



# Platelet Antibody Testing in Ontario: The right test at the right time

**D**isorders of platelet number and activation can cause thrombocytopenia and thrombosis. Both can be caused by antibodies that bind to the platelet, resulting in increased clearance by the reticuloendothelial system or uncontrolled activation leading to microthrombi and thrombosis. Sufficient numbers of platelets to enable efficient adhesion to subendothelium and cross-linking by plasma factors, accompanied by controlled activation, are pivotal to hemostasis.

Testing for the anti-platelet antibodies requires specialized laboratories that have technologies to process platelets for different testing methods. Some assays require that platelets and their surface proteins be maintained in native conformations to measure antibody binding to glycoproteins. Others demand that platelets be maintained in a resting state, but retain the ability to activate and release granule contents when exposed to specific antibodies. For these reasons, anti-platelet antibody testing is usually referred to specialized laboratories.

For over twenty-five years, the McMaster University Platelet Immunology Laboratory, directed by Dr. John Kelton, has performed basic and applied research into immune causes of thrombocytopenia and thrombosis. Results of this research include many landmark papers and discoveries describing novel antibody-mediated mechanisms of disease<sup>1</sup>, identification of new platelet alloantigens<sup>2,3</sup>, and clarification of the usefulness of platelet antibody testing<sup>4</sup>. An important development was the serotonin release assay (SRA), now the gold standard for testing for heparin-induced thrombocytopenia (HIT)<sup>5</sup>. Different diseases require specific testing for each type of platelet antibody. The McMaster laboratory is in the unique position of blending developmental research with clinical investigation of neonatal alloimmune thrombocytopenia (NAT), idiopathic thrombocytopenic purpura (ITP), and heparin-induced thrombocytopenia.

The McMaster Platelet Immunology Laboratory is the Ontario reference laboratory for the investigation of alloimmune platelet disorders, including NAT and post-transfusion purpura. NAT is caused by maternal antibodies directed to alloantigens on fetal platelets. The antibodies can cross the placenta in the first trimester, resulting in thrombocytopenia, bleeding, intra-cranial haemorrhaging or death. NAT occurs in about 1:1000 births, affecting about 300 pregnancies annually in Ontario. NAT is analogous to haemolytic disease of the newborn (HDN). However, unlike HDN, thrombocytopenia and morbidity in NAT often occur unexpectedly in the first pregnancy.

least one antigen based on gene frequencies. Therefore, an antigen incompatibility alone is not informative.

It is important that sensitive testing is performed to identify maternal alloantibodies directed to paternal/fetal alloantigens. Glycoprotein-specific assays are used to test for maternal alloantibodies to discriminate platelet-specific alloantibodies from other antibodies often found in maternal sera (anti-HLA; anti-ABH). Enzyme immunoassay (EIA) using immobilized platelet glycoproteins and radioimmuno-precipitation assays are used to investigate maternal alloantibodies. Therefore, comprehensive alloantigen and antibody test-

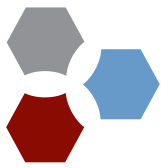
The McMaster laboratory is in the unique position of blending developmental research with clinical investigation of neonatal alloimmune thrombocytopenia (NAT), idiopathic thrombocytopenic purpura (ITP), and heparin-induced thrombocytopenia.

Investigation of NAT requires identification of an incompatible paternal-fetal platelet antigen and the presence of maternal alloantibody that binds paternal platelets. Platelet alloantigens are conformational epitopes on glycoproteins caused by single nucleotide polymorphisms resulting in an amino acid change. Testing requires that the mother and father be investigated for an antigen incompatibility and a maternal alloantibody. The most commonly implicated antigen is HPA-1a. A homozygous HPA-1bb mother is exposed to the HPA-1a antigen inherited by her fetus from the father. Although 2% of women are HPA-1bb, other factors play a role in the immune response, reducing the risk in the general population by about 20 fold. HPA-1a accounts for about 85% of NAT, followed by the HPA-5 and -15 antigens. However, with over 16 platelet antigen systems identified, it is probable that most couples will be incompatible for at

ing must be used to provide information for the management of the affected child in current and future pregnancies.

Glycoprotein-based assays also are used for detecting autoantibodies for the investigation of ITP. Unlike NAT, it is difficult to identify anti-platelet antibodies in serum or plasma of ITP patients using indirect assays. The most informative tests measure autoantibodies directly on the patient's platelets, however, it is important to understand that simply measuring platelet-associated IgG is not useful. As the platelet count decreases, the amount of immunoglobulin associated with platelets increases non-specifically. This is observed in both immune and non-immune causes of thrombocytopenia, giving these tests poor specificity. More informative assays measure the amount of antibody bound specifically to platelet glycoproteins, usually GP IIb/IIIa and IbIX.

*Continued on page 22*



### *Continued from page 21*

These assays use monoclonal antibodies to capture proteins from patient platelets. Autoantibody bound to these proteins is measured using enzyme-conjugated anti-human IgG or IgM. A positive result in glycoprotein-specific assays is highly specific for the disease.

**It was not until the SRA was developed that a sensitive and specific test was available to measure HIT antibodies, eliminating artifacts caused by testing platelets in citrated plasma.**

HIT is one of the most common drug-induced immunologic causes of thrombocytopenia. It is caused by antibodies that bind neo-epitopes on platelet factor 4 induced by binding of heparin. Although moderate thrombocytopenia is commonly observed, a frequent complication is thrombosis caused by platelet activation via crosslinking of Fc receptors by HIT antibodies. Early assays using platelet aggregation tests suffered from poor sensitivity. It was not until the SRA was developed that a sensitive and specific test was available to measure HIT antibodies, eliminating artifacts caused by testing platelets in citrated plasma. The SRA measures HIT antibody-induced platelet activa-

tion at therapeutic heparin concentrations. Numerous controls are included to ensure the specificity of activation, including inhibition of activation by blocking Fc receptors with monoclonal antibodies. It is important to note that without recent prior heparin exposure, HIT develops over the course of 5 to 10 days from initiation of therapy, and that testing for HIT antibodies should be

performed within this time frame in conjunction with pre-test probability analysis<sup>6</sup>. Other tests available to measure HIT antibodies, such as the PF4/heparin EIA (heparin enzyme-immunoassay), are less specific than the SRA, overcalling a positive result in over 10% of samples. Positive results with the EIA should be confirmed using the SRA to ensure heparin anticoagulation is not unnecessarily withdrawn from the patient.

The methods and issues described here emphasize the importance of the type of testing and analysis used for each platelet disorder. New platelet antibody assays, including point-of-care testing, are being developed. It will be important to ensure that appropriate

methods are used for the unique requirements for investigation of immune-mediated thrombocytopenia and thrombosis. ❖

### **References:**

1. Kelton JG, Sheridan D, Santos A, et al. Heparin induced thrombocytopenia: Laboratory studies. *Blood* 1988 72:925-930
2. Smith JW, Kelton JG, Horsewood P, et al. Platelet-specific alloantigens on the platelet glycoprotein IaIIa complex. *Br J Haematol* 1989 72:534-538
3. Kelton JG, Smith JW, Horsewood P, et al. Gov-a/b alloantigen system on human platelets. *Blood* 1990 75:2172-2176
4. Kelton JG. The serological investigation of patients with autoimmune thrombocytopenia. *Thromb Haemost* 1995 74:228-233
5. Sheridan D, Carter C, Kelton JG. A diagnostic test for heparin-induced thrombocytopenia. *Blood* 1986 67:27-30
6. Lo GK, Sigouin CS, Warkentin TE. What is the potential of over diagnosis of heparin-induced thrombocytopenia. *Am J Hematol* 2007 82:1037-1043

### *Feature, continued from page 17*

Infection control departments are another key ingredient to controlling the spread of antibiotic resistance. They impose physical barriers of space, rooms with controlled airflow, as well as policies and procedures such as proper hand hygiene, and contact precautions between an infected patient and non-colonized people. This prevents the spread of antibiotic resistant organisms. The nurses and patients must understand the importance of the barriers to comply with the necessary restrictions. This involves the last aspect of

antibiotic stewardship to be discussed: education.

Key premises behind antibiotic stewardship need to be passed on to students in the medical field, physicians, nurses, laboratory technologists, infection control practitioners, pharmacists, nurses, other staff and patients. Education will allow everyone to buy into, and support, antibiotic stewardship.

In short, the entire community needs to become a gatekeeper to slow the development of antibiotic resistance and ensure a useful stock of effective antibiotics for future use. ❖

### *Viewpoint, continued from page 18*

the use of cell cultures instead of eggs. This is referred to as the manufacture of DNA vaccines; the development of the H5N1 (Avian flu) vaccine is an example. This technology will allow for quicker, more cost-effective procedures of new vaccine production. Other anti-viral and antibiotic drugs will be developed by targeting the genome of the emerging zoonotic disease. There are two antiviral drugs that are approved for use: Tamiflu® (oseltamivir phosphate) and Relenza® (Zanamivir). No doubt more of these drugs are being developed. JLB ❖