75 Half the Sky in Hematopathology: Diagnosis of Lymphoma With Limited Resources

Rajan Dewar MD
Dennis O'Malley MD
Ranjana Advani MD

2011 Annual Meeting – Las Vegas, NV

AMERICAN SOCIETY FOR CLINICAL PATHOLOGY
33 W. Monroe, Ste. 1600
Chicago, IL 60603
This session will feature an overview of the WHO classification for lymphoma, including the laboratory tools necessary for diagnosing some common hematopoietic disorders. Topics covered will be: resource restrictions in resource-poor countries, how to make a diagnosis of lymphoma in a resource-poor environment, modifying tests for efficient use in resource-poor countries, and management of lymphoma and its effect on diagnostic approaches. Also presented will be the "bare bones" needs to diagnose lymphoma and effectively treat patients in resource-poor countries.

- Recognize the disparities in diagnostic tools used in resource poor countries.
- Maximize the utility of the ancillary techniques and develop a diagnostic approach using limited resources.
- Identify opportunities to curb costs without compromising diagnostic accuracy, based on new approaches applied in resource poor countries.

FACULTY:

Rajan Dewar MD  
Dennis O'Malley MD  
Ranjana Advani MD  
Entire Pathology Team  
Hematopathology  
Hematopathology  
2.0 CME/CMLE Credits

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HALF THE SKY IN HEMATOLOGY
Diagnosis of lymphoma with limited resources

Rajan Dewar MD PhD
Staff Hematopathologist
Beth Israel Deaconess Medical Center
Harvard Medical School
Boston, MA

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HALF THE SKY concept

- 'Women hold up half the sky'
- Hematopathology – Diagnosis of lymphoma
  - Challenges
  - Limited resources

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In our world...

Pathology & Genetics
Tumours of Haematopoietic and Lymphoid Tissues

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

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‘Resources…’

Molecular

FISH

Flow Cytometry

Immunohistochemistry

Good quality - H&E

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Diagnosis of lymphoma with limited resources

Overall approach to the diagnosis of lymphoma

Abnormal Morphology

Diagnosis of Lymphoma

Proof of clonality

Aberrant antigen expression

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The process…

Benign / reactive process

Malignant Lymphoma

Sub-type

Prognosticate

Clinical decision making

***IN A LOW RESOURCE SETTING, THE WORK-UP AND MARKERS ARE DETERMINED BY CLINICAL RELEVANCE***
Outline

- Specific diagnostic entities: Rajan Dewar
  - Distinguishing reactive – Malignant Lymphoma
  - Distinguishing various nodular lymphoma
- Specific diagnostic entities: Dennis O’Malley
  - Distinguishing subtypes of malignant lymphoma
- Clinical framework
  - Dr. Ranjana Advani

Using morphological features as the basis for diagnosis of lymphoma

Predominantly nodular morphology

Case example - using morphological criteria & tools: Follicular lymphoma

- Follicular lymphoma
  - 20% of all lymphoma
  - Highest incidence in USA & Europe
  - Rest of world – much lower incidence
  - Abnormal lymphoid follicles due to failure of apoptosis & not increase in proliferation
  - BCL2 over-expression – KI67 generally low
  - Graded based on Mann-Berard (1-3): proportion of centroblast
  - Clinically significant grades – Low grade (1-2) and High grade (3)
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B-cell lymphoma

CD10 positive
Kappa light chain restricted

- Pre B-Acute lymphoblastic leukemia
- Follicular lymphoma
- Diffuse Large B-cell lymphoma
- Burkitt Lymphoma

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Follicular lymphoma

Diagnosis of lymphoma with limited resources

Mimics of Follicular lymphoma - FRFH
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Polarized follicles

Polarized ‘germinal centers’ – Ki67

Utility of Morphology in distinguishing FRFH & FL

- Follicular lymphoma
- Florid reactive follicular hyperplasia

Nodules
- Back to back
- Interspersed

Tingible body macrophages
- Rare-absent
- Prominent

Polarization
- Rare-absent
- Present

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Mimics of Nodular architecture - 2

Nodular architecture – low power view

Cell shape – non-cleaved; mitotic figures
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High proliferation & atypical mitotic figures

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Nodular lymphoma – ‘pink histiocytes’

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Cyclin D1 – useful adjunct to distinguish Mantle cell lymphoma
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Nodular mimics - 3

Pseudo follicles – ‘polarization centers’

Cell shape – cell type

Paraimmunoblasts of CLL / SLL
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B-cell lymphoma
- CD5 positive
- Kappa light chain restricted
- • CLL/SLL
  • MCL

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Nodular lymphoma -4

Diagnosis of lymphoma with limited resources

RS variants
Diagnosis of lymphoma with limited resources

Binucleate – RS cells

Nodules & sclerosis

Eosinophils at the trailing edge of sclerotic bands
Nodular architecture - 4

Identification of RS cells – key & essential

PAX5 expression in Hodgkin Lymphoma
Diagnosis of lymphoma with limited resources

Utility of PAX-5

<table>
<thead>
<tr>
<th></th>
<th>Hodgkin</th>
<th>NLPHEL</th>
<th>Reactive</th>
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<tbody>
<tr>
<td>CD30</td>
<td>POS</td>
<td>Neg</td>
<td>+/-*</td>
</tr>
<tr>
<td>PAX5</td>
<td>Dim +</td>
<td>Br++</td>
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</table>

* IMMUNOBLASTS

Diagnostic utility of the B-cell lineage markers CD20, CD79a, PAX5, and CD19 in paraffin-embedded tissues from lymphoid neoplasms.

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Sub-typing Hodgkin lymphoma

- Nodules – separated by sclerosis – NS
- Not much lymphocytes/leukocytes (LD)
- Lymphocytes, neutrophils, plasma cells (MC)
- Only lymphocytes (LR)

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Clinically significant entities – Hodgkin camp

- NLPHEL from CHL ***
- LR from NS/MC/LD WHO 2008

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Diagnosis of lymphoma with limited resources

Key take home points...

» Nodular lymphoma diagnosis
  » Differentiate between reactive and lymphoma
  » Polarization, Proliferation and Presence of TBM
  » Clinically significant subgroup
  » Distinguishing Mantle cell lymphoma
    » Mitosis, pink histiocytes, cell morphology
    » BCL1 immunostains

» Hodgkin lymphoma -- diagnosis
  » PAX-5 distinguishes Hodgkin/RS cells from immunoblasts

Dennis P. O’Malley, M.D.
Clarient Inc./GE Healthcare
Aliso Viejo, California, USA
Adjunct Associate Professor
MD Anderson Cancer Center/University of Texas
Houston, Texas, USA
AFRICA:

- Fewer than 50% of cancers are diagnosed by histopathology*
- Presumptive clinical diagnosis (of Burkitt lymphoma) accurate in 75% of cases (but could be as low as 53%)
- Issues: small biopsy size, inadequate preservation, processing, suboptimal staining


WHO Classification, Mumbai

- "Suboptimal quality of tissue in many of the cases"
- "less than optimal workup of some cases in terms of immunostains and molecular studies...mostly due to economic constraints"
- "technical hurdles... small biopsy size, inadequate tissue fixation, suboptimal processing"


Classification

- Clinical Findings
- Morphology
- Immunophenotype
- Genetics
- Prognosis
- Therapy
2008 WHO Classification

★ Excellent job of detailing specific clinico-pathologic entities
★ BUT, what if resources are limited?
  • Classification should still be used, WHEN POSSIBLE
★ Primary decisions are based on serving the patient’s best interests

Does there need to be an alternate classification
★ No, but…..
★ When possible, diagnosis should be made as completely as possible
★ In the context of limited tests available, diagnosis should be especially focused on therapeutic decisions

2008 WHO Classification: Lymphoid B-Cell

“Precursor” (lymphoblastic) considered separately

- CLL/SLL
- B cell Prolymphocytic leukemia
- Splenic B cell marginal zone ML
- Hairy cell leukemia
- Splenic B cell lymphoma, unclassifiable
- Lymphoplasmacytic lymphoma
- Heavy chain diseases
- Plasma cell neoplasms
- Extramedullary marginal zone lymphoma of MALT
- Nodal marginal zone lymphoma
- Follicular lymphoma
- Primary cutaneous FCL
- Mantel cell lymphoma
- Diffuse large B-Cell lymphoma
- T/lymphocyte rich LBCL
- Primary CNS DLBCL
- Primary cutaneous DLBCL, leg type
- EBV+ DLBCL of elderly
- DLBCL assoc with chronic inflammation
- Lymphomatoid granulomatosis
- Primary mediastinal LBCL
- Intravascular LBCL
- ALK+ LBCL
- Plasmablastic lymphoma
- LBCL, HHV8+ from m-Castleman
- Primary effusion lymphoma
- Burkitt lymphoma
2008 WHO Classification: Lymphoid T-Cell

- Precursor (lymphoblastic) considered separately
- T cell Prolymphocytic leukemia
- T cell large granular lymphocytic leukemia
- Chronic lymphoproliferative disorder of NK cells
- Aggressive NK cell leukemia
- EBV positive T cell LPD of childhood
  - Systemic EBV+ LPD of Childhood
  - Primary effusion lymphoma
- Adult T cell leukemia/lymphoma
- Extramedullary NK/T cell lymphoma, nasal type
- Enteropathy type T cell lymphoma
- Hepatosplenic T cell lymphoma
- Subcutaneous panniculitis-like T cell lymphoma
- Mycosis fungoides
- Sezary syndrome
- Primary cutaneous CD30 positive T cell LPD

Primary cutaneous peripheral T cell lymphomas, new subtypes
- Peripheral T cell lymphoma, NOS
- Angioimmunoblastic T cell lymphoma
- Anaplastic large cell lymphoma, ALK positive
- Anaplastic large cell lymphoma, ALK negative

Hodgkin Lymphoma
- Nodular lymphocyte predominant Hodgkin lymphoma
- Nodular sclerosis CHL
- Mixed cellularity CHL
- Lymphocyte-rich CHL
- Lymphocyte-depleted CHL

LIMITED RESOURCES

- Personnel
- Training
- Technology

Cost

Improving Lymphoma Diagnosis
Cost & Lymphoma Diagnosis

- NEEDLE BIOPSY
  - Diagnosis: 77% of cases (sensitivity)
    - With flow cytometry and immunohistochemistry
    - No grading of follicular lymphomas
    - 40% of cases were DLBCL
    - 14 cases of lymphoma

Charges: FNAB/Biopsy

<table>
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<tr>
<th>Procedure</th>
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<th>Radiologist</th>
<th>Surgeon</th>
<th>Anesthesia</th>
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<td>FNAB</td>
<td>$587</td>
<td>$235</td>
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<td>$0</td>
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<td>$235</td>
<td>$348</td>
<td>$488</td>
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The Other Side

- Primary diagnosis: 29% reported correctly using classification system
- 14% non-diagnostic
- Recurrence: 41% reported correctly using classification system
- 9% non-diagnostic
- 6.3% of DLBCL diagnosed correctly
- Sensitivity: 12%
**Tissue Sampling**

**NEEDLE BIOPSY/FNA**
- Small sample
- No architecture
- Lower morbidity
- Requires more ancillary studies for diagnosis
- Cost:
  - Sample: lower
  - Diagnosis: higher

**NODE BIOPSY**
- Large sample
- Architecture present
- Higher morbidity
- Diagnosis without ancillary studies increased
- Cost:
  - Sample: higher
  - Diagnosis: lower

---

**Larger Biopsies**

Which would you rather diagnose?

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**Maximize Resources Available**

- Clinical Findings
- Morphology
- Immunophenotype
- Genetics
- Prognosis
- Therapy
Maximize Resources

- **Therapy/Clinical approach**
  - Optimize communication with clinicians
  - Comparison of *what if* in terms of subclassification and therapeutic choices (more later)

Maximize Resources: Clinical Findings

- Insist on clinical history!
- Site, number of nodes/sites involved, B symptoms.
- IPI (International prognostic index)
  - age in years (≤ 60 versus >60), tumor stage I or II (localized) versus III or IV (advanced); number of extranodal sites of involvement (0 or 1 versus >1); patient's performance status (0 or 1 versus 2 to 4); and serum LDH (normal versus abnormal).
- FLIPI (Follicular lymphoma international prognostic index)
  - factors including age (>60 years versus ≤ 60 years), Ann Arbor stage (II to IV versus I to II), hemoglobin level (<120 g/L versus ≥ 120 g/L), number of nodal areas (>4 versus ≤ 4) and serum LDH level (above normal level versus normal or below)
- B Symptoms
  - systemic symptoms of fever (greater than 38°C), unexplained weight loss (more than 10% body weight in the 6 months before diagnosis, and drenching night sweats are used to define 2 categories for each stage of NHL: A (symptoms absent) and B (symptoms present). The presence of B symptoms is known to correlate with extent of disease (stage and tumor bulk), but symptoms also have been shown to have prognostic significance for cause-specific survival that is independent of stage.

Maximize resources available

- Clinical Findings
- **Morphology**
- Immunophenotype
- Genetics
- Prognosis
- Therapy
Maximize Resources: Morphology

- Morphology
  - Larger biopsies
  - Optimize histologic preparation
  - Optimum fixation
    - Universal fixative? ..... formalin
    - Adequate time in fixative (12-24 hours)
  - Thinner sections
  - Multiple sections
    (consider Giemsa in addition to H&E)

$1 worth of fixative is worth $$$ of special studies

- Optimum fixation is critical in all circumstances
- It is more important in resource limited circumstances, as diagnosis may be
  - Based entirely on morphology
  - May be sent away to other locations for additional studies
- The potential loss of outcome at this earliest step is CRITICAL

Maximize resources available

- Clinical Findings
- Morphology
- Immunophenotype
- Genetics
- Prognosis
- Therapy
DLBCL AND THE 2008 WHO: WHAT DOES SUBCLASSIFICATION COST?

AS Chang, C Guidice, D Chang, TS Barry, S Chen, MK Hibbard, R Chen, DP O’Malley
Clari@nt Inc.
Aliso Viejo, California

Materials & Methods

- 100 large B cell lymphomas evaluated
  - Cases included DLBCL, Burkitt, EBV+DLBCL, plasmablastic, TC/HRBCL, and aggressive B cell lymphoma*
- Panel of IHC stains
  - CD3, CD20, CD5, CD10, BCL2, BCL6, MUM1, Ki67
  - In situ EBV (EBER)
- Panel of FISH studies
  - IgH/BCL2, BCL6 break-apart, C-MYC break-apart, IgH/C-MYC

*Defined for this study as cases with features intermediate between DLBCL and Burkitt

Costs of Tests*

<table>
<thead>
<tr>
<th>Test</th>
<th>SNOMED CODE</th>
<th>COST</th>
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<tbody>
<tr>
<td>Surgical Pathology</td>
<td>88305</td>
<td>$125</td>
</tr>
<tr>
<td>IHC per stain</td>
<td>88342</td>
<td>$115</td>
</tr>
<tr>
<td>In situ stain</td>
<td>88365</td>
<td>$185</td>
</tr>
<tr>
<td>Paraffin-FISH per probe</td>
<td>88274 + 88365 + 88291</td>
<td>$540</td>
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*Costs based on 2009 Medicare fee schedule for Orange County, California
Conclusions, 1:

- Wide variability in costs associated with diagnosis, prognosis and therapy.
- However, difference between expensive work-up (~$2500) and inexpensive work-up (~$1000) are relatively minimal in face of costs associated with therapeutic/prognostic decisions.

The AWP of rituximab for a 10mg/ml 10ml vial is $582.19. Therefore, the medication AWP for a two dose course of therapy at 1,000 mg per course would be $11,643.80.

American College of Rheumatology:  
http://www.rheumatology.org/publications/hotline/0506newdrugs.asp

TIME as a limited resource

- In practice, time can be considered a resource that is exchangeable for cost in some circumstance.
- Immediate decisions which may have a time sensitive nature (ex. promyelocytic leukemia) may require a more rapid turn-around.
- Conversely, if immediacy is not needed then testing can be sequential and selective.
Selective and Sequential Testing

- Testing is performed in a very focused way. A single critical question is "asked"
  - If diagnosis is resolved, then process is finished
  - If diagnosis is unresolved, then add test(s) to resolve next query
  - Longer turn around, but fewer tests leading to less cost

Example Sequential testing: Mantle Cell Lymphoma

- Small cell lymphoma
  - Characteristic morphology
    - YES
    - Diagnosis
    - FISH: t(11;14), B CELL?
      - YES
      - CyclinD1
      - CD5+
      - OR
      - B CELL?
      - YES
      - CD5+
      - OR
      - DIAGNOSIS = MANTLE CELL LYMPHOMA
    - NO
      - T cell
      - DIAGNOSIS = SMALL CELL LYMPHOMA
  - NO
Do I believe that flow cytometry is cost effective for lymphoma diagnosis in a system of limited resources?

YES
- Cost analysis based on an FNA/F.C. system required
- All negative cases would then be triaged for further evaluation based on clinical concern?
- ADVANTAGES:
  - Speed

NO
- No specific findings in addition to morphology/IHC
- Added cost/repeated tests
- DISADVANTAGES:
  - Added cost
  - Repeated tests
  - Additional testing if negative

Low Grade B cell lymphoma
- Chronic lymphocytic leukemia/Small lymphocytic lymphoma
- Extranodal marginal zone lymphoma (“MALT lymphoma”)
- Splenic marginal zone lymphoma
- Nodal marginal zone lymphoma
- Lymphoplasmacytic lymphoma
- Follicular lymphoma, grade 1 & 2

Characteristic Morphologic Features
- Chronic lymphocytic leukemia/Small lymphocytic lymphoma
  - Architecture: Proliferation centers
  - Cytology: Small round cells
- Follicular lymphoma
  - Architecture: follicles!
  - Cytology: small irregular lymphocytes with admixed larger transformed cells
Characteristic Morphologic Features

- Marginal Zone lymphoma
- Lymphoplasmacytic lymphoma
- Mantle cell lymphoma

One Approach to Small B-cell Lymphomas

MORPHOLOGY BIASED EVALUATION

- Monomorphic? No large cells?
- Pseudofollicles? Follicles? Grade?
- MCL, MZL or LPL
Maximize resources available

- Clinical Findings
- Morphology
- Immunophenotype
- Genetics
- Prognosis
- Therapy – COVERED IN NEXT SECTION
OPTIMIZE

- Clinical information
- Optimal fixation
- H&E histology
- Training in lymphoma diagnosis
- Communication with clinical colleagues regarding therapeutic choices

What about Molecular Testing?

- Molecular testing can be cost effective
- However, it should only be used in the context of specific clinical questions
- It should supplement, or replace other ancillary studies for cost-effectiveness

ADDRESSING PRACTICAL DIAGNOSTIC CONCERNS
<table>
<thead>
<tr>
<th>Non-Hodgkin Lymphoma Classification Project</th>
<th>Morphology</th>
<th>+IHC (Increase)</th>
<th>+IHC/clinical (Increase)</th>
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<tr>
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<td>94 (1)</td>
<td>94 (0)</td>
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<tr>
<td>Extramedullar marginal zone lymphoma</td>
<td>84</td>
<td>86 (2)</td>
<td>86 (0)</td>
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<tr>
<td>CLL/SLL</td>
<td>84</td>
<td>87 (0)</td>
<td>87 (0)</td>
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<tr>
<td>LPL</td>
<td>53</td>
<td>56 (3)</td>
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<td>High grade B-cell</td>
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<td>53 (6)</td>
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<td>Medialinal large B-cell</td>
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<td>80 (17)</td>
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<td>Nodal MZL</td>
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<td>63 (0)</td>
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<td>Mantle cell lymphoma</td>
<td>77</td>
<td>87 (10)</td>
<td>87 (0)</td>
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<tr>
<td>DLBCL</td>
<td>73</td>
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<tr>
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<td>PTCL</td>
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WHO Classification, Mumbai

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<th>Diagnosis</th>
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<td>Primary review</td>
<td>70%</td>
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<tr>
<td>Consensus review</td>
<td>82% (+12)</td>
</tr>
<tr>
<td>Added IHC</td>
<td>90% (+8)</td>
</tr>
<tr>
<td>Advanced IHC studies</td>
<td>100% (+10)</td>
</tr>
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</table>

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AREAS of INTEREST

- DLBCL
- Small B cell lymphomas
- Hodgkin lymphoma
- Other diagnoses

Problem Areas

1. Differences in Therapy
2. Differences in Prognosis

Observations

- Points:
  - Emphasizing therapeutic differences:
    - Benign versus malignant
      - Low grade lymphoma, subtypes
      - Identification of mantle cell lymphoma
      - Identification of diffuse large B cell lymphoma
      - Distinction of Hodgkin lymphoma; some variation of subtypes
      - Distinction of T cell lymphomas
**Immunohistochemistry**

- CD3/CD20
  - Lineage assignment
- CD15/CD30
  - Hodgkin lymphoma for difficult cases
  - ALCL
- Cyclin D1
  - Exclude mantle cell in small B cell lymphomas
- Ki67
  - High-grade lymphomas
  - Pattern may allow for additional diagnosis

**Limited Use**

- CD5
- CD23
- CD10
- MUM1
- CD138
- Kappa/lambda

**DLBCL**

- Morphology +
- Limited IHC
  - CD3 and CD20 (lineage identification)

  - Prognostication:
    - Ki67?
    - GC markers: CD10, bcl6?
    - Non-GC markers: MUM1?

**Clues: DLBCL**

- Identify large cell lymphoma as B cell
  - CD20 staining
- Exclude morphologic diagnosis of Burkitt lymphoma
- No other subclassification necessary to "stratify"
PROBLEMS
Small B cell lymphoma
- Evaluation Based on Therapy
  - Mantle cell lymphoma
    - Not "low grade" in clinical behavior
    - Fairly characteristic morphology
    - Distinction should be based on confirmatory added study
  - Limited IHC:
    - CD3, CD20, cyclin D1

Small B cell lymphomas
- FL
- CLL
- LPL
- EMZL

Differences: Small B cell lymphomas
- Follicular lymphoma
  - Morphology
- Chronic lymphocytic leukemia/small lymphocytic lymphoma
  - Morphology, clinical findings
- Nodal marginal zone lymphoma
  - Morphology, exclusion of other types
Clues: Small B cell lymphomas

- Identification as B cells
- Correlation of clinical findings (peripheral blood/bone marrow findings)
  - CLL/SLL, splenic marginal zone lymphoma and lymphoplasmacytic lymphoma
  - Exclusion of mantle cell lymphoma

Follicular Lymphoma

- Older patients, male:female.
- Indolent onset with painless, slow growing adenopathy.
- Often stage IV at diagnosis.
- Cytology: May have varying amounts of centrocytes and centroblasts (grading based on proportion of centroblasts), lack features of reactive follicles
- Pattern: Nodular growth pattern with variable diffuse areas, extend outside node capsule
- CD20+, CD10+, bcl-6+, CD5-, CD21 or CD23 may help to highlight follicular dendritic meshwork
- t(14;18)(q32;q21) in 70-95%

Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

- Older patients, indolent onset, peripheral blood involvement frequent
- Nodes in any site
- Cytology: small mature lymphocytes with dense, cracked earth chromatin, round nuclei; larger cells with large round nuclei, prominent central nucleoli; moderate amounts of cytoplasm (prolymphocytes/paraimmunoblasts)
- Pattern: diffuse effacement of nodes, or extranodal tissue; predominant small, round lymphs; focal pale, nodular areas with ill-defined borders (proliferation centers or "pseudofollicles")
- t(11;14)(q21;q32)
- IHC: CD5+, CD20+, CD23+, Ki-67 hotspots
- FISH: Prognosis
Mantle Cell Lymphoma

- Older patients, M>F, GI involvement frequent, Waldeyer’s ring common
- Cytology: small mature lymphocytes with mature chromatin, irregular nuclei; scattered large pink histiocytes; mitotic figures can be seen
- Pattern: Mantle zone (rare) - associated with better prognosis; nodular or diffuse
- CD5+, CD20+, CD23-, cyclin D1+
- Cytogen/FISH: t(11;14)

Extranodal Marginal Zone Lymphoma (“MALT lymphoma”)

- Usually indolent presentation
- Symptoms depend on site. Sites of involvement include: stomach, pulmonary, ocular, skin
- Cytology often mixed with predominant small cells and variable numbers of large, transformed lymph. Small cells may have increased clear cytoplasm (monocytoid B cells). Plasma cell differentiation is common, and may be prominent
- Histologic pattern may be diffuse of involve residual, reactive follicle + colonization. Lymphoepithelial lesions are common
- CD20+, CD5-, CD10-, CD23-
- Cytogen/FISH: +3, t(14;18), t(11;18), t(3;14), t(1;14)

Nodal Marginal Zone Lymphoma

- Elderly patients with localized adenopathy, indolent growth. One third have more disseminated disease. Females more frequently affected
- Morphologic diversity with small cells, small cleaved cells, plasma cells, monocytoid B-cells and large cells
- Diffuse nodal effacement
- Reactive components
- CD20+, CD5-, CD10-. Will co-express CD43 (30.50%) and bcl-2
- May be able to show light chain restriction
Lymphoplasmacytic Lymphoma
- May present as nodal mass, disseminated disease or leukemia
- Association with Waldenstrom macroglobulinemia
- Infiltrate of small lymphocytes, plasmacytoid lymphocytes, plasma cells.
- May see Dutcher bodies in plasma cells.
- Diffuse or follicular effacement.
- Must exclude other low-grade NHL with plasmacytoid differentiation
- CD20 (variable) and CD79a+, CD5- (usually), CD10- (usually), CD23+/-
- CD43 co-expressed in plasma cells.
- Monoclonal.

Classical Hodgkin lymphoma
- Appropriate morphology:
  - "Classic"
- Ancillary studies
  - Flow of no benefit
  - CD20
    - If strongly positive, then context changes
  - CD30
- CD20+ CD20- CD30+
  * CHL versus other
  CHL
  TCRBCL Not Hodgkin*

Clues: Hodgkin lymphoma
- In straightforward cases (NLPHL, nodular sclerosis CHL, mixed cellularity CHL) NO stains necessary
- In difficult cases:
  - CD30, CD20
  - Rule out T cell rich/histiocyte rich large B cell lymphoma
PROBLEMS
Hodgkin lymphoma versus DLBCL

- Classical Hodgkin lymphoma versus T cell/histiocyte rich large B cell lymphoma
  - Morphology: in most cases, the distinction cannot be reliably made between these two based on histology alone
  - IHC:
    - CD15, CD30, CD3, CD20

PROBLEMS
Classical Hodgkin Lymphoma versus NLPHL

- Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL)
- Distinction between NLPHL and lymphocyte-rich classical Hodgkin lymphoma (LR-CHL) is probably not possible without IHC
NLPHL versus Progressive Transformation of Germinal Centers

- Distinction of NLPHL from a benign mimic progressively transformed germinal centers (PTGC)
- Some significant morphologic clues*
- Limited IHC probably can clarify most cases
  - CD3, CD20
  - CD15, CD30 (to r/o lymphocyte rich-classical Hodgkin lymphoma)

MORPHOLOGY

<table>
<thead>
<tr>
<th>PTGC</th>
<th>NLPHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usually only partial nodal involvement</td>
<td>Usually complete nodal involvement</td>
</tr>
<tr>
<td>Reactive GC present</td>
<td>No reactive GC</td>
</tr>
<tr>
<td>Occasional interfollicular eosinophils</td>
<td>Almost never see eosinophils</td>
</tr>
<tr>
<td>Cells within large nodules are mantle type cells with GC type cells</td>
<td>Nodules include large atypical cells – LP cells – which are neoplastic</td>
</tr>
<tr>
<td>LP cells have rosettes of T cells</td>
<td>LP cells are strongly positive for CD20 and PAX5</td>
</tr>
</tbody>
</table>

Clues: T cell lymphomas

- Identify T cell lineage
- Be familiar with subtypes and prognosis
  - Non-aggressive: mycosis fungoides, ? Others
- Identify anaplastic large cell lymphoma (ALCL)
  - CD30 staining in suspect cases
PTCL
- Clinical suspicion +
- Morphology +
- IHC
  - CD3 and CD20 (lineage and exclusion of B cell)
  - Subclassification difficult without extensive panels
  - Not necessarily relevant to prognosis or therapy

Extranodal NK/T, nasal type
- AKA - Lethal midline granuloma, angiocentric T-cell lymphoma
- Formerly considered a vasculitis
- More common in Asia, South America
- Linked to EBV
- Extranodal, usually midline facial structures
- Aggressive
- Occasional hemophagocytic syndrome

Extranodal NK/T, nasal type
- CD2, CD43, CD56, CD57
- CD5+/–, CD7+/–
- TCR α/β +, TCR γδ –
- EBV LMP +, EBER+
- Extensive necrosis
  - Acute inflammation/abscess
  - Vasculo-centric necrosis
SUMMARY

- Optimize biopsy, fixation, processing, sectioning and H&E morphology
- Optimize training in lymphoma diagnosis
- Appropriate clinical histories
- Limited immunohistochemical staining in clinically relevant cases
- Testing decisions made based on clinical outcomes

Thank you for your kind attention!

domalley@clarientinc.com

"Half the Sky" in Hematopathology
Diagnosis of Lymphoma With Limited Resources
What A Clinician Needs To Know From A Pathologist

Ranjana Advani, MD
Associate Professor
Stanford University Medical Center
Classification of Lymphomas

“Nowhere in pathology has a chaos of names so clouded clear concepts as in the subject of lymphoid tumors.”

R.A. Willis, 1948
Pathology of Tumors

Lymphoma Classification

<table>
<thead>
<tr>
<th>Classification</th>
<th>Years</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rappaport</td>
<td>1960s</td>
<td>morphology</td>
</tr>
<tr>
<td>Lukes &amp; Collins (US)</td>
<td>1970s</td>
<td>morphology, cell lineage</td>
</tr>
<tr>
<td>Kiel (Europe)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working Formulation</td>
<td>1980s</td>
<td>morphology, cell lineage</td>
</tr>
<tr>
<td>REAL/WHO</td>
<td>1990s</td>
<td>morphology, cell lineage, clinical, genotype</td>
</tr>
</tbody>
</table>

WHO Classification 2001

Fits on one slide

<table>
<thead>
<tr>
<th>Precursor cell lymphoma</th>
<th>Peripheral T &amp; NK (15%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoblastic lymphoma, T cell</td>
<td>T-cell granular lymphocytic leukemia</td>
</tr>
<tr>
<td>Lymphoblastic lymphoma, B cell</td>
<td>Aggressive NK cell leukemia</td>
</tr>
<tr>
<td>B-CLL/SLL</td>
<td>Mycosis fungoides/Sézary syndrome</td>
</tr>
<tr>
<td>B-prolymphocytic leukemia</td>
<td>Peripheral T-cell lymphoma, NOS</td>
</tr>
<tr>
<td>Lymphoplasmacytoid lymphoma</td>
<td>Angiomeroblastic T-cell lymphoma</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>Extramedullary NK/T-cell, nasal/nasal-type</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>Entopathy-type T-cell lymphoma</td>
</tr>
<tr>
<td>Extramedullary marginal zone lymphoma</td>
<td>Hepatosplenic pilocytic T-cell lymphoma</td>
</tr>
<tr>
<td>Nodal marginal zone lymphoma</td>
<td>Subcutaneous panniculitis-like T-cell</td>
</tr>
<tr>
<td>Splenic marginal zone lymphoma</td>
<td>Anaplastic large cell lymphoma, syn.</td>
</tr>
<tr>
<td>Lymphomatoid granulomatosis</td>
<td>Anaplastic large cell lymphoma, cutan.</td>
</tr>
<tr>
<td>Hairy cell leukemia</td>
<td>Adult T-cell lymphoma/leukemia</td>
</tr>
<tr>
<td>Diffuse large cell lymphoma</td>
<td>Primary effusion lymphoma</td>
</tr>
<tr>
<td>Burkitt lymphoma</td>
<td></td>
</tr>
</tbody>
</table>
WHO 2008: Large B-Cell Lymphomas

11 Entities
1. Diffuse large B-cell lymphoma, NOS and its subtypes/variants
2. Primary mediastinal large B-cell lymphoma
3. Intravascular large B-cell lymphoma
4. ALK-positive large B-cell lymphoma
5. Plasmablastic lymphoma
6. Large B-cell lymphoma arising in HHV8+ multicentric Castleman
7. Lymphomatoid granulomatosis
8. DLBCL associated with chronic inflammation
9. B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma
10. B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

Mature T and NK Lymphoma

WHO Classification (2008) More entities added

Nodal
- Peripheral TCL, NOS
- Angioimmunoblastic TCL
- Anaplastic large cell lymphoma, ALK+
- Anaplastic large cell lymphoma, ALK-
- Adult T-cell leukemia/lymphoma

Leukemic
- T-cell prolymphocytic leukemia
- T-cell LGL leukemia
- Chronic LPD of NK cells
- Aggressive NK-cell leukemia
- Sezary syndrome

Extranodal
- NK/T-cell lymphoma, nasal type
- Enteropathy-associated TCL
- Hepatosplenic TCL
- Subcutaneous Panniculitis-like TCL
- EBV+ T-cell LPD of childhood
- Hydroa vacciniforme like lymphoma
- EBV+ T-cell LPD of childhood
- Adult T-cell leukemia/lymphoma

Cutaneous
- Mycosis Fungoides
- Primary cutaneous CD30+ T-cell LPD
- Primary cutaneous peripheral gd
- Primary cutaneous CD4+ aggressive
- Primary cutaneous CD4+ small/mume

Current Paradigms in B-cell Lymphomas

- Early pre-B
- Immature B cell
- Mature B cell
- Plasma cell
- Bone Marrow
- Lymph Nodes/Spleen/MALT
- Germinal Center
- Marginal Zone
- Mantle Zone
- Burkitt’s Lymphoma
- Mantle Cell Lymphoma
- Multiple Myeloma
- Early pre-B
- Pre-B
- Immature B cell
- Mature B cell
- Plasma cell
- Bone Marrow
- Lymph Nodes/Spleen/MALT
- Germinal Center
- Marginal Zone
- Mantle Zone
- Burkitt’s Lymphoma
- Mantle Cell Lymphoma
- Multiple Myeloma

- t(14;18)
- BCL-2
- IgH
- t(11;14)
- BCL-1
- IgH
- c-MYC
- BCL-6
- API2-MLT
- t(11;18)
- MALT
- 3q27
- Diffuse Large B-cell Lymphoma
Impact on a Clinician

Resource Rich or Poor
Lymphoma is COMPLEX
- Hard for a clinician to keep up with changing classifications and emerging entities
- Prognostic tools often contain variables that are not clinical
- Therapy getting more complex
- Emerging role of targeted therapy

Diagnosis of Lymphoma with Limited Resources
- What basic information is necessary
  - Impact on selecting primary therapy
- When is additional information necessary
  - Impact on refining primary therapy
- Icing on the cake
  - Academic pursuit, research, unlimited resources
Relative Incidence of NHL Subtypes

≈66,120 new NHL cases in 2008

2011 ASCP Annual Meeting

Fundamental Level:
Need to know B vs T cell

- Rituxan®, MabThera®
  - 1st FDA-approved MoAb for cancer (1997)
  - Standard of care single agent or with chemotherapy for all B cell NHL

Diffuse Large B Cell Lymphoma
**Recommended panel for paraffin IHC:**
- CD20, CD3, CD5, CD10, CD45, BCL2, BCL6, Ki67, MUM1
- OR
- Cell surface marker by flow cytometry: kappa/lambda, CD45, CD3, CD5, CD19, CD10, CD20

**Useful under certain circumstances:**
- Additional IHC for lymphoma subtype
  - Paraffin panel: cyclin D1, kappa/lambda, CD138, ALK, HTLV
  - Molecular: BCL1, BCL2, MYC rearrangements
  - Cytogenetics or FISH: t(14;18), t(3;v), t(8;14)
- **Essential for basic therapy choices**
  - CD20 +, CD3 -, CD5 -, kappa/lambda
- **Potentially useful for refining therapy**
  - CD10, CD30, BCL6, Ki67, MUM1

**Additional IHC for lymphoma subtype**
- CD138, ALK, HTLV, EBV, Oct 1, Rel
- Molecular: BCL1, BCL2, MYC rearrangements
- Cytogenetics or FISH: t(14;18), t(3;v), t(8;14)

**Icing on the cake:**
- Additional IHC for lymphoma subtype: CD138, ALK, HTLV, EBV, Oct 1, Rel
Gene Expression Signatures Predict OS in DLBCL

Icing on the cake!


Immunohistochemical Algorithms and OS in DLBCL

Which model??


Icing on the Cake: DLBCL
One Diagnosis-Multiple Diseases?

Is there therapy which may be better than R-CHOP?
**DA-EPOCH-R in DLBCL: PFS**

Analysis of Biomarkers

N=72, Median age 50 y, Primary Mediastinal excluded, HI/H IPI: 40%

- **GCB (81%)**
  - BCL-2+ (83%)
  - BCL-2- (78%)
  - P=0.81

- **Non-GCB (74%)**
  - BCL-6+ (88%)
  - BCL-6- (68%)
  - P=0.04

**Ongoing US Intergroup Trial**

- **CALGB Phase III Randomized Study of B-CHOP vs DA-EPOCHR with Milotuzumab**

- **NCN Guideline® Version 4.2011**
  - Diffuse Large B-Cell Lymphoma
  - Primary Mediastinal Large B-Cell Lymphoma (PMBL)

  - PMBL can be defined as a context entry presenting with primary site or disease in mediastinum with or without other sites and has histology of DLBCL.
  - Clinical pathological correlation is required to establish diagnosis.

  - **Optimal frontline therapy is more controversial than other subtypes of NHL**

  - **Because of relative rarity of PMBL, the role of R-CHOP 21 is not established as the definitive treatment option for this disease. However, R-CHOP 21 is widely used in NCN in institutions based on data in DLBCL and other regimens have been used (see BCL-6).**

  - **There are data suggesting that more intense therapy may be better based on non-randomized comparisons.**

  - **Role of Rit is controversial. If PET-CT scan negative at the end of treatment, may be observed.**

  - **Residual mediastinal masses are common. PET-CT scan is essential post-treatment.**

  - **Staging of PET-CT scan positive mass is recommended if additional treatment is contemplated.**

2011 ASCP Annual Meeting
Primary Mediastinal DLBCL
Gene Expression Profiling
Todeschini et al. Br J Ca 90:372, 04

Primary Mediastinal NHL
Non Randomized Comparison

Outcome of CHOP-Based Regimens in MYC + DLBCL
MYC in DLBCL
Event-Free Survival: DA-EPOCH-R in DLBCL
Median follow-up 48 months

Mantle Cell Lymphoma

Recommended panel for paraffin section IHC:
CD20, CD3, CD5, cyclin D1, CD10, CD21, CD23, BCL2, BCL6, Ki67

Or
Cell surface marker analysis by flow cytometry:
kappa/lambda, CD19, CD20, CD5, CD23, CD10

Useful under certain circumstances:
Molecular genetic analysis to detect: antigen receptor gene rearrangements; BCL1 rearrangements
Cytogenetics or FISH: t(11;14); t(14;18); CLL panel
Essential for basic therapy choice:
- CD20 +, CD3-, CD5+, cyclin D1+

Useful for refraining therapy:
- Ki67

Icing on the cake:
- Molecular genetic analysis to detect: antigen receptor gene rearrangements; BCL1 rearrangements
- Cytogenetics or FISH: t(11;14); t(14;18); CLL panel

MCL Subtypes:

- Prognosis by Morphology

  - Retrospective analysis of 46 patients (1986–1992)
  - Patients had various treatments
    - CHOP, VAD, DHAP/CHOP, observation

<table>
<thead>
<tr>
<th>Morphologic Pattern</th>
<th>% Total Patients</th>
<th>Treatment CR</th>
<th>3-Year Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mantle Zone</td>
<td>26%</td>
<td>73%</td>
<td>100%</td>
</tr>
<tr>
<td>Nodular</td>
<td>13%</td>
<td>25%</td>
<td>50%</td>
</tr>
<tr>
<td>Diffuse</td>
<td>61%</td>
<td>19%</td>
<td>55%</td>
</tr>
</tbody>
</table>


MCL: Prognosis

- Blastic Variant is an Adverse Prognostic Factor
Refining Therapy
MCL: Proliferation Signature Genes

Proliferation signature is prognostic:
- Translate to alternative platforms
- Additional prognostic information
- Directed therapy


MCL: Prognosis

Ki67: A Prognostic Marker
- Retrospective analyses from clinical trial in MCL Network
- 116 CHOP and 96 R-CHOP pt
- Cells counted (1000) and scored as <10, 10-30, >30% positive
- Differences in OS observed in pts treated with R-CHOP
- Ki67 is not a reproducible marker


OS; Observation Versus Early Treatment

Overall From start of 1st systemic therapy

MCL Prognosis: MIPI Score

Mathematical model includes Ki67

Overall Survival

<table>
<thead>
<tr>
<th>MiPI Risk Score</th>
<th>Low (0-3)</th>
<th>Intermed (4-5)</th>
<th>High (6-11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Points</td>
<td>Age</td>
<td>ECOG</td>
<td>LDH/ULN</td>
</tr>
<tr>
<td>0</td>
<td>&lt; 50</td>
<td>0-1</td>
<td>&lt;0.67</td>
</tr>
<tr>
<td>1</td>
<td>50-59</td>
<td>-</td>
<td>0.67-0.99</td>
</tr>
<tr>
<td>2</td>
<td>60-69</td>
<td>2-4</td>
<td>1-1.49</td>
</tr>
<tr>
<td>3</td>
<td>70+</td>
<td>-</td>
<td>1.5+</td>
</tr>
</tbody>
</table>

Hypothetical Models of Two Different Molecular Subtypes of MCL: Icing on the cake (Sox 11)

Follicular Lymphoma
Recommended panel for paraffin IHC:
CD 20, CD3, CD5, CD 10, BCL2, BCL6, cyclin D1, CD 21 or 23
OR
Cell surface marker by flow cytometry: kappa/lambda, CD23, CD5, CD19, CD 10, CD 20

Useful under certain circumstances:
Paraffin IHC: Ki67
Molecular: BCL 2 rearrangements
Cyto genetics or FISH: t (14;18), t(8;14) or variants

Essential for basic therapy choices:
CD 20+, CD3-, CD5-, CD10+ kappa/lambda

Potentially useful for refining therapy:
Ki 67

Icing on the cake
Molecular: BCL 2 rearrangements
Cyto genetics or FISH: t (14;18), t(8;14) or variants

WHO Histologic Grading of Follicular Lymphoma

<table>
<thead>
<tr>
<th>Grade</th>
<th>Histology</th>
<th>Clinical Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0–5 centroblasts/HPF</td>
<td>Indolent</td>
</tr>
<tr>
<td>2</td>
<td>6–15 centroblasts/HPF</td>
<td>Indolent</td>
</tr>
<tr>
<td>3a</td>
<td>&gt; 15 centroblasts/HPF, centrocytes present</td>
<td>Indolent-Aggressive</td>
</tr>
<tr>
<td>3b</td>
<td>&gt; 15 centroblasts/HPF, centrocytes absent; centroblasts in large sheets</td>
<td>Aggressive (similar to DLBCL)</td>
</tr>
</tbody>
</table>
Indolent Lymphoma: Indications for therapy

- Symptoms of disease
  - cytopenias, pain, SOB, B symptoms
- Tumor burden
  - > 3 LN's larger than 3 cm or a single mass > 7cm
- Impending involvement of critical organ
- Steady progression during a period of observation
- Evidence of histologic transformation
  - rapid progression, elevated LDH, histologic proof
- Patient preference

Observation vs Initial Treatment in Low Grade Lymphoma

BNLI, n=309
Randomized to observation vs chlorambucil
Median f/u 16 years
No difference in overall survival (A) or DSS (B)

Phase III Rituximab + Chemotherapy: FL

<table>
<thead>
<tr>
<th>Chemotherapy</th>
<th>N</th>
<th>Therapy required</th>
<th>% PFS R-CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVP Marcus 2008</td>
<td>321</td>
<td>Y</td>
<td>50% at 3y</td>
</tr>
<tr>
<td>MOP + IFN Herold 2007</td>
<td>196</td>
<td>Y</td>
<td>71% at 4y</td>
</tr>
<tr>
<td>CHOP + IFN/HCT Buze ASH 2008</td>
<td>428</td>
<td>Y</td>
<td>69% at 5 y</td>
</tr>
<tr>
<td>DNP+IFN Salles 2008</td>
<td>359</td>
<td>Y</td>
<td>59% at 5 y</td>
</tr>
<tr>
<td>FND-RM Vito ASH 2008</td>
<td>234</td>
<td>Y</td>
<td>73% at 2 y</td>
</tr>
<tr>
<td>PRIMA (R-CT/RM) Salles ASH 2010</td>
<td>1217</td>
<td>Y</td>
<td>75% at 3 yrs</td>
</tr>
<tr>
<td>FIT Hagenbeek ASH 2010</td>
<td>409</td>
<td>Y</td>
<td>48% at 5 yrs</td>
</tr>
</tbody>
</table>
B Cell NHL: Confusing Terminology

- Follicle center cell lymphoma
- Low grade lymphoma either a CD 10 negative FL or a MZL
- SLL or a cyclin D1 negative MCL
- B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma
- B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

Diagnosis of Lymphoma with Limited Resources

Summary: B cell Lymphoma

- Basic: Is it aggressive or indolent and is CD 20 expressed
  - Aggressive
  - Is it a DLBCL, MCL or BL
  - Indolent
  - Is it FL, SLL or MZL
  - Grade of FL

- Additional:
  - Mediastinal mass: Can this be a primary mediastinal NHL
  - Any features to suggest it may be BL
  - Any features that suggest transformation from an indolent to a more aggressive type

T Cell Lymphoma
Challenges in PTCL

- Rare: 10-15% of NHL so clinical studies difficult
- Multiple subtypes which are morphologically heterogeneous
- Geographic variation
- Lack of immunophenotypic markers for clonality
- Variable clinical behavior
  - Multiple subtypes each with specific characteristics and prognosis
- Suboptimal risk stratification tools
- Outcome generally worse than B cell NHL
- No improvement in outcome in 2 decades
PTCL

Entities where the exact subtype impacts choice of therapy

- ALCL
- ENKT lymphoma

Anaplastic Large T-cell lymphoma

- 2% of NHL
- CD 30 (Ki-1 positive)
- TCR (γ/δ) gene rearrangements 90%
- Frequently t(2;5) and ALK positive
- Undifferentiated carcinomas, Hodgkin's disease and malignant histiocytosis
- Two clinical entities ALK+ and ALK-

ALCL t(2;5): NPM-ALK gene

- Anaplastic lymphoma kinase gene (2p23) fused to several partners, most frequently with Nucleophosmin (NPM) gene (5q35)
- Kutok J. JCO 02
- Stein: Blood 00

ALK gene under control of the NPM promoter
- Results in ALK fusion proteins
  - Bind multiple adapter proteins
  - Linked to diverse pathways
  - Regulate cell proliferation, survival and transformation
**ALCL: ALK + vs -**

- Outcome
- Depends on IPI

**ENKTL: Clinical Characteristics**
- Predominantly EN lymphoma
- High prevalence Asia, Central and South America - 17%
- Median age 43 y, males
- H/o nasal obstruction, discharge, loss of olfaction
- Often midline: nasal cavity/sinuses and nasopharyngeal regions
- Orbital extension
- Extra-nasal sites involved
  - testes, GI, skin, BM
- B symptoms even with localized disease
- Strong association with EBV

**Nasal vs Extra nasal NK Lymphoma**

- International T cell Lymphoma Project
- Nasal does better than Extra nasal
- Stage: Nasal does better
ENKTL: Prognosis

- Overall survival 30-40%
- Unfavorable prognostic factors
  - Advanced stage
  - Invasion of bone or skin
  - Extra-nasal disease
  - High circulating EBV DNA levels/EBV+ cells in BM
- NK-IPI, Korean IPI

L-Asparaginase
Selective Apoptosis of Natural Killer-Cell Tumours
L-Asparaginase in Relapsed NK-T NHL

33 pt CHOP failures treated with L-Asparaginase: CR 51%

PTCL: Cannot depend on any one test

Pathologists and clinicians need to collaborate so that diagnosis is based in context of clinical presentation.

Hodgkin Lymphoma
Pathologic Diagnosis:
Classical Hodgkin’s Disease

Hodgkin’s & Reed-Sternberg cells

Nodular Lymphocyte Predominant
Hodgkin Lymphoma (NLPHL)

Distinct Clinical Entity
- Male predominance: 65-85% cases
- Pediatric and adult (~ median 35 y)
- Asymptomatic, Early stage (70-80% I-II)
- Peripheral nodes - mediastinum spared,
  Non-bulky
- Indolent behavior with late relapses
- Deaths related to treatment (older series)
- Histologic transformation to DLBCL, TCR-BCL (2-15%)

Histopathology
- Nodular architecture
- L & H “popcorn” cells
- CD20+

NCCN Guidelines
When Advances in Therapy are Substantial Certain Tests Become Very Important

**CD 30 expression**

Routine: HL, ALCL

Now: All T Cell NHL, Mediastinal NHL

**SGN35 (Brentuximab Vedotin)**

- Ongoing studies:
  - Upfront treatment for HL
  - Upfront treatment for ALCL
  - CD30+ ref/rel NHL
Limited Resources and the Diagnosis of Lymphoma

There are a variety of reasons why there may be a limit on the resources available to evaluate and diagnose lymphomas. They mostly have to do with cost, although other causes such as lack of information, skills or other specific resources (availability of technology or tests) can be contributing factors as well.

I also like to consider time as one of the resources that can be a limiting factor in the diagnosis of lymphomas. If given an infinite amount of time, we could hold all diagnoses until a disease either progressed, the patient got better or we evaluated the autopsy findings to finalize a diagnosis. Likewise, a frozen section diagnosis is an attempt to balance a shorter amount of time with histologic quality in order to make surgical decisions, an example of limiting this resource.

As such, we are constantly finding a balance between the resources available and the decision we need to make based on them.

OVERVIEW OF PROBLEMS:

It has been noted in several studies from resource poor communities that the problems that plague pathology diagnosis are small biopsy size, inadequate tissue fixation and suboptimal processing. These problems are not limited to the resource poor world and are in fact a problem at all levels of practice. Further, the absence of appropriate clinical history is a frequent area that is a limitation in adequate diagnosis.

Getting to know the clinical needs is an important factor. While the classification of hematolymphoid neoplasms tend to get extensive, and sometimes complex needing more and more newer markers, the real need of the practicing clinician may actually be limited. Thus knowing what the clinician wants is an important determinant. For example, if the ‘Hans classifier’ is needed to prognosticate diffuse large B-cell lymphoma in a given center, then a work up that includes CD10, BCL6 etc., will be needed. But if a morphological diagnosis and grading based on histology is sufficient for the clinician, then this will save resources. Thus knowing the local clinical practice can be paramount. This also highlights the un-uniformity in practice – both by the clinician and the pathologist.

It is in these areas that the most benefit can be gained by improving the pathologic diagnosis in limited resource areas. Obtaining adequate tissue, for diagnosis remains a cornerstone in the diagnosis of lymphoma. Core needle biopsies or fine needle aspirates, while can provide material for flow cytometry and at times diagnostic, can sometimes need extensive work-up, and sometimes not a definitive diagnosis. Tissue adequacy and minimal invasive procedures thus compromise and balance each other.

Turn around time can be an additional factor in the diagnosis of lymphoma. If a pathologist is required to provide a diagnosis in order to institute specific therapy, he/she
is likely to use a shot gun approach, and order many stains or tests upfront. Thus, redundant flow cytometry findings and immuno-histochemistry, which give identical information sometimes may be ordered upfront. On the other hand, a algorithmic panel based approach, will take few additional rounds of test ordering, but conserves resources. So, in the subsequent discussion, I am going to assume that time is NOT a limiting factor in diagnosis in the following way: that a reasonable amount of time may pass, say 1-7 days, before a complete pathologic diagnosis is rendered. In almost all cases, this would be considered adequate in the management of most lymphomas, excluding those that present as medical emergencies.

The cornerstone in the diagnosis of lymphoma is based on a morphologic evaluation of a well-prepared H&E section.

As noted by several publications on the subject, there are locations in the world where this fundamental step is not met for several reasons. It is my sincere hope that improving these individual steps would allow for overall improvement, with minimal resources, to the diagnosis of lymphoma.

BIOPSY:

The quality and size of the tissue sampling are critical. There is some merit to obtaining a cytologic diagnosis, in response to lessening patient morbidity. It may be an appropriate method to triage cases as to: 1) infection or not, 2) carcinoma or not, or 3) possible lymphoma. However, in most cases, by itself, it is quite difficult to adequate subclassify or diagnosis lymphoproliferative disorders by cytologic examination. Further, a cytology based system usually causes an increase in the number of ancillary tests. And so, it is my (biased) conclusion that when possible, an excisional biopsy of tissue should be obtained. A small gauge needle core biopsy has many of the same disadvantages as FNA, not allowing for examination of architecture, thereby increasing the need for ancillary studies for complete classification.

If lymph node biopsy is performed, then the site and type of node chosen are also of import to the diagnosis. In almost all circumstances, the largest, but most superficially placed (to minimize surgical morbidity) should be removed. If there are several sites involved, then inguinal or groin nodes should be avoided as they often have fibrosis which may obscure some architectural details.

FIXATION:

Allow me to be redundant in saying I am fixated on fixation. This is a crucial step in optimizing lymphoma diagnosis. There is no more critical stage as getting freshly excised tissue of an appropriate size into an appropriate amount of fixative for an appropriate amount of time. Or: fresh tissue, thinly sectioned, in a good fixative, for a good amount of time.
The exactitudes of the preceding are where difficulty can arise. If surgeons or other practitioners are removing tissue, they need to have a supply of fixative available for immediate use. While apportionment of fresh lymphoid tissue (for flow, frozen material, etc.) may be at issue in locations where additional testing is available, it is more critical that tissue be well-fixed rather poorly preserved by delay of fixation for ancillary testing. Keep in mind that most immunophenotypic analysis can be done in paraffin and PCR testing can also be done on formalin fixed, paraffin embedded tissue. In my mind, it is the obligation of the pathologist to supply appropriate fixative, in appropriate amounts to doctors removing tissue. Attempts at saving cost by diluting, using very small amounts or reusing fixative will only lead to poor results and cost savings will be lost to ancillary testing.

The appropriate amount of fixative to tissue is \( \textit{at least 10 times the volume of fixative to the volume of tissue present} \). If any sample barely fits in a jar of fixative, then the quality of fixation will be poor. Share this knowledge with any who obtain tissue, as this may not be common knowledge.

Fixation time is dependent on a number of factors, most importantly the type of fixative and the thickness of the tissue to penetrate. Intact lymph nodes should be sectioned to 2-5 mm before placing in fixative, if possible. Penetration of fixative is typically only about 1 mm/hour (a gross estimation). This would suggest that a 5 mm thick slice would take at least 2 ½ hours (it would penetrate from two directions!). However, it should be noted that when tissue is thicker, autolytic processes may already be degenerating the tissue present so that delayed fixation, no matter how thorough, will not improve the quality. So, cut them thin! Fixation time should be at least 4 hours, and if possible, 8 or even 24 hours in a formalin-based fixative.

And while we’re on the subject, my strong opinion is that formalin is really the \textit{universal fixative} for lymphoma evaluation. It is associated with excellent consistent results,, when used in appropriate amounts on appropriate tissue (see above). It is also important in that most ancillary tests (immunohistochemistry, in situ stains) have been optimized for formalin fixed tissues, and that formalin fixed tissues can be evaluated by PCR studies. A number of other fixatives, while perhaps marginally improving the histologic examination, will often interfere with one or more of the possible ancillary tests, making their merit dubious, at best.

\textbf{CLINICAL COMMUNICATION}

The lack of communication between pathologists and surgeons or treating clinicians is not a resource question, but is definitely a worldwide problem. Its remedy is as simple as it is difficult. We as pathologists need to find the time to communicate with our colleagues. If appropriate clinical history is not provided, we need to gently and repeatedly ask the providing physician to provide this information. If it helps, generate a “history form” with check boxes of the most common presenting symptoms.
Likewise, it is our obligation to contact treating physicians if there are problems or difficulties with the diagnosis. If there is a lack of surety, it should be discussed with the clinician as they may have important insights which would bias the pathology results.

SELECTIVE AND SEQUENTIAL TESTING

As mentioned above, time can often be a factor in testing choices. In some practices in order to optimize turn-around-time (TAT), many tests are ordered up front, in order to maximize the number of cases that can be signed without additional studies. In a limited resource setting, in almost all cases, time cannot be considered a critical factor. If it is, then likely more testing will be performed.

It is possible to optimize testing by performing tests sequentially. The basic methodology is to evaluate an initial specimen (either H&E or based on both clinical and gross findings) and ordered minimal testing for answer very specific differential diagnostic considerations. (ex. lymphoid polyps in GI tract – order cyclin D1 to exclude mantel cell lymphoma/Lymphomatoid polyposis). Only if the initial round of testing is negative or non-informative, is additional testing requested.

This testing procedure is dependent on a high level of skill of the operator. That is, the reviewing pathologist must have knowledge of the possible diagnoses, the relevant testing, and the simplest way to distinguish the differential diagnoses. Still, it is incumbent on the pathologist to use either internal or externally established diagnostic algorithms, and by using these, one can optimize the efficient use of special studies.

THERAPY

When speaking of clinical applicability of pathology diagnoses, it is important to realize that resource limited settings raise questions associated with therapy. Frequently, pathologic subclassification may be far more complex than is required by the treating clinicians. It is important to communicate closely with oncologists. If there is no therapeutic difference between two lymphoma types (say nodal marginal zone lymphoma and lymphoplasmacytic lymphoma, for example), then doing additional testing, beyond establishing the diagnosis may not be necessary or even relevant. It is not unreasonable to consider that a diagnosis of “low grade B cell lymphoma:” may be sufficient to establish an appropriate treatment plan for a patient, requiring only minimal ancillary testing.

SPECIFIC DIAGNOSES:

It is beyond the scope of this course or handout to cover all of the diagnostic subtleties of each possibly lymphoma type. Rather, a few highlights will be covered to emphasize certain point in the diagnostic evaluation of lymphoma and how that relates to limited resources.

1) Morphology, morphology, morphology
As mentioned above, optimal histology is the most important diagnostic tool available to the pathologist. Careful examination of the key features of architectural pattern and cytologic features will make many diagnoses more apparent, and reduce the number of ancillary studies necessary.

2) Diffuse Large B cell Lymphoma
DLBCL is, in many cases, a relatively easy morphologic diagnosis. The only absolutely required ancillary study is a CD20 stain to confirm the B cell nature of the neoplastic cells. If this is accomplished, then the therapy in most cases (a CHOP or CHOP+rituximab) is also relatively straightforward. In difficult cases, other studies may be necessary for specific diagnoses. However, the tools associated with prognosis, such as establishing germinal center (GC) versus non-germinal center types (non-GC) are not necessary.

3) Mantle cell and everything else….
In the case of small B cell lymphomas, one key feature is to exclude mantle cell lymphoma. While there are some strong morphologic clues to each small B cell subtype, the presence or absence of cyclin D1 immunohistochemical staining is really a cornerstone to this evaluation.

4) Everything else (small B cell)
Well-established morphologic pattern can be relied upon to accurately distinguish many subtypes of small B cell lymphomas in up to 85% cases. Immunohistochemical studies (or other ancillary tests) are probably not really required, if there is a limitation of resources.

5) Hodgkin lymphoma
One of the more common diagnoses in hematopathology, Hodgkin lymphoma can be quite straightforward in many cases. While in a litigious situation, immunohistochemical stains are probably required, in situations lacking this restraint, morphology can often be relied on to make many diagnoses. If there are diagnostic questions, difficult cases can be resolved with a minimum of studies. Most valuable are CD20 and Cd30 staining. As outline in the lecture slides, this combination allows distinction of the main mimics as follows: CD30+CD20- good for Hodgkin lymphoma; CD30-CD20+ large B cell lymphoma; possible T cell/histiocyte rich large B cell lymphoma; CD30+(strong, uniform)CD20+(weak, variable) good for Hodgkin; CD30- not Hodgkin, CD30-CD20- neither Hodgkin nor usual B cell lymphoma. As a reminder, CD20 is weakly and variably positive in up to 40% of cases of Hodgkin lymphoma. However, strong and uniform CD20 expression is rare in classical Hodgkin lymphoma.

SHORT LIST OF “CHARACTERISTIC FEATURES OF SOME LYMPHOMAS

Chronic lymphocytic leukemia/small lymphocytic lymphoma
Architecture: Diffuse with proliferation centers. Also called Pseudofollicles, these areas are composed of increased numbers of intermediate to large lymphocytes with prominent central nucleoli and small to moderate amounts of cytoplasm. While not seen in every case, their presence is highly characteristic of CLL/SLL
Cytology: Predominantly small round to slightly irregular nuclei with dense chromatin and scant cytoplasm. Invariably, there is another population of
intermediate to large cells (prolymphocytes, paraimmunoblasts) which are described above. Mitotic figures are almost never seen. Immunohistochemistry: In limited diagnosis, proving the combination of CD5 and CD20 with the appropriate morphology is adequate. In select cases, it may be necessary to exclude mantle cell lymphoma, by demonstrating a lack of cyclin D1 staining.
Molecular/Genetic: No molecular or genetic findings are characteristic for CLL/SLL. Some findings are common, including trisomy 12, but can be seen in other lymphoma types.

Mantle cell lymphoma
Architecture: Three patterns: diffuse, nodular or mantle zone (rare).
Cytology: Small irregular lymphocytes with dense mature chromatin and scant cytoplasm. No significant population of admixed large lymphocytes. Background will have scattered single or small clusters of epithelioid histiocytes with ample pink cytoplasm and bland nuclei. Mitotic figures are not usually seen.
Immunohistochemistry: In limited evaluation, a positive cyclin D1 can confirm a diagnosis without other stains, with appropriate morphology. Confirming B cell phenotype by CD20 may also be useful. CD5 expression is not entirely necessary.
Molecular/genetic: If cyclin D1 immunohistochemistry is not available, evidence of the characteristic t(11;14) (BCL1/IGH) by FISH can be of benefit in confirming the diagnosis.

Follicular lymphoma
Architecture: Nodal architecture is effaced by a proliferation of abnormal follicular structures. These lack tangible body macrophages, polarization and well-formed mantle zones. They are often relatively uniform in size, with a back-to-back arrangement.
Cytology: The cytologic composition is predominantly small round to irregular lymphocytes with dense chromatin and scant cytoplasm. There are varying numbers of admixed, larger transformed lymphocytes (e.g. centroblasts). Mitotic figures are rare, but can be seen.
Immunohistochemistry: As architecture is often distinctive, excluding a major differential of reactive follicles form neoplastic is often necessary. In this case if follicles are positive for bcl2, this is more supportive of a follicular lymphoma compared to follicular hyperplasia. Likewise, a low proliferation rate by Ki67 is more supportive of follicular lymphoma versus follicular hyperplasia. If the distinction form other lymphomas is necessary, bcl6 is good at identifying small cells as being of follicles center origin.
Molecular/genetic: Demonstration of the (14;18) of follicular lymphoma by either FISH studies or PCR can confirm a diagnosis, although is not necessary for confirmation, and is not present in a subset (5-20%) of cases of follicular lymphoma.

Extranodal marginal zone lymphoma (“MALT lymphoma”)
Architecture:
Cytology:
Immunohistochemistry: There is no immunophenotype that is specific for NMZL. It will lack CD5, cyclin D1 and bcl6 staining.
Molecular/genetic: No characteristic molecular or genetic defects are present.

Nodal marginal zone lymphoma
Architectural:
Cytology:
Immunohistochemistry: There is no immunophenotype that is specific for NMZL. It will lack CD5, cyclin D1 and bcl6 staining.
Molecular/genetic: No characteristic molecular or genetic defects are present.