Practical FNA in Lymphoma Diagnosis: What the Clinician wants to Know and How to Get There!

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Presentation Bullet Points

• Diagnosis of lymphoma using WHO Classification
• Grade of Lymphoma
• Proliferation Index
• Transformation
• FISH
• Complementary Papanicolaou and Romanowsky stains
• Immunophenotyping by Cytospin or FCM or both

“An Approach to the Diagnosis of Lymphoma by Fine Needle Aspiration: What the Clinician Needs to Know”

By

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INTRODUCTION

In the past, the role of fine-needle aspiration (FNA) biopsy of lymph nodes was basically to evaluate for metastatic disease; however, over the last two decades there has been a gradual increase in the number of publications documenting the value of FNA in the diagnosis and subclassification of non-Hodgkin lymphomas, particularly in institutions which use cytomorphology in conjunction with ancillary studies. At M.D. Anderson Cancer Center highly accurate lymphoma diagnoses can be made when compared to the corresponding histology, when FNAs are combined with immunophenotyping and Ki-67, and classified by the WHO classification. In many institutions, FNA of lymphomas is used primarily to document residual or recurrent disease or to stage the patient; however, with the advent of the WHO classification for lymphoma combined with sophisticated phenotyping by flow cytometry and molecular studies such as fluorescence-in-situ hybridization (FISH) for non-random chromosomal translocations, it is possible to use FNA as a primary modality for classifying some subtypes of non-Hodgkin’s lymphoma. To some extent, the role of FNA in rendering a primary diagnosis of lymphoma remains controversial. Many of the limitations in diagnosing lymphoproliferative disorders by FNA have been addressed and center particularly on the lack of architecture provided by tissue smears to further subclassify certain lymphomas. In many instances of follicular lymphoma (FL) careful examination of the smear will reveal recognizable follicular structures that may represent the neoplastic follicles of FL. Moreover, the absence of architecture can be overcome to a significant extent with the use of immunophenotyping to assess B-cell and T-cell differentiation, FISH or PCR to assess cytogenetic abnormalities and Ki-67 for proliferation activity as most subtypes of lymphoma have distinctive cytomorphologic, immunophenotypic, molecular and proliferation profiles. However, a subsequent biopsy should always be performed if there is inadequate material, ambiguous results, or if the clinical and radiographic findings are not in keeping with the cytologic interpretation.

This handout emphasizes the most important features that the pathologist performing the FNA should provide in the report, namely:

- Diagnosis by the WHO classification
- Grade of lymphoma
- Immunophenotype
- Proliferation status (Ki-67)
HANDLING OF THE LYMPHOCYTE-RICH FNA SPECIMEN

It is strongly recommended that a standardized approach be implemented in the handling and triaging of FNA specimens that includes cytomorphology along with immunophenotyping and proliferation markers as together they have excellent potential for a cytology-based diagnosis. These lymphocyte-rich, highly cellular specimens are easily obtained and if they are handled according to the schema presented below, more than 90% of the aspirates can be diagnosed accurately.

If a patient presents with a clinical history suggestive of a lymphoproliferative disorder, the pathologist should make adequate preparations before performing FNA. It is important to locate a laboratory that can handle immunophenotyping of FNA specimens using flow cytometry or immunocytochemical stains on cytospin preparations. An adequate portion of the aspirate should always be saved in tissue culture medium (RPMI) or other transport media for immunophenotyping. It is helpful to perform a cell count to determine if there is adequate cellularity for ancillary studies. At MD Anderson Cancer Center we use a Coulter Counter to quantitate the rinsed cells that have been placed in RPMI media until a total of at least 10 million cells have been collected.

When evaluating a lymphoproliferative disorder, one should assess the cellular features and cell composition. The cell size may be small (equal or slightly larger than a normal resting lymphocyte), intermediate (one and one-half times the size of a normal lymphocyte, and no greater than the nucleus of a histiocyte), or large (two to three times the size of a normal lymphocyte). The nuclei may be round, cleaved, or lobulated. The cell population may be monomorphous with one cell type predominating, or polymorphous containing a mixture of cells. Depending on the cytologic evaluation and the clinical history, appropriate ancillary studies can be requested. Our approach to the work-up of lymphoproliferative disorders always includes the following whenever possible:

1. Cytomorphology assessed on Papanicolaou stain (alcohol-fixed) and Diff-Quik (air-dried) smears and classified according to the WHO classification
2. Immunophenotyping by flow cytometry or immunocytochemistry on cytospin preparations
3. Proliferation index determined by image analysis on Feulgen stained slides, or immunostaining with Ki-67 and used in conjunction with morphology to grade lymphomas, or rule out transformation.
4. Genotyping by polymerase chain reaction (PCR) is rarely performed except for non-marking lymphomas, low-grade T-cell lymphomas, partially involved nodes, or equivocal marker studies; cytogentictics, or fluorescence-in-situ-hybridization (FISH) can be used to confirm lymphomas with characteristic chromosomal abnormalities
5. Aspirated material for cultures and special stains (Gomori methenamine-silver, acid fast bacilli (GMS), and gram stain) if an infectious process is suspected

Cytospin preparations for marker studies are made from the aspirated cells that are collected in a preservation medium such as RPMI and concentrated by a density gradient technique. A panel of immunomarkers for small B cell lymphomas should include kappa, lambda, CD3, CD5, CD10, CD19, CD20, CD23, bcl-6 and Ki-67 with additional cytospins made and stored at -80 degrees C in the event that further studies are needed. Immunocytochemistry is performed on air-dried cytospins that are left to air-dry under the hood overnight, and then fixed in acetone for 10 minutes just prior to commencement of immunostaining. Immunophenotyping and proliferative activity can also be determined by flow cytometry. A minimum of 10 million cells is needed for most of these studies, and this amount can usually be obtained in two to three passes.
Flow Cytometric Immunophenotyping (FCI)

Three- or four-color flow cytometric analysis is used on the cells from FNAs rinsed in RPMI, and is an excellent method for evaluating low cellularity specimens (less than 5 million cells). Sensitivity is very high and may detect aberrant cells at 1/1000 to 1/10,000. Cells are stained in the RPMI solution with appropriate antibodies (see below) and the specimens are subjected to whole blood lysis following incubation with antibodies using NH4Cl and washing with PBS. Cells are then fixed with 1% paraformaldehyde and analyzed on a FACScan (Becton-Dickinson) using CELLQuest software (Becton-Dickinson). All antibodies are purchased from Becton Dickinson (San Jose, CA), except for CD10 (Coulter) and FMC7 (Immunotech). The following antibody combinations tailored specifically for FNA usage are as follows: CD3-APC/CD45-PerCP/CD23-PE/FMC7-FITC, CD45-PerCP/CD19-APC/CD10-FITC, CD20-APC/CD45-PerCP/Lambda-PE/Kappa-FITC, CD45-PerCP/CD-11C-PE/CD-22-FITC. Controls are isotype controls using IgG1- or IgG2-PerCP/IgG1-PE/IgG1-FITC, while monocytes are excluded from the gate using a CD45/CD14 combination.

For each tube a minimum of 500,000 cells are incubated with 10 microliters of antibody at 2-8 C for 15 minutes in the dark. The minimal requirement is to have 50,000 cells per tube to analyze 10,000 events and this is better performed if there is an almost pure population of lymphoma cells such as one frequently obtains by FNA. For analysis 10,000 cells are acquired from each tube and lymphoid cells are gated based on CD45 antigen density and right angle light scatter pattern. Results are expressed as the percentage of cells expressing antigen within the gated region.

Disadvantages of FCI compared to immunocytochemistry on slides include poorer assessment of certain nuclear or cytoplasmic markers than by immunocytochemistry, loss of cytoplasm on large cell lymphoid processes resulting in false negative results (need to check forward light scatter on histograms to see if large cell population is present), and no direct correlation between morphology and phenotype, which of course is possible by slide based immunocytochemistry.

Interpretation of the patterns generated by the flow data is critical to accurate interpretation of a malignant versus a reactive population. For an excellent discussion of the applications of FCI the reader is referred to the paper by Jeffrey L. Jorgensen, Cancer Cytopathology, Dec 25, 2005, Vol 105, Number 6.

Proliferation Studies

Proliferation indices can be determined by flow cytometry, image analysis, or immunocytochemistry using Ki-67 % labeling index. DNA image analysis can also determine the DNA ploidy and the proliferative index, which has shown a strong correlation with the grade and outcome of lymphoma. This technique is particularly useful in aspirates that yield low cellularity and can be performed on a single cytospin preparation prepared in a similar manner as for immunocytochemical analysis, but fixed in image fixative and Feulgen stained. At our institution, a cutoff of 26% Ki-67 labeling index is used to separate low-grade versus high-grade lymphoma.

Ki-67 is very helpful in grading follicular lymphoma (see table below).
The distribution of Ki67 (%) by groups of follicular lymphoma.

<table>
<thead>
<tr>
<th>Group of Follicular Lymphoma</th>
<th>N</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>7</td>
<td>10 (2 - 26)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>10</td>
<td>15 (8 - 25)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>21</td>
<td>40 (5 - 90)</td>
</tr>
<tr>
<td>Transformed</td>
<td>14</td>
<td>50 (25 - 90)</td>
</tr>
</tbody>
</table>

**P-value < 0.0001** (Kruskal-Wallis test) between Grades 1 and 2, and Grade 3 and 1

**Reactive Hyperplasia (Chronic Lymphadenitis)**

A number of conditions are associated with characteristic architectural patterns in reactive hyperplasia. Unfortunately, a compartmentalized architectural pattern (follicular versus inter-follicular versus sinusoidal) cannot be assessed by FNA. Usually, the purpose of the FNA is not to determine the specific cause of the reactive hyperplasia, but to exclude malignancy and infectious organisms.

Chronic Lymphadenitis, more commonly referred to as reactive hyperplasia, is the most common diagnosis rendered on lymph node aspirates because most enlarged lymph nodes are benign and reactive. Clinically, chronic lymphadenitis can occur at any age, but is usually seen in children. The cervical, axillary, and inguinal lymph nodes are more often involved, since they drain large areas of the body. Reactive nodes are usually less than 3 cm in diameter; however, they may be larger in children.

**Cytomorphology:** Reactive hyperplasia is characterized by a polymorphous cell population comprising the whole range of follicular center cells (small and large, cleaved and noncleaved), immunoblasts, small lymphocytes, plasmacytoid lymphocytes, neutrophils, tingible body macrophages, and reactive follicular center fragments. These are best recognized at low power on Diff-Quik smears, in which the cells of different sizes cling together, held in place by follicular dendritic cells or antigen-presenting cells. Apoptotic fragments and mitoses may be seen in these cell groups.

**Ancillary Studies:** Immunophenotyping typically shows a polyclonal population with absence of light chain restriction. Reactive T-cells may constitute a large component; however, if they exceed 80%, then a low-grade T-cell lymphoma should be considered.

**Differential Diagnosis:** Follicular lymphoma lacks a well-defined transition of small to large cells; two neoplastic subpopulations, one comprising small to intermediate or large cleaved or centrocytic cells, and a second large centroblastic population, are seen. However this morphology may be complicated by up to 30-50% smaller lymphocytes, representing residual T-cells, that may be present. In general, follicular lymphomas show many more, and larger, atypical centroblasts than even the most florid reactive hyperplasia. Monoclonality for B-cell derivation and CD10 positivity is demonstrated by marker studies. T-cell lymphomas also may contain plasma cells, eosinophils, and epithelioid histiocytes. Demonstration of T-cell derivation may be more difficult because of the bland nature of the cells, and genotyping may be required.

Reactive hyperplasia may be also be confused with marginal zone lymphoma which may present with a mixed cell population of small to intermediate-sized cells with bland histioyte-
LYMPHOMAS

Classification of Lymphomas

In the last decade there has been remarkable progress in the understanding of lymphomas based on laboratory and clinical findings, i.e., morphologic features, immunophenotype, cytogenetic analysis, molecular findings, and clinical manifestations and course. This information has been used to help define entities in the Revised European-American Classification of Lymphoid Neoplasms (REAL) as proposed by the International Lymphoma Study Group in 1994. This classification system categorizes entities based on the neoplasm’s cell of origin. Since the REAL classification has a greater emphasis on cytomorphologic features in conjunction with immunophenotyping and molecular studies, it can be easily applied to aspiration specimens using a multiparameter approach. The WHO classification for lymphomas is similar to the REAL classification for lymphomas (Table 1) with minor modifications as subsequent data has become available. This handout highlights the small B cell non-Hodgkin’s lymphomas which are encountered in everyday practice, and not all the lymphomas in the WHO classification are discussed.

Certain lymphoma entities can be cytologically graded within entities: hence, follicular lymphoma may be graded as Grade 1, 2, 3 based on the number of large transformed cells within follicular structures noted on the smears; mantle cell lymphoma may be defined as classical or blastoid, based on presence of blasts cells in the latter. Diffuse large cell B-cell lymphoma encompasses entities previously known as centroblastic, immunoblastic, T-cell or histiocyte rich, and anaplastic large B-cell.

Burkitt’s-like lymphoma (BLL) defines a lymphoma which formerly fell between the morphological categories of Burkitt’s lymphoma (BL) and diffuse large cell lymphoma, and frequently shared the same translocation of t(8;14), or variant translocations t(2;8) and t(8;22) as BL, or t(14;18) seen in a small subset of diffuse-large cell lymphoma. In order to call a lymphoma BLL by the WHO classification, it is now a requirement that at least 99% of cells be cycling as demonstrated by Ki-67.

NON-HODGKIN LYMPHOMA

Small Lymphocytic Lymphoma

In the WHO classification, B-cell chronic lymphocytic leukemia and small lymphocytic lymphoma are grouped together as mature B cell neoplasms. The majority of cases occur in older adults. Most patients present with bone marrow and blood involvement; however, occasionally they are aleukemic with nodal involvement. Many of the latter cases will eventually develop disease in the bone marrow and blood. The clinical course is indolent, but not curable. It may transform to prolymphocytic lymphoma, large cell lymphoma (Richter’s syndrome), and rarely to Hodgkin lymphoma.

Cytomorphology: Small lymphocytic lymphoma is composed of a monotonous population of small round lymphocytes with nuclei containing a coarsely clumped “checkerboard” chromatin pattern. Scattered in the background are large cells representing prolymphocytes and paraimmunoblasts. Prolymphocytes are slightly larger than paraimmunoblasts, but they both have round nuclei, with prominent nucleoli and gray-blue cytoplasm.
An increase in the numbers of paraimmunoblasts may indicate a more aggressive clinical course than the typical small lymphocytic lymphoma, while a predominance of large cells is indicative of a transformation to large cell lymphoma (Richter’s syndrome).

Ancillary Studies: Immunophenotyping shows light chain restriction and positivity for cell-associated antigens (CD19, CD20, CD79a), CD5, and CD23, while CD10 and FMC7 are negative.

Cytokinetic studies of small lymphocytic lymphoma show a diploid population with low proliferative activity (Ki-67 has a mean labeling index of 5%). In contrast, transformed large cell lymphoma (Richter’s syndrome) has a high proliferation index.

Trisomy for chromosome 12 and abnormalities for 13q14, Lamp1, p53 and ATM gene(11q22-23) may be detected by FISH on cytospin preparations (7).

Differential Diagnosis: Although both small lymphocytic lymphoma and mantle cell lymphoma show aberrant co-expression of CD5 and lack CD10, small lymphocytic lymphoma is positive for CD23, while mantle cell lymphoma is not. Both follicular lymphoma and marginal zone lymphoma are CD5 and CD10 negative. Lymphocyte predominant Hodgkin Lymphoma should be considered if there are polyploid cells (so-called L & H cells or popcorn cells) in a background of mature lymphocytes instead of paraimmunoblasts.

**Mantle Cell Lymphoma**

Mantle cell lymphoma usually occurs in older adults with a predilection for males. At the time of diagnosis, the disease is often widespread and can involve lymph nodes, spleen, bone marrow, blood, and extranodal sites. It is more aggressive than the other B-cell non-Hodgkin’s lymphomas composed of small cells, and has a median survival of 3 to 5 years.

Cytomorphology: Mantle cell lymphoma shows a variety of morphologies ranging from the classic variant, to a pleomorphic variant and a blastic variant. The classic variant shows a monotonous population of uniform, small-to-intermediate sized cells, with slight to marked nuclear membrane irregularities and indentations. The chromatin pattern is fine, giving a lighter appearance to the nuclei, compared with those seen in small lymphocytic lymphoma. Nucleoli generally are small and inconspicuous, and the cytoplasm is scanty. In most cases, minor populations of cells with small round nuclei, as well as cells with angulated irregular nuclei are noted. The cells of the blast variant are intermediate in size and resemble blasts of lymphoblastic T-cell lymphoma. The pleomorphic variant has a range of cell types, including the small angulated cells and larger transformed cells. Monocytoid cells and histiocytes with ingested debris may be seen. Mitoses may be seen.

Ancillary Studies: The cells show positive immunostaining for B-cell associated antigens (CD19, CD20, CD22), CD5, and FMC7, and negative for CD10, and CD23. Usually lambda-light-chain restriction is more common than kappa-light-chain restriction.

Cytokinetic studies of the classic variant usually show a diploid population with low to intermediate proliferative activity. The blastic variant typically is tetraploid, with very high Ki-67 labeling index.

A characteristic chromosomal t(11;14)(q13;32q) translocation has been demonstrated involving the Ig heavy chain locus and the Bcl-1 locus on the long arm of chromosome 11. This translocation results in overexpression of the PRAD/bcl-1 gene that encodes for cyclin D1, a cell-cycle protein. Immunostaining in our laboratory for cyclin D1 on cytospin preparations has been variable but if a cell block is performed, antigen retrieval and immunocytochemistry for Cyclin-D1 antibody will be successful. The polymerase chain reaction detects breakpoints in the major translocation cluster (MTC) region of bcl-1; however, this is positive in only 30-50% of mantle cell lymphomas (74). The t (11; 14) translocation can be demonstrated by FISH in a high percentage of MCL on cytology specimens.
Differential Diagnosis: Differentiating mantle cell lymphoma from small lymphocytic lymphoma and follicular center lymphoma is based on the absence of “blast” cells (paraimmunoblasts and centroblasts) and a homogeneous population of cells. In addition, immunophenotyping is very important in distinguishing the B-cell lymphomas composed on small cells. While both mantle cell lymphoma and small lymphocytic lymphoma are CD5 positive, and CD10 negative, only the latter is positive for CD23. Follicular lymphoma shows CD10 expression. Mantle cell lymphoma may be confused with the centrocyte-like cells of marginal zone lymphoma; however, the latter shows a far more heterogeneous population of cells including monocytoid B-cells, plasma cells, and centroblasts. Like mantle cell lymphoma, marginal zone lymphoma is negative for CD3 and CD10, but it can be differentiated by its lack of CD5.

Marginal Zone Lymphoma

Marginal zone lymphoma is rare, low grade, B-cell neoplasm that has two major clinical presentations, extranodal and nodal. Extranodal marginal zone lymphoma is also known as mucosal-associated lymphoid tissue (MALT) and the majority of these cases have localized disease.

Cytomorphology: There is a heterogeneous population composed of cleaved forms (centrocytes), mature cells, small lymphoid, plasma cells, and monocyteid cells.

Ancillary Studies: Immunophenotyping shows cells positive for B-cell-associated antigens (CD19, CD20, CD22, CD79a), but negative for CD10, CD23, CD103, and CD5 (rare cases are positive).

Cytogenetics: t (11;18) AP12 (apoptosis inhibitor gene); 18q 21 (MLT).

Differential Diagnosis: Reactive processes can be differentiated by demonstration of a polyclonal cell population. By cytology, hairy cell leukemia can be difficult to distinguish from the monocytoid B-cells of marginal zone lymphoma. MZL may occur in the spleen and produce a syndrome of splenic lymphoma, with villous lymphocytes mimicking hairy cell leukemia. Small lymphocytic lymphoma and mantle cell lymphoma can be differentiated by CD5 positivity, while follicular lymphomas are positive for CD10.

Follicular Lymphoma (FL): Cytologic Grades—1 (Predominantly Small Cells), 2 (Mixed Small and Large Cells), and 3 (Predominantly Large Cells)

Follicular lymphomas are B-cell neoplasms composed of a mixture of cleaved follicular center cells (centrocytes) and large noncleaved follicular center cells (centroblasts). FL has the ability to recapitulate the follicular architecture, at least focally; however, diffuse areas may also be present. We use a cytologic classification loosely based on the histologic criteria of Mann and Berard . We initially look for follicular structures in which to base our estimation of cell types. The numbers of large, noncleaved (centroblasts) as a percentage of small centrocytic cells per high power field within a follicular structure are counted. In the absence of follicles, we count representative fields. The presence of confluent areas of large cells may indicate a partial transformation to a higher grade FL.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Follicles cell count %</th>
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<tbody>
<tr>
<td>Grade 1 (n = 31)</td>
<td>9.7 (5.1 – 15.7)</td>
</tr>
<tr>
<td>Grade 2 (n = 46)</td>
<td>24.7 (15.9 – 35.5) *</td>
</tr>
<tr>
<td>Grade 3 (n = 10)</td>
<td>48.3 (37.5 – 60.8) **#</td>
</tr>
</tbody>
</table>
**Cytomorphology:** Follicular lymphoma, Grade 1, is composed predominantly of small cells that are slightly larger than mature lymphocytes. Nuclei have moderately to markedly irregular or wrinkled nuclear contours and inconspicuous nucleoli. Less than 15% of the cell population is composed of large noncleaved cells, which are characterized by large round nuclei with fine chromatin and two to three peripherally placed nucleoli.

Follicular lymphoma, Grade 2 shows an admixture of small cleaved and large cleaved cells, and between 16% and 36% of large noncleaved cells. Small cleaved and large cleaved cells are present in relatively equal numbers.

Follicular lymphoma, Grade 3, shows predominantly large cells (>37%), with irregular nuclear contours, vesicular chromatin, and inconspicuous nucleoli, as well as variable numbers of large noncleaved cells.

**Ancillary Studies:** Immunophenotyping is positive for B-cell-associated antigens (CD19, CD20, CD22, CD79a) and bcl-2 and bcl-6 proteins. There is variable staining for CD10 and CD23. CD5 and CD43 are negative. Cytogenetic analysis usually shows the t(14;18)(q32;21) translocation resulting in the rearrangement of the bcl-2 gene. This is easily performed by FISH on a single cytospin.

**Differential Diagnosis:** The small-cleaved cells of mantle cell lymphoma stain with CD5, and are not CD10 positive. SLL shows CD19/CD5 co-expression and CD23 positive, and CD10 negative. FL Grade 1 cells co-express CD10 and CD19.

**CONCLUSION**

As more pathologists become aware of the potential of FNA diagnosis of lymphoma, when combined with immunophenotyping and adjunctive studies, more of these procedures will be performed. The ultimate beneficiaries of the FNA procedure will be the patients, who, in some instances, may still require a formal surgical excision to subclassify their lymphomas, but will know in advance what to expect, and will have the appropriate staging studies performed without delay. Many patients, however, will not require any further surgical procedure and will be treated according to the FNA diagnosis and the clinical presentation. In the presence of unexplained lymphadenopathy, unresponsive to antibiotics, FNA and ancillary studies of a lymphoid-rich aspirate should become the initial diagnostic procedure. If performed properly with ancillary studies, it will yield valuable diagnostic information that will facilitate appropriate patient management at a lower cost.

**TABLE 1**

**Entities from the updated WHO Classification of Lymphoid Neoplasms that may be amenable to Cytological Diagnosis.**

B-cell Neoplasms
Precursor B-cell neoplasm: Precursor B-lymphoblastic leukemia/lymphoma
Mature B-cell Neoplasms
B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma
Lymphoplasmacytic lymphoma
Plasma cell myeloma/plasmacytoma
Nodal marginal zone B-cell lymphoma
Follicular lymphoma
Mantle cell lymphoma
Diffuse large B-cell lymphoma
Mediastinal large B-cell lymphoma
Primary effusion lymphoma
Burkitt’s lymphoma (includes Burkitt’s like)
T- and NK cell neoplasms
Precursor T-cell neoplasm: Precursor T-lymphoblastic lymphoma/leukemia (precursor T-cell acute lymphoblastic leukemia)
Mature T cell neoplasms
Extra-nodal NK/T-cell lymphoma, nasal type
Mycosis fungoides
Anaplastic large cell lymphoma, T/null-cell, primary cutaneous type
Peripheral T-cell lymphoma, not otherwise characterized
Angioblastic T-cell lymphoma
Anaplastic large cell lymphoma, T/null cell, primary systemic type
Hodgkin lymphoma
Lymphocyte predominance Hodgkin lymphoma
Classical Hodgkin lymphoma
(encompasses nodular sclerosis Hodgkin’s lymphoma (grades 1 and 2), lymphocyte-rich classical HL and lymphocyte-depleted HL)

REFERENCES


Please also see Dr. Katz’ PowerPoint presentation for her further handout information.

**References**


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USCAP, San Diego, 2007
What does the treating clinician need to know from the FNA in order to treat?

Valuable not Redundant procedure!
Indications for Fine Needle Aspiration of Lymphoproliferative Disorders at University of Texas M. D. Anderson Cancer Center

• Diagnosis
  – To firmly establish relapse and cell type
  – For primary diagnosis of selected cases
• To rule out transformation of low grade lymphoma
• To diagnose second primaries, i.e., LCL in patients with previous Hodgkin’s disease, new non-lymphoid primaries
• To obtain tissue for immunophenotyping or FISH
• To clarify stage of disease
• To rule out divergent histologies
• To obtain fresh material for research purposes such as DNA microarrays
What the treating physician needs to know and what the Diagnosis should contain

- Diagnosis of lymphoma, WHO Classification
- Grade of Lymphoma
- Immunophenotype
- Proliferation index
- ? Transformation
How to get sufficient cells!

- Aspiration without suction (French technique) using 25 gauge needle
- **Suction introduces blood!**
- Move needle in rapid iterations up and down 250 times (takes about 90 seconds)
- Look for creamy white tissue arising in hub of needle (Radiologists use same method)
- Place tiny drop to make smears (gently), air dried (Diff Quik) and Alcohol fixed Pap smears
Tricks of the Trade

• Flush most of specimen by reattaching syringe with air to needle and flush into RPMI 1640
• Carry on aspirating and rinsing needle until a minimum of 10 million cells obtained by Coulter Count, hematocytometer or visual inspection. Usually 2-3 passes sufficient
• Examine smears and triage for ancillary studies based on morphology
Establishing a standardized approach to the work up of lymphocyte-rich FNA is Key to Success!
• Complementary Papanicolaou and Romanowsky stains
• Classify by WHO classification
• Immunophenotyping by Cytospin or FCM or both
• FISH on selected cases
MYTH

In order to make a diagnosis of lymphoma, the whole lymph node should be excised and examined histologically.
Lymphoma With “Architecture”

- Follicular center cell
- Mantle cell (follic/diffuse/mz)
- Marginal zone
Cell Counterparts of Follicular, Mantle Zone and Marginal Zone Patterns

MANTLE ZONE PATTERN
- Mantle cell proliferation
- Follicular center cell

NORMAL FOLLICLE
- Mantle cell
- Follicular center cell

MARGINAL ZONE PATTERN
- Mantle zone
- Marginal zone benign/malignant
- Monocytoid B-cell Proliferation
- Follicular center cell
Diffuse large Cell Lymphoma
CLL/SLL

MZ

MCL

MALT

MargZ

GC

BL

DLBCL

FL

P53 INACTIVATION
Methods:

Cell Count (10 million)

- Flow Cytometry
  - Kappa & Lambda
  - B-cell markers
  - CD-19/CD10
  - CD-19/CD5
  - CD5/CD20
- Immunocytochemistry
- Labeling Index (Ki-67)
  - Cytospins
  - Ficoll-Hypaque density gradient
  - FISH
  - DAKO (Ki-67)
  - % of staining 200 cells

Specific markers are performed by either FC/IHC or both depending on each case.
POST–CENTRIFUGATION TUBE
FICOLL GRADIENT PROCEDURE
Ficoll-Hypaque Technique

Standard Centrifugation Technique
Pre-Ficoll Hypaque Pleural Fluid, SLL, Plasmacytoid

Post-Ficoll Hypaque Pleural Fluid, SLL, Plasmacytoid
Immunophenotyping General Statements FCM vs. Cytospins

- High degree of concordance irrespective of method
- Tailored panel vs. larger battery
- Spurious or inconclusive results by both methods if necrotic, or large lymphoma cells with fragile cytoplasm
- Need to interpret results in conjunction with the morphology on smears and the pattern on the flow histogram
Problems with Immunophenotyping
Flow Cytometry

- Gating on wrong populations by FCM (miss a small malignant subpopulation, example;
- Reporting markers on a predominant reactive small lymphocytic subpopulation without noting tight clonal distribution or “trailing” seen in a benign process
- Sampling problems, excessive peripheral blood contamination
Reactive vs. Lymphoma? Note large cells

Kappa

Lambda
Problems in Flow Cytometry Related to Improper Gating

All lymphoid cells regardless of size gated

Net result - looks polyclonal

Small cells

Large cells
FCM With Correct Gating

Gated on larger lymphoid cells

Monotypic Lambda “tight”
IDENTIFY PREDOMINANT CELL TYPE

NON HODGKIN LYMPHOMA

SMALL CELLS
- SMALL LYMPHOCYTIC/PLASMACYTOID
- MANTLE CELL, FOLLICULAR LYMPHOMA GRADE1, MARGINAL ZONE,
- LP HODGKIN’S LYMPHOMA

MEDIUM SIZED CELLS
- LYMPHOBLASTIC
- MANTLE CELL LYMPHOMA, BLASTOID VARIANT
- BURKITT’S LYMPHOMA

LARGE CELLS
- LARGE CELL LYMPHOMA
- IMMUNOBLASTIC LYMPHOMA
- ANAPLASTIC LARGE CELL LYMPHOMA (Ki-1)
WHO CLASSIFICATION B-Cell Neoplasms

Precursor B-cell neoplasm: precursor B-lymphoblastic leukemia, lymphoma

Peripheral B-cell neoplasm
  B-cell chronic lymphocytic leukemia, prolymphocytic leukemia, small lymphocytic lymphoma

Lymphoplasmacytoid lymphoma, immunocytoma

Mantle cell lymphoma

Follicle center lymphoma, Grades 1, 2, 3

Marginal zone B-cell lymphoma
  Extranodal (MALT-type with or without monocytoid B cells)

Hairy cell leukemia
  Plasmacytoma, plasma cell myeloma

Diffuse large B-cell lymphoma

Burkitt’s lymphoma
Lymphomas Composed of Small Cells
Differential diagnosis of Small Cell Lymphomas

- SLL/CLL, B or T cell
- Lymphoplasmacytic lymphoma
- Mantle Cell lymphoma
- Marginal zone lymphoma/MALT/Splenic lymphoma with villous lymphocytes
- Follicular lymphoma
- Lymphocyte rich Hodgkin Lymphoma
- Progressive transformation of Germinal Centers
## Low Grade B-Cell Lymphoma

<table>
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<tr>
<th></th>
<th>Small Cells</th>
<th>Large Cells</th>
<th>Phenotype</th>
<th>FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B-CLL/SLL</strong></td>
<td>Round</td>
<td>Prolymphocyte</td>
<td>CD5+, CD23+, cep 12, p53, LSI13,</td>
<td>LAMP1, ATM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Paraimmunoblast</td>
<td>slg+(k, lambda), CD10-, Co-express</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD5/CD19</td>
<td></td>
</tr>
<tr>
<td><strong>Mantle Cell</strong></td>
<td>Cleaved, notched (+- round/oval)</td>
<td>None</td>
<td>slg+(k, lambda)</td>
<td></td>
</tr>
<tr>
<td><strong>Lymphoma</strong></td>
<td></td>
<td></td>
<td>CD5+, CD10-/+ CD23-</td>
<td>t(11;14) (q13;q32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Co-express</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD5/CD19</td>
<td></td>
</tr>
</tbody>
</table>
Small Lymphocytic Lymphoma

SLL, transforming, prolymphocytes and centroblasts
SLL, paraimmunoblasts
Small Lymphocytic Lymphoma transformed to large cell Lymphoma
(Richter’s Syndrome)

56 YO woman with history of SLL, and recent posterior chest wall mass
Small Lymphocytic Lymphoma transformed to Large Cell Lymphoma (Richter’s Syndrome)

TRISOMY 12, DEL13q14, DEL13q34

TRISOMY 12, DEL13q14, DEL13q34

DEL P53, POLYSOMY OF ATM
Results of FISH Panel for SLL derived from 42 FNAs

**SLL PANEL**

<table>
<thead>
<tr>
<th>GRADE</th>
<th>NO:OF CASES</th>
<th>12</th>
<th>13q14</th>
<th>LAMP1</th>
<th>P53</th>
<th>ATM 11q22-23</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLL LOW GRADE</td>
<td>20</td>
<td>3/20 (15%)</td>
<td>8/20 (40%)</td>
<td>1/20 (5%)</td>
<td>3/20 (15%)</td>
<td>8/20 (40%)</td>
</tr>
<tr>
<td>SLL HIGH GRADE*</td>
<td>22</td>
<td>2/22 (9%)</td>
<td>14/22 (63.6%)</td>
<td>5/22 (22.7%)</td>
<td>11/22 (50%)</td>
<td>8/22 (36.3%)</td>
</tr>
</tbody>
</table>

*SLL High grade, Accelerated phase, Richter’s syndrome

P53 deletion associated with poor survival
Mantle Cell Lymphoma: Cytomorphologic Features

- Monotonous population of small, atypical lymphoid cells
- Slightly irregular eccentrically placed nuclei, condensed chromatin, absent or small nucleoli, scant pale cytoplasm
- Prolymphocytes, paraimmunoblasts, and large noncleaved cells are absent
Morphologic Variants of MCL

- Blastic (blastoid, anaplastic, pleomorphic, Centrocytoid-centroblastic) – important to diagnose
- Lymphoblastic-like cells with very dispersed chromatin, usually inconspicuous nucleoli and a high mitotic rare
- Large cell-like with more prominent nucleoli
- Predominance of small round lymphocytes
Mantle Cell Lymphoma, classic variant
Mantle Cell Lymphoma higher proliferation with tingible body macrophages
Mantle Cell Lymphoma, pleomorphic
Mantle Cell Blastoid Variant
Interphase FISH positive for bcl-1 (t 11;14) performed on Cytospin of Mantle Cell Lymphoma showing Multiple Fusion signals (preferred to Cyclin D1 immunostain)
Marginal Zone vs Mantle Cell

• Features favoring Mantle cell include:
  – Monomorphic cellular population
  – Prominent nuclear irregularities
  – Scattered pink histiocytes
  – Frequent mitoses

• Features favoring Marginal Zone include:
  – Polymorphous cell population
  – Frequent plasma cells
  – Centrocyte-like cells with clear cytoplasm

• Overlapping morphologic features, immunophenotyping is essential
Seventy three year old lady with decreased appetite, fatigue, 30 lb weight loss, night sweats, CT scan showed massive splenomegaly and abdominal lymphadenopathy.

Previous excisional biopsy of lymph node in inguinal region was non-diagnostic. Outside FNA suggested low grade B cell lymphoma.

At MDACC, a periportal lymph node FNA was performed.
Well spaced monotonous small to intermediate lymphoid cells. FCM showed monoclonal kappa, CD19, CD22, CD11c, CD23 dim, negative CD5 and negative CD10, c/w MZL.
Marginal Zone Lymphoma
Spleen, (weight 2390 grams)

Effacement of architecture, and replacement by monotonous population of both medium and large cells
Ki-67, 25% to 50% of cells are positive for Ki-67, and Bcl-2 positive cells are present. Spleen with Marginal Zone Lymphoma, and focal large cell transformation, increased Ki-67 labeling index.
Follow up: Patient developed a large right pleural effusion, hypotension, and renal failure. Pleural Fluid, Large B cell lymphoma transformed from MZL
Ki67, 50%

Monotypic kappa

Pleural fluid, Large cell lymphoma, transformed from MZL. Patient expired due to lymphoma.
Differential Diagnosis of a Polymorphous Mixed Cell Population

- Reactive Hyperplasia
- Lymphoma

- MZL / MALT
- Follicular lymphoma, grade 2
- Peripheral T-Cell lymphoma
- Immunocytoma, (transformed SLL plasmacytoid)
Reactive Lymphoid Hyperplasia, spectrum of lymphoid cells

Mitosis and Apoptosis
Follicular Lymphoma, grade 2, admixture of large centroblasts and smaller centrocytic cells
Reactive Hyperplasia: Germinal center cells, and apoptosis with tingible body macrophages X40

Follicular Lymphoma versus reactive hyperplasia?
Reactive Hyperplasia

Here we rely on cytospin immunophenotyping to resolve the problem...advantage of morphology together with phenotyping and Ki-67.
Florid Reactive Hyperplasia..Pitfall!!
Reactive Hyperplasia:

- Polymorphous infiltrate: small round, small cleaved, small and large non-cleaved
- Plasmacytoid lymphocytes, immunoblasts, tingible body macrophages
- Florid immunoblastic reaction may confuse with large cell lymphoma
- Apoptosis
- Frequently high Ki-67
- Polyclonal, lacks CD10
Follicular Lymphoma, Approach to Diagnosis by FNA
FOLLICULAR LYMPHOMA

- Follicular Lymphoma most prevalent subtype of NHL in the Western Hemisphere, is very frequently aspirated in retroperitoneum
- A B cell neoplasm compared of a mixture of cleaved follicular center cells (centrocytes) and large non-cleaved follicular center cells (centroblasts).
- FL may have a pure follicular pattern or it may have a partial follicular and diffuse pattern on histology
- WHO classification grades FL from 1-3 to reflect the reality of a cytologic continuum
- Grade 3 lymphomas are associated with poorer survival compared to grade 1 and 2, if treated with regimens not containing anthracyclines
Grading of Follicular Lymphoma
Follicular Lymphoma:
Grading on histology according to WHO classification

<table>
<thead>
<tr>
<th>Grading</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>0-5 centroblasts per hpf*</td>
</tr>
<tr>
<td>Grade 2</td>
<td>6-15 centroblasts per hpf*</td>
</tr>
<tr>
<td>Grade 3</td>
<td>&gt;15 centroblasts per hpf*</td>
</tr>
</tbody>
</table>

3a Centrocytes present
3b Solid sheets of centroblasts
# Follicular Lymphoma Grading as Used in Cytopathology*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Centroblast cell count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1 (n = 31)</td>
<td>9.7 (5.1 - 15.7)</td>
</tr>
<tr>
<td>Grade 2 (n = 46)</td>
<td>24.7 (15.9 - 35.5) *</td>
</tr>
<tr>
<td>Grade 3 (n = 10)</td>
<td>48.3 (37.5 - 60.8) * #</td>
</tr>
</tbody>
</table>

* = p < 0.05 vs. grade 1 group  
# = p < 0.05 vs grade 2 group  
+Grading is based on centroblasts within follicles, Easier to perform on Pap smears
FL grade 1

Centroblast count within follicles, 10%

Pap

Ki-67

DQ
FL grade 2

Pap

DQ

Centroblast count within follicles 25%

Ki-67
FL grade 3a

Centroblast count within follicles >50%,
Note residual centrocytes
FL grade 3b

>90% centroblasts within follicles, and forming sheets
Follicular Lymphoma, Grade 3, Transformed from Grade 1 FL

Diff Quik Pap

Kappa Ki-67 80%
Ki-67 = 80.00%
PI = 8.10%, tetraploid, cells
%>5c = 46.0%

Transformed Follicular Lymphoma
The Distribution of Ki67 (%) by Grade of Follicular Lymphoma. A Clinical Outcome Study of 57 Patients (40/57 relapsed FL)

<table>
<thead>
<tr>
<th>Follicular Lymphoma</th>
<th>N</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>7</td>
<td>10 (2-26)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>10</td>
<td>15 (8-25)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>21</td>
<td>40 (5-90)</td>
</tr>
<tr>
<td>Transformed</td>
<td>14</td>
<td>50 (25-90)</td>
</tr>
</tbody>
</table>

SD (P-value < 0.0001) between combined grades 1 and 2, and grade 3 and grade 3 transformed.

Cut off between LG (1 and 2) and HG (3 inc TL) is 25%

No SD between FL1 and FL2 by Ki67.
Kaplan-Meir Estimate for Overall Survival by Ki-67 (N=51), using >/< 40% cut-off

- Ki-67 <= 40 (2/33)
- Ki-67 > 40 (9/18)

p-value = 0.004

- PI <= 2.5 (4/36)
- PI > 2.5 (7/20)

p-value = 0.06
It is possible to determine the grade of FL on an FNA by determining the percentage of centroblasts within the follicular structures. This should be done after clonality has been established by FCM or immunocytochemistry.
FNA

Morphology + Immuno (Flow/ICC) + Labeling Index and grading

WHO

Cytologic Diagnoses = Histologic Diagnoses
When do we request a biopsy?

- Insufficient numbers of cells to do immunophenotyping
- New cases of lymphoma where tissue has never been obtained, is easy to obtain and diagnosis by FNA is unclear
- Composite lymphoma (MZL lymphoma) or when architecture is important for treatment such as mantle zone lymphoma
- For classifying Hodgkin Lymphoma
- For unusual benign lymphadenopathies, such as Castleman’s disease when excision may be curative
- When diagnosis is not straightforward or does not fit clinical history or presentation
- For some suspected low grade T cell lymphomas where gene rearrangement studies are equivocal
Using Multiparameter Testing on FNA, Will Enable The Treating Physician To Have:

- Reasonable approach to first evaluation of lymphadenopathy
- Can be used confirm recurrent disease in patients with lymphoma
- May be used as definitive diagnosis particularly when contraindication to biopsy such as palliative therapy in a compromised patient
- Access to sites such as the retroperitoneum and mediastinum without resorting to open biopsy
Conclusions

• Collection technique and on-site documentation of adequacy (quantity and quality) optimizes FNA Dx outcome

• Case by case allocation of cytologic material for ancillary studies remains critical for successful cytologic evaluation

• Above all, understand the limitations of the technique, and be an advocate for the patient rather than for a particular technique of diagnosis!!