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Laboratory diagnosis of inherited platelet function disorders

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ABSTRACT

Platelets respond to vessel wall injury by forming a primary hemostatic plug to arrest blood loss. Hemostatic plug formation is complex, and involves platelet adhesion to the subendothelium that results in platelet activation and ultimately, aggregation. If any of these processes are deficient, primary hemostasis is impaired. Inherited platelet function disorders (IPFDs) are a heterogeneous group of defects in these processes, with patients experiencing mainly mucocutaneous bleeding symptoms that can range from very mild to life threatening, depending on the specific disorder. Here, we review the approach to an initial patient assessment required to inform laboratory testing, and the frequently used clinical laboratory assays for diagnostic evaluation of IPFDs. Newer testing approaches that may improve laboratory diagnosis in the near future are described.

1. Introduction

1.1. Platelet activation and aggregation

The primary physiological role of platelets is to support hemostasis at sites of vascular injury by forming platelet plugs that arrest blood loss. Circulating platelets monitor the integrity of the endothelial lining of blood vessels. When the blood vessel wall is injured and the endothelium is damaged, platelets adhere to the exposed subendothelial matrix proteins, initiating activation events that result in formation of a hemostatic plug [1]. Platelets adhere to collagen in the subendothelium directly, via their glycoprotein (GP)VI and integrin α2β1 membrane receptors, and to collagen-bound von Willebrand factor (VWF), at sites of higher shear ($> 1000\ s^{-1}$) such as small arteries and the microvasculature, via GPIbα of the GPIb-IX-V membrane receptor complex. Intracellular signaling events initiated by platelet adhesion result in: reorganization of the platelet cytoskeleton leading to platelet shape change to irregular spheres with extended filopodia and spreading on the subendothelium; secretion of the contents of storage granules (e.g., ADP and serotonin from dense granules, and fibrinogen and growth factors from α-granules); formation of thromboxane A_2 (TxA_2) from arachidonic acid via cyclooxygenase-1 and thromboxane synthase; and procoagulant phosphatidylserine (PS) exposure on the platelet surface that accelerates thrombin generation. By binding to their specific membrane receptors, platelet agonists (ADP, TxA_2 and thrombin) initiate signaling pathways that convert integrin αIIbβ3 (GPIIb-IIIa) to a high affinity conformation that binds divalent fibrinogen and, at high shear, multivalent VWF. These ligands function as bridges between αIIbβ3 on adjacent activated platelets, mediating aggregation. (See Fig. 2 in Pryzdial et al in this issue for a schematic of the process of platelet plug formation.) Primary hemostasis is impaired if any of the adhesion, activation or aggregation processes are deficient [2].

1.2. Inherited platelet function disorders

Inherited platelet function disorders (IPFDs) encompass defects in: platelet adhesion, with deficiencies or dysfunction of receptors for subendothelial VWF or collagen; platelet activation, including deficiencies or dysfunction of receptors for soluble agonists, of cytoskeletal proteins, of signaling pathways, of dense and α-granules, and procoagulant surface exposure; and platelet aggregation, with deficiencies or dysfunction of αIIbβ3.

The bleeding symptoms experienced by patients with IPFDs are primarily mucocutaneous, including epistaxis, bruising, bleeding from the oropharynx or gastrointestinal tract, menorrhagia, and postpartum and surgical (including dental) bleeding; these symptoms can range from very mild to life threatening, depending on the disorder (severity may vary among individuals with the same disorder). The prevalence of these disorders is unknown, as there are no population-based data. While severe disorders such as Glanzmann thrombasthenia and

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Bernard-Soulier syndrome are relatively easy to diagnose but rare, milder disorders are more difficult to characterize and it is likely that they are more common than previously appreciated (Table 1) [3]. Mild disorders may go undetected unless a family history prompts early testing or until a hemostatic challenge (e.g., surgery involving mucous membranes) results in bleeding.

Inherited platelet disorders include both qualitative and quantitative defects. This review will focus on the laboratory investigation of IPFDs (excluding disorders primarily due to thrombocytopenia; readers are referred to recent reviews on the diagnosis of inherited thrombocytopenias [4,5]).

2. Laboratory testing

The inherited platelet function disorders (IPFDs) are diverse in their laboratory presentation, and diagnostic testing can be complex [6,7]. Laboratory testing does not always lead to a definitive diagnosis, particularly in mild and moderate platelet disorders. An algorithmic approach can be helpful in organizing the clinical and laboratory investigation of patients with IPFDs, and several algorithms for the diagnosis of IPFDs have been published [6,8–10]. Ideally, platelet function testing should be conducted in laboratories with specialized expertise and an adequate frequency/volume of testing to maintain technological proficiency. Laboratory results should be interpreted in the context of clinical information.

As VWD is the most common disorder of primary hemostasis, specific testing of VWF antigen and activity levels either prior to, or concurrently with, testing for IPFDs is recommended [6]. Also, VWD type 2B and platelet-type VWD can present with macrothrombocytopenia, and so these subtypes of VWD should be considered in patients with low platelet counts.

2.1. Clinical assessment

To facilitate establishing a correct diagnosis, a detailed medical history, including bleeding, family and medication histories, and a careful physical examination are key. Assessment of bleeding symptoms can be aided by the use of one of several validated bleeding assessment tools (BATs), useful in standardizing information obtained from the patient history and accurately recording the severity and frequency of bleeding symptoms [11]. The high negative predictive value of some BATs may, in the future, make it possible to use them as a screen prior to laboratory testing. However, existing tools have low specificity and will not provide a definitive diagnosis [11,12]. In addition to the patient's bleeding history, the family history may provide important clues about the potential inheritance of an underlying bleeding disorder; most IPFDs are inherited in an autosomal-recessive fashion (Table 1). Evaluation of the bleeding history in family members by a BAT could be useful for appreciating the significance of the family bleeding history. A detailed medication history is required to rule out medication-associated acquired platelet dysfunction [13], which is more common than inherited defects. Examples of common drugs that lead to acquired platelet dysfunction are the non-steroidal anti-inflammatory drugs (NSAIDs), e.g., aspirin, ibuprofen, and supplements/herbal remedies such as garlic and feverfew. On physical examination, evidence of mucocutaneous bleeding, as described in Section 1.1, suggests a disorder of primary hemostasis, i.e., VWD or an IPFD. Additional clinical abnormalities may suggest the presence of a syndromic IPFD, e.g., oculocutaneous albinism in Hermansky-Pudlak syndrome (see Table 1 for further syndromic manifestations of specific IPFDs).

2.2. Platelet count and volume, and blood film

Initial testing to investigate for IPFDs should include an automated complete blood count (CBC) and a peripheral blood film analysis; these are sensitive and specific for abnormalities of platelet number and can guide further laboratory testing [14]. Standard automated impedance and optical platelet counting methods provide accurate counts within a broad range, but are less accurate when platelet counts are $< 50 \times 10^9$/L or when platelets are so large that they are outside the established reference interval of size set in the automated counter. In these cases, immuno-counting using flow cytometric detection of platelets labelled with a fluorescent antibody should be considered [14]. The mean platelet volume (MPV) determined by automated cell counters is influenced by blood sample collection and analysis method [14]. Even if these are standardized, obtaining an accurate MPV can be problematic for samples with macrothrombocytopenia.

Examination of a peripheral blood film stained with Wright's or May-Grunwald-Giemsa by light microscopy is critical, as it provides information about platelet number, size, clumping and granularity [6]. For example, large, pale (due to the absence of α-granules) platelets in low number are characteristic of gray platelet syndrome, while large, granulated platelets in low number are characteristic of Bernard-Soulier syndrome. Pseudothrombocytopenia resulting from clumping of platelets collected in EDTA anticoagulant can also be identified by examining the blood film, and can be confirmed by re-collecting a specimen in citrateanticoagulant in which clumping will not occur. It must be noted that the presence of thrombocytopenia does not rule out platelet dysfunction, as some inherited platelet disorders are characterized by both a decreased platelet count and abnormal function [15].

2.3. Tests of primary hemostasis: the bleeding time and the PFA-100/200 closure time

There is no ideal simple, inexpensive, sensitive screening test that reliably identifies patients requiring specialized testing of platelet function. Although the bleeding time and the Platelet Function Analyzer (PFA-100/200) have been used for this purpose, these tests are not adequately sensitive to rule out the need for further testing in patients with mucocutaneous bleeding [16,17] and should be considered as optional initial tests.

The oldest test of platelet function is the bleeding time. In its most recent refinement, testing is accomplished by applying a blood pressure cuff to the upper arm to a pressure of 40 mm Hg, making standardized incisions on the volar surface of the forearm with a spring-loaded device containing 2 sterile blades, and recording the time taken for bleeding to cease due to the formation of a platelet plug [18]. Its advantage is that it is a simple test that informs on all aspects of primary hemostasis including vascular abnormalities. It is the only test of hemostasis that does so. However, the test is highly operator-dependent, poorly reproducible, invasive, and insensitive to mild platelet defects [17], and its routine use in most clinical laboratories is on the decline [19] as it is difficult to maintain quality assurance.

The PFA-100 was developed as a rapid, simple, and reproducible test that measures primary platelet-related hemostasis in small samples of anticoagulated whole blood under conditions of high shear, with platelet plug formation, therefore, being dependent on VWF rather than fibrinogen. The recently released PFA-200 operates on the identical principal as its predecessor, such that test results are essentially identical with the 2 instruments [20]. To perform the test, 0.8 mL of citrated whole blood that has been placed into the reservoir of a disposable test cartridge is drawn up under vacuum through a 200 µm diameter stainless steel capillary and a 150 µm diameter aperture in a nitrocellulose membrane coated with collagen and epinephrine (Col/Epi) or collagen and ADP (Col/ADP). In response to the high shear rates of 5000–6000 s^{-1} and the agonists, a platelet aggregate forms that blocks the flow of blood through the aperture; the time taken to occlude the aperture is reported as the closure time (CT) and is measured to a maximum of 300 s. Platelet plug formation is dependent on platelet adhesion to collagen and aggregation mediated via VWF. CTs are very sensitive to VWF levels and are prolonged under conditions of