The updated (2008) WHO lymphoma classification

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Why do we need a lymphoma classification?

- There are several subtypes of lymphoma
- Not all lymphomas share the same clinical behaviour and prognosis
- Treatment depends on specific histologic features
- Allows the comparison of clinical trial results
- Essential to study etiology, pathogenesis, risk factors, and epidemiology of lymphomas
Requisites of a classification

- Easy to apply
- Minimal intra- and interobserver variability
- Must give relevant clinical information relating to pathogenesis and prognosis
- Must be validated in prospective studies
- Must be a dynamic process that can integrate clinical (prognosis, therapy) and pathological advances (immunology, genetics)
Evolution of lymphoma classification

- Rappaport (considered cytological and architectural features)
- Lukes and Collins (immunophenotype)
- Kiel Classification (Europe)
- Working Formulation (USA)
- REAL Classification (1992)
- WHO classification (2001)
- Update of WHO classification (2008)
Kiel Classification

- Cytologically based scheme based on the characteristics of the neoplastic cells (centrocytes-centroblasts)
- Attempted to reflect the stage of lymphocyte maturation and the putative cell of origin
- Led to confusion in certain areas (i.e., mantle cell lymphomas)
- Inflexibility (extrapolation to extranodal lymphoma was incorrect)
- Poorly reproducible and misleading in the classification of T-cell lymphomas
The Working Formulation

- Originally designed to stratify lymphomas according to **clinical outcome** (attempt to compare results from clinical trials)
- Subsequently used as classification (requested by clinicians)
- Most categories were heterogeneous
- Poor reproducibility
- Did not include T-cell lymphomas
REAL Classification (Revised European-American Classification of Lymphoid Neoplasms)

- Based on the consensus of a group of 19 expert hematopathologists
- Used data from published literature (did not reflect personal opinions)
- Focused on „real disease“ and incorporated principles of the Kiel Classification and of the Working Formulation
- Identified entities on the basis of morphological characteristics supported by immunophenotype and genetic features
## WHO Classification

<table>
<thead>
<tr>
<th>2001</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic myeloproliferative diseases</td>
<td>Myeloproliferative neoplasms (including mastocytosis)</td>
</tr>
<tr>
<td>Myelodysplastic/myeloproliferative disease</td>
<td>Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of</td>
</tr>
<tr>
<td>Myelodysplastic syndromes</td>
<td>PDGFRA, PDGFRB or FGFR1</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>Myelodysplastic/myeloproliferative neoplasms</td>
</tr>
<tr>
<td></td>
<td>Myelodysplastic syndromes</td>
</tr>
<tr>
<td></td>
<td>Acute myeloid leukemia (AML) and related precursor neoplasms</td>
</tr>
<tr>
<td></td>
<td>Acute leukemia of ambiguous lineage</td>
</tr>
</tbody>
</table>
WHO Classification

2001 = 2008

- Precursor B- and T-cell neoplasms
- Mature B cell neoplasms
- Mature T-cell and NK neoplasms
- Hodgkin lymphoma
- Immunodeficiency associated lymphoproliferative disorders
- Histiocytic and dendritic cell neoplasms
B-cell differentiation

Precursor B-lymphoblast

Bone marrow

Precursor B lymphoblastic Leukemia/lymphoma

Naïve B-cell (sIgM e sIgD, CD5+)

Bone marrow, peripheral Blood, primary lymphoid follicles

Mantle cell lymphoma

ANTIGEN
Germinal center neoplasms

Centroblast
Somatic mutations
bcl2-, bcl6+

Centrocyte
Bcl6+, CD10+

Follicular lymphoma
DLBCL
Burkitt (from memory cells?)
Hodgkin lymphoma

ANTIGEN
Memory B-cell
sIgM, CD5-, CD10-, IRF4+, MUM1+

Marginal zone
Marginal zone B cell
Lymphoma, CLL/SLL, DLBCL

Plasma cell
CD79a+, CD138+, CD20-
marrow
Plasmacytoma
Lymphatic follicle with germinal center
- Centroblasts (BCL2 -) and their progeny are susceptible to death through apoptosis.
- BCL6 mutation serve as a marker of cells that have been through the germinal center.
Principles of the WHO classification

1. Morphology
2. Immunophenotype
3. Molecular biology
4. Genetic
5. Clinical presentation and course

I love pathologists who can diagnose lymphomas without immunohistochemistry!
## Immunophenotype

<table>
<thead>
<tr>
<th></th>
<th>CD5</th>
<th>CD23</th>
<th>CD10</th>
<th>Cyclin D1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Marginal zone lymphoma</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Immunohistochemistry can indirectly detect specific translocation

- Bcl-2, t (14;18)
- Cyclin D1, t (11;14)
- ALK, t (2;5), t (1;2)
Immunohistochemistry is laboratory specific

- Tissue preservation
- Fixative
- Tissue pretreatment
- Antibody (clone)
- Immunohistochemistry method
- Evaluation method
- Subjective interpretation
Molecular biology

FISH
# Chromosomal translocations in B-NHL

<table>
<thead>
<tr>
<th>Lymphoma Type</th>
<th>Chromosomal Alteration</th>
<th>Oncogene Involved</th>
<th>Mechanism of Oncogene Activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular</td>
<td>t(14;18)(q32;q21)</td>
<td>BCL2</td>
<td>Transcriptional deregulation</td>
</tr>
<tr>
<td>MALT</td>
<td>t(11;18)(q21;q21)</td>
<td>API2/MALT1</td>
<td>Fusion protein</td>
</tr>
<tr>
<td></td>
<td>t(1;14)(p22;q32)</td>
<td>BCL10</td>
<td>Transcriptional deregulation</td>
</tr>
<tr>
<td></td>
<td>t(14;18)(q32;q21)</td>
<td>MALT1</td>
<td>Transcriptional deregulation</td>
</tr>
<tr>
<td>Mantle cell</td>
<td>t(11;14)(q13;q32)</td>
<td>BCL1</td>
<td>Transcriptional deregulation</td>
</tr>
<tr>
<td>B-DLCL</td>
<td>3q27 translocations</td>
<td>BCL6</td>
<td>Transcriptional deregulation</td>
</tr>
<tr>
<td>Burkitt's</td>
<td>t(8;14)(q24;q32)</td>
<td>c-MYC</td>
<td>Transcriptional deregulation</td>
</tr>
</tbody>
</table>
# Chromosomal translocations in T-NHL

<table>
<thead>
<tr>
<th>Lymphoma Type</th>
<th>Chromosomal Alteration</th>
<th>Oncogene Involved</th>
<th>Mechanism of Oncogene Activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaplastic large cell</td>
<td>t(2;5)(p23;35) t(1;5)(q25;p23) t(2,3)(p23;q35)</td>
<td>ALKNPM</td>
<td>Fusion protein</td>
</tr>
</tbody>
</table>
MALT1 (18q21) Dual Color, Break Apart

Normal interphase cell

MALAT1 rearrangement

18q21
Potential pitfalls in microchip technology

- Need snap-frozen or fresh tissue
- Time consuming
- No experience in large prospective studies
- Poor reproducibility among research groups
- May be misleading in biopsies with heterogeneous tissue or with only minimal involvement by lymphoma
Revised WHO classification (2008)
What changed?

<table>
<thead>
<tr>
<th>Hodgkin lymphoma</th>
<th>Minor changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature B cell neoplasms</td>
<td>Some changes!</td>
</tr>
<tr>
<td>Mature T-cell and NK-cell neoplasms</td>
<td>Minor changes (unfortunately!)</td>
</tr>
</tbody>
</table>
Mature B- cell neoplasms (2008)

- Chronic lymphocytic leukemia/SLL
- B-cell prolymphocytic leukemia
- Splenic B-cell marginal zone lymphoma
- Hairy cell leukemia
- Heavy chain disease
- **Splenic B-cell marginal zone lymphoma unclassifiable**
  - Splenic diffuse red pulp small B-cell lymphoma
  - Hairy cell leukemia variant
- Lymphoplasmacytic lymphoma
- Plasma cell myeloma
- Solitary plasmacytoma of bone
- Extraosseous plasmacytoma
- Extranodal marginal zone B-cell lymphoma (MALT)
- Nodal marginal zone B-cell lymphoma
- Follicular lymphoma
- Primary cutaneous follicle center lymphoma
- Mantle cell lymphoma
- Diffuse large B-cell lymphoma, NOS
  - T-cell/histiocyte rich large cell lymphoma
  - Primary DLBCL of the CNS
  - Primary cutaneous DLBCL, leg type
  - *EBV positive DLBCL of the elderly*
- DLBCL associated with chronic inflammation
- Lymphomatoid granulomatosis
- Primary mediastinal (thymic) large B-cell lymphoma
- Primary intravascular large B-cell lymphoma
- ALK positive large cell lymphoma
- Plasmablastic lymphoma
- Large B-cell lymphoma arising in HHV8 associated multicentric Castleman disease
- Primary effusion lymphoma
- Burkitt lymphoma
- B-cell lymphoma, unclassifiable, with features between DLBCL and BL
- B-cell lymphoma, unclassifiable, with features between DLBCL and CHL
Chronic lymphocytic leukemia/small lymphocytic lymphoma

- Definition of minimal criteria for the diagnosis of CLL (5 x 10^9/L monoclonal lymphocytes with CLL phenotype in the PB)
- Definition of monoclonal B lymphocytosis (MBL)
- Mutated and unmutated CLL are different subtypes
- VH mutation status and genomic aberrations are prognostic and predictive factors
- ZAP70 and CD38 are both associated with adverse prognosis
- CLL and B-cell prolymphocytic leukemia are two entities
CLL/SL: unresolved issues

- Clinical significance of monoclonal B lymphocytosis (MBL) remains to be determined
- Significance of CD5 neg. CLL/SL
- Definition of atypical CLL
- Should we recognize aggressive forms of CLL (for instance with c-myc mutation)?
Splenic B-cell lymphoma/leukemia, unclassifiable

- Splenic diffuse red pulp small B-cell lymphoma and hairy cell leukemia-variant are introduced in the classification as provisional entities.
- Both lymphomas are clinical indolent.
- Splenic diffuse red pulp small B-cell lymphoma is characterized by a very large spleen and intrasinusoidal infiltration of the bone marrow sinusoid, and villous cytology.
Lymphoplasmacytic lymphoma, extranodal marginal zone lymphoma of mucosa associated lymphoid tissue (MALT) lymphoma, nodal marginal lymphoma

- Remain as distinct entities
- Share many clinical features
- Better definition of diagnostic criteria of MALT lymphoma (role of molecular biology in the differential diagnosis from reactive proliferations)
- Definition of cytogenetic abnormalities in MALT lymphomas
Extranodal marginal zone B-cell lymphoma. Unresolved issues

- Should the number of blasts be reported?
- How do we define „sheet of blasts“ to support the diagnosis of DLBCL
- How do we evaluate follow-up biopsies of gastric MALT lymphoma? Do we need to routinely perform molecular studies?
- How do we interpret synchronous marginal lymphoma in lymph nodes and extranodal sites
- How should MALT lymphoma with plasmacytic differentiation be separated from solitary extraosseous plasmocytoma (for instance in head and neck area?)
# Follicular lymphoma (2001=2008)

<table>
<thead>
<tr>
<th>Grading</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1-2 (low grade)</td>
<td>0-15 centroblasts per hpf</td>
</tr>
<tr>
<td>Grade 1</td>
<td>0-5 centroblasts per hpf</td>
</tr>
<tr>
<td>Grade 2</td>
<td>6-15 centroblasts per upf</td>
</tr>
<tr>
<td>Grade 3</td>
<td>&gt;15 centroblasts per hpf</td>
</tr>
<tr>
<td>Grade 3A</td>
<td>centrocytes present</td>
</tr>
<tr>
<td>Grade 3B</td>
<td>Solid sheets of centroblasts</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reporting of pattern</th>
<th>Proportion follicular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular</td>
<td>&gt;75%</td>
</tr>
<tr>
<td>Follicular and diffuse</td>
<td>20-75%</td>
</tr>
<tr>
<td>Focally follicular</td>
<td>&lt;25%</td>
</tr>
<tr>
<td>Diffuse</td>
<td>0%</td>
</tr>
</tbody>
</table>
Grading FL

- FL with more large cells behave more aggressively and have a higher likelihood of progression to DLBCL than those with fewer larger cells.
- No grading method has shown high reproducibility.
- Distinction of low grade (grades 1 and 2) from grade 3 is clinically relevant.
- There is insufficient evidence at this time to recommend an alternative method or to recommend eliminating grading altogether.
- Any area of DLBCL in a FL should be reported as primary diagnosis.
FL Variants

- Pediatric follicular lymphoma
- Primary intestinal follicular lymphoma
- Other extranodal follicular lymphoma
- Intrafollicular neoplasia/“in situ“ follicular lymphoma
FL in situ
FL in situ: bcl2
Intestinal FL
Mantle cell lymphoma

- **Aggressive variants**
  - Blastoid (cells resemble lymphoblasts). Mitosis and ki-67 index are prognostic important
  - Pleomorphic

- **Other variants**
  - Small cell (cells resemble cells of CLL)
  - Marginal zone-like
WHO classification 2001: Mature aggressive B-cell lymphoma

**DLBCL variants**
- Centroblastic
- Immunoblastic
- T-cell/histiocyte rich
- Anaplastic

**DLBCL subtypes**
- Mediastinal (thymic) large B-cell lymphoma
- Intravascular large B-cell lymphoma
- Primary effusion lymphoma

**Burkitt lymphoma**
- Burkitt-like
Diffuse large B-cell lymphoma

- Common morphologic variants
  - Centroblastic
  - Immunoblastic
  - Anaplastic
- Rare morphologic variants
- Molecular subgroups
  - Germinal centre B-cell-like (GCB)
  - Activated B-cell-like (ABC)
- Immunohistochemical subgroups
  - CD5-positive DLBCL
  - Germinal centre B-cell-like (GCB)
  - Non-germinal centre B-cell-like (non-GCB)
Diffuse large B-cell lymphoma

## Immunophenotypic surrogates of gene expression profile in DLBCL

<table>
<thead>
<tr>
<th></th>
<th>GCB</th>
<th>ABC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD10</td>
<td>Pos.</td>
<td>Neg.</td>
</tr>
<tr>
<td>Bcl6</td>
<td>Pos.</td>
<td>Neg./Pos.</td>
</tr>
<tr>
<td>MUM-1</td>
<td>Neg.</td>
<td>Pos.</td>
</tr>
<tr>
<td>Fox-P1</td>
<td>Neg.</td>
<td>Pos.</td>
</tr>
<tr>
<td>Bcl2</td>
<td>Neg./Pos.</td>
<td>Pos. (strong)</td>
</tr>
<tr>
<td>5 yr OS</td>
<td>76%</td>
<td>34%</td>
</tr>
</tbody>
</table>

Hans et al. Blood 2004
Decision tree for classification of DLBCL based on immunohistochemistry

Hans et al. Blood 2004
DLBCL subtypes

- T-cell/histiocyte rich large B-cell lymphoma
- Primary DLBCL of the CNS
- Primary cutaneous DLBCL, leg-type
- *EBV*-positive *DLBCL of the elderly*

Other lymphomas

- ALK+ B-cell lymphoma
- Plasmablastic lymphoma
- Primary effusion lymphoma
- DLBCL associated with HHV8+, multicentric Castleman's Disease
- Intravascular large B-cell lymphoma
- DLBCL associated with chronic inflammation
Burkitt lymphoma (WHO 2001)

- Classical Burkitt Lymphoma
  - Endemic
  - Sporadic
- Variant Burkitt Lymphoma with plasmacytoid appearance (HIV+)
- Atypical Burkitt lymphoma and Burkitt-like Lymphoma
Will we need micro-chips technology for the diagnosis of Burkitt lymphoma?

A Biologic Definition of Burkitt’s Lymphoma from Transcriptional and Genomic Profiling

Molecular Diagnosis of Burkitt’s Lymphoma
Burkitt lymphoma (WHO 2008)

- No single parameter can be used as a gold standard for the diagnosis (morphology, immunophenotype, genetic analysis)
- CD10+, Bcl2-, bcl6+, ki67~ 100%,
- Most cases (90%) have a MYC translocation
- Gene profiling studies have demonstrated a consistent gene signature for BL, which is clearly distinct from DLBCL. However intermediate cases were also found
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and BL

- This is a heterogeneous category that is not considered a distinct disease entity, but is useful in allowing the classification of cases not meeting criteria for classical BL or DLBCL
- This diagnosis should not be made in morphological typical DLBCL with MYC translocation
- This diagnosis should not be made in morphological typical BL without MYC translocation
- 30-50% have non-IG-MYC translocation and 15% have BCL2 translocation
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and CHL

- Overlapping clinical, morphological, and/or immunophenotypic features, between classical HL and DLBCL
- Mostly primary mediastinal large B-cell but also in peripheral lymph nodes
- Primary mediastinal large B-cell lymphoma has gene expression signature that distinguishes it from other DLBCL and many elements of that signature are shared by classical Hodgkin lymphoma cells (Rosenwald, J Exp Med 2003)
PMLBCL and CHL

- Similar age and clinical presentation
- Composite and sequential PMLBCL and CHL
- Overlapping histology and Immunophenotype

GRAY ZONE LYMPHOMA

Conclusions

- There are still a lot of open questions in lymphoma pathology
- The revised classification (2008) does not clarify all issues
- The 2001 classification was a huge step forwards in lymphoma classification and contributed to better diagnosis worldwide
- Hopefully we will not make a step backwards!
Biologic gray zones in HL and NHLs

HL is derived from an altered B lymphocyte. The precise molecular events that result in the Reed-Sternberg cell are not fully elucidated; however, it is likely that these events can occur de novo, in a normal B cell, or secondarily, in a neoplastic B cell. Therefore, biologic interfaces are identified between HL and diverse subtypes of B-cell lymphoma. NLPHEL, nodular lymphocyte-predominant HL; TCRLBCL, T-cell-rich B-cell lymphoma.

From E.S. Jaffe and W.H. Wilson, 2004
In contrast to true biologic interfaces, morphologic interfaces occur between HL and other non-Hodgkin lymphomas. These morphologic interfaces may cause problems in differential diagnosis, but they do not reflect an underlying biologic relationship.

From E.S. Jaffe and W.H. Wilson, 2004