicillin and other Penicillin, as well as the Cephalosporin moxolactam, prolong the bleeding time in a dose-related fashion. This effect begins within hours after drug administration and may last for several days. All phases of platelet function—adhesion, aggregation, and secretion— may be affected. In vitro, the Penicillin impairs the interaction of the VWF and aggregation agonists with the platelet surface membrane.

The plasma expanders' Dextran and Hydroxyethyl starch can prolong the bleeding time and cause abnormalities of platelet function comparable to those observed in dysproteinemias. Numerous other agents used in clinical medicine adversely affect normal haemostasis. Included among these are the anti-inflammatory agents (e.g. Diclofenac, Indomethacine,) and others like:

- ? Non-aspirin NSAIDs (non-steroidal anti-inflammatory drugs)
- ? Dipyridamole
- ? Clopidogrel and ticlopidine e.t.c[18].

THERAPY

All patients should avoid aspirin and other drugs that can affect platelets until the condition clears. Those whose only symptoms are petechiae may need no additional treatment.For people with more serious bleeding disorders, transfusions of platelets are given (if no detectable anti-platelet antibodies are present) until the underlying defect is corrected and the body produces enough healthy platelets on its own. Those with chronic or inherited forms of the disease may require transfusions whenever bleeding problems arise or before surgical or dental procedures are performed[19].If a drug is found to be the cause, discontinuation of the drug usually resolves the problem quickly.

Corticosteroid drugs may be prescribed to suppress the destruction of platelets by the immune system. The disorder often improves within several weeks and may disappear completely. Other immunosuppressive drugs may be tried as well.Those who continue to have platelet

defects despite corticosteroids or other forms of therapy may benefit from surgical removal of the spleen (splenectomy). The spleen acts to produce helpful antibodies and to remove worn-out blood cells, including platelets. But in those with platelet disorders, it can become enlarged and overactive and thus stall recovery. Splenectomy—used especially in those with ITP—brings about long-term remissions in many patients without causing other long-term side effects.

Therapeutic modalities that have been employed with questionable results include Desmopressin (DDAVP), corticosteroids, and oral contraceptive therapy to decrease menstrual bleeding. In a life-threatening bleeding complication also the recombinant factor VIIa may be effective.

In case of bleeding the therapy of choice is platelet transfusions, which are successful initially but are followed as a rule by subsequent immunization. According to recent case reports, the recombinant Factor VIIa concentrate (NovoSeven™) seems to be a potential alternative to platelet transfusion in Glanzmann'sthrombasthenia patients, particularly in those with antiplatelet antibodies and/or platelet refractoriness[20].

- ? Desmopressin
- ? Cyklokapron and Amicar
- ? Platelet transfusion
- ? Anti-fibrinolytic agents
- ? Other treatment

- Recombinant factor VIIa[20].

CONCLUSION

Congenital and Acquired Platelet disorders lead to defects in primary haemostasis and produce signs and symptoms (Petechiae and Purpuric spots) different from disorders of secondary haemostasis (coagulation factor deficiencies) like haemophilia. However, the assessment and distinguishing of these disorders can be carried out in the laboratory.

The manifestation of platelet disorders can be due to the fact that a patient inherits the abnormality from the parent as referred to as CONGENITAL/INHERITED or due to the fact that certain environmental conditions or disease conditions resulted in the disorder of platelet function referred to as ACQUIRED. Furthermore, care should also be taken when regarding certain chemical substances as the commonest disorders of platelet functions are acquired.

Research however, has helped medical personnel to design different therapeutic options for platelet disorders and more research is still on to further improve on medical laboratory investigations, innovative tests and the management of these physiological disorders.

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