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A Review on Platelets and Coagulation

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Abstract

Platelets are the smallest of the blood cells, yet they are one of the main players during the process of thrombus formation. This paper discussed platelets from the synthesis to function and haemostasis with coagulation factors together with the pathways. It also discussed coagulation disorders.

Keywords: Platelets, coagulations factor, coagulation pathways, coagulation disorders

Platelets

According to Gregory (2003) platelets are the smallest of the blood cells, yet they are one of the main players during the process of thrombus formation. Also, the traditional belief that the endothelium exists simply to provide an inert interface between the blood and the vessel wall is no longer accurate. Indeed, the endothelium produces a large number of substances that affect blood flow and in turn are affected by changes in the blood and the pressure of blood flow. Platelets play a central role in maintaining hemostasis and must be present in adequate number and have normal function. Platelets undergo a complex series of morphological and biochemical changes when activated. Platelets have the ability to bind to non-endothelial surfaces (adhesion), bind to other platelets (aggregation) and secrete substances that are stored in internal granules (secretion) (Aydilek et al., 2005).

Evaluation of Platelet Disorders

- Platelet count and smear evaluation
- Platelet Function Screen
- May be abnormal if platelet counts less than 150,000/uL or Hct less than 30% Sample submitted to lab within 1 - 2 hrs of collection or patient comes to labsite for blood collection.
- Platelet aggregation studies may be abnormal if plateletcount less than 150,000/uL or

Hct less than 30%Patient must not be taking aspirin or NSAID containing medication.

- Test requires patient to come to UWMC blood draw for blood collection. Platelet and vascular disorders are characterized by petechiae and/or small superficial ecchymosis, mucosal hemorrhage (e.g. gingival bleeding, GI bleeding, menorrhagia) and immediate profuse bleeding from small cuts. This immediate bleeding distinguishes platelet disorders from a coagulation protein deficiency where the bleeding is typically delayed.
- Platelet disorders can be quantitative or qualitative.

Qualitative platelet disorders can be inherited or acquired and are further classified into disorders of adhesion, aggregation or secretion. The inherited disorders usually have normal platelet counts(Okoroiwu *et al.*,2015).

The most common adhesion disorder is Von Willebrand disease, a quantitative and/or qualitative defect of Von Willebrand factor (VWF). VWF serves as a bridge between platelets and collagen, permitting adhesion of platelets to injured vessels. Bernard Soulier is a rare adhesive disorder where the patient's platelets lack part of the GPIb receptor complex that is required for platelets to bind to von Willebrand factor. This group of patients may present with mild to moderate thrombocytopenia and abnormally large platelets on smear evaluation. Thereare inherited aggregation disorders include Glanzmann'sThrombasthemia (missing or defective fibrinogenreceptor, GPIIbllla) and afibrinogenemia. Defective secretion disorders include storage pool abnormalities, Wiskott-Aldrich syndrome, Hermansky-Pudlak syndrome and Chediak-Higashi. Of this group, storage pool abnormalities are the most common (Kartaloglu *et al*., 2005).

Acquired platelet function abnormalities are more common than inherited defects and can be associated with decreased number and/or abnormal function. Drugs such as aspirin, penicillin and alcohol affect platelet function.Uremia, disseminated intravascular coagulation and myeloproliferative disorders are associated with abnormal platelet function as well. Thrombocytopenia can be a result of a production defect, non-immune destruction, immune platelet destruction or splenic sequestration. The severity of bleeding is usually related to the degree of thrombocytopenia and may be more severe when there is a rapid loss of platelets. Some drugs may cause thrombocytopenia through a variety of mechanisms. Platelet counts usually return to normal within 7-10 days once the offending drug is discontinued. Heparin-induced thrombocytopenia is a potentially life threatening form of acquired immune thrombocytopenia, caused by development of an antibody to the complex of PF4 and heparin on the platelet surface. At the same time, the PF4-heparincomplex also binds to the endothelial surface where it is thought to promote potentially life threatening arterial and venous thrombosis. The first indication of the development of this antibody is a rapid unexplained drop in the platelet count after the administration of heparin (Aydilek *et al.,* 2005). Thrombocytosis (marked increase in platelet count) may be primary or secondary. Primary thrombocytosisis observed in myeloproliferative disorders such as polycythemia vera, essential thrombocythernia and chronic granulocytic leukemia. Polycythemia vera and essential thrombocythernia are associated with prolongedelevations of the platelet count and thrombosis due to abnormal platelet number and function (Besses *et al.,* 1999). In secondary or reactive thrombocytosis the platelets have normal function and the elevated platelet count is usually transient (Harrison *et al.,* 2002).

Hemostasis

Hemostasis is a complex interaction between vessels, platelets and coagulation proteins that, when working properly, stops bleeding while maintaining blood flow in the vessel. Medical evaluation of the hemostasis system began with visual observation of the clotting process. During the time of medical bloodletting, observation of the size of the clot in a basin (clot retraction) was used to determine when bloodletting had to be

decreased. In the early 20th century, manual timing of whole blood clotting (i.e., Lee-White Whole Blood Clotting Time) and later plasma, in glass tubes permitted a more accurate measurement of blood clotting. Further discoveries about hemostasis in the 1930's and 1940's led to more sophisticated laboratory tests, including the prothrombin time, activated partial thromboplastin time, and specific assays of platelet function and fibrinolysis. The advent of the monoclonal antibody, molecular analysis, and the microcomputer in the 1980s led to an explosion of knowledge about hemostasis and hemostasis testing that is still growing (Mesa *et al.,* 1999).

Hemostasis involves four distinct but at the same time interrelated functions: vessel wall function, platelet function, coagulation and fibrinolysis (Briere, 2007). Specific tests are available to evaluate platelet function, coagulation proteins, natural occurring inhibitors and fibrinolysis (Barbui *and Finazi*, 2003).

Platelets play an immediate and central role in hemostasis. Qualitative and/or quantitative defects may exist which lead to excessive bleeding. When vascular injury occurs, the sub endothelium is exposed and in the presence of von Willebrand factor, platelets adhere to collagen. Stimulated platelets release ADP (which potentiates platelet aggregation), expose anionic phospholipid, and release factor V and fibrinogen, which promotes coagulation. The fragile primary platelet clot is quickly stabilized by fibrin formation via the coagulation cascade (Lee 1997).

Factors Affecting Prothrobin Time Test

i. Difficult in obtaining a various blood smaller resulting in hemolysis or small clot in the sample
ii. Using tubes or pipette which are not clean and dry or contaminated with detergent
iii. Not pipetting correct volumes of the plasma as well as well as the plasma as well as the reagent
iv. Not putting the sample in water bath bring it to body temperature
v. Use of unsatisfactory regents and inadequate timing

Blood coagulation

The blood coagulation system is composed of series of functionally specific plasma proteins (coagulation factors) which interact in a highly ordered and predetermined sequence there by resulting in the formation of an insoluble fibrin mesh which acts to consolidate and stabilize the primary hemostatic plug (Baker and Silverton, 2003).

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Process of Blood Coagulation

Blood coagulation can simply be defined as a host defence system which maintains the integrity of the high pressure closed circulatory system and this process may be considered a mechanism for rapid replacement of unstable platelet plug with a chemically stable fibrin clot following tissue damage (Kasper et al., 2000). The process of blood coagulation occurs mainly in three essential steps

a. In response to rapture of the vessel or damage to the blood vessel itself, there is complex ceasceda of chemical reactions which occurs in the blood involving more than millions of blood coagulation factors with the each result of formation of prothrombin activator from trauma to the blood, or contact of the blood with damaged endothelial cells or with collagen and other tissue elements outside the blood vessel.
b. The prothrombin activator formed catalyses the conversion of prothrombin into thrombin. This occurs in the presence of sufficient amount of calcium ions, platelets also play vital role in the conversion of prothrombin to thrombin because much of the thrombin first attaches to

prothrombin receptors on the platelets that have already bound to the damaged tissue, thereby accelerating more formation or thrombin from the prothrombin through this occurs in the specific tissue where the doth is needed

c. The thrombin acts as an enzyme thus causing polymerizations of fibrinogen molecules into fibrin fibers that enmesh platelet, blood cells and plasma to form the clot. This thrombin is a protein and functions by removing four low molecular weight peptides from each molecules of fibrinogen forming to polymerize with other fibrin monomer molecule thereby needing to fibrin which constitute the reticulum of the clot.

For many years it was thought that coagulation proceeded via two pathways, intrinsic and extrinsic, with the difference between the two being method of activation. Today, we understand that only a single pathway exists coagulation activation that occurs in two phases (Khamesiu *et al.,* 2007).

The first phase of coagulation activation, termed the initiation phase depend in the hemostasis laboratory, automated assays have replaced many of the manual procedure of the past, and there is increasing interest in rapid, point of care haemostasis assays for perioperative and critical care, as well as self-testing to support the millions of patients now receiving oral anticoagulation for hypercoagulation diseases. Interestingly, measurement of clot retraction is kill focus of a variety of these techniques, a fact that would no doubt be appreciated bythe early physicians. This paper presents a global overview of the techniques presently used in the haemostasis laboratory, with the realization that many of these may be quickly surpassed by new information, developments, and applications the near future on exposure or transport of tissue factor to the site of the wound(Lee 1997).

Thrombin generation is slow during the initiation phase. As thrombin levels increase, thrombin activates additional platelets and factors V, VIII and XI which dramatically increases the rate of thrombin generation leading to fibrin and clot formation, this is termed the propagation phase.

In vivo, almost all coagulation reactions arc initiated by exposure of tissue factor and platelet activation. Tissue factor autocatalyzes factor VII to VIIa. Factor Vila in turn activates factor IX, IXa activates X, Xa converts prothrombin to thrombin, finally resulting in conversion of fibrinogen to fibrin (Mirsaledi *et al.,* 2007).

In the activated partial thromboplastin time (APTT) test, the contact system is activated which in turn initiates the "intrinsic" coagulation pathway through factor Xlla. Contact phase factors include prekallikrein, high molecular weight kininogen, and factor XII, which when activated convert factor XI to XIa. Factor Xia converts IX to IXa, which then activates factor X which in turn converts prothrombin into thrombin (Dacie and Lewis, 2001).

In vivo there are a variety of control mechanisms to limit thrombus formation through the naturally occurring inhibitors protein C, protein S and \ antithrombin. Thrombomodulin present on the endothelial surface binds thrombin, "modulating" its specificity and turning thrombin into an activator of protein C. Activated protein C, with its cofactor protein S, proteolytically degrades factors Va and VIIIa. Thrombin in plasma is inhibited by antithrombin. This reaction is accelerated by heparinoids on the endothelial surface and heparin given therapeutically. Activated Protein C is regulated by activated protein C inhibitor (APCI). Protein C exists in two forms in plasma: tree and bound to C4b-binding protein. Only the free form of protein S is active (Lee 1997).

The fibrinolytic system plays an important role in regulating the formation and removal of thrombin. Fibrinolysisis initiated by the release of tissue plasminogcn activator (tPA) from vascular endothelial cells. Tissue plasmainogen activator (tPA) in the presence of fibrin, converts plasminogen to ptasmin, which in turn lyses fibrin in the thrombus. The concentration of tPA in circulating blood is regulated by the secretion of tPA by the vascular endothelium,clearance of tPA by the liver arid the inhibition of tPA by plasminogen activator inhibitor type 1. The concentration of plasmin in the blood is regulated

by antiplasmin (aka plasmin inhibitor). Increased levels of fibrinolytic activity in blood are associated with bleeding, while decreased levels are associated with thrombosis. Bleeding disorders may be due to abnormalities of the coagulation, platelet, vascular or fibrinolytic systems (Schumacher *et al.*, 2004).

Hereditary disorders are usually due to an abnormality of a single system, whereas acquired abnormalities may involve two or all of the systems listed above. Clinical laboratory tests are available to evaluate platelets, coagulation and fibrinolysis (Kartaloglu *et al*., 2005).

Coagulation screening test

The Coagulation Screen includes PT, APTT, Thrombin time, and Clauses Fibrinogen assays. The DIC panel includes these same tests plus a quantitative D-Dimer and platelet count. Prothrombin time (PT) test is used as a screening test to evaluate the integrity of the extrinsic coagulation pathway. The test measures the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system. The test is among the first line investigation of defects of coagulation preformed in the laboratory, which attempt to reproduce in-vitro process that normally occurs in the body. If heparin is discovered in either of these testing packages it will be removed and the APTT result will be reported for the sample containing heparin as well as for the sample after heparin removal. The APTT on the sample after heparin removal is a separate test that will be automatically added on in the event heparin is detected in the sample (Okutan *et al*., 2000). Mixing studies may be ordered to determine if bleeding disorders are factor deficiency- or inhibitor- based (Schumacher *et al.*, 2004).

Coagulation pathways and its disorders

Coagulation Pathways

Traditionally, the blood coagulation scheme has been divided into intrinsic and extrinsic pathways which converge at a point where factor *X* is activated. The intrinsic path way may be initiated in the clinical laboratory by activation of Hageman factor (factor Xii) while the extensive pathway is activated by issue factor a circular incorporation present at the of issue injury.

The Extrinsic Pathway

When blood clothing is activated through the intrinsic pathway is plays a dominant physiologic role in haemostatic, (Furie *et al.*, 1909) in this processtraumatized issue release a complex of several factor called tissue factor or tissue thromboplasin, which is a cellular receptor for factor VI1 and via and is present on f most cell surfaces. "The expression of issue factor activity is constitutive on most non-vascular cell and can be inducible through denovo synthesis in cell within the blood vessel wall including monocytes and endothelial cells. This tissue factor is composed of phospholipids from the membrane of the tissue plus a proproteins complex which further complexes with factor will in the presence of calcium ions and then acts enzymatically a factor X thereby generating factor iXa and factor Xa respectively. Similarly, the mechanism by which a small amount of factor vii is converted to its active from is unknown in the absence of ongoing blood clotting, plasma contains factor villa at levels of 0.5-08mgml (Morisey *et al.*, 1993).

Factor Xa that is formed is able to convert further factor vll to factor vlla, thus amplifying the imitation of clothing. The activated factor X formed combines immediately with tissue phospholipids that are part of tissue factor or phospholipids released from platelets as well as with factor Va which is extrinsic membrane binding protein leading to the formation of prothrombinase complex or prothrombin activator that acts on prothrombin as a substrate. The prothrombin activator, in the presence of sufficient amount of calcium ions causes conversion of prothrombin to thrombin within seconds. This thrombin then acts on fibrinogen to remove four low molecular weight peptides (two fibrin peptide A and two fibrin peptide B) to form fibrin monomer which polymerizes to form long

strands known as proto fibril which through the action of factor xiiia, a transglutaminase arc covalently cross-linked to yield an insoluble fibrin which is not easily disrupted (Kasper *et al.*, 2001).

The Intrinsic Pathway of Coagulation

In the pathway all the necessary components are found within the circulating blood. When blood comes in contact with a foreign surface e.g. exposed collagen fiber fibrin in the well of the blood vessel or a glass surface, a series of reaction which is mediated by enzymes start. On contact with a foreign surface prekallikrein and high molecular weight kininogen (HMWK) participates in the activation of factor Xii to xiiia. Xiia in turn activates factors xi to from xia to xiia this process continues further involving factor xi, viii and xi factor x is converted to Xa by the action of calcium ions and phospholipids on platelets.

Common Pathway of Coagulation

The common pathway involves the activation of factor x to xa through the extrinsic pathway factor xa in the presence of calcium ions, platelet ii and factor iii and factor v concerts factor ii (prothrombin) to the active enzyme thrombin. Thrombin act on factor xiii helps in the formation of stabilized stronger clot.

Screening Tests of Extrinsic Coagulation

The prothrombin time (PT) test is used as a screening test to evaluate the integrity of the extrinsic coagulation pathway.

The test measures the clotting time of plasma in the presence of an optimal concentration tissue extract (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system. It depends on prothrombin factor V, VIT, X and on the fibrinogen concentration of the plasma (Dacie *and Lewis*, 2001).

Bleeding associated with coagulation abnormalities is characterized by the formation of large hematomas, hemarthrosis, large single ecchymosis (either spontaneous or following minor trauma) or delayed bleeding following trauma, surgical or dental procedures. Petechiae and mucosal hemorrhage are rare unless there is an associated platelet disorder (Aydilek *et al.*, 2005).

Coagulation disorders may be congenital or acquired

Congenital coagulation disorders: are often associated with a positive family history. A moderate to severe deficiency usually presents in early infancy through adolescence. A mild deficiency may not be detected until the patient is challenged with surgery or trauma. The most common of the congenital disorders is von Willebrand disease. It is a platelet-like bleeding disorder with a quantitative and/or qualitative defect of VonWillebrand factor and a borderline to decreased factor VIII activity. Von Willebrand disease is Hemophilia A (VIII deficiency) is the next most common, followed by hemophilia B (IX deficiency) (Kartaloglu *etal.,2005).* Both are inherited as sex-linked recessive disorders. Hemophilia C (XI deficiency) is inherited as an autosomal recessive trait. Factor XII deficiency is not associated with a bleeding disorder. Von Willebrand Disease typel may present with a mild to marked prolongation of the APTT but other testing more specific for defining the disease is available as the von Willebrand Disease Panel. All of the Hemophilias and the Factor XJI deficiency will present with a mild to marked prolongation of the APTT with all other screening procedures normal. The other congenital coagulation factor deficiencies (II, V, VII, and X) are extremely rare. Factor II, V, or X deficiency will have a moderate to marked prolongation of the prothrombin time and mild to moderate prolongation of the APTT. An isolated Factor Vll deficiency has a prolonged prothrombin time with all other screen assays normal (Schumacher *et al.*, 2004).

Acquired coagulation disorders: are more common in hospitalized patients and can be life threatening. Acquired disorders are associated with acute and chronic disseminated intravascular coagulation (DIG), liver disease, vitamin K. deficiency (dietary, wide spectrum antibiotic

therapy, and/or oral anticoagulant therapy), heparin therapy and the use of fluid for volume replacement as in trauma patients or dilutional as in massive blood transfusion. The patients may have multiple abnormalities including variable prolongation of the prothrombin time, APTT, thrombin time and decreased fibrinogen and platelets (Aydilek et al, 2005).

Bleeding can be associated with acquired factor inhibitors. Inhibitors have been described for all coagulation proteins. Although inhibitors are rare, the most common have been described against factor Vlfl and factor V. A prolonged APTT which does not correct in a 1:1 mix and where the 1:1 mix APTT increases as the mix: In 37°C water bath is suggestive of a factor VIII inhibitor in a bleeding patient. A patient previously exposed to topical thrombin with a prolonged prothrombin time, which does not correct on a 1:1 mix, should be evaluated for a possible inhibitor against factor V. These same patients may demonstrate a prolonged thrombin time when bovine thrombin is used in the test procedure. The patient thrombin time is normal if human thrombin is used (Kartaloglu *et al.,* 2005).

Conclusion

Platelets are the smallest of the blood cells, yet they are one of the main players during the process of thrombus formation. Platelets undergo a complex series of morphological and biochemical changes when activated. Platelets have the ability to bind to non-endothelial surfaces, bind to other platelets and secrete substances that are stored in internal granules.

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