PLATELET FUNCTION TESTS

Platelet function tests are designed to detect qualitative (functional) platelet abnormalities in patients who are experiencing the symptoms of mucocutaneous bleeding (Chapter 41). A platelet count is performed, and the blood film is reviewed before platelet function tests are begun, because thrombocytopenia is a common cause of hemorrhage (Chapter 40).[24] Qualitative platelet abnormalities are suspected only when bleeding symptoms are present and the platelet count exceeds 50,000/μL.

Although hereditary platelet function disorders are rare, acquired defects are common.[25] Acquired platelet defects are associated with liver disease, renal disease, myeloproliferative neoplasms, myelodysplastic syndromes, myeloma, uremia, autoimmune disorders, anemias, and drug therapy. Platelet morphology is often a clue; for instance, in Bernard-Soulier syndrome, the blood film reveals mild thrombocytopenia and large gray platelets (Figure 41-3). Similarly, the presence of large platelets on the blood film associated with elevated mean platelet volume often indicates rapid platelet turnover, such as what occurs in *immune thrombocytopenic purpura* or *thrombotic thrombocytopenic purpura*. Giant or dysplastic platelets are seen in myeloproliferative neoplasms, acute leukemia, and myelodysplastic syndromes.

Bleeding Time Test for Platelet Function

The *bleeding time* test was the original test of platelet function, although it is now largely replaced by near-patient analysis of platelet function using the PFA-100 (Siemens Healthcare Diagnostics, Inc., Deerfield, IL), the Multiplate (DiaPharma, West Chester, OH), or platelet aggregometry.[26] To perform the test, the phlebotomist uses a lancet to make a small, controlled puncture wound and records the duration of bleeding, comparing the results with the universally accepted reference interval of 2 to 9 minutes. The bleeding time test was first described by Duke[27] in 1912 and modified by Ivy[28] in 1941. In 1978 some standardization was attempted. A blood pressure cuff was inflated to 40 mm Hg, a calibrated spring-loaded lancet (Surgicutt Bleeding Time Device; International Technidyne Corp., Edison, NJ) was triggered on the volar surface of the forearm a few inches distal to the antecubital crease, and the resulting wound was blotted every 30 seconds with filter paper until bleeding stopped.[29,30]

A prolonged bleeding time could theoretically signal a functional platelet disorder such as von Willebrand disease (VWD) or a vascular disorder such as scurvy or vasculitis, and was a characteristic result of therapy with aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs). Measurement of the bleeding time was often requested by surgeons at admission in an attempt to predict surgical bleeding, but a series of studies in the 1990s revealed that the test has inadequate predictive value. The bleeding time is affected by the nonplatelet variables of intracapillary pressure, skin thickness at the puncture site, and size and depth of the wound, all of which interfere with accurate interpretation of the test results. Owing to its poor predictive value for bleeding and its tendency to scar the forearm, use of the bleeding time assay has been discontinued at most institutions.

Platelet Aggregometry and Lumiaggregometry

Functional platelets *adhere* to subendothelial collagen, *aggregate* with one another, and *secrete* the contents of their α-granules and dense granules (Chapter 13). Normal adhesion requires intact platelet membranes and functional plasma VWF. Normal aggregation requires that platelet membranes and platelet activation pathways are intact, that the plasma fibrinogen concentration is normal, and that normal secretions are released from platelet granules. Platelet adhesion, aggregation, and secretion are assessed using in vitro platelet aggregometry.

An aggregometer is an instrument designed to measure platelet aggregation in a suspension of citrated whole blood or PRP. Specimens are collected and managed in compliance with standard laboratory protocol as described in the section entitled Preparation of Hemostasis Specimens for Assay, and maintained at ambient temperature (18° C to 24° C) until testing begins. Specimens for PRP-based light-transmittance aggregometry must stand undisturbed for 30 minutes after centrifugation while the platelets regain their responsiveness. Specimens for impedance whole blood aggregometry are diluted 1:1 with normal saline and tested immediately. Specimens must be tested within 4 hours of collection to avoid spontaneous in vitro platelet activation and loss of normal activity. Platelet aggregometry is a high-complexity laboratory test requiring a skilled, experienced operator.

Platelet Aggregometry Using Platelet-Rich Plasma

PRP aggregometry is performed using a specialized photometer called a *light-transmittance aggregometer* (PAP-8E Platelet Aggregation Profiler; Bio/Data Corp., Horsham, PA).[31] After calibrating the instrument in accordance with manufacturer instructions, the operator pipettes the PRP to instrument-compatible cuvettes, usually 500 μL; drops in one clean plasticized stir bar per sample; places the cuvettes in incubation wells; and allows the samples to warm to 37° C for 5 minutes. The operator then transfers the first cuvette, containing specimen and stir bar, to the instrument's reaction well and starts the stirring device and the recording computer. The stirring device turns the stir bar at 800 to 1200 rpm, a gentle speed that keeps the platelets in suspension. The instrument directs focused light through the sample cuvette to a photodetector (Figure 42-3). As the PRP is stirred, the recorder tracing first stabilizes to generate a baseline, near 0% light transmittance. After a few seconds, the operator pipettes an agonist (aggregating agent) directly into the sample to trigger aggregation. In a normal specimen, after the agonist is added, the shape of the suspended platelets changes from discoid to spherical, and the intensity of light transmittance initially (and briefly) decreases, then increases in proportion to the degree of shape change. Percent light transmittance is monitored continuously and recorded (Figure 42-4). As platelet aggregates form, more light passes through the PRP, and the tracing begins to move toward 100% light transmittance. Platelet function deficiencies are reflected in diminished or absent aggregation; many laboratory directors choose 40% aggregation as the lower limit of normal.