Platelets Function Assays for Clinical Management

By: Paul Lindholm, MD, Associate Professor of Pathology

Platelet function assays may be performed prior to surgery or invasive procedure to determine whether a patient may be at risk to bleed excessively due to platelet dysfunction. During complex or prolonged surgical procedures, platelet function can be monitored to determine the need for platelet replacement therapy. Platelet function can be used to identify and diagnose platelet dysfunction in a patient with a defect in primary hemostasis. Platelet function assays can also be used to monitor antiplatelet therapy in a patient who is taking a medication that can inhibit platelet function and blood clotting.

Acquired platelet function defects are most common and are frequently caused by medications including aspirin, NSAIDs and selective serotonin reuptake inhibitors [SSRIs]. Acquired platelet function defects can also occur with uremia and hematopoietic disorders including myeloproliferative and myelodysplastic syndromes. Less commonly, hereditary disorders that lead to defects in primary hemostasis include: von Willebrand disease (VWD), Glanzmann thrombasthenia (GT), Bernard–Soulier syndrome (BSS), Platelet storage pool disease, Gray platelet syndrome (GPS), Wiskott–Aldrich syndrome (WAS) and May–Hegglin anomaly.

Although the bleeding time (BT) was once the initial test to evaluate primary hemostasis, it falls short in diagnosis of platelet disorders, predicting bleeding tendency and monitoring treatments to correct bleeding problems. Standardized, automated techniques such as the platelet function analyzer (PFA–100) have largely replaced the bleeding time [1]. The PFA–100 measures the ability of platelets activated in a flowing high–shear environment to occlude an aperture in a membrane treated with collagen and epinephrine (CEPI, PFA–I) or collagen and adenosine diphosphate (CADP, PFA–II) [2].

The closure time in the CEPI column is tested first and if it is abnormally prolonged, then the CADP column is also used. A prolonged closure time for only the PFA–I (>171 seconds) indicates an ‘aspirin–like’ type of platelet dysfunction. If both the PFA–I and PFA–II (>134 seconds) are prolonged, this result suggests a more significant congenital or acquired platelet dysfunction or von Willebrand factor deficiency [2–5]. An abnormal PFA–100 result should be considered with careful
Platelets Function Assays for Clinical Management (contd.)

clinical evaluation and history and may include further platelet function testing with platelet aggregation. In most clinical situations, the PFA–100 has a high negative predictive value; normal results pointing towards normal primary hemostasis. The PFA–100 is fairly sensitive to aspirin–induced platelet dysfunction and von Willebrand factor deficiency. Several medications can prolong the PFA including NSAIDs and some antibiotics, antidepressants, antihypertensives and antihistamines. However, the PFA is less sensitive to platelet secretion defects, storage pool disorder and mild type I von Willebrand factor deficiency [4]. Other conditions that may prolong the PFA closure times include uremia and myeloproliferative disorders including chronic myelogenous leukemia (CML) and essential thrombocythemia (ET). An abnormal PFA closure time does not always indicate that a patient has increased bleeding risk. Anemia, thrombocytopenia, and uremia can cause prolonged platelet function assay closure times. A careful patient history and clinical evaluation is always central to the evaluation of bleeding risk.

When there is evidence of platelet dysfunction, platelet aggregation assays can help to identify the nature of platelet dysfunction. In light transmission platelet aggregometry, a suspension of platelets is stimulated to aggregate in vitro and the extent of aggregation is quantified as the increase in light transmission through the platelet–rich plasma. Platelet aggregation studies can identify defects in platelet fibrinogen or von Willebrand factor receptors or von Willebrand factor deficiency, storage pool disorders and certain drug effects [5, 6]. When normal, a standard platelet aggregation assay will exclude most qualitative platelet defects; however, it may not detect a mild von Willebrand factor deficiency. In some cases, spontaneous platelet hyperaggregability may be detected in the absence of added in vitro agonist. Platelet aggregation studies require preanalytical preparation and are time consuming and labor intensive and it is therefore best to schedule this test ahead of time with the laboratory.

To specifically monitor the effects of aspirin and P2Y12 receptor blocking therapies on platelet function, VerifyNow assays may be employed [7]. These assays measure platelet function based upon the ability of activated platelets to bind to fibrinogen in a modified whole blood aggregation assay. The activated platelets bind through activated GPIIbIIa to fibrinogen coated beads. The antiplatelet therapies aspirin and clopidogrel will cause a decrease in aggregation which is detected as a change in the optical signal caused by platelet aggregation. The platelet aspirin assay reports the extent of platelet aggregation that occurs after addition of an arachidonic acid and is reported as Aspirin Reaction Units [ARU]. A result of less than 550 ARU indicates that platelet dysfunction consistent with aspirin effect has been detected. The P2Y12 assay reports the extent of P2Y12–mediated platelet aggregation P2Y12 Reaction Units [PRU] following addition of an ADP homolog to the assay. Clinical studies are being performed to determine the levels of ARU and PRU that are associated with low risk of thrombosis recurrence. Further studies are needed to validate these assays in randomized prospectively controlled studies.

The thromboelastograph (TEG) whole blood coagulation test measures and records visco–elastic changes in whole blood as the sample clots, retracts or lyases. It is sensitive to all of the interacting cellular and plasma components in the blood and can give an indication of platelet function which is most closely related to the maximum amplitude of the developed clot. A TEG that shows low maximum amplitude may indicate low platelet function, while high maximum amplitude may indicate high levels of platelet function. The TEG has been most often used in liver transplant surgery to help the anesthesiologist manage the complex coagulopathy and replacement therapy during the procedure. In newer applications, the TEG may also be used in cardiovascular surgery, complicated obstetrics and trauma surgeries [8, 9]. Platelet mapping is a special application of the TEG designed to monitor aspirin and P2Y12 receptor blocking therapies [10]. In platelet mapping, the degree of platelet inhibition is measured for aspirin with arachidonic acid agonist and for ADP receptor inhibiting drugs with addition of the ADP agonist. As with the VerifyNow, clinical studies are needed to validate these assays in randomized prospectively controlled studies.
Platelets Function Assays for Clinical Management
(contd.)

Platelet function testing is performed in the NMH hemostasis laboratory in blood freshly collected in 3.2 percent sodium citrate with gentle inversion to prevent premature clot formation. Platelet testing has strict specimen handling requirements. To preserve platelet function, the specimen must be kept at room temperature and not centrifuged. Most platelet function assays should be performed after the blood has rested for 10–15 minutes and within 4 hours of collection (within 2 hours for TEG). Platelet function assays are most informative when the hemoglobin and platelet counts are as close to normal as possible.

References

Crossmatch Testing of Red Blood Cells

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Crossmatching a red blood cell (RBC) unit is one element of pretransfusion compatibility testing performed between the RBC unit and the intended recipient’s plasma. According to the Food and Drug Administration (FDA) regulations, transfusion facilities are required to perform certain pretransfusion tests for compliance with Current Good Manufacturing Practice [1, 2]. Pretransfusion testing in a transfusion facility is performed to determine the group ABO/Rh (D) of the donor and recipient, and if the patient has been immunized to antigenic determinants present on the red blood cell membrane. RBC antigen immunization may be a result of allogeneic, self-present, bacterial, viral, or medication-mediated determinants.

In 2011, the FDA published a guidance for streamlined computer crossmatches [3]. This type of crossmatch requires three main elements. The first element pertains to the ABO group of the recipient. The recipient is to be group ABO/Rh (D) tested two times. A historical group ABO/Rh (D) test is acceptable for one, but a current sample drawn and tested within the past three days is required regardless of patient history. The second element pertains to unexpected antibodies. The patient must have no prior history or current identification of non–group ABO antibodies. Thirdly, the RBC unit is to be typed for group ABO/Rh (D) twice, by the blood supplier and by the hospital. With these elements in place, and with an FDA–approved and validated computer system, the crossmatching testing may be performed electronically, rather than serologically. Benefits of computer crossmatching include: a faster crossmatch, no exhaustion of the patient’s sample requiring subsequent blood draws, decreased exposure of the testing personnel to blood pathogens, and better inventory management. If an electronic system is unavailable to logically exclude incompatible crossmatches, such as in a downtime situation, then the recipient’s plasma and donor unit cell suspension must be tested by immediate–spin centrifugation to confirm group ABO/Rh (D) compatibility.

Crossmatch testing must be performed serologically when a patient has historical or current clinically significant non–group ABO alloantibodies. Following the identification of an allogeneic antibody, RBC units must be screened in order to find units lacking the corresponding antigen. Different RBC antigens have different frequencies of prevalence, and multiple antibodies compound the difficulty of finding antigen negative RBC units. Without including ABO/Rh (D) typing and donor ethnicity, if one antigen has a frequency of 80% in a random population, 20% of units would be compatible with a patient producing the corresponding antibody. If a patient produced two antibodies in different blood groups, one antibody to an antigen of 80% prevalence and one to an antigen of 65% prevalence, only 20% x 35% = 7% of RBCs would be compatible. In this scenario, to find one RBC compatible unit, the blood bank would have to phenotype approximately 14 units. Once antigen negative units have been identified, an aliquot of the patient’s plasma is tested against a suspension of the RBC units in an antibody enhancement medium. To identify group ABO/Rh (D) and clinically significant antibody incompatibility, this testing requires a period of incubation at 37°C followed by addition of anti–human globulin (AHG).

Patients with warm autoantibodies present extra difficulty because the warm autoantibody may obscure the detection of underlying alloantibodies, and may cause serological crossmatch incompatibility. After extra testing to seek underlying alloantibodies, least incompatible units are often issued. For the purpose of clinician awareness, a physician will need to sign a document prior to release of the least–incompatible unit(s) acknowledging the unit(s) to be transfused are incompatible at the AHG phase of testing. If there are no underlying alloantibodies, known or unknown, warm autoantibodies do not cause hemolytic transfusion reactions by themselves; the survival rate of transfused RBCs is similar to that of the warm autoimmune hemolytic anemia patient’s own red blood cells [1].

The concept of a universal donor blood type is a misnomer. There are multiple antigens on RBCs beyond the group A and B carbohydrate epitopes with which clinically significant antibodies may interact, which could result in a transfusion reaction. All benefits and possible adverse reactions should be weighed prior to any transfusion.
Crossmatch Testing of Red Blood Cells (contd.)

If you have any questions, please contact the NMH Blood Bank at 312.926.2513 or BloodBank@nm.org.

Definitions:
Current: drawn within three days of transfusion
Unexpected antibodies: Non-group A or B specific antibodies

References:
Lipid Risk Panel

By: Elizabeth Bertsch, MD, Resident in Pathology

Dyslipidemia is both the most prevalent and the most important modifiable risk factor of atherosclerosis, the leading cause of morbidity and mortality in the United States. Effective treatment of atherosclerosis requires managing levels of plasma lipids, including cholesterol/cholesterol esters, triglycerides (TG) and phospholipids. TG are composed of 3 fatty acids, each of which is esterified to a glycerol backbone. The fatty acids can be short, medium or long chain. The chains, in turn, can be saturated, mono–unsaturated or polyunsaturated. Phospholipids are composed of 2 fatty acids and a phosphate–containing ‘head’ group, each extending also from a glycerol backbone. Cholesterol is composed of 4 fused hydrocarbon rings, an extended hydrocarbon side chain and a single hydroxyl group. Due to their hydrophobic nature, the pure lipids are insoluble in plasma and are, therefore, packaged into lipoprotein particles for distribution. The lipoprotein particles include chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). Each lipoprotein is composed of a phospholipid coat, the other major lipids, i.e., TG and cholesterol/cholesterol ester, and apolipoproteins.

Ingested lipids are internalized by small bowel enterocytes, where they are packaged into chylomicrons. The chylomicrons transport lipids to somatic cells, predominantly liver cells. In the liver, cholesterol and TG are metabolized and packaged as VLDL for secretion into the blood. In blood, TG are progressively removed from VLDL, thereby increasing the density of the lipoprotein to IDL and, eventually, to LDL. On a weight percentage basis, LDL has the most cholesterol/cholesterol ester of the lipoproteins. Because it is the main vehicle for transporting cholesterol from blood to various cells of the body, LDL and its associated cholesterol, i.e., LDL–cholesterol, has become known as ‘bad cholesterol’. Importantly, the liver also produces HDL, the lipoprotein responsible for the transport of cholesterol from peripheral tissues to the liver. Because it removes excess cholesterol from circulation, HDL and its associated cholesterol, i.e., HDL–cholesterol, has become known as ‘good cholesterol’.

Given the involvement of lipids in atherosclerosis, it follows that knowledge of plasma lipid levels should facilitate monitoring and treatment of the disease and its healthcare consequences. Indeed, lipid panels performed by clinical laboratories do play a major role in mitigating the effects of atherosclerosis. The lipid risk panel performed by the NMH Clinical Laboratory includes total cholesterol, HDL–cholesterol, total TG, LDL–cholesterol (calculated), and non–HDL–cholesterol (calculated). Total cholesterol, HDL–cholesterol and TG are measured directly, using enzymatic means. LDL–cholesterol is a calculated value, determined using the Friedewald equation, i.e., LDL–cholesterol = total cholesterol – HDL cholesterol – (TG ÷ 5). Unfortunately, the Friedewald relationship loses accuracy when TG exceed 400 mg/dL. In such cases, the lab advises clinicians to order a directly measured LDL, which is performed by a reference facility. Blood samples for lipid panel testing should be collected into a light green–top (lithium heparin) evacuated tube. Patients should have fasted and, ideally, rested 12–14 hours prior to specimen collection. This is important because lipid levels are affected by diet, alcohol consumption and physical stress, e.g., exercise and smoking. Here at NMH, lipid risk panels are performed using samples as they are received, 24 hours per day, seven days per week.

Reference values applicable to the battery members of the NMH lipid risk panel are adopted from the 2013 ACC/AHA Blood Cholesterol Guideline [1]. They include optimal values and, for LDL–cholesterol, TG and non–HDL–cholesterol, highly abnormal values. The values are as follows: total cholesterol < 170 mg/dL (optimal), TG < 100 mg/dL (optimal) and > 499 mg/dL (highly abnormal), HDL–cholesterol > 50 mg/dL (optimal), calculated LDL–cholesterol < 100 mg/dL (optimal) and > 189 mg/dL (highly abnormal), and non–HDL–cholesterol < 120 mg/dL (optimal) and > 219 mg/dL (highly abnormal). Of special note, optimal values are not intended to be construed as targets for drug therapy. They can, however, be used by otherwise healthy individuals to direct lifestyle changes. Highly abnormal values require prompt attention and are expected to be reviewed by the patient’s medical team. They are especially meaningful to persons with certain risk factors, e.g., smoking, diabetes or hypertension.

Reference
Laboratory Test Utilization: A Focus on the Paraneoplastic Panel

By: Todd DeJulio, MD, Resident in Pathology

Although the percentage of total healthcare dollars spent on clinical laboratory testing is relatively small, the absolute dollar figure is actually rather impressive, given the trillions of dollars spent on healthcare annually in the United States. Lab testing is an integral part of patient care, and a majority of ordered tests are necessary to provide optimal care. Unfortunately, however, a very significant minority of tests is ordered: 1) excessively, 2) inappropriately, or 3) in an inappropriate venue. Therefore, significant efforts continue to be made/need to be made to reduce excessive and/or inappropriate lab test utilization.

At Northwestern Memorial Hospital (NMH), when a send-out, i.e., referred, test is ordered by a clinician, it is sometimes appropriate for a Pathology representative to contact the ordering clinician to discuss the test’s necessity and/or appropriateness. If the referred test is intended to be done using a specimen that was either difficult to obtain or irretrievable, e.g., a surgical biopsy or cerebrospinal fluid (CSF), then the test is usually approved because specimen collection involved significant risk to the patient. Ideally, however, testing should, in all instances, be dictated by clinical appropriateness alone.

In daily practice, issues related to appropriate test utilization are frequently associated with CSF specimens. As mentioned, CSF is precious and cannot be/should not be easily re-collected. Patients undergoing lumbar puncture are typically very ill, experiencing one or more significant neurological defect. Often, the etiology of the neurological defect(s) is unknown at the time of the procedure, so the differential diagnosis may be broad. That being the case, extensive testing is sometimes ordered, often exhausting the volume of CSF collected for testing. Some of the most common ancillary studies performed using CSF are flow cytometric analyses and paraneoplastic antibody panels. They’ll be discussed next, in turn.

The ability to perform flow cytometric analyses necessitates that a minimum number of leukocytes be present in the CSF, which frequently is not the case. Therefore, cancellation of such studies is often automatic (and not unexpected), a consequence of very limited sample cellularity. In situations where there are adequate leukocytes, morphologic examination of the fluid can provide further information relevant to the necessity of flow cytometric studies.

Unfortunately, such criteria and examinations are not applicable to CSF samples intended to be assayed for paraneoplastic antibodies. Paraneoplastic syndromes (PNS) include signs and/or symptoms that are secondary to a substance produced by cancer cells and that are not attributable to direct invasion, metastasis or treatment of the cancer. Although rare, several PNS are well-documented, e.g., Cushing syndrome secondary to small cell lung cancer, or hypercalcemia secondary to secretion of PTH-related protein by squamous cell lung cancer.

On behalf of NMH, paraneoplastic antibody panels are performed by an outside reference facility that utilizes immunofluorescence and radioimmunoprecipitation assays to screen for the presence of autoantibodies commonly encountered in paraneoplastic neurologic syndromes. The cost of a base paraneoplastic antibody panel is ~ $400. Because the reference facility does not third-party bill, the expense of the panel is incurred by the Hospital.

There are several scenarios for which the work-up of a paraneoplastic antibody is useful: 1) evaluation of acute/subacute neurologic symptoms in a patient with significant risk factors for malignancy, 2) investigation of neurologic symptoms that emerge during the course of treatment of a known malignancy, and/or 3) monitoring for recurrence of malignancy in a previously seropositive patient. Unfortunately, the number of requests for paraneoplastic antibody panels is far greater than the number of specific clinical scenarios that warrant such testing. Indeed, the test is most often ordered – inappropriately – on behalf of patients with known ischemic or hemorrhagic stroke, on behalf of patients with known dementia, or as an add-on test on behalf of patients with vague neurologic symptoms.

Given that the paraneoplastic antibody panel is a rather expensive test that is most often ordered inappropriately, a rational
Laboratory Test Utilization: A Focus on the Paraneoplastic Panel (contd.)

systematic approach should direct its use. One such approach is that of Mahta, et al. The first step involves ruling out other etiologies, including ones related to infection, metabolic derangement or drug use. Serum tumor markers and whole-body imaging should follow. If their work-up is unremarkable, a PNS is unlikely. If symptoms persist, repeat testing and imaging should be considered, to search for occult malignancy. Conversely, if the information gleaned from clinical history, physical exam, other directed laboratory testing and imaging is highly suggestive and classic for a PNS, paraneoplastic antibody evaluation is not necessary. Only in those situations where the clinical suspicion of a PNS is high but the presentation is not classic should paraneoplastic antibody evaluation be considered.

In conclusion, the inappropriate ordering of paraneoplastic antibody panels is representative of a common – and expensive – laboratory utilization problem. The ordering of a panel is especially problematic when the specimen intended for analysis is one that placed the patient at some risk during its collection, e.g., CSF. Unfortunately, such collection often unduly ‘obliges’ the laboratory to perform the test, even when the test’s performance is not clinically indicated. The use of rational diagnostic approaches/algorithms is, perhaps, the best means by which clinicians can self-limit their ordering of laboratory tests inappropriately.

Reference

Toxicology Testing in the Special Chemistry Laboratory at NMH

By: Mark D. Ball, Ph.D., SC(ASCP)CM

By the end of 2015, the Special Chemistry Division of the NMH Clinical Laboratory will have launched three new toxicology tests and upgraded an existing one. Each of the tests is intended for the qualitative detection of selected drugs and indicator metabolites in urine. For clinicians, the tests will have at least three purposes: (1) to confirm compliance with therapy, (2) to reveal ingestion of controlled substances, and/or (3) to identify substances present in cases of suspected overdose. None of the tests will be orderable on a stat basis.

Three New Tests
The first new test (name to be determined) detects 32 analytes across five pharmacological categories: antidepressants, stimulants, hallucinogens, opiate agonists, and antitussives. The second test (name to be determined) detects six barbiturates. The third test (Nicotine Metabolite and Anabasine), by detecting the major metabolite of nicotine and a tobacco-specific alkaloid, is intended for the monitoring of nicotine-replacement therapy. It reveals the recent ingestion of nicotine, while showing whether the patient has recently used tobacco.

An Upgraded Test
Since late 2014, the laboratory has offered Drugs of Abuse, Opiate Confirmation (order code: OPCONF), a test used clinically to monitor pain management and addiction treatment. The current test, which detects 10 selected opioids, is being upgraded to detect 14 opioid-related analytes (nine drugs and five metabolites). Among those metabolites is 6-acetylmorphine, the presence of which indicates recent heroin ingestion.

Technology
Each test employs the technique of liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS). The procedure begins with the extraction of the compounds of interest from a urine specimen. The resulting extract is then subjected to chromatography, which separates the various components of the mixture. Those separated components then enter the mass spectrometer, which causes the molecules to break apart into smaller fragments with discrete mass-to-charge ratios. Because the fragmentation pattern is reproducible under a given set of conditions, it allows for identification of the original molecule, which, in our toxicology tests, is the drug or metabolite of interest.
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