LAB Connections

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HAMILTON REGIONAL LABORATORY MEDICINE PROGRAM

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HIT Antibody Testing in HRLMP

Heparin-induced thrombocytopenia, or HIT, is one of the most important adverse drug reactions in medicine. Approximately 20 to 30 patients per year develop confirmed HIT in one of our Hamilton hospitals (1). Despite receiving an anticoagulant (heparin) and having thrombocytopenia (low platelet count), patients with HIT paradoxically develop a highly prothrombotic disorder; the relative risk for thrombosis in HIT is 12−15× non-HIT controls, and since HIT typically occurs in patients at high risk for thrombosis - and who therefore are given heparin for thrombosis prophylaxis or treatment - the result is that 50% to 75% of patients with HIT develop clinically-evident thrombosis (2). Approximately 5% to 10% of HIT patients will die of HIT-related thrombosis (e.g., pulmonary embolism, thrombotic stroke, myocardial infarction) and 5% develop ischemic limb loss(es) (3).

Since heparin exposure is ubiquitous in hospitals and thrombocytopenia of diverse non-HIT etiology is common, many patients undergo laboratory testing for HIT antibodies, of whom only 5-10% are confirmed as having HIT (1). Since the McMaster Platelet Immunology Laboratory offers the only definitive test for HIT in Canada - the platelet serotonin-release assay (SRA) - we receive many hundreds of blood samples yearly.

HIT is caused by platelet-activating antibodies of IgG class that recognize antigens on a cationic (positively-charged) protein, platelet factor 4 (PF4), when it forms multimolecular complexes with (anionic) heparin (or certain other polyanions, such as polyvinylsulfonate [PVS]).

Although formation of anti-PF4/heparin antibodies among heparin-exposed patients is common, only a minority of patients form high-titer antibodies of IgG class ("HIT antibodies") that are able to activate platelets via their IgG Fc receptors (4). Thus, whereas many hospital and commercial labs offer enzyme-immunoassays (EIAs) that can detect anti-PF4/heparin antibodies, only Hamilton offers both EIA analysis and the definitive test (SRA) for detecting platelet-activating antibodies. The SRA, which measures the ability of patient serum to induce release of serotonin from normal donor platelets in a heparin-dependent fashion, was invented at McMaster by Dr. John Kelton in 1986 (5), and even today the SRA remains the reference test for HIT (6).

The McMaster Platelet Immunology Laboratory tests more than 1500 samples annually for HIT from ~50 centers within Ontario and across Canada (7). Figure 1 shows how samples for HIT testing are handled from patients in Hamilton (left part of figure); the right part of the figure shows how samples are handled from centres that perform initial immunoassay screening for HIT antibodies. About 50% of referring centres only send samples for HIT testing that have tested positive using on-site EIAs or other commercial immunoassays. For previously unscreened patient samples, we now provide a 2-step HIT testing protocol in order to provide optimal sensitivity and specificity while minimizing turnaround time.

STEP 1: Anti-PF4/PVS EIA screening test. For patients in Hamilton, as well as non-Hamilton patients for which initial screening has not been performed, we test for anti-PF4/heparin antibodies by using a commercial polyspecific anti-PF4/PVS EIA that detects antibodies of the three major immunoglobulin classes (IgG, IgA, IgM). The EIA is a semi-quantitative test that detects antibodies against PF4/heparin complexes but does not discern between (platelet-activating) HIT antibodies and other non-activating anti-PF4/heparin antibodies. The EIA is ~99% sensitive, but only 25-50% specific (depending on the patient population), and is most useful for excluding HIT when the test is negative. Because of its high sensitivity, a negative EIA result essentially rules out HIT (likelihood <1%). EIA screening will exclude 65% of all referred samples from needing an SRA test. We run the screening EIA daily, Monday-Friday. All negative results are reported that day, with no further testing required. All positive EIA results are also reported same day, with the optical density (OD405) result provided along with the corresponding probability of a positive SRA result. For example, a weak-positive EIA (0.4 to <1.0 units) predicts for a 2.7% probability of a positive SRA, whereas a strong EIA (OD405 ≥ 2.0 units) predicts a ~90% probability. This interim information (pending results of SRA testing) assists the clinician in clinical decision-making (7).

STEP 2: Serotonin-release assay (SRA). The SRA is a functional test that identifies samples with pathogenic anti-PF4/heparin antibodies based on their ability to activate platelets in-vitro (1,4-7). HIT antibodies activate platelets strongly at pharmacologic concentrations of heparin (0.1 and 0.3 U/mL), whereas platelet activation is inhibited by high levels of heparin (100 U/mL) (as high heparin concentrations disrupt the antigen-containing complexes). For greater specificity, we also add a platelet Fc receptor-blocking monoclonal antibody, as this inhibits platelet activation by HIT antibodies. In our hands, a positive SRA confers a diagnostic specificity for HIT of ~95%. Thus, in most circumstances, a positive SRA indicates a diagnosis of HIT. Approximately 35% of samples referred for HIT testing will have a positive EIA and require testing by SRA. A positive SRA result strongly correlates with HIT, whereas a negative SRA result generally excludes HIT even if the EIA was positive. The SRA is performed on Tuesdays and Thursdays.

HIT is a preventable adverse reaction. Its risk is 10-fold lower with low-molecular-weight heparin (versus standard unfractionated heparin (8); the risk is believed to be negligible (but not zero) with fondaparinux (9). Although not triggered by the newer non-heparin anticoagulants, such as rivaroxaban or apixaban, HIT is sufficiently common that it can occur during treatment with rivaroxaban or apixaban, as a result of preceding exposure to heparin or even due to "spontaneous" generation of HIT antibodies, as is reported following knee replacement surgery (10). Recently, hematologists at our Juravinski Cancer Centre identified HIT in 4

multiple myeloma patients undergoing preparation for stem cell transplantation; the use of heparin "flushes" for apheresis catheter management was identified as the triggering event (submitted for publication).

Jane C. Moore, MLT, ART, BSc, Platelet Immunology Laboratory
Theodore (Ted) E. Warkentin, MD
Ishac Nazy, PhD, Assistant Professor, Medicine

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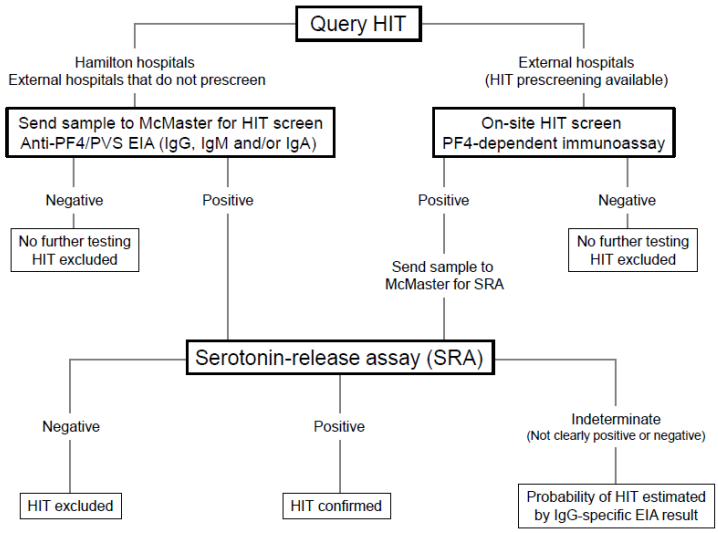

News from HRLMP

News from Administration

The HRLMP is excited to introduce the **Medical Laboratory Technology Excellence Award.** This annual award will acknowledge clinical, educational, leadership and professional excellence in the field of Medical Laboratory Technology.

The award is intended for individuals who:
• Demonstrate clinical excellence through outstanding knowledge, skill and judgment in laboratory medicine
• Demonstrate excellence in teaching or education
• Use innovative thinking and problem solving when faced with challenges in the laboratory
• Demonstrate leadership qualities
• Demonstrate commitment to quality improvement initiatives and projects within the laboratory
• Identify individuals in need and provide them with help
• Accept change and facilitate the implementation

of change in the laboratory
• Demonstrate respect and caring towards patients and colleagues.

The award will be presented annually to one laboratory professional in each of the following disciplines: Anatomic Pathology, Chemistry, Hematology, Genetics, Microbiology, and Transfusion Medicine.

Individuals will be nominated by peers and nominations will be accepted four weeks before National Medical Laboratory Week. Nominations should be submitted to the Education and Safety Manager, HRLMP.

The Terms of Reference and nomination form can be found at:
www.hrlmp.ca

News from Genetics

Good Luck and Good Bye!

With mixed feelings we say farewell to several staff members who have left or are leaving the HRLMP Genetics Laboratories. **Karen Elmer-Sharp** worked as a MLA in the Cancer Genetics Laboratories. **Lem Pitters** and **Amanda Cocca**, both MLTs, worked in the molecular oncology lab and performed molecular diagnostic tests for hematological cancers and solid tumors. Amanda was also active on the Quality team and was a safety representative for the Cancer Genetics Laboratories at HRLMP.

We would like to thank Karen, Lem and Amanda for their contributions to the Genetics Laboratories and wish them well in their new roles! We will miss you!

We also announce the retirement of our cytogenetics technologist **Coral Wichert**. Coral worked for many years in the Cytogenetics lab preparing slides for karyotype analysis and analyzing karyotypes in both constitutional disorders and cancer. Coral will be missed by her colleagues and we wish her well in the future as she starts this new chapter in her life.

The HRLMP Genetics laboratory also extends a warm welcome to our new laboratory staff starting in February/March: **Daniella Peach** (MLT) and two recent MLT graduates from the Genetics Technology Program at Michener Institute, **Courtney Schmude** and **Yvette Kuo**.

We would like to congratulate **Dr. Elizabeth McCready** (principal investigator) and **Drs. John Provias, Boleslaw Lach, Guillaume Pare, Hal Hirte** (co-investigators) with reception of ***Cancer Pathology Translational Research Stream 1 Grant*** from the Ontario Molecular Pathology Research Network. Dr. McCready and co-investigators will receive $34,845 to access clinical utility of novel genomic and epigenomic methodologies for detection of prognostic and predictive biomarkers in gliomas.

Hematology News

CONGRATULATIONS to **Dr. Ronan Foley** and his team in the stem cell processing facility! This team purified the stem cells for the groundbreaking multicenter CIHR project on experimental gene therapy.

Below are a couple of articles that report on this experimental gene therapy.

Calgary man becomes world's first to receive experimental gene therapy

CALGARY — A team of Canadian physicians and researchers is believed to be the first in the world to have used gene therapy to treat a patient with Fabry disease, a rare inherited enzyme deficiency that can damage major organs and shorten lifespan.

People with Fabry disease have a gene called GLA that doesn't function as it should; as a result their bodies are unable to make the correct version of a particular enzyme that breaks down a fat called Gb3. A buildup of Gb3 can lead to problems in the kidneys, heart and brain.

In the trial, researchers collected a quantity of a Fabry patient's own blood stem cells then used a specially engineered virus to augment those cells with copies of the fully functional gene that is responsible for the enzyme. The altered stem cells were then transplanted back into the patient on Jan. 11, 2017.

http://www.albertahealthservices.ca/news/Page13691.aspx

http://www.albertahealthservices.ca/assets/news/rls/ne-rls-2017-02-16-farby-bkg.pdf

February 16, 2017

Partnerships make first Fabry gene therapy trial a reality

CALGARY —Many clinicians and organizations helped make possible the first Fabry gene therapy trial in the world.

Dr. Jeffrey Medin is the overall principal investigator for the project. He began the background studies more than 20 years ago that eventually led to this trial. He worked with Dr. Roscoe Brady at the National Institutes of Health in Bethesda, Md. (Dr. Brady discovered the enzymatic defect in Fabry disease.) Groundwork for this present trial started in 2012 when Dr. Medin was a professor at the University of Toronto and affiliated with the University Health Network. Dr. Aneal Khan is the site lead for the Calgary arm of the project. He is an Alberta Health Services (AHS) medical geneticist and Associate Professor of Medical Genetics and Pediatrics at the Cumming School of Medicine, University of Calgary.

The trial is a true pan-Canadian effort. **Key components of the trial were completed in Hamilton, Ont., under the leadership of Dr. Ronan Foley**; in Toronto, under the leadership of Dr. Armand Keating; in London, Ont., under the leadership of Dr. Tony Rupar; in Sherbrooke, Que., under the leadership of Dr. Christiane Auray-Blais; and in Halifax, N.S., under the leadership of Dr. Michael West.

Also ….

Many of us see **Nate** on a daily basis in the Core Laboratory at the McMaster site. His interaction and friendly demeanor towards staff and patients is evident each day. His role as a ***Child Life Specialist*** in the health care journey of a pediatric patient was recently published in a Share article. *Well done!!*

Click on the link below for the full story:
http://hhsshare.ca/2016/05/strategy-patients/

Microbiology News

Recently, our own Deborah Johnson, Supervisor, Microbiology, was featured in a ***Faces of HHS*** article.

Click on the link below to read the full story and what's happening in the Microbiology laboratory:
http://hhsshare.ca/2017/01/deborah-johnson/

News from Pathology

Congratulations to **Dr. Monalisa Sur** who received a Certificate of Recognition from the Royal College of Pathologists recognizing her contributions in developing International education in pathology!!


The Royal College of Pathologists
Pathology: the science behind the cure

Certificate of Recognition
2016

Professor Monalisa Sur

is awarded this certificate in recognition of their invaluable contribution and efforts in furthering the College's international aims, strategy and work programme in 2016.

In 2016, Professor Monalisa Sur undertook the following activities on behalf of the College:

- Serving as a member of the College's International Committee
- Acting as the College's Country Advisor for Canada
- Co-Chairing the International Committee's Policy, Advocacy & Communications Working Group
- Coordinated the attendance and participation of College representatives (Dr Maadh Aldouri, Miss Rosemary Emodi and Miss Uno Young) at the CAP-ACP Conference, which was held in Vancouver, Canada in July
- Facilitating the establishment of a partnership between the College and the Canadian Association of Pathologists (CAP)
- Attended the College's Pathology Diaspora Forum meeting in May
- Participated as a contributor in the College's International Pathology Day Roundtable in November

Dr Maadh Aldouri
Director of International Affairs
The Royal College of Pathologists

Dr Suzy Lishman
President
The Royal College of Pathologists

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INVESTORS IN PEOPLE

We are saddened to announce the passing of Pam Leishman, a retired HRLMP cytotechnologist.

Click on the link below for further information:
http://turnerfamilyfuneralhome.ca/2017/02/leishman-pamela-joan-pam/

Quality News

We would like to welcome **Jim St. Marie** to the HRLMP as a Program Analyst. Jim will support the HRLMP's Quality Management System through data management and decision support, facilitating evidence-based decision making.

Welcome Jim and we wish you every success in your new role.

Research News

Now that the Holiday season is over, the HRLMP has a number of new studies starting up. We're looking forward to being involved with one which will analyze results for the immature platelet fraction (IPF), one of the parameters available on the new CBC analyzer from Sysmex used in the Core Laboratories of the HRLMP.

A Hamilton study has been named among the most influential of 2016 by one of the world's leading medical journals, the New England Journal of Medicine. The Heart Outcomes Prevention Evaluation (HOPE)-3 trial was chosen as one of 14 influential papers "being the most meaningful in improving medical practice and patient care."

Click on the link below to read the full story:
http://www.thespec.com/news-story/7075758-hamilton-study-named-best-of-2016-by-medical-journal/

Click on the link below to access the NEJM article:
http://www.nejm.org/doi/full/10.1056/NEJMoa1600176