EDUCATIONAL COMMENTARY - PLATELET FUNCTION TESTS AND ASPIRIN INHIBITION

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Learning Outcomes
Upon completion of this exercise, participants will be able to:

- describe precautions to take when collecting a blood sample for platelet function testing.
- list the types of platelet function analyses currently used.
- explain the role of aspirin resistance in treatment for cardiovascular and thrombotic disorders.

Platelets are tiny cell fragments circulating in the blood whose function is to assist with stopping bleeding after an injury. Platelets are evaluated in the laboratory in two general ways: quantitatively by the platelet count and qualitatively by functional testing. Disorders due to quantitative platelet abnormalities are much more common than those caused by qualitative abnormalities. Also, it is much more difficult to test for platelet function disorders in the laboratory than to count platelets. If a platelet count is >50,000/µL (50 ×10^9/L; reference range: 150,000-450,000/µL [150-450 ×10^9/L]) and a patient is exhibiting symptoms of mucocutaneous bleeding and/or bruising, platelet function tests are indicated.¹

Preanalytic Considerations
Blood collection for all coagulation testing must be done in a special manner. The anticoagulant of choice is 3.2% sodium citrate, and the ratio of anticoagulant to blood must be precise. Underfilled or overfilled evacuated tubes or tubes with even the tiniest clot are unacceptable. For platelet function testing (PFT), the above rules also apply, and most tests should be performed within 3 hours of collection. Samples should be stored at 18° to 24°C because colder storage will affect platelet function.²

A wide variety of drugs, including aspirin and any aspirin-containing drugs, can affect platelet function for the entire life span of the platelet, which is 7 to 10 days. PFT should not be performed unless the patient has been informed to cease taking any of these drugs for 7 to 10 days prior to testing. A comprehensive list of drugs containing aspirin should be given to the patient, and the patient should follow the instructions precisely. In addition, a platelet count should be performed and a stained blood film examined prior to PFT. If the platelet count is significantly decreased, PFT will not be accurate and should be discontinued.³ Morphologically abnormal platelets on the smear may help to identify the type of disorder.
Platelet Function Testing

Platelet function testing has in the past been limited to specialty laboratories because test methods have been difficult and time-consuming. It was a challenge to produce accurate and reliable results. With the recent introduction of new test methods and analyzers, PFT has become more affordable and practical for the busy clinical laboratory.

Screening Tests

Historically, the bleeding time test was the only screening test to evaluate platelet function. It began as the Duke Bleeding Time, which was an in vivo measure of the time required for a superficial injury to the earlobe to stop bleeding. It was subject to many sources of error including the depth and width of the injury, skin thickness, capillary pressure, and the experience of the person performing the test. At best, it was a crude measure of platelet function and was also affected by platelet number and the integrity of the vascular system. Over the years, the method was improved and became the Modified Ivy Bleeding time, which used a template to decrease the variation in size and depth of the cut and used the forearm in place of the earlobe. This test was modified further by using disposable devices that are spring-loaded to standardize the cut. The Modified Ivy Bleeding time is the most commonly used method today if it has not been replaced by another more specific test. Even with these improvements, the test is susceptible to numerous sources of error and is time-consuming. The bleeding time has been replaced in most laboratories by other more reliable tests as described below.1

The Platelet Function Analyzer (PFA-100) was introduced by Dade Behring, now known as Siemens Healthcare Diagnostics, Deerfield, IL, in the mid-1990s. This is a quick, reliable, and easy-to-use analyzer to screen for platelet function abnormalities. It is an in vitro test, uses whole blood, and eliminates many of the errors inherent in the bleeding time test.4 Whole citrated blood is introduced into each of two cartridges containing a membrane coated with collagen/epinephrine or collagen/adenosine diphosphate (ADP). These substances activate platelets to adhere to the membrane and form a platelet plug. The time for the platelet plug to occlude a tiny aperture in each membrane is measured in seconds as the “closure time.”

Another small instrument currently being marketed is the VerifyNow System distributed by Accumetrics, San Diego, CA. A change in optical density is measured as the platelets respond to an added substance that causes aggregation (agonist). This instrument is designed to detect a patient’s response to platelet function inhibiting drugs, such as aspirin. It is not designed for use in patients with suspected congenital or non-aspirin induced acquired platelet abnormalities.
EDUCATIONAL COMMENTARY - PLATELET FUNCTION TESTS AND ASPIRIN INHIBITION (cont.)

Definitive Tests
The “gold standard” test for platelet function is the platelet aggregation test using platelet-rich plasma (PRP).\(^5\,^6\) This test is usually performed in a special coagulation laboratory. It is time-consuming and requires strict adherence to protocol. PRP is obtained by spinning citrated blood for 10 minutes at 200g with the tube stopper in place to maintain proper pH.\(^3\) Red and white blood cells spin down to the bottom of the tube leaving the smaller, lighter platelets suspended in the plasma. The platelets in the PRP sample are counted and adjusted to a platelet count of approximately 200,000/µL. The PRP is then placed in a specialized photometer called an aggregometer, where it is warmed to 37°C and stirred constantly to keep the platelets in suspension. Light is focused through the sample cup to a photomultiplier tube. Agonists are added to the PRP and cause a change in optical density as the platelets aggregate. The instrument plots a graph of the pattern of aggregation with each agonist. Commonly used agonists are thrombin, ADP, collagen, epinephrine, arachidonic acid, and ristocetin. If the patient’s platelets are resistant to a certain agonist, they will not produce reaction in the form of a curve at all, only a straight line showing no response. Some agonists normally cause a monophasic curve, and others are known to form a biphasic curve. The pattern of aggregation with each of these agonists is used to help identify specific disorders. For example, a patient with von Willebrand disease will produce a normal curve with all agonists except ristocetin.

Platelet aggregation testing using whole blood is quicker and requires less sample than the method just described. There is less manipulation of sample because preparation of PRP is eliminated. However, platelet numbers are not standardized as in the plasma method. Both patient and control samples are diluted with saline, and each is placed into a well containing two probes. A small amount of electric current is generated, and platelets will begin to coat the probes. As various agonists are added, the platelets aggregate and the electrical resistance between the probes increases. The time required to cause a change in resistance (impedance) is measured as the end-point. Amplitude is displayed in ohms on the front panel of the instrument, with an option to interface a chart recorder for graphs as in the previous method.

Clinical Applications
Platelet function testing is useful to identify hereditary disorders of platelet function such as von Willebrand disease, Bernard-Soulier syndrome, Glanzmann thrombasthenia, and storage pool disease. Acquired disorders in which platelet function may be abnormal are liver disease, renal disease, myeloproliferative disorders, myelodysplastic syndromes, multiple myeloma, and drug therapy. Another common use is to screen a high-risk patient for bleeding prior to a major surgical procedure.
EDUCATIONAL COMMENTARY - PLATELET FUNCTION TESTS AND ASPIRIN INHIBITION (cont.)

Aspirin Resistance
Because of aspirin’s ability to interfere with platelet function, it is widely used as therapy after an acute coronary syndrome or ischemic stroke to prevent recurrence. It is also prescribed to prevent cardiovascular disease in high-risk patients. Some patients experience a recurrent cardiovascular event or stroke despite treatment with aspirin therapy. This phenomenon is often called “aspirin resistance.” PFT is sometimes used to monitor a patient’s response to this antiplatelet therapy.

Aspirin interferes with platelet function permanently by inactivating the enzyme, cyclooxygenase-1 (COX-1), preventing the conversion of arachidonic acid to thromboxane A2 in platelets. The reported prevalence of aspirin resistance varies from 5% to 60%, depending upon the types of patients studied, other diseases present, assay used, aspirin dosage, duration of treatment, etc. Another variable that is difficult to monitor is patient compliance with prescribed aspirin dosage. Most researchers believe that multiple mechanisms, including but not limited to inadequate inhibition of COX-1, are responsible for poor clinical outcomes in patients treated with aspirin. Many studies need to be performed to identify the best PFT method, its availability, and cost-effectiveness, before PFT is recommended to evaluate aspirin resistance in clinical practice. The International Society on Thrombosis and Haemostasis (ISTH) Scientific Standardization Committee concurs that the clinical usefulness of platelet aggregation testing to monitor antiplatelet therapy requires further evaluation.

References


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