HAMILTON REGIONAL LABORATORY MEDICINE PROGRAM

LAB CONNECTIONS

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IN THIS ISSUE:

Molecular classification of breast cancer may provide enhanced prognostic information and predict patient response to adjuvant therapies. Dr. Anita Bane, HRLMP Pathologist and Clinician Scientist, reviews the research done in this area and explores the future role of molecular classification in patient care.

WHAT'S NEW?:

- Dr. Elena Bulakhtina has officially joined Forensic Pathology at the Hamilton General Hospital. She is looking forward to sharing her knowledge with students in our new, Royal College recognized, Forensic Pathology training program.
- There have been some changes to our Editorial Board. Dr. Vijay Grey has stepped down as the Newsletter Editor after 3 years of dedicated service. During this time, she created the Editorial Board and transformed the Newsletter. Dr. Cheryl Main will become the new Editor and Dr. Cynthia Balion will join the Editorial Board for Chemistry.

Molecular Classification of Breast Cancer for the Surgical Pathologist

Traditionally a number of clinical and tumour characteristics have been used to determine the prognosis of a patient presenting with early stage breast cancer. These variables include patient age, tumour size, grade, hormone receptor status, HER2 status, the presence or absence of lymphovascular space invasion and the extent of lymph node involvement. For many years these clinicopathological characteristics have guided physicians in their recommendations for adjuvant systemic therapy (chemotherapy and/or endocrine therapy) administered post-operatively in patients with early stage breast cancer.

In reality these traditional variables are limited in their ability to predict who will develop recurrent cancer (prognostic capacity) and who will benefit from adjuvant therapy (predictive utility). In an effort to better predict breast cancer behavior, researchers have utilized a number of experimental approaches to identify and characterize the genetic alterations that underpin breast cancer development and prognosis. One of these approaches, termed gene expression profiling, has provided a new *molecular classification* of breast cancer that adds new and exciting prognostic and predictive information for breast patient management.

In 2000, Perou and colleagues at Stanford University conducted gene expression profiling experiments on ~100 invasive breast cancers (1, 2). Using hierarchical clustering, these investigators provided a *new molecular classification* for breast cancer based on the relative expression of the ~500 genes, known as an 'intrinsic' gene set. They discovered that breast cancers could be classified into five molecular subgroups. Two of these are estrogen receptor positive (ER+), whereas three are estrogen receptor negative (ER−). The ER+ subgroups, termed Luminal A and

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YOUR FEEDBACK IS VALUED!

Your feedback, suggestions and new ideas are most welcome!

Luminal B, are identified based on their relative expression of the ER gene, ER regulated genes and other genes expressed by normal breast 'luminal' cells. The ER− subgroup consists of the HER2 overexpressing, normal breast-like and the basal-like subgroups. The HER2 overexpressing subgroup is characterized by the overexpression of the HER2 and other genes on the 17q amplicon, such as GRB7. The normal breast-like subgroup expresses genes characteristic of adipose tissue which suggests that this subgroup could be a technical artifact resulting from low tumour cellularity. Lastly, the basal-like subgroup represents a *distinct and novel* class of tumours characterized by the lack of expression of ER, PR and HER2, hence the alternative designation of 'triple negative' (TN) tumours. Additional characteristics are the high expression of cytokeratins (CK) 5, and/or CK 17 (amongst other genes) which are characteristic of the basal cell layer of the normal breast epithelium. These study findings have been subsequently reproduced by other groups of investigators using similar platforms. More recent studies uncovered the existence of additional less common subtypes to include 'claudin-low' and 'molecular apocrine'.

Most importantly, the initial gene expression profiling experiments demonstrated that the basal-like subtype together with the HER2 overexpressing subtype were associated with a particularly poor prognosis. By comparison, luminal A type tumours displayed an excellent prognosis. *Thus, the molecular classification of luminal A, luminal B, basal-like and HER2 added new and important prognostic information beyond that provided by standard clinicopathologic predictors*.

Gene expression profiling studies such as those detailed above require fresh frozen tumour tissue; however, these types of specimens are rarely available outside of the research or clinical trial setting. Using a surrogate panel of six immunohistochemical (IHC) antibodies (ER, PR, HER2, CK5, EGFR and Ki67), a number of investigators have demonstrated that it is feasible to reliably identify the molecular subtypes of breast cancer in formalin fixed paraffin embedded (FFPE) tumour tissue, which constitutes the bulk of patient samples and clinical trial archives (3, 4). While no universally accepted immunohistochemical surrogate 'definition' of the molecular subtypes is available, the growing consensus in the literature would suggest that;

- Luminal A type tumours are ER+ and/or PR+, HER2−, with a low Ki67 labeling index (<14%)
- Luminal B type tumours are ER+ and/or PR+, HER2+ (3+ or amplified by ISH) and/or have a high Ki67 labelling index (>14%)
- HER2+ tumours are ER−, PR−, HER2+ (3+)
- Basal-like tumours are ER−, PR−, HER2-, CK5 + and/or HER1(EGFR) +

The expression pattern of these six tumour markers appears to be highly specific for basal-like breast cancer, and moderately sensitive and specific for luminal A/B and HER2 overexpressing types, with patient survival curves closely approximating those reported for the gene expression studies described above (5, 6).

In addition to providing enhanced prognostic information, the intrinsic molecular subtype of a breast cancer may be able to predict response to adjuvant therapies (chemotherapy and radiation therapy). This has been best demonstrated in the neoadjuvant setting where combined results from a number of trials suggest that luminal A type tumours achieve a very low rate of pathologic complete response (pCR) (~7%) when treated with neoadjuvant therapy, whereas basal-like and HER2 subtypes exhibit a high pCR rate (30-40%). Luminal B type tumours have a pCR rate (~17%) which is intermediate between that of luminal A and the HER2 & basal-like groups. There is accumulating evidence that the basal-like and HER2 subtypes are associated with an increased incidence of loco-regional recurrence after breast conserving surgery and whole breast irradiation and in high-risk women treated with post-mastectomy radiation(7-9).

The molecular classification of breast cancer is not routinely performed by the Pathology Department of Hamilton Health Sciences or elsewhere and remains to date a research tool, although the current standardized reporting of ER, PR and HER2 on all newly diagnosed breast cancers allows an approximation of the molecular subtypes and is often used by our clinical colleagues in managing their patients. In the future, it is likely that some or all of the additional immunohistochemical markers (Ki67, CK5/6 and EGFR) will come into clinical practice, or alternative assays to determine the molecular subtypes will be implemented. One such assay is PAM50, a gene expression assay that can be performed on FFPE tumour material. This test

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measures the expression of 50 classifier genes and five control genes to identify the 5 molecular subtypes of breast cancer. The test has already been shown to have prognostic significance analogous to the original gene expression profiling studies, and multiple studies are ongoing to demonstrate its predictive utility (10, 11). The company that markets the PAM50 assay is currently seeking FDA approval for the test.

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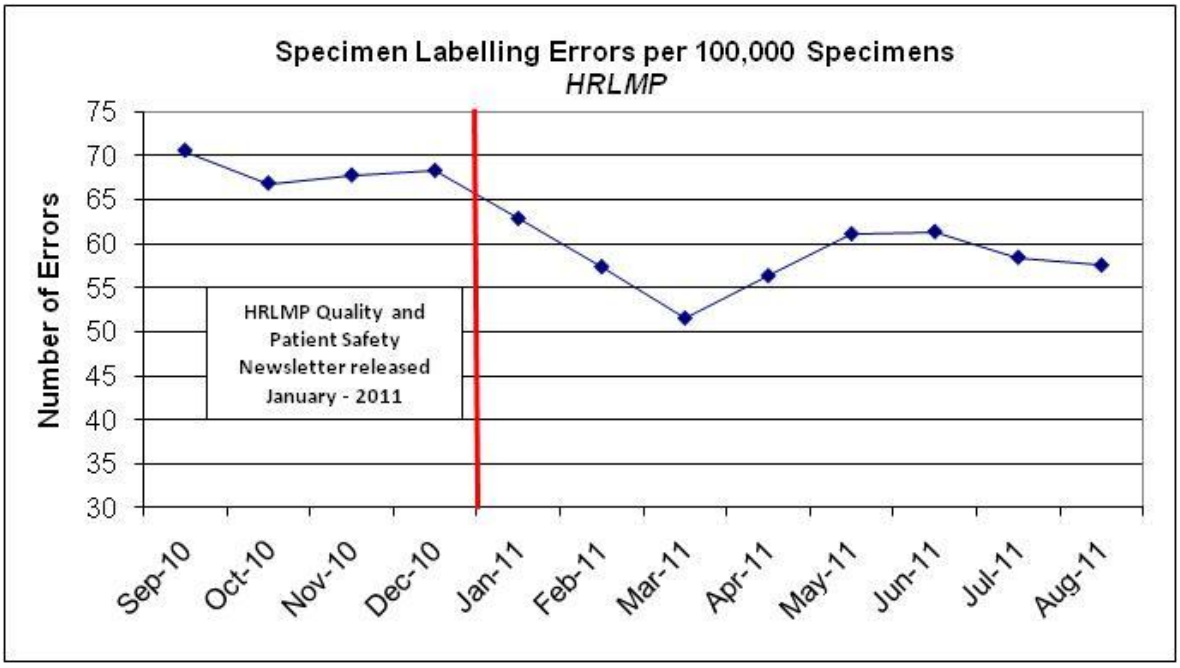
QUALITY SNAPSHOT: Specimen Labelling Errors

Correct patient identification is a critical step for ensuring patient safety and quality laboratory results. Proper specimen labelling is critical to this process and poses a challenge for many laboratories - including the HRLMP.

In order to address this concern, an educational tool was created that illustrated several types of labelling errors, including incorrect patient/specimen identification, improper label positioning on specimen containers, illegible specimen labels, and unlabelled specimens. This tool can be found by clicking: The Usual Suspects Specimen Labelling Guide.

The tool, along with an internal Quality and Patient Safety Newsletter discussing the topic, was distributed to all Clinical Educators in Hamilton. Recipients were encouraged to share the information with their teams and, as a result, we saw an initial decrease in the frequency of specimen labelling errors in the three months following our intervention. The improvement does not appear to have been sustained; however, the HRLMP continues to work with our partners to improve this quality attribute.



Cathie McCallum, Quality Manager, HRLMP, and
Tom Dorland, Quality Specialist, HRLMP

EDUCATION:

In January 2011, the Royal College of Physicians and Surgeons approved the application for recognition of forensic pathology training at McMaster University, Hamilton Health Sciences Centre. The Forensic Pathology Unit performs approximately 700 autopsies annually for the purpose of medicolegal death investigation. Our service area covers a large geographical area and a population base of approximately 2.5 million. The Coroner's Act was amended to recognize the role of forensic pathologists in June of 2009. With this recognition came the need for formalized training of forensic pathologists within Canada. The first educational program started in Toronto, and McMaster becomes the second recognized program. We are expecting that our first candidate will start in the near future and annually we hope to accept one PGY6 resident. In the long term, this will help to address the needs for service and provision of an academically driven scientifically focused approach to death investigation.

The **Annual Resident Research Day for Pathology and Molecular Medicine and HRLMP** will be held on May 24, 2012. We are fortunate to have two exceptional keynote speakers presenting:

Dr. Jennifer Hunt, Professor of Pathology and Director of Molecular Diagnostics, Harvard Medical School, Harvard University, Boston

Dr. Guillermo J. Tearney, Professor of Pathology, Harvard Medical School and Harvard-MIT Division of Health Sciences and Technology and Director of the Wellman Center of Photomedicine and Optical Diagnostics Program Leader at the Center for the Integration of Medicine and Innovative Technology

More detailed information will be provided closer to the event.

The Medical Biochemistry Resident Training program is pleased to welcome Mohammed Rehan who is starting his basic clinical training year.

For information and the latest news on our residency training programs please follow the link:
http://www.fhs.mcmaster.ca/pathres/news/index.html
Information on the postdoctoral fellowship training program can be obtained by following the link:
http://fhs.mcmaster.ca/pathology/education/postdoctoralfellowshiptraining.html

ANATOMICAL PATHOLOGY
GRAND ROUNDS - 2011

TIME: 12:30 - 1:30 p.m. 2011	SPEAKER:	TOPIC:
October 20th MDCL – 2232	Dr. J. Parfitt London Health Sciences	GI Pathology
November 10th MDCL – 2232	Dr. Robin Edwards McMaster University	Professionalism in Postgraduate Medical Education: Policies and Procedures.
December, 2011	**Holiday Season**	**No Rounds**

ANATOMICAL PATHOLOGY GRAND ROUNDS ARE SPONSORED BY THE DEPARTMENT OF PATHOLOGY AND MOLECULAR MEDICINE
ROYAL COLLEGE ACCREDITATION

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