Classification of Aggressive B-cell Lymphomas

by Lauren Smith M.D., Assistant Professor of Pathology with Bryan Betz, Ph.D., Assistant Professor of Pathology & Technical Director of Molecular Diagnostics Laboratory

The most recent edition of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (2008) includes a new provisional entity entitled “B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma.” This category is intended to include highly proliferative B-cell lymphomas with an immunophenotype or morphologic features suggestive of Burkitt lymphoma; however, it can be difficult to determine which cases to include in this category. In prior classification schemes, many of these cases were classified as “Burkitt-like” or simply called diffuse large B-cell lymphoma (DLBCL). 9

Many of these cases have a MYC rearrangement in the context of a complex karyotype with or without juxtaposition of MYC to one of the immunoglobulin genes. 5, 6 Burkitt lymphoma, on the other hand, typically has a simple karyotype involving the immunoglobulin loci. 4, 7 Studies have shown that some of these cases had additional BCL2/IGH translocations or, less frequently, BCL6 rearrangements. Many hematopathologists began to refer to cases as “double-hit” if they harbor a MYC rearrangement and either a BCL2/IGH translocation or a BCL6 rearrangement. If all three are present, it is called “triple-hit,” although these cases are rare. 1, 2, 8

While there has been no definite national or international consensus to date on which cases should be included in the new B-cell lymphoma, unclassifiable category, I have developed an approach based on more recent literature with the intention of identifying the majority of cases that are “double or triple hit.” These cases indicate an aggressive lymphoma with a poor prognosis and they are the most important subset to classify differently. 9 While current treatment approaches differ depending on the institution, identifying these cases will allow studies that will ultimately determine the optimal treatment approach.

While it is difficult to identify these cases with certainty on routine histology, as they may not morphologically resemble Burkitt lymphoma at all, I use a morphologic and immunohistochemical approach to determine which cases can benefit from paraffin fluorescence in situ hybridization (FISH) studies. If a case has a high mitotic rate and numerous tingible body macrophages, immunostains for Ki-67 and
CD10 are ordered. If the case fits morphologic and immunophenotypic criteria for classic Burkitt lymphoma, the case is typically sent for MYC rearrangement only. If the lymphoma does not fit classic Burkitt lymphoma but has a high proliferative fraction and expresses CD10 (the immunophenotype typical of Burkitt lymphoma), the case is sent for the three FISH studies (MYC rearrangement, BCL2/IGH translocation, and BCL6 rearrangement). While there is no definite threshold for Ki-67 that will identify all cases of the “double-hit” lymphomas, I have decided to use greater than 80% as a reasonable triage strategy, although this will miss some cases. Bcl-2 immunostain can be helpful in excluding classic Burkitt lymphoma which should be negative. Many of these “double-hit” cases are Bcl-2 positive (87% in one large series). Preliminarily, the case will be signed out as “diffuse aggressive B-cell lymphoma” with a differential diagnosis which may or may not include classic Burkitt lymphoma. While occasional cases of “double-hit” lymphomas have been identified in the literature with lower proliferative fractions, these cases are in the minority and the median Ki-67 expression was 80-90% in one large series. CD10 negative cases exist; however, these are rare. Greater than 98% (all but one case) were CD10 positive in one large series and all of the cases expressed CD10 in two smaller studies. Additional research is needed before I can support performing these FISH tests on every case of large B-cell lymphoma. As this is my own personal approach to identifying these cases in a cost-effective manner, it will require revision as research advances allow more precise guidelines for testing.

In the event that we receive a consult case that may fit into this new category, additional work-up is initiated. If the morphology and immunophenotype are suggestive of a “double-hit” lymphoma, the client will be contacted to approve the FISH studies before they are ordered. If these are not deemed to be appropriate based on clinical or other considerations, the case will be signed out as “diffuse aggressive B-cell lymphoma” with a differential diagnosis. This may be perfectly appropriate in certain clinical situations, including elderly patients or individuals who may elect palliative care. In addition, in some hospitals these patients may be treated with the same chemotherapeutic agents regardless of this distinction, as the best therapy for these patients has yet to be determined. Certainly, in these situations, this distinction may not yet have clinical relevance and the studies may be unnecessary.

The FISH studies will take approximately 5 working days for results. In the event that only a MYC rearrangement is found, the pathologist will need to determine whether it is best classified as diffuse large B-cell lymphoma or Burkitt lymphoma based primarily on morphology and immunophenotype (see Figure 1). MYC rearrangement does not exclude DLBCL.

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**Figure 1:** Algorithm for ordering and interpreting paraffin FISH tests on diffuse aggressive B-cell lymphomas.

**Figure 2:** Identification of a double-hit lymphoma by fluorescence in situ hybridization (FISH). MYC rearrangements are detected with a break-apart probe strategy. With this design, a normal MYC gene is observed as overlapping or adjacent red/green signals; while a rearranged MYC gene is indicated by split red and green signals. BCL6 rearrangements are identified using a similar break-apart probe strategy. IGH/BCL2 rearrangements are detected with a dual-fusion probe strategy. With this design, colocalization of the probes is observed when an IGH/BCL2 translocation is present. This case harbored both a MYC rearrangement and an IGH/BCL2 rearrangement.
While some cases with MYC gene rearrangement may be appropriate for the intermediate category, many respond to conventional therapy, and therefore DLBCL is the preferred diagnosis if the morphology fits DLBCL. If MYC rearrangement is present with IGH/BCL2 and/or BCL6 rearrangement, this case will most likely be classified as "B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL." If all three FISH studies are negative, the diagnosis will most likely be diffuse large B-cell lymphoma.

If a client is working up a diffuse aggressive B-cell lymphoma, two options exist through our MLabs program. The case can be sent to our hematopathology consultation service as an anatomic pathology consult or the paraffin block can be sent directly for FISH studies in our molecular laboratory.

When FISH studies are requested, unstained tissue sections are prepared and FISH is performed on tissue regions selected by our pathologists. Detection of MYC gene rearrangements is accomplished through the use of break-apart FISH probes specific to the MYC gene locus at 8q24. The probe mixture includes a red probe and a green probe that flank the MYC gene breakpoint region. Splitting of the probes is observed when a rearrangement involving MYC is present (Figure 2). This strategy offers the ability to detect any MYC rearrangement, including the common MYC/IGH, MYC/IGL, and MYC/IGK translocations as well as variant MYC rearrangements that sometimes occur in double-hit lymphomas. Detection of BCL6 rearrangements is accomplished through a similar break-apart FISH probe strategy since BCL6 translocations occur with a diverse spectrum of different partner genes (Figure 2).

In contrast to the variable partner genes found in MYC and BCL6 rearrangements, BCL2 rearrangements almost always occur with IGH. Therefore, a dual-fusion FISH probe strategy is used to specifically detect the IGH/BCL2 translocation for these cases. The dual-fusion probe mixture includes one specific to the IGH gene and another to the BCL2 gene, each spanning their respective translocation breakpoint regions. Colocalization of the probes is observed when an IGH/BCL2 translocation is present (Figure 2).
TO SEND A SPECIMEN

FISH for MYC rearrangement, BCL6 rearrangement, and BCL2/IGH translocation is performed on formalin-fixed paraffin-embedded tissues. Tissue blocks can be sent at room temperature.

For assistance 24 hours per day, 7 days per week, call MLabs at 800-862-7284 or visit our website at www.mlabs.umich.edu.

REFERENCES


New Service at U-M: Fewer Women Need Repeat Breast Cancer Surgeries

PATHOLOGY EVALUATIONS DONE ON-SITE CUT OPERATING TIME, REDUCED COST, STUDY SHOWS

by UM News

Nearly one in three women who have breast cancer surgery will need to return to the operating room for additional surgery after the tumor is evaluated by a pathologist.

A new service at the University of Michigan Comprehensive Cancer Center cuts that number drastically by having pathologists on-site in the operating suite to assess tumors and lymph nodes immediately after they are removed. Meanwhile, the surgeon and patient remain in the operating room until the results are back, and any additional operating can be done immediately.

This cut the number of second surgeries needed by 64 percent, to one of every 10 women.

U-M began offering the service about two years ago at its East Ann Arbor Ambulatory Surgery Center, where the majority of outpatient breast cancer surgeries now occur. A study evaluating 271 patients treated eight months before and 278 treated eight months after this program began appears in the American Journal of Surgery.

“The frequent need for second surgeries among patients undergoing breast cancer surgery represents a tremendous burden for patients. Beyond the inconvenience and additional time away from work, additional surgeries can result in worse cosmetic outcomes and increased complication rates. Our experience shows that offering on-site pathology consultation has a substantial impact on quality of care,” says lead study author Michael S. Sabel, M.D., associate professor of surgery at the U-M Medical School.

Patients must return to the operating room for two primary reasons: to remove additional tissue when the cancer cells are too close to the margin of tissue removed; and in some cases, to remove additional lymph nodes if the initial sentinel lymph node biopsy tests positive for cancer.

Before the on-site pathology, 25 percent of patients needed a second operation to remove more tissue,
compared to 11 percent after the service began. Among patients with cancerous lymph nodes, 93 percent of them avoided a second surgery with on-site pathology.

In addition to reducing second surgeries, the study found that assessing the margins in the OR allowed more women to conserve their breasts. The study authors suggest that women who have positive margins requiring additional surgery are more likely to choose mastectomy because they fear their cancer will return or that they’ll need a third operation.

Establishing on-site pathology requires a different technique for preserving and evaluating the cells, called frozen section analysis. After this is completed, U-M pathologists then process the tumors for standard testing using traditional methods. The study showed consistent results across both types of analysis.

On-site pathology using frozen tissue sections is offered at a handful of academic medical centers across the country.

“In large part, routine intraoperative analysis of lumpectomy margins is rare because of logistical issues, especially as breast surgery is more commonly performed at outpatient surgical centers,” Sabel says.

Obstacles include transporting the tissue samples, building a pathology facility, and staffing it appropriately at an offsite surgical center.

“Despite these obstacles, we found that not only is this beneficial for our patients, but it reduced the costs of caring for patients with breast cancer,” Sabel adds.

The study authors also considered new guidelines that suggest fewer women need to have their lymph nodes removed if the sentinel lymph node biopsy is positive. The authors factored in that reduction and still found that intraoperative analysis was highly cost-effective.

“Establishing an intraoperative pathology consultation service is feasible, highly efficient and extremely beneficial to patients, surgeons and reducing the costs of cancer care,” Sabel says.

Additional U-M authors: Julie M. Jorns, M.D.; Angela Wu, M.D.; Jeffrey Myers, M.D.; Lisa A. Newman, M.D., M.P.H.; and Tara Breslin, M.D., M.S.


Test Updates

New Tests

CFTR GENE SEQUENCING

The Molecular Genetics Laboratory began sequencing the CFTR gene (MIM 602421) to detect mutations associated with the autosomal recessive disorders cystic fibrosis (MIM 219700) and congenital bilateral aplasia of the vas deferens [MIM 277180, which is also referred to as Congenital Absence of the Vas Deferens (CAVD)] effective March 7, 2012. This full gene sequencing assay is used to detect the presence of CFTR mutations in patients with CFTR-related disorders affecting the respiratory tract, pancreas, sweat glands, intestine, male genital tract, and liver, and for the confirmation of a diagnosis of Cystic Fibrosis in patients who had no mutations detected in a targeted mutation analysis for the 23 CF mutations recommended by ACMG/ACOG but have a clinical presentation consistent with Cystic Fibrosis. Please note that ordering the Cystic Fibrosis Diagnostic Mutation Detection assay is recommended prior to the CFTR Gene Sequencing assay, especially for patients of Western European ancestry.

KETOACIDS, URINE BY DNPH

The Biochemical Genetics section of the MMGL Laboratory began processing semi-quantitative urine ketoacids using a DNPH spot test methodology, beginning January 9, 2012. This test is used to determine urine ketoacid excretion in MSUD (maple syrup urine disease) patients and is also positive in patients with PKU (phenylketonuria). Collection Instructions: Please notify MLabs Client Services Center to schedule testing prior to collecting specimen. Collect random urine specimen and freeze. Freeze each specimen immediately after collection if multiple collections are needed to reach the minimum volume (10 mL). Include the patient’s family history, clinical condition (asymptomatic or acute), diet, and a list of current medications with the test requisition.

Patients excreting material including low solubility drugs and x-ray contrast dye may yield a false positive result. Ketotic patients will also test positive. Positive results in previously undiagnosed patients should be confirmed by urine organic acids testing. Plasma
amino acid testing is required to confirm a diagnosis of MSUD. Dilute urine may show a false negative result.

**PDGFRA MUTATION FOR GIST**

The MLabs Molecular Diagnostics Laboratory began offering PDGFRA Mutation for GIST testing by Polymerase Chain Reaction (PCR) followed by DNA sequence analysis, effective January 16, 2012.

PDGFRA gene mutations occur in approximately 30% of gastrointestinal stromal tumors (GISTs) that are wild type for KIT mutation, and in 7% of GISTs overall. PDGFRA and KIT mutations are mutually exclusive in GISTs. The vast majority of PDGFRA gene mutations involve exons 12 and 18, and invariably result in constitutive activation of the PDGFRA protein. The most common PDGFRA mutation, D842V, is associated with imatinib therapy resistance. This DNA sequencing test will detect mutations within exons 12 and 18 of the PDGFRA gene. The tested regions correspond to PDGFRA amino acids 552-585 (exon 12) and 826-848 (exon 18). Appropriate specimens include formalin fixed paraffin-embedded blocks, unstained paraffin sections on slides, and fresh/frozen tissue. The submitted specimen should contain an adequate proportion of tumor nuclei (>40%) to enable mutation detection.

Collection Instructions: Send fresh, frozen, or formalin-fixed paraffin-embedded tissue containing greater than 40% tumor. Fresh tissue should be sent on a piece of gauze in saline, or in RPMI, within 24 hours of collection; refrigerate. Frozen tissue should be stored at -80 degrees C; do not allow to thaw at any time. Paraffin-embedded tissue should be stored at room temperature. Please provide an estimate of tumor percentage with paraffin-embedded tissue specimens.

**Test Methodology, Reference Range, and Specimen Handling Changes**

**ANDROSTENEDIONE, SERUM**

Effective February 15, 2012, the test methodology for the Androstenedione assay has changed from RIA (radioimmunoassay) to Chemiluminescence. Specimen collection and handling requirements have not changed, with the exception that the requirement that no radioisotopes should be administered 24 hours prior to venipuncture no longer applies. The reference range has not changed.

**ANTIBIOTIC SUSCEPTIBILITY, FUNGUS (MIC)**

Effective January 20, 2012, fungal susceptibility testing sent by Mayo Medical Laboratories to the U.T. Health Science Center at San Antonio are no longer available unless the organism identification is first performed at Mayo Medical Laboratories. This change is due to CDC regulations relating to Select Agents or Toxins. Select Agents or Toxins listed by the CDC (e.g., Coccidioides immitis or Francisella tularensis) require specific documentation from the CDC and should be shipped directly to the testing laboratory.

**CHROMOSOMAL MICROARRAY ANALYSIS**

Effective February 6, 2012, the MMGL Molecular Genetics Laboratory has transitioned to performing a higher density Illumina Human CytoSNP-12 chromosomal microarray (330K markers) from the current EmArray Cyto600 chromosomal microarray (44k features). The Illumina Human CytoSNP-12 chromosomal microarray combines genotype and intensity information to detect various types and sizes of structural genomic variation in the human genome including deletions, amplifications, copy-neutral Loss of Heterozygosity (LOH), Uniparental Disomy (UPD), and mosaicism. This Illumina SNP array includes a panel of genome-wide tag SNPs and CNV markers targeting regions of known clinical importance, including approximately 250 subtelomeric and pericentromeric regions, as well as approximately 400 disease-related genes and sex chromosomes.

The Human CytoSNP-12 chromosomal microarray replaces the EmArray Cyto600 chromosomal microarray.
**FLECAINIDE**

Warde Medical Laboratory has discontinued their Flecainide assay effective March 12, 2012. MLabs will refer requests for this test to Mayo Medical Laboratories.

Collection Instructions: Collect specimen in a red top tube. Centrifuge, aliquot 3 mL (minimum 1.1 mL) of serum into a plastic vial and refrigerate.

Reference Range: 0.2 – 1.0 mcg/mL

**GLUCOSE, CSF**

Effective February 13, 2012, a Critical Value flag has been added for CSF Glucose values <30 mg/dL or >300 mg/dL.

**LEFLUNOMIDE**

Effective January 3, 2012, Mayo test #57113 Leflunomide (Arava) as Teriflunomide Metabolite referred to MedTox has been replaced by Mayo test #60292 Leflunomide Metabolite (Teriflunomide), Serum.

Collection Instructions: Collect specimen in a red top or SST tube. Centrifuge and aliquot 1 mL of serum into a plastic vial within 2 hour of collection. Store and transport at room temperature (preferred); specimen may be stored refrigerated or frozen for up to 14 days.

Reference Range: Therapeutic: >40 mcg/mL; Elimination: <0.020 mcg/mL.

**LEISHMANIA ANTIBODY**

Effective April 5, 2012, the preferred specimen storage and shipping temperature for Mayo Medical Laboratories Leishmania Antibody assay will change to Refrigerated. Frozen specimens will be accepted.

Collection Instructions: Collect specimen in a red top tube. Centrifuge, aliquot serum into a plastic vial and refrigerate.

**Thyroid Peroxidase Antibodies, Serum**

Thyroglobulin Antibody testing adds slightly to the power of TPO antibody detection in autoimmune thyroid disease and predict risk of future hypothyroidism. This is especially true in iodine deficient areas. The quantitative ELISA for Thyroglobulin antibody correlates well with the previously reported titers from the agglutination assay.


**VITAMIN D 25-HYDROXY**

Effective February 23, 2012, the 25-hydroxy Vitamin D assay has moved from the special chemistry area to the automation area of chemistry where it is performed on the Siemens Centaur XP using a chemiluminescence test methodology. This change allows MLabs to increase the production schedule for this testing to 24 hours per day, 7 days per week, and to reduce the number of draw tubes required when ordered with other automation line serum testing.

Collection Instruction: Collect specimen in an SST tube. Centrifuge, aliquot 0.5 mL (minimum 0.25 mL) of serum into a plastic vial and refrigerate up to 6 days or freeze for longer storage. Red top, lavender top (EDTA) and green top (sodium heparin) tubes are also acceptable.

Reference Range: Optimal: 25 – 100 ng/mL; Deficiency: <10 ng/mL; Toxicity: >150 ng/mL.
MLabs News

U-M DEPARTMENT OF PATHOLOGY NEWS

Congratulations are to be extended to Yali Dou, Ph.D., Assistant Professor of Pathology who has been selected as a Scholar of the Leukemia & Lymphoma Society for a five year period beginning July 1, 2012.

Congratulations are to be extended to Kojo Elenitoba-Johnson, M.D., who received the Outstanding Researcher Award for 2012 from the American Society of Investigative Pathology (ASIP). This award recognizes mid-career investigators who demonstrate excellence in experimental pathology.

David Lombard, Ph.D., Assistant Professor of Pathology and Richard Miller, Ph.D., Professor of Pathology are authors of “Sorting Out Sirtuins,” a News and Views in the February 22, 2012 issue of Nature.

MLabs is pleased to announce that Andrew Muntean, Ph.D. will join the faculty as an Assistant Professor in the Division of Sponsored Research. Dr. Muntean, who is currently a postdoctoral fellow in Dr. Jay Hess’ laboratory, will be working on mechanisms of transformation in acute leukemia and on the development of new therapeutic strategies.

An article in the December 19, 2011 issue of the American Journal of Surgery reports how a collaborative effort between the University of Michigan Departments of Pathology and Surgery and the East Ann Arbor Ambulatory Surgery Center to provide intraoperative frozen section guidance led to a dramatic reduction in reoperation rates for breast cancer patients. The authors included Michael Sabel M.D., Julie M. Jorns, M.D., Angela Wu, M.D., Jeffrey Myers, M.D., Lisa A. Newman, M.D., M.P.H., and Tara Breslin, M.D., M.S. For more information see the article on page 4 of this issue of the MLabs Spectrum.

MLabs is pleased to announce that Scott Tomlins, M.D., Ph.D. will join the Department of Pathology with a joint appointment in the Department of Urology. Dr. Tomlins will be a member of the Michigan Center for Translational Pathology and pursue research in the molecular genetics of bladder cancer and the application of sequencing-base diagnostics to cancer care.