Update of WHO Classifications of Tumors of the Lymphoid Tissues

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Learning Objectives

1. Overview of previous and current hematopoietic and lymphoid tumors classifications
2. Touching on new and controversial entities and clarification of current diagnostic criteria
3. New ancillary studies (e.g., immunohistochemical markers and genetic signatures) helpful in working up a diagnosis
4. Highlights probable changes/additions in the next edition of the “BLUE BOOK” tumor classification
Introduction
How did we get here

• “Consistent with the trends we see in the larger world where governments and major corporations adhere to international standards, we have moved from multiple competing classifications to just one generally acceptable classification”

• While some bemoan the complexity of our modern classification and long for earlier times, one should realize that this field has actually never been simple

Courtesy of Dr. Steven Swerdlow, U. Pittsburgh
Milestones 1960s-1980s


Tumours of the hematopoietic and lymphoid tissues

• Gall and Mallory classification
• Rappaport classification
• Kiel classification
• Working Formulation
• French-American-British (FAB) system
• Lukes-Collins classification
• Revised European-American classification of Lymphoid neoplasms (REAL)
Classifications

The REAL Classification:


The “WHO ERA” 3rd and 4th editions:


Developed jointly by the Society for Hematopathology (SH) and the European Association for Haematopathology (EAHP).

The work of a large number of hematopathologists along with the advice and consent of clinicians.

The WHO classification emphasized the identification of distinct clinicopathologic entities, rather than just being a "cell of origin" classification.

The concept was that one should incorporate key available information including morphology, immunophenotype, molecular and cytogenetic findings and clinical features.

Referred to as “integrated approach” to disease definition.
Small B-cell lymphoproliferative disorders
Monoclonal B-cell (MBC) Lymphocytosis

• B-cells ≤ 5 x 10⁹/L
• Monoclonal:
  • K:L < 0.3:1 or > 3:1
  • Do not fulfill CLL criteria: Absence of features/symptoms of other lymphoproliferative disorder, cytopenia or autoimmune disease
• MBCL can be seen in up to 12% of adults with normal blood counts (CLL-type)
MBCL Subtypes:

**CLL-like (70%)**
- CD5+, CD20 (dim), Ig (dim)

**Atypical CLL (15%)**
- CD5+, CD20 (bright) or CD23-

**Non-CLL (15%)**
- Phenotype/Genotype suggestive of splenic lymphoma:
  - CD49d+/CD38-, CD10-, 20% CD5+ weak
- 80% develop lymphocytosis more than 4 X 10⁹/L
- BM involvement (sinusoidal)
- 72% aberrant karyotypes: del(7q) 15%, t(7q) 13%, i(17q)
- 17% progression with splenomegaly and other lymphoma (MZL)

**Low count (<50/µl):**
- Very low risk of progression
- No monitoring required

**High count (500-5000/µl):**
- High risk cytogenetic alterations (5-9%)
- Annual progression requiring treatment 1-2%
- Require clinical monitoring

Dagklis et al, Blood, 2009
Karube et al, Sem Cancer Biol 2014
Xochelli A et al, Blood, 2014
MBCL: nodal counterpart

- Atypical cells with CLL phenotype in otherwise reactive LN
- Absence of proliferation centers
- Normal size or slightly enlarged (< 1.5cm nodes)
- Some regressed

Gibson SE et al, Haematologica, 2011
Histologic progression in CLL (clinical significance of proliferation centers)

- Additional factors affecting CLL survival:
  - Expanded/ confluent proliferation centers
  - High proliferation (Ki67 >30%)
  - Del(11q) 25%, del(17p) 16% and ?t(14q) 13%
- Survival intermediate between CLL and Richter transformation
- Histological criteria not standardized

M Ciccone et al, Leukemia, 2012
New markers in CLL/SLL

**LEF1** (Lymphoid Enhancer binding factor 1)

- Transcription factor, WNT/β-catenin pathway
- Role in lymphopoiesis
- Normally expressed in some T and pro-B cells but not mature B cells.
- 100% expression in CLL, not in other small B-Cell LPD (total of 290 B-LPD)
  - 38% in DLBCL
- 88% Neg for β-catenin expression

B Tandon et al, Modern Path, 2011
Tonsils

Richter’s

B Tandon et al, Modern Path, 2011
New markers in CLL

CD200

- A transmembrane type Ia glycoprotein
- Expressed in various cell types (B cells, activated T cells, thymocytes, endothelial cells and neurons) as well as CLL, HCL and FL
- Negative/ dim in MCL but positive in 24% indolent SOX11-Neg MCL

challagundla et al, AJCP, 2014
New markers in CLL

CD49d

- Integrin family (α subunit), surface molecule
- Promotes microenvironment-mediated proliferation of CLL leukemic cells
- Prognostic value independent of CD38/ZAP70
- Predictive value for B-Cell receptor targeted therapies?

Bulian et al, A Soci C Onc, 2014
Role of MYD88 mutation: Lymphoma with plasmacytic differentiation

- Crucial adaptor protein in IL-1, IL-8 and TLRs signaling pathway
- **L265P**, most common mutation, detectable in FFPE tissue
- Useful information in the differential diagnosis
- Interpret with caution

<table>
<thead>
<tr>
<th>Condition</th>
<th>MYD88 Mutation</th>
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<tr>
<td>&gt;90% Waldenstrom M/ LPL</td>
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<td>&gt;50% IgM MGUS</td>
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<td>70% LBCL (CNS &amp; Testis)</td>
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<td>29% DLBCL-ABC</td>
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<td>7-9% MALT</td>
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<td>0-10% SMZL</td>
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<td>0-24% nMZL</td>
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<td>3% CLL</td>
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Landgren & Tageja, Leukemia, 2014
Early & In-Situ Follicular Lymphoma

“in Situ” FL

- Incidental finding
- Low incidence of progression (<5%)
- Need to exclude systemic lymphoma

Partial involvement by FL

- 50% progress to overt FL

<table>
<thead>
<tr>
<th>FLIS</th>
<th>PFL</th>
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<tr>
<td>Architecture intact</td>
<td>Altered architecture</td>
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<td>Folicle size normal</td>
<td>Folicle size often expanded</td>
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<td>Involved follicles widely scattered</td>
<td>Involved follicles grouped together in LN</td>
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<td>Intact cuff with sharp edge to GC</td>
<td>Blurred edge to GC and attenuated cuff</td>
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<tr>
<td>Very strong expression of BCL2 and CD10</td>
<td>BCL2 and CD10 more variable in intensity</td>
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<tr>
<td>Almost pure centrocytes</td>
<td>Centrocytes with few centroblasts</td>
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<tr>
<td>Atypical cells confined to GC</td>
<td>Atypical cells (CD10+/BCL2+ B cells), may be found outside the GC</td>
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</table>

Table Diagnostic features of FLIS and PFL

Jegalian et al, Blood, 2010
Adam et al, Am J Surg Pathol, 2005
IRF4 rearrangement (MUM1)

- Interferon regulatory factor 4 (IRF4) is a transcription factor protein encoded by the IRF4 gene located at 6p25-p23
- Presentation: Waldeyer’s ring, head and neck nodal and bowel
- Most commonly in children/ young adults
- Germinal center phenotype (CD10/bcl6)
- Bcl2 expression but no t(14;18)
- Strong IRF4 expression and IRF4 translocation
- Distinct entity within both DLBCL (most) and FL 3b (some)
- Treatment is often required but good prognosis

Salaverria et al, Blood, 2014
Mantle cell lymphoma
Indolent Variants

- In situ MCL, Mantle zone pattern, low proliferation index (SOX11+/-)
- Non-nodal subtype of MCL
  - Leukemic disease/ splenomegaly
  - SOX11-
  - May transform into blastoid MCL (TP53 mutation)

Jares et al, J Clin Inv, 2012
Large/ Aggressive B-cell lymphoproliferative disorders
WHO 4th ed Diffuse Large B-cell Lymphomas (DLBCL)

- Diffuse large B-cell lymphoma, not otherwise specified
- DLBCL associated with chronic inflammation
- Lymphomatoid granulomatosis
- Primary mediastinal (thymic) large B-cell Lymphoma
- Intravascular large B-cell lymphoma
- ALK+ DLBCL
- Plasmablastic lymphoma
- Primary effusion lymphoma
- Large B-cell lymphoma arising in HHV8-associated multicentric Castleman Disease
Classification should have a greater emphasis on the importance of cell of origin (COO)

- Many clinical trials are based on COO
- GCB and ABC/ non-GCB
- IHC algorithms should be used and stated in the diagnosis
- Move to mRNA gene expression based platforms soon
- Now COO is important for prognosis and probably will be important for treatment choices in the future

Clinical subtypes (CNS, leg-type) are typically non-GCB/ABC type

- Clinical types are important and take precedence over COO
- Again, COO should be stated if known
Double hit Lymphoma (DHL) Clinical Advisory Committee

- Consensus that DHL is defined as translocation of MYC and BCL2 or BCL6
- Cases may have different morphology:
  - B-cell lymphoma, unclassifiable with features intermediate between BL and DLBCL, (60-67%), BCL-U
  - DLBCL (31-35%)
  - Lymphoblastic lymphoma (20%)
  - FL (2%)
- DLBCL morphology predicts better outcome (OS = 3 yrs Vs. 4 mon (p˂0.00001))
- FISH for BCL2 and MYC in all cases is preferred by clinicians
  - May not be a practical recommendation
  - Two steps might be a better approach (IHC:MYC, BCL-2 and Ki67)
- Controversy is where DHL should be classified (DLBCL or BCL-U)
  - Clinicians prefer a new category for both DLBCL and BCL-U with DH

20-30% of DLBCL are MYC+ BCL2+ by IHC (double expression)

Not all have MYC/BCL2 rearrangement

IHC for MYC and BCL2 (cutoff 40%)

Worse prognosis than non double expression cases

Not as bad as genetic “double hit”, figure1
FIGURE 2. OS and PFS among randomized patients stratified by MYC IHC and morphology.

Cook et al, Am J Pathol 2014
DHL and BCL-U: WHO update, current thinking

WHO 4th ed:

- B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma (BCL-U)
  - Cases morphologically resembling BL but that don’t “fit” (morphology, immunophenotype, genetics [DH])
  - Includes but not limited to DH cases; does not include DLBCL-DH

WHO 4th ed Update:

- High grade B-cell lymphoma, NOS
  - High grade B-cell lymphoma with MYC and BCL2 or BCL6 rearrangements (“double hit”)
    - Specify whether DLBCL or BCL-U morphology
    - If FISH or Cytogenetic is pending, sign out as “B-cell lymphoma with high grade features, pending genetic studies”
    - Cases of FL or LBL with DH are not included in this category
  - High grade B-cell lymphoma, NOS
    - Cases with BCL-U morphology or other high grade features and no DH
Burkitt Lymphoma: ID3 mutation (new tumor suppressor gene)

- Inhibitor of DNA binding-3 (ID-3) protein regulate normal cellular development.
- Mutations in ID3 (alone) seem unlikely to have a clear oncogenic role in most cancers.
- MYC deregulation along with inactivating ID3 mutations might have a role by significantly amplifying the actions of these oncogenes in BL.
- Wild-type ID3 in BL decrease cell proliferation (potential therapeutic approach)

Leucci et al, J Pathol, 2008
MYC Negative Burkitt lymphoma

- Rare
- Younger than 40
- More frequently with nodal presentation
- Morphologically and phenotypically similar to BL
- All high grade morphology
- Complex karyotype
- 11q aberrations may be seen. *Figure*
MYC Negative Burkitt lymphoma
- Rare
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- Complex karyotype
- 11q aberrations may be seen. *Figure*

? New variant: “Burkitt-like lymphoma with 11q aberrations”
CHL-DLBCL
Gray Zone Lymphoma

WHO 4th ed:

- Chapter acknowledged that non-mediastinal cases can occur, but their features were not described in details

WHO 4th ed Update:

- Studies (Eberle et al, Modern path, 2011): 33 cases
  - Patients with mediastinal disease were significantly younger, M>F
  - Genetic: aberrations (2p16.1, 9p24.1 and 8q24) were observed (33%, 55%, 27%)
  - Genetic aberrations were seen more with mediastinal involvement (Similar survival)
  - About half resemble CHL and other are of a DLBCL
- Two subtypes now distinguished: mediastinal Vs. non-mediastinal
  - ?No change in name
Histopathologic NLPHL variants according to Fan et al.:

**Pattern A:** B cell–rich nodular, tumor cell–rich case with high numbers of LP cells in the nodules

**Pattern B:** Serpiginous/interconnected

**Pattern C:** Nodular with prominent extranodular LP cells

**Pattern D:** T cell–rich nodular pattern

**Pattern E:** Diffuse T cell/histiocyte–rich B-cell lymphoma–like pattern

**Pattern F:** Diffuse B-cell rich, moth-eaten, pattern.
Nodular Lymphocytic Predominant Hodgkin Lymphoma (NLPHL), variants

Histopathologic NLPHL variants according to Fan et al.:

**Pattern A**: B cell–rich nodular, tumor cell–rich with high numbers of LP cells in the nodules

**Patterns A&B = 75%**

**Pattern B**: Serpiginous/interconnected

**Patterns C-F = 25%**

**Pattern C**: Nodular with prominent extranodular LP cells

**Pattern D**: T cell–rich nodular

**Variant patterns associated with:**
- High stage (p=0.0012)
- IPS score (p=0.0005)
- Early relapse (p=0.0009)
- But not worse overall survival (p=0.1751)

Hartmann et al, Blood, 2013
“Diffuse” NLPHL Vs. THRLBCL

• Distinction may be difficult:
  • Some make a diagnosis of relapsed NLPHL with diffuse pattern
  • Others make diagnosis of progression to THRLBCL

Clinical Advisory Committee:

• Distinction is clinically important (NLPHL with diffuse areas has worse prognosis but not as bad as de novo THRLBCL)
• NLPHL with histological progression or diffuse areas should not be classified as THRLBCL

WHO 2008 Updates:

• Diagnosis of THRLBCL should be restricted to primary/de novo cases
• Occurrence or relapse of NLPHL with a partially or entirely diffuse pattern should be called either diffuse LPHL or “NLPHL, THRLBCL-like”
• Careful search for focal NLPHL important in de novo cases of THRLBCL (One nodule of NLPHL rules out THRLBCL)
Plasma Cell Neoplasms
WHO 4th ed Update

- IMWG genetic testing recommendations (2009):
  - Recommend that at a minimum baseline genetic information should be obtained in all PCM cases.
Plasma Cell Neoplasms
WHO 4th ed Update

• New provisional category: “PC neoplasm with associated paraneoplastic syndrome”
• TEMPI (telangiectasias, elevated erythropoietin/ erythrocytosis, monoclonal gammopathy (IgG MGUS), perinephric fluid collections, intrapulmonary shunting)
• Dramatic responses to Bortezomib

Sykes, NEJM 2011
T/ NK Cell Lymphoproliferative Disorders
Primary cutaneous CD4 positive small/medium T-cell Lymphoma

- Was provisional in 2008
- Vast majority of patients have an isolated single lesion
- 75% head and neck area
- Follicular helper T cells derived neoplasm, may express markers (PD1, CXCL13 and BCL6)
- TCR gene rearrangement (monoclonal)
- Excellent prognosis following simple excision
- Only patient with multiple lesions had an aggressive clinical course

Grogg et al, Mod Pathol, 2008
Primary cutaneous CD4 positive small/medium T-cell Lymphoma

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Proposal: Primary cutaneous CD4+ T-cell LPD (not lymphoma)

Grogg et al, Mod Pathol, 2008
Enteropathy associated T-cell lymphoma. Type I & II are distinct

EATL I:

- Northern European
- Celiac disease
- Medium – Large sized
- CD5-, CD8-/+, CD4-, CD103+
- Usually αβ
- Frequently +1q and +5q

EATL II:

- Asian and Hispanic
- Medium sized cells with clear cytoplasm, epitheliotropic
- CD56+, CD8+, CD4-, MAT kinase +
- Usually δγ
- 8q24(myc) amplifications

Tan SY, et al. Leukemia 2013
Enteropathy associated T-cell lymphoma. Type I & II are distinct

**EATL I:**
- Northern European
- Celiac disease
- Medium–Large sized
- CD5−, CD8−/+, CD4−, CD103+
- Usually αβ
- Frequently +1q

**EATL II:**
- Asian and Hispanic
- Medium sized cells with clear cytoplasm, epitheliotropic
- CD56+, CD8+, CD4−, MAT kinase +
- Usually δγ
- 8q24(myc) amplifications

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EATL II = Monomorphc epitheliotropic intestinal T-cell lymphoma γδ

Tan SY, et al. Leukemia 2013
T & NK cell lymphoma of the Gastrointestinal Tract

- EATL, classical (αβ)
- Monomorphic epitheliotropic intestinal T-cell lymphoma (γδ)
- Extranodal NK/T EBV+ (Mainly Asian)
- PTCL, NOS (α, γδ or TCR silent)

All Clinically Aggressive
Indolent GI T-cell OR NK cell LPD of low malignant potential

Features:

- Superficial infiltrate confined to mucosa. No invasion of the wall (non destructive)
- Very low proliferation rate.
- Bland infiltrate (may show atypia).
- Do not response to chemotherapy
- Most commonly affects colon and small bowel

Perry et al, blood, 2013
Mansoor et al, blood, 2011
Indolent T-cell LPD of the GI tract

Perry et al, blood, 2013
Indolent CD8+ lymphoid proliferation of the ear

- Dense monomorphous medium-sized, non-epidermotropic clonal proliferation
- Treated with local radiotherapy or excision
- Local recurrence in some but no progression
- Also involves other acral cutaneous sites (face, nose, etc)

Indolent CD8+ lymphoid proliferation of the ear

- Dense monomorphous medium-sized, non-epidermotropic clonal proliferation
- Treated with local radiotherapy or excision
- Local recurrence in some but no progression
- Also involves other acral cutaneous sites (face, nose, etc.)

**Primary cutaneous acral CD8+ T-cell lymphoma**

TABLE 2. Immunohistochemical Results

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<tr>
<th></th>
<th>CD20</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>CD2</th>
<th>CD5</th>
<th>CD7</th>
<th>CD30</th>
<th>TIA-1</th>
<th>Granzyme B</th>
<th>CD56</th>
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<td>ND</td>
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Hepatosplenic T-cell lymphoma γδ

- New recurrent mutations:
  - STAT5B (33%); STAT3 (10%)

- Signal transducer and activator of transcription (STAT) factors 3&5:
  - Implicated in lymphocyte development, survival and growth
  - STAT5 activation has been linked to T-cell development and homeostasis, as well as to the initiation of γδ T-cell differentiation.

Nicolae et al, Leukemia, 2014
Others:

- T-LGL (STAT3 (40%); STAT5B (2%))
- T-ALL (JAK1, JAK3, STAT5B (subset))
- T-PLL (STAT5B (36%))
- γδ TCL (STAT5B (33%); STAT3 (8%))
- EATL II (STAT5B (36%))
- NK TCL (STAT3 (6%); STAT5B (6%))
ALK negative ALCL

• No longer a provisional entity
• Should have very similar morphology and phenotype as ALK+ ALCL
• Patients older than ALK+
• Worse prognosis
• Required: cohesive growth pattern with hallmark-like cells. Strong and uniform CD30 expression
• Desirable: EMA+, cytotoxic+, sinusoidal growth, loss of “T-cell antigen”

Castellar et al, blood, 2014
ALCL, ALK-: DUSP22 & TP63 rearrangements

Castellar et al, blood, 2014
Breast implant associated anaplastic large cell lymphoma. Long term follow up in 60 patients

- 93% CR in patients with disease confined to the capsule
- 72% CR in patients with a mass, *Figure*
- No difference in OS or PFS in patients who had chemotherapy
- Recommend: implant removal with capsulectomy

WHO 2008

• 4th Edition is now over 7 years old and out of date

• Multiple meetings of editors (2012-2014)
  • March 2014, Chicago: Clinical Advisory Committees (Lymphoid and Myeloid)
  • December 2014, Chicago: Myeloid editor/advisors meeting
  • March 2015, Boston: Editors/advisors meeting
  • Mid April 2015: Authors invited to update chapters

• Update to 4th edition will be allowed
  • Available both online and in print and eBook
  • Anticipated in 2016
What to expect

• New significant information published over the last 7 years related to existing entities will be incorporated.

• ?New entities

• ?Shocking changes

• Classification should simply serve to:
  • Codify practices
  • Bring on a consensus about potentially controversial topics
  • Provide a basis for guidelines.
References:

Small B-cell LPD:


Aggressive B-cell LPD:


References:

T and NK-cell LPD:


