CURRENT CHALLENGES IN LYMPHOMA DIAGNOSIS AND CLASSIFICATION

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R.A. Willis (1948):
"Nowhere in pathology has a chaos of names so clouded clear concepts as in the subject of lymphoid tumors."

Centroblastic-centrocytic lymphoma
Nodular poorly differentiated lymphocytic lymphoma
Small cleaved cell lymphoma

But the Working Formulation caught on as a popular classification worldwide in the 1980's and early 1990's, although it was intended only for facilitating translation of terminologies among the different classifications.
Problems of the Working Formulation

- Most of the categories are heterogeneous
- Disregards immunophenotype (B, T, NK)
- Delineation of prognostic groups was based on survival data from patients treated in 60's and 70's. Clinical outcome may have changed nowadays
- "New" entities continue to be recognized

The International Lymphoma Study Group (ILSG)

Revised European-American Lymphoma (REAL) Classification

- List of distinctive clinicopathologic entities that can be recognized with available techniques
- Commonly used terms are retained
- A new paradigm in lymphoma classification: "Biologic" entities defined by clinical, morphologic, immunophenotypic and genotypic features

Peripheral B cell neoplasms

- B-cell SLL/CLL/PLL
- Lymphoplasmacytoid lymphoma
- Mantle cell lymphoma
- Follicle center lymphoma
- Marginal zone B cell lymphoma
  - Extramedullary (MALT)
  - Nodal*
- Splenic marginal zone B cell lymphoma*
- Hairy cell leukemia
- Plasmacytoma
- Diffuse large B cell lymphoma
- Burkitt's lymphoma
- High grade B cell lymphoma, Burkitt-like*

Peripheral T cell and postulated NK cell neoplasms

- T-cell CLL/PLL
- Large granular lymphocyte leukemia
  - T cell or NK cell type
- Mycosis fungoides
- Peripheral T cell lymphoma unspecified
- Angioimmunoblastic T cell lymphoma
- Angiocentric lymphoma
- Intestinal T cell lymphoma
- Adult T cell lymphoma-leukemia, HTLV1+
- Anaplastic large cell lymphoma, T & null-cell
- Anaplastic large cell lymphoma, Hodgkin-like*
The ideal classification

Should be:
- Scientifically accurate
- Reproducible
- Clinically relevant

How well does the REAL Classification fare?

- No problem with scientific accuracy (based on published data)
- Multi-centered retrospective study: reproducibility and clinical relevance validated by a study on 1,378 cases contributed by 9 institutions from 8 countries (NHL Classification Project, Blood 1997;89:3909-18)
- The entities do show distinctive clinical features (e.g. age, sex, stage) and behavior
- Better separation of outcome than previous classifications

DNA MICROARRAY

- DNA microarray studies (analyzing tens of thousands of genes simultaneously) are providing exciting new information to enhance the understanding of lymphomas

The REAL Classification was modified into W.H.O. Classification 2001

Widely accepted and used classification

![Graph showing overall survival over years for different grades of lymphomas](image)
Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling

- Analysis of gene expression profiles (signatures) of diffuse large B-cell lymphomas reveals two groups:
  - Germinal center B-like
  - Activated B-cell-like

5-yr overall survival
76% vs 16%

Use of molecular profiling to predict survival after chemotherapy for diffuse large B-cell lymphoma
Rosenwald A, et al

Traditional approach

Newer approach

CAC meeting, Airlie House 2007
New WHO Classification of lymphomas

THE DILEMMAS

• Are there entities in the WHO classification that are “impure” that require splitting?
• Are there entities that are biologically closely related that perhaps should be lumped together (e.g. extranodal NK/T-cell lymphoma and aggressive NK cell leukemia)?

Immunophenotype?

• Some lymphoma types still lack distinctive immunophenotypic profiles (e.g. marginal zone B-cell lymphoma, peripheral T-cell lymphoma)
• Not all cases of those lymphoma types with specific immunophenotype show the typical profile

THE DILEMMAS

• Entities defined by:
  – Clinical features (including site of disease)
  – Morphology
  – Immunophenotype
  – Genetic/ molecular features

But which is the most important parameter?

Cyclin D1 expression in mantle cell lymphoma

• Cyclin D1 expression, while characteristic of mantle cell lymphoma, is not 100% specific:
  – Rare cases of B-CLL can be positive
  – A proportion of cases of myeloma and hairy cell leukemia are positive
• Cyclin D1 expression is also not a pre-requisite for diagnosis of mantle cell lymphoma, since microarray studies have shown that ~7% of mantle cell lymphomas are cyclin D1 negative
Cytogenetic features?

- Many lymphoma types lack distinctive cytogenetic features.
- For lymphoma types with specific cytogenetic features:
  - Only variable proportions of cases show the changes.
  - One type of lymphoma may show several different types of cytogenetic alterations.

Extranodal marginal zone B-cell lymphoma of MALT

- At least four distinctive types of chromosomal translocations are identified:
  - t(11;18)(q21;q21): API2/MALT1 ~30%
  - t(1;14)(p22;q32): BCL10/IGH
  - t(14;18)(q32;q21): IGH/MALT1 ~15%
  - t(3;14)(p14.1;q32): FOXP1/IGH

<table>
<thead>
<tr>
<th>Gene Translocation</th>
<th>Stomach</th>
<th>Lung</th>
<th>Thyroid</th>
<th>Salivary gland</th>
<th>Eye</th>
<th>Skin</th>
<th>Liver</th>
<th>Breast</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(11;18)</td>
<td>24-35%</td>
<td>0-1%</td>
<td>0-5%</td>
<td>0%</td>
<td>0%</td>
<td>0-2%</td>
<td>0%</td>
<td>0%</td>
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<tr>
<td>t(14;18)</td>
<td>36-56%</td>
<td>0-7%</td>
<td>7-19%</td>
<td>12-22%</td>
<td>24-37%</td>
<td>14-33%</td>
<td>100%</td>
<td>0%</td>
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<tr>
<td>t(1;14)</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>?</td>
</tr>
<tr>
<td>t(3;14)</td>
<td>0%</td>
<td>0%</td>
<td>57%</td>
<td>?</td>
<td>?</td>
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Gene expression profile?

- In recent years, gene expression profiling studies have provided an enormous amount of information on lymphoma, especially for diagnosis and prognostication.
- While promising, it is unclear how the test will perform outside the research laboratory setting.
- Most diagnostic laboratories currently do not have facilities to perform microarray assay.

Examples

- Diffuse large B-cell lymphoma 33%
  - Nodal
  - Extranodal
Diffuse large B-cell lymphoma: Morphologic subclassification

- While some studies report immunoblastic lymphoma to have a worse prognosis than centroblastic lymphoma, many studies report no difference
- Greatest problem: Lack of reproducible morphologic criteria to distinguish between the two

Are there better ways to delineate entities within this waste-basket category?

Proposed types of diffuse large B-cell lymphoma in new WHO classification

- Diffuse large B-cell lymphoma, unspecified
- Primary mediastinal large B-cell lymphoma
- T-cell/histiocyte-rich large B-cell lymphoma
- Intravascular large B-cell lymphoma
- Plasmablastic lymphoma
- Diffuse large B-cell lymphoma associated with chronic inflammation
- Primary DLBCL of the central nervous system
- Primary cutaneous DLBCL, leg-type

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Newly separated out
Primary mediastinal large B-cell lymphoma:
Distinctive clinical features vs LBCL unspecified

- Young adults (median 37 yrs vs 64 yrs)
- Female predominance (M:F = 1:2 vs 1.2:1)
- Often bulky disease >10 cm (52% vs 30%)  
- Marrow involvement very rare (3% vs 17%)
- LDH often raised (75%), but β2-microglobulin not raised

Mediastinal large B cell lymphoma: Pathology

- Common histologic features:
  - Prominent sclerosis
  - Clear cells
- Distinctive immunophenotype:
  - Commonly lacks surface Ig expression
  - Deficient MHC molecule expression  
  - CD23+ in 70%
  - MAL+

Mediastinal large B cell lymphoma: Pathology

<table>
<thead>
<tr>
<th></th>
<th>Mediastinal LBCL</th>
<th>DLBCL unspecified</th>
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<tbody>
<tr>
<td>BCL6 rearrangement</td>
<td>4 - 6%</td>
<td>35%</td>
</tr>
<tr>
<td>BCL6 point mutation</td>
<td>10% - &gt;50%</td>
<td>&gt;50%</td>
</tr>
<tr>
<td>BCL2 rearrangement</td>
<td>5%</td>
<td>20 - 30%</td>
</tr>
<tr>
<td>MAL gene overexpression</td>
<td>70%</td>
<td>Rare</td>
</tr>
</tbody>
</table>

Gene expression profiling studies show greater similarity to classical Hodgkin lymphoma than conventional large B-cell lymphoma!
No wonder there are:

- Composite tumors of classical Hodgkin lymphoma + large B-cell lymphoma of mediastinum (synchronous or metachronous)
- Grey zone lymphomas (borderline cases sharing features of both)

**BURKITT LYMPHOMA**

- An oncologic emergency!
- Most rapid doubling time among tumors!

**Burkitt lymphoma: Immunogenetics**

- Pan-B+
- CD10+, Bcl-6+
- Bcl-2-
- Ki67 index approaches 100%
- t(8;14) or variants, with translocation between C-MYC and Ig gene
Atypical Burkitt lymphoma

- Morphologically resembles Burkitt lymphoma, except:
  - greater variation of nuclear size and shape
  - nucleoli more prominent and fewer in number

But distinction between atypical Burkitt lymphoma and diffuse large B-cell lymphoma can be difficult and non-reproducible!
Even experts don't perform too well in diagnosis of Burkitt lymphoma based on morphology, immunophenotype +/- information on MYC.

Burkitt lymphoma

- Some cases of molecular BL may deviate from the prototypic profile
  - Up to 21% of cases can express bcl-2 (although often weak)
  - Up to 34% of cases have Ki67 index <95%
  - 9% of cases lack MYC translocation
Molecular diagnosis of Burkitt lymphoma yields a more precise definition of this entity than do current diagnostic criteria.

Practical issues in diagnosis
- Using currently available techniques, we are likely to misdiagnose some cases of Burkitt lymphoma as large B-cell lymphoma (or vice versa).
- In future, application of antibodies against gene products shown to identify molecular Burkitt lymphoma may help.

B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)
- The most common chronic leukemia of lymphoid lineage.
- Mostly old patients.
- Patients are asymptomatic or present with symptoms of anemia or lymphadenopathy.
- Typically indolent course, but the disease can be more aggressive in some patients.

B-CLL: Prognostication
- Cases with unmutated Ig gene fare much worse than those with mutated Ig gene.
- Immunostaining for ZAP-70 can serve as a surrogate marker for the unmutated group.
How to deal with the category “B-CLL”?

• One disease with two subtypes?
  – Mutated Ig gene
  – Unmutated Ig gene (worse prognosis)

• Two separate diseases with different histogenesis?

ENTEROPATHY-TYPE T-CELL LYMPHOMA

• A tumor of intestinal intraepithelial T lymphocytes
• Presentation: small intestinal mass/ucler, often multifocal. Intestinal perforation, obstruction or hemorrhage.
• Relationship with celiac disease
  – Long history
  – Short history of adult celiac disease
  – History of dermatitis herpetiformis
  – No history of celiac disease

Enteropathy-type T cell lymphoma: Pathology

• Cytology: Usually large cells, but can be small or medium-sized
• Epitheliotropism is common
• Some cases may have many admixed eosinophils and histiocytes
• Surrounding mucosa: villous atrophy, crypt hyperplasia, intraepithelial lymphocytosis (i.e. features of celiac disease)
Enteropathy-type T cell lymphoma: Immuno-genetic features

- T-lineage markers positive, but CD5 often -
- CD103+ (marker for intraepithelial T lymphocyte)
- Commonly CD4- CD8-, but some are CD8+
- CD56 can be positive (usually monotonous medium-sized cells, and often CD8+)
- EBV negative

Two histogenetic types of enteropathy-type T-cell lymphoma

<table>
<thead>
<tr>
<th></th>
<th>CD56+</th>
<th>CD56-</th>
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<tbody>
<tr>
<td>Frequency</td>
<td>20%</td>
<td>80%</td>
</tr>
<tr>
<td>Monomorphic histology</td>
<td>73%</td>
<td>7%</td>
</tr>
<tr>
<td>Clinical evidence of celiac disease</td>
<td>0%</td>
<td>32%</td>
</tr>
<tr>
<td>Enteropathy histology</td>
<td>50%</td>
<td>85%</td>
</tr>
<tr>
<td>CD8+</td>
<td>80%</td>
<td>19%</td>
</tr>
</tbody>
</table>
90% Celiac disease-associated HLA haplotype
80% +5q34-q35
73% +1q32-q41
27% +8q24
67% +9q

CD56- CD56+
+9q 67% 67%
+8q24 73% 27%
+1q32-q41 20% 73%
+5q34-q35 20% 80%
Celiac disease-associated HLA haplotype 50% 90%

Practically never seen in Asians!

What to do with the entity “enteropathy-type T-cell lymphoma”?
• To split into two separate entities?
  – Classical enteropathy T-cell lymphoma: association with celiac disease, CD56-
  – Monomorphic intestinal T-cell lymphoma: should not carry the prefix ‘enteropathy’, CD56+
• Still maintaining one category with two subtypes?
  Adopted for the time being. WHO does not want premature creation of “entities” because of ICD coding issues

CURRENT LYMPHOMA DIAGNOSIS
• Clinical, morphologic and immunohistochemical information are still important
• But increasingly genetic/molecular data may be required to support or fine-tune the diagnosis -- of course, molecular studies have to be used judiciously and interpreted in the appropriate context; otherwise the information can be misleading rather than helpful

Fluorescence in situ hybridization (FISH)
• For detection of chromosomal translocation, FISH shows much higher sensitivity and specificity than PCR
• For example, Burkitt lymphoma vs large B-cell lymphoma, use FISH to look for:
  – C-MYC rearrangement (preferably MYC-IG)
  – Lack of BCL2 and BCL6 rearrangement

Burkitt lymphoma: FISH
Break-apart probe of C-MYC

DNA microarray: now and the future?
• While DNA microarray is a powerful tool, the reproducibility issues have not been adequately addressed, and the technology is still very expensive
• In future, perhaps analysis of a smaller number of discriminatory genes (mRNA) by RT-PCR or a scaled-down version of microarray assay, or proteins (immunohistochemistry) may suffice for diagnostic purposes