HAMILTON REGIONAL LABORATORY MEDICINE PROGRAM

LAB CONNECTIONS

June 2013 /
Issue #125

IN THIS ISSUE

WHAT'S NEW

Editorial Office:
Dr Cheryl Main, Editor
Email: mainc@hhsc.ca
Michelina Bozzo, Editorial Assistant
Email: bozzom@hhsc.ca

Newborn Screening for Severe Combined Immunodeficiency

Severe Combined Immune Deficiency (SCID) is a condition that, despite its rarity, has had high profile attention in the scientific and popular media over several decades. In the 1970's and 1980's the "Bubble Boy", David Vetter, captured the attention of American and world press as his life in a sterile isolator was documented over the entire 12 years he survived. In 1990, another SCID patient, Ashanti DeSilva, became the first human recipient of gene therapy. SCID was again in the news when serious safety concerns led the FDA to temporarily halt all retroviral gene therapy trials after oncogene activation caused cancer in a SCID gene therapy trial in 2003[i]. In January 2013, Newborn Screening Ontario (NSO) announced that Ontario will be expanding its program in mid-2013 to include SCID. Though certainly less high-profile than some of the other SCID news, this means that as of this summer, every newborn in Ontario will be tested for this rare but potentially fatal condition.

Severe combined immune deficiency is actually a diverse group of genetic disorders that cause combined deficiency of T-cell and B-cell function. Genetic mutations in more than 30 genes have now been identified in 80% of SCID cases[ii]. Children with SCID typically present within the first 2 years of life with recurrent or unusual infections, chronic viral diarrhea and failure to thrive. Most children die from infection during the first 2 years of life unless they receive hematopoietic stem cell transplantation (HSCT)[iii]. Early treatment by HSCT (at <3.5 months of age) has a much higher survival rate than later treatment (94% survival versus 69%, $p<0.001$)[iv]. One of the disorders, adenosine deaminase deficiency, can also be treated with regular infusions of replacement enzyme. Occasionally, milder "hypomorphic" forms of SCID can lead to later presentation with chronic lung disease and chronic autoimmune disorders like severe colitis. "Hypomorphic SCID" or "variant SCID" can be caused by milder mutations in the genes known to cause SCID or by mutations in one of several other genes that leads to a lesser impairment of T-cell number and function. Without specific treatment these patients live longer than classical SCID – typically into their second decade of life – but ultimately die of severe target organ damage.

In 2010, the United States Secretary of the Department of Health and Human Services accepted the Secretary's Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC) recommendation to add SCID to the Recommended Uniform Screening Panel[v]. In 2012, a similar recommendation was made in Ontario by the Newborn and Childhood Screening Subcommittee, with Government of Ontario acceptance of the recommendation widely announced in January 2013[vi]. A significant factor in these recommendations was that a suitable screening test using a dried blood spot was now available. The screening test is a quantitative polymerase chain reaction (qPCR) analysis of the T-cell receptor excision circles (TRECs)[vii]. These small circles of DNA are normal by-products of the T-cell maturation process when the T-cell receptor gene undergoes rearrangements to produce the final unique T-cell receptor for that T-cell. As the name implies, qPCR allows quantitation of this DNA, which in turn is highly correlated with the number of T-cells in peripheral blood. Very low concentrations of TRECs are seen in SCID, typically <25 TRECs/uL.

An important consideration in any screening program is the sensitivity and specificity of the screening test. Newborn screening programs have several unique challenges in accurately determining these parameters. Typically, these programs define "primary targets", which are a set list of disorders that meet the overall criteria for inclusion in a newborn screening program. These criteria include disease-specific considerations (significant health problem, pre-symptomatic or early intervention leads to better clinical outcomes, etc.) and test-specific criteria (cost, feasibility, sensitivity, specificity). However, often the screening test also detects other clinically significant disorders which might not meet strict screening criteria, or, conversely, mild variants of the primary target that are not clinically significant. For SCID, with over 30 known genetic disorders accounting for 80% of cases (20% of cases from unknown causes), plus the existence of mild forms of several of the known disorders, it is particularly challenging.

Pilot programs in Wisconsin, California, Louisiana, New York, Massachusetts, Puerto Rico have provided valuable data to help both the US and NSO decide to add SCID to their newborn screening panels. These pilots have also highlighted the difficulty in quantifying the screening test performance. At the time of the SACHDNC report, the combined screening experience from the pilots was 961,925 newborns screened for SCID by TRECs. There were no reports of any missed cases of SCID, indicating a sensitivity of close to 100%. The screen positive rate was 0.04%, with 364 newborns having a TRECs level below the cutoff and, therefore, requiring further diagnostic testing. Of these, 60 infants were identified with some form of "immune deficiency", for an incidence of 1 in 16,032, but only 14 of those met the primary target definition of SCID, for an estimated incidence of 1: 68,000. This minor difference in case definitions of what a "true positive" is, has a negligible effect on the calculation specificity of the TRECs screening test: 99.968% for a more liberal definition of an immunodeficient newborn versus 99.964% for the more-strict "classical SCID" newborn. However, because the conditions are rare, the positive predictive value of the test drops from 16.5% in the former scenario to only 3.8% in the latter, a more than 4 fold decrease in performance of the test. Expressed another way, the newborn screening program would have to retrieve and perform diagnostic testing on 26 babies to find just one newborn with classical SCID but only 6 babies need to be retrieved to find one with immunodeficiency in general.

In the pilot programs, 80% (11/14) of the "classical SCID" cases underwent bone marrow transplantations and the remaining 20% (3/14) had adenosine deaminase deficiency and are on enzyme replacement therapy. The other 44 immune deficient infants had "variant SCID" (6 cases) or non-SCID T-cell lymphopenia (40 cases). The most common causes of non-SCID detected by

the newborn screening pilots were DiGeorge syndrome/ del22q11.2 (30%) and Down syndrome/ trisomy21 (5%), but there was a long list of other known conditions (30%) and a large proportion with idiopathic lymphopenia (35%). Many of these conditions also require life-saving treatment such as HSCT or supportive care with intravenous immunoglobulin (IVIgG) and antibiotics; therefore, classifying these cases as "false positive" screens would underestimate the clinical benefit of the screening program.

Based on the experience of the US pilot projects and with advice from immunology experts in Ontario, NSO devised a screening protocol and a screen-positive clinical follow-up protocol. When fully implemented, the internal screening protocol will include TRECs as the first-tier screening test to identify SCID and will also include other second-tier tests to identify certain specific SCID diagnoses as well as non-SCID conditions such as 22q11 deletion syndrome. Applying the pilot study results to Ontario's newborn population, one can estimate approximately 53 newborns to screen positive every year with 2 to 9 of those babies having immunodeficiency ranging from classical SCID to a less severe but still clinically significant immunodeficiency. Screen-positives will be referred to one of the five Newborn Screening Treatment Centres (each located in an academic children's hospital) who will retrieve the newborn and arrange diagnostic testing. While most babies with SCID do not begin showing symptoms until after maternal immunoprotection wanes at 6 months of age, clinical safety precautions such as using only CMV-free irradiated blood products, avoiding infectious contacts and public places, and avoiding live vaccines will immediately be put into place pending final resolution of the screening result. The diagnostic testing will include CBC and differential, chromosomal analysis for a deletion in the 22q11 region, repeat TRECs, and immunophenotyping. Newborns with confirmed SCID will be urgently referred to the Hospital for Sick Children for treatment initiation and assessment for possible HSCT. If progress continues in gene therapy research, perhaps the treatment options will soon include gene therapy as well.

Murray Potter, MD, FRCPC, FCCMG
Head – Newborn Screening Treatment Centre,
McMaster Children's Hospital

References:

[i] Hacein-Bey-Abina S, et al (2003, Jan). A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. N Engl J Med 348(3): 255-6.
[ii] Cosso F (2010). Genetics of SCID. Ital J Pediatrics 36: 76.
[iii] Van der Burg M and Gennery A (2011). Educational paper. The expanding clinical and immunological spectrum of severe combined immunodeficiency. Eur J Pediatr 170: 561-571.
[iv] Buckley RH (2011). Transplantation of hematopoietic stem cells in human severe combined immunodeficiency: Long-term outcomes. Immunologic Research 49: 25–43.
[v] http://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/recommendations/correspondence/combinedimmunodeficiency.pdf
[vi] http://www.newbornscreening.on.ca/data/1/rec_docs/500_Bulletin_62.pdf
[vii] Chan K and Puck JM (2005, Feb). Development of population-based newborn screening for severe combined immunodeficiency. Journal of Allergy and Clinical Immunology 115(2): 391–398.

Education News

The 2013 **Laboratory Medicine Resident Research Day** was an overwhelming success. Congratulations to the following laboratory medicine residents who won awards at the event:

Award for Original Research
Saranya Kittanakom, CC Fellow

Best Platform Presentation
1st Place Miranda Schell, AP Resident

Best Case Report/Series Presentation
1st Place Lei Jiao, MM Resident
2nd Place Tarek Ezzat, AP Resident

Laboratory Medicine Best Poster
1st Place Yang Yu, MM Resident
2nd Place Ipshita Kak, GP Resident
3rd Place Linda Kocovski, GP Resident

Best Paper Presentation
Angela Rutledge - CC

HRLMP Award - Jeremy Daniels, AP Resident

New Program Director for the Infectious Disease Resident Training Program

Dr. Cheryl Main, Medical Microbiologist in the HRLMP, will be taking on the role of Program Director for the Infectious Diseases Training Program at McMaster on July 1, 2013. Dr. Main is currently the Program Director for the Medical Microbiology Resident Training Program.

For information and the latest news on our residency training programs follow the link: http://fhs.mcmaster.ca/pathres/news/index.html

Information on the postdoctoral fellowship: http://fhs.mcmaster.ca/pathology/education/postdoctoralfellowshiptraining.html

News from Genetics

New Prenatal Cytogenetic Testing Algorithm

In 2011, the Society of Obstetricians and Gynaecologists of Canada (SOGC) and the Canadian College of Medical Geneticists (CCMG) approved a joint clinical practice guideline for the use of quantitative fluorescence PCR (QF-PCR) in the prenatal diagnosis of fetal aneuploidy (ie., Down syndrome, trisomy 13, trisomy 18, Turner syndrome). The guidelines recommend that QF-PCR "should replace conventional cytogenetic analysis whenever prenatal testing is performed solely because of an increased risk of aneuploidy in chromosomes 13, 18, 21, X or Y"[1]. More recently, studies have shown that chromosome microarray testing can detect potentially pathogenic chromosome rearrangements in as many as 6-7% of prenatal cases with an otherwise normal cytogenetic chromosome analysis, depending on the clinical indication[2,3].

The HRLMP Molecular Cytogenetics Laboratory, in discussion with the Prenatal, Maternal Fetal Medicine and Clinical Genetics Clinics, has developed a new algorithm for the prenatal cytogenetic testing that incorporates the SOGC-CCMG guidelines and enables improved sensitivity by incorporating chromosome microarray testing for some clinical indications. In the new testing, algorithm QF-PCR will continue to be performed for all amniocentesis, but reflex chromosome testing will only be performed in specific instances. For those cases with an abnormal QF-PCR result, chromosome testing will continue to be available to assess for rearrangements that may impact recurrence risks. For cases with fetal ultrasound anomalies, chromosome testing will be performed using a microarray assay rather than classical methods to ensure higher detection rates of potentially pathogenic chromosome rearrangements. For remaining cases in which an amniocentesis has been performed solely because of a risk for abnormal amounts of chromosomes 13, 18, 21, X or Y (ie. positive biochemical prenatal screen results, or advanced maternal age) DNA will be banked for a 6 month period but no other testing will be performed unless clinically indicated. It is anticipated that this new testing algorithm will enhance the current standard of care for prenatal cytogenetic testing within the LHIN, while improving laboratory efficiency. Launch of the new test algorithm is anticipated to occur July 15, 2013.

References:

1. Langlois et al. 2011. JOGC, 265:955-960.
2. Wapner et al. 2012. N Engl J Med, 367(23):2175-2184.
3. Shaffer et al. 2012. Prenat Diag, 32:976-985.

FFPE FISH: Expanded Services Available from Cancer Genetics Laboratory

Solid tumor tissue analysis by fluorescence in situ hybridization (FISH) has recently been introduced in the Cytogenetics laboratory. The assays can help differentiate solid tumors, soft tissue sarcomas, and lymphomas, and provide pathologists with a valuable tool for diagnosing tumors at the molecular level. Utilization of FISH technology for formalin fixed, paraffin embedded (FFPE) tissues involves development of specific DNA probe sets that can identify genetic aberrations such as translocations, deletions and copy number changes of disease specific genetic markers. The FISH probe sets can be grouped in five categories: deletion, duplication, fusion, break-apart, and enumeration probes. Deletion and duplication probe sets are designed to assess for a loss or gain of copy number of a genetic locus, while enumeration probe sets are designed to assess relative copy number of a particular gene locus including high-level amplifications. Fusion probe sets are used to detect common translocations; presence of a fusion gene product is detected by the juxtaposition of fluorescence signals from two normally separated gene loci. When a particular gene can be involved in fusion rearrangements with multiple different gene partners, a break-apart probe set is commonly used, and provides a better diagnostic yield. Break-apart probe sets label one end of a gene red and another end green. In normal cells, the proximity of the red and

green probe signals will appear as yellow under the fluorescence microscope. In an abnormal cell, the gene rearrangement (e.g. fusion translocation) separates the two ends of the gene, so the probe signals then separate into red and green signals. While break-apart probe sets are excellent for detecting a rearrangement of a specific target gene, they do not show the fusion partner for that gene; in some cases, reflex follow-up testing with a gene fusion probe set or a molecular assay may be added.

We have validated a number of break-apart FFPE FISH assays. The MYC break-apart probe set is used to characterize Burkitt's lymphoma, and is designed to detect any of the MYC locus rearrangements including t(8;14), t(2;8), and t(8;22). EWSR1 gene locus break-apart probe set are associated with Ewing's sarcoma and primitive neuroectodermal tumors. Other validated break-apart FFPE FISH probe sets include SS18/SYT for synovial sarcoma, FUS for fibromyxoid sarcoma, PAX 3 and PAX 7 for differentiation of alveolar rhabdomyosarcomas and ALK for non small cell lung cancer and lymphoma. We are currently in the process of validating other lymphoma probes for FFPE applications including BCL2 (follicular), CCND1 (mantle) and MALT (marginal). Additional probes to be validated in the near future include MDM2 (liposarcomas), TFE3 (RCC) and PTEN (prostate & other CA).

Dianna Munavish Joschko and Bekim Sadikovic

Improvements in TATs for Genetic Testing

The past year has seen drastic improvements in turn around times for major components of Clinical Genetics service. The most significant improvements were seen in the constitutional and cancer cytogenetic G-band analysis, which are the most commonly ordered clinical cytogenetic tests. The TAT for constitutional G-band analysis in July 2012 was 29 weeks, and bone marrow / oncology G-band analysis TAT was 14 weeks. As a result of a focused strategy to improve the TATs and very hard work by the Genetics Laboratory technologists over the past 11 months, we currently improved our constitutional G-band TATs to 11 weeks, and bone marrow G-bands TATs to 4.5 weeks. Quantitative analysis of the BCR/ABL levels used to measure response to chemotherapy in the CML patients is another high-volume molecular genetic test which has seen a very significant improvement in TATs, starting with 12 weeks in July 2012 to currently being at 4 weeks. These improvements are largely a result of the hard work and dedication of our Genetics Laboratory staff, and we look forward to continued improvements of our laboratory services along the same trajectory.

News from Hematology

10 Colour Flow Cytometry Testing in Malignant Hematology

On Monday, June 3, 2013, the Malignant Hematology Laboratory at the Juravinski Hospital went live with 10 colour flow cytometry testing. This opens a world of new diagnostic possibilities. Flow cytometry uses antibodies that when conjugated to molecules, fluoresce in unique colours. This allows multiple surface and cytoplasmic antigens to be analyzed simultaneously, using a single aliquot of patient cells. By staining each cell with up to twelve antibodies at a time, all normal cell populations in complex specimens such as bone marrows, can now be fully characterized using a single tube.

The advantages of ten colour are several. When specimens are sent for flow such as small core biopsies, it will no longer be necessary to split those few precious cells into several aliquots in order to fully characterize the cells within the specimen. In specimens with abnormal cell populations such as leukemia or lymphoma, it may be necessary to run 20, or often more markers in numerous combinations in order to fully describe the population. With 10 colour flow, we can do this in far fewer tubes than our previous methods. We expect a reduction of approximately 60% in the number of tubes processed on a daily basis. However, 10 colour fluorescent data is an order of magnitude more complex than current methods requiring time and care to analyze. To help us achieve this, we have adopted a data analysis program called Kaluza that we have heavily customized to suit the needs of a busy clinical laboratory and regional reference center that uses high colour flow cytometry. Kaluza allows us to visualize our data in new ways allowing us to focus on all normal and abnormal cell populations efficiently. 10 colour flow also means a significant increase in sensitivity: the more antibodies present, the greater the likelihood of identifying small populations of abnormal cells. We are now able to detect extremely small leukemic populations, opening

the door to new diagnostic applications such as minimal residual disease detection. While the changes to the methodologies are extensive, flow cytometry reports will remain unchanged. In the future, it will be possible to enhance the reports with additional and more detailed comments as 10 colour evolves.

On the morning of June 3, the technologists in Malignant Hematology walked in to a completely redesigned laboratory. New instrumentation and analysis software went live, old instrumentation was reconfigured, and new processes and procedures were put into action. The implementation of process improvements as widespread as these can be stressful and rarely go live without a few unforeseen wrinkles to iron out. Not only did the entire staff of Malignant Hematology rise to the challenge, they embraced the changes, took ownership of their new procedures adding enhancements of their own. They did so in the very best of spirits and as consummate professionals. David Kimmel, Technical Specialist, Malignant Hematology, has been instrumental in the development, testing and validation required to move this project forward and would like to express his deepest appreciation and respect to every member of the staff in Malignant Hematology, without whom, this project would never have moved beyond the earliest planning stages. We are extraordinarily proud of what we have managed to accomplish together.
Anyone up for 20 colours? ☺

Medical Leadership Changes in the Tissue Typing Laboratory

Since March 2012, Dr Jianping Li, Assistant Professor, Department of Pathology and Laboratory Medicine, University of Ottawa, has been the interim ASHI (American Society for Histocompatibility and Immunogenetics) accredited Laboratory Director in the Tissue Typing Laboratory. **Dr. Kylie Lepic** and Dr. **Christine Ribic** are acting as directors in training.

Dr. Kylie Lepic will be the future Director for the Bone Marrow Division. She received her MD from McMaster University in 2006 and completed Internal Medicine and Hematology residency training at McMaster. This was followed by a fellowship in Blood and Marrow Transplantation at the Fred Hutchinson Cancer Research Center in Seattle, WA. Dr. Lepic is currently an Assistant Professor in the Department of Medicine at McMaster and is based at the Juravinski Hospital and Cancer Centre.

Dr. Christine Ribic will be the future Director for the Renal Division. Dr. Christine Ribic is a Clinical Transplant Nephrologist and an Assistant Professor at McMaster University. She obtained her MD from the University of Toronto and completed her Internal Medicine and Nephrology residency at McMaster University. She also completed additional training after her Nephrology Fellowship in Renal Transplantation at both academic Ontario transplant centres and in the United States. She completed a Master of Science in Pharmacology and Cancer Genetics at the University of Toronto in 2001 and is currently completing a second Master of Science thesis in Clinical Epidemiology and Biostatistics in the Health Research Methodology Program at McMaster University

News from Microbiology

Automation Comes to Bacteriology

Microbiology is embarking on an exciting journey as we introduce automation into our Bacteriology Laboratory. Automation offers the advantages of standardizing laboratory processes, providing more rapid organism identification and susceptibility test results, and potentially reduces the risk of laboratory acquired infections. HRLMP is the first microbiology laboratory in Ontario to introduce this advanced technology. Laboratory construction will begin in early July, but Bacteriology will remain fully operational throughout this time. Our dedicated staff will be working under very challenging circumstances during these renovations, and your patience will be greatly appreciated.

News from Pathology

After an extensive optimization and validation process, the Immunohistochemistry Lab in Anatomical Pathology has switched over to a new staining system for its entire set of more then 200 stains, using the identical primary antibodies but employing a novel detection system with greatly improved quality. The DAKO Polymer system is automated to ensure constant and reproducible results.

News from the HRLMP

After 36 years of dedicated service, **Connie Lester** has made the decision to retire from the HRLMP. Connie began her career at the Henderson General Hospital as a technologist in "Blood Bank". She has held several leadership positions within Transfusion Medicine and is currently the Supervisor for the four sites and the Stem Cell Laboratory. We would all like to thank her for her tireless effort and wonderful work ethic! Connie will retire on June 21, 2013.

Check out the new websites for HRLMP and LRC Hamilton

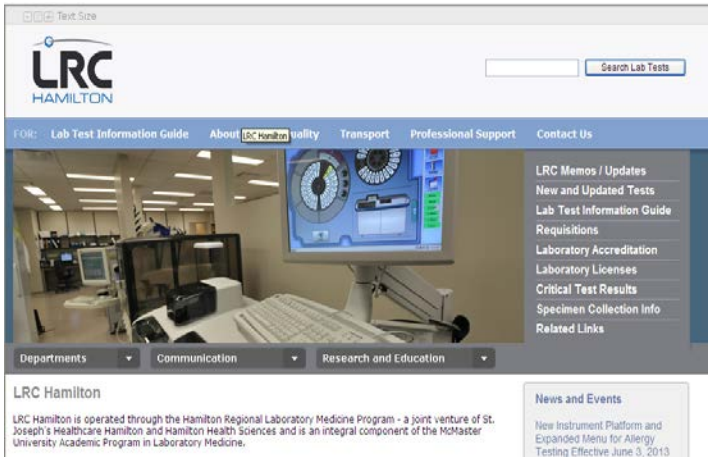
HRLMP and LRC Hamilton (previously Laboratory Reference Centre) have launched new websites.

HRLMP is pleased to offer an updated more user-friendly website for our staff and external clients.

Some of the features to be found on the new site include:

- Requisitions accessible from the home page and from individual discipline pages
- Link to the Lab Test Information Guide (currently undergoing a re-design)
- Access to HRLMP newsletters
- Up-to-date information about the HRLMP and the laboratory services we provide.

LRC Hamilton (previously Laboratory Reference Centre) is excited to announce the launch of their new logo and website.



Some of the new features and improved functionality on the website include:

- Improved Lab Test Information Guide with new functionality
- Detailed lists of test offerings by department
- Easily accessible requisition forms