Two New Genetic Tests – QF-PCR and FLT3/NPM1

Quantitative Fluorescence Polymerase Chain Reaction (QF-PCR) Analysis for Detection of Fetal Aneusomies

Genetics is introducing a new test, Prenatal QF-PCR, to screen for major numerical chromosome disorders, i.e. trisomy 21, 18, 13 and sex chromosomes aneuploidies (1). The Prenatal QFPCR test is intended as a replacement for an existing test (FISH testing for aneusomies, also known as Aneuvision). The cytogenetics laboratory has provided the prenatal FISH (fluorescence in-situ hybridization) testing for over 5 years, but it is a microscopic technique with a high cost for both reagents and technical time and has been restricted to women who meet high-risk criteria. The molecular technology used in the prenatal QFPCR assay offers significant cost advantages; partial automation of the procedure permits higher throughput of samples and reagent costs are significantly lower. As a result, we expect to be able to offer QFPCR screening as a rapid and cost-effective option for all pregnant women. Access to testing will follow our usual process of referral by health care professionals to the high-risk obstetrics clinic at the McMaster Medical Centre.

We will be using a commercial kit, provided by Aneufast™ QF-PCR kit (Genomed Ltd, Kemsing, Kent, United Kingdom). Genomic DNA is extracted from either amniotic fluid cells or tissues from chorionic villus sampling. The Aneufast™ QF-PCR kit uses a five-dye fluorescent system for automated DNA fragment analysis. The kit contains six multiplex marker sets of short tandem repeats (STRs). In the routine setting, two multiplex QF-PCR sets (S1 and S2) are first performed and analyzed by a single electrophoresis. In cases where only one or none of the
markers in S1 and S2 are informative, four chromosome-specific marker sets (M21, M13, M18 and MXY) can be used as a reflex test panel to provide a conclusive result.

The validation project at the HRLMP started in December 2010. Since then, 80 cases (70 amniotic fluids and 10 CVS) have been successfully tested. Three cases with trisomy 21, two with trisomy 18, one with trisomy 13 and one with 69,XXY were identified. The success rate, sensitivity and specificity met expected standards for reporting. We are programming the reporting format and working with the high-risk obstetrical clinic staff to develop educational materials and updates for patients and referring health care professionals.

The target date for offering the test in service is May 1, 2011. Test results will be reported twice weekly with an average reporting time of about 3 days from receipt of the amniotic fluid or CVS specimen.

**FLT3/NPM1 for Acute Myeloid Leukemias**

The molecular hematology and cancer genetic services have completed the validation and implementation of FLT3/NPM1, a new diagnostic test for patients with acute myeloid leukemia (AML) (2). This test protocol will be based upon extraction of DNA or RNA and will automatically be performed as a reflex PCR-based test for patients with a confirmed clinical diagnosis of acute leukemia and normal cytogenetic results. The primary purpose of the test is to provide more refined information in regard to prognosis (and therefore options in management) for patients who do not have clonal chromosomal abnormalities at diagnosis (e.g. trisomy 8, deletion 5q) or a positive result from the usual molecular screening panel (PML-RARa, AML1-ETO, and CBFB).

One component of the testing examines the FLT3 gene (a tyrosine kinase) for the presence of internal tandem duplications. The duplications are a type of acquired mutation of FLT3 that is detected in about 25% of all adult cases of AML. The size of the duplicated sequences can vary from 6 - 50 bp, the number of duplications can vary from 1 to 3, the site of the duplications in the gene can vary and there is some evidence that these details are prognostically relevant. The test protocol will provide an indication of the size and number of duplications.

A second component of the test will be to evaluate another gene called NPM1, which codes for a nucleolar phosphoprotein with multiple functions in the nucleus, the cytoplasm and ribosomal activity. Acquired mutations of NPM1 are detected in about 25% of adult AML (particularly M5, M4 and M2 subtypes).

AML patients with normal cytogenetics and negative for the major fusion rearrangements (e.g. PML-RARa, AML1-ETO, CBFB, MLL) are generally determined to have an intermediate prognosis. In this group, mutations of FLT3 are associated with a more adverse prognosis. Mutations of NPM1 are associated with a more favourable prognosis in the absence of an FLT3 mutation. Concurrent mutation of both FLT3 and NPM1 is associated with an intermediate prognosis.

This test is expected to be in service by May 1, 2011.

References:

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Ron Carter, PhD, FCCMG, Head of Cancer Genetics
Brian Leber, MD, FRCPC, Head of Service, Molecular Hematology
**EDUCATION:**
Congratulations to Lori Edwards, Anatomical Pathology resident. Lori won the Stowell Orbison Award (best project presentation) for best poster competing with over 250 other projects in this category at the 100th Annual United States and Canadian Academy of Pathology Conference.

**Training Programs:**
For information and the latest news on our residency training programs please follow the link: [http://www.fhs.mcmaster.ca/pathres/news/index.html](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](http://fhs.mcmaster.ca/pathology/education/postdoctoralfellowshiptraining.html)

**Anatomical Pathology Lectures:**
Rounds Schedule for remainder of the year:

**TIME: 12:30 – 1:30 P.M.**

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<td>June 16th</td>
<td>Dr. M. Sur, HRLMP – McMaster University</td>
<td>Hematopathology - TBA</td>
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