The next lymphoma classification
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Evolution of lymphoma classification
- Rappaport
- Lukes and Collins (immunophenotype)
- Kiel Classification (Europe)
- Working Formulation (USA)
- REAL Classification (1992)
- WHO classification (2001)
- Update of WHO classification (2008)
- Next lymphoma classification (?)

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)

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

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
- RA61/2 mutations
- Mantle cell lymphoma
- ANTI GEN
Germinal center Neoplasms
activation induced cytidine deaminase (ACIDA) mutations

Centroblast
Somatic mutations (ACIDA)
bcl2-, bcl6+

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

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

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!

Immunohistochemistry is ..... laboratory specific

- Preanalytic
  - Time to fixation
  - Time of fixation
  - Type of fixation

- Analytic
  - Test validation
  - Type of antigen retrieval
  - Test reagents
  - Standardized procedures
  - Automated methods

- Postanalytics
  - Interpretation criteria
  - Reporting elements
  - Quality assurance procedures

Molecular biology

SPLIT SIGNAL O BREAK APART

IgH FR2
MYC (8q24) break apart

DUAL COLOUR DUAL FUSION

IGH/BCL2 dual color dual fusion

DLBCL categories

1. DLBCL specified by site
2. DLBCL with characteristic histologic, immunophenotypic or genotypic features
3. DLBCL associated with EBV or HHV8 infection
4. DLBCL, NOS
5. Lymphoma non classifiable

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- EBVpositive DLBCL of the elderly
- DLBCL associated with chronic infection
- DLBCL with pyothorax
- Plasmablastic lymphoma
- Lymphomatoid granulomatosis
- Primary effusion lymphoma
- Large B-cell lymphoma arising in HHV8 associated multienteric Castleman disease
- Post-transplant lymphoma

Common morphologic variants
- centroblastic
- immunoblastic
- anaplastic

Molecular subgroups
- Germinal centre B-cell-like (GCB)
- Activated B-cell-like (ABC)
- Unclassified

Cytogenetic alterations
- Bcl2
- Bcl6
- C-myc

#### Diffuse large B-cell lymphoma


#### Immunophenotypic surrogates of gene expression profile in DLBCL

<table>
<thead>
<tr>
<th>Marker</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>MUM1</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>
</tbody>
</table>

5 yr OS: 76% vs 34%  
Hans et al. Blood 2004

#### Decision tree for classification of DLBCL based on immunohistochemistry

- **GCB**
  - CD10
  - MUM1
  - bcl6
- **Non-GCB**
  - CD10
  - MUM1
  - bcl6

Hans et al. Blood 2004

#### Immunohistochemical algorithms (Hans, Blood 2004) in the CHOP-treatment era

- **It works...**
  - Blood 2004; 103:275
  - Mod Pathol 2005; 18:1113
  - Arch Pathol Lab Med 2006; 130:1819
  - J Pathol 2006; 208:714
  - Blood 2007; 109:4930
  - Eur J Haematol 2007; 79:501
  - J Clin Oncol 2006; 24:4135

- **It doesn't work...**
  - Histopathology 2007; 51:70
  - Blood 2003; 101:78
  - J Clin Oncol 2005; 23:2760
  - Haematologica 2007; 92:778
  - J Clin Oncol 2008; 26:447
Gene expression-based distinction of DLBCL in subgroups


Gene expression-based distinction between GCB and ABC DLBCL carried a prognostic impact in the CHOP and R-CHOP treatment era.

A New Immunostain Algorithm Classifies Diffuse Large B-Cell Lymphoma into Molecular Subtypes with High Accuracy

Immunoblastic morphology but not the immunohistochemical GCB/non-GCB classifier predicts outcome in diffuse large B-cell lymphoma in the RICOVER-60 trial of the DSHNHL.

G Ott et al. Blood 2010, prepublished online August 24

Extension of the study published in Haematologica 2009; 15:5494
Gene expression profiling may divide DLBCLs in prognostically important subgroups.

The application of the Hans's immunohistochemical algorithm for OS prediction gives contradictory results.

The application of new immunohistochemical algorithms must be validated in prospective studies.

Immunoblastic morphology may predict outcome of DLBCL in CHOP and R-CHOP treated patients (multivariate analyses, but validation in prospective studies is still lacking).

1. DLBCL specified by site
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- centroblastic
- immunoblastic
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Molecular subgroups
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Cytogenetic alterations
- Bcl2
- Bcl6
- C-myc

**MYC**

- MYC is a transcription factor controlling the expression of a large set of target genes involved in cell cycle regulation, metabolism, DNA repair, stress response and protein synthesis.
- MYC is involved in the regulation of miRNA expression.
- MYC expression in germinal center is lower than in memory or naive B-cells.
- Genomic alterations of the MYC gene include chromosomal translocations, mutations affecting regulatory and promoter regions, as well as copy number increase.
- Most chromosomal breakpoints involving MYC are mediated by activation induced cytidine deaminase (ACIDA) and not by RAG1/2 gene.

303 DLBCL, all treated with R-CHOP
35 (14%) with MYC-R
Combination with other rearrangements (BCL2, BCL6)
MYC-R is a strongly adverse prognostic factor (in combination with age and IPI).
MYC-R occurs in 3-16% of DLBCL. The presence of a MYC-R is a strong adverse prognostic factor in CHOP and R-CHOP treated patients. MYC-R in combination with IPI and patient's age accurately predict clinical outcome. The presence of MYC-R can not be predicted by lymphoma's morphology. MYC is rarely found as the sole genetic abnormality (Double-Hit lymphoma).

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Double hit B-cell lymphomas

- B-cell lymphomas characterized by a recurrent chromosomal translocation in combination with a MYC/B2q24 breakpoint
- DH lymphomas are rare (0-12%)
- Most DH lymphomas have a BCL2/MYC combination
- There are no unifying morphological features of DH-lymphomas
- Most DH lymphomas have a GC phenotype (CD10+, bcl6+, bcl2+, high ki67)

Double hit B-cell lymphomas

- Complex karyotypes
- Gene expression profile intermediate between Burkitt lymphoma and DLBCL
- Frequent non IGH partner of MYC
- Median age 51-65 years
- Highly aggressive clinical behaviour
- Elevated LDH, bone marrow and CNS involvement, high IPI score
- Resistant to chemotherapy (median survival of only 0.2-1.5 years)

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>
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<tbody>
<tr>
<td>Follicular</td>
<td>t(14;18)(q32;q21)</td>
<td>BCL2</td>
<td>Transcriptional deregulation</td>
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<td>MALT</td>
<td>t(11;18)(p11;q21)</td>
<td>BCL10</td>
<td>Transcriptional deregulation</td>
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<td>t(11;14)(q21;q32)</td>
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<td></td>
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<td>Mantle cell</td>
<td>t(11;14)(q13;q32)</td>
<td>BCL1</td>
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<tr>
<td>Burkitt's</td>
<td>t(8;14)(q24;q32) c-MYC</td>
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<td>Transcriptional deregulation</td>
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Table 1. Incidence of chromosomal breakpoints in unselected series of diffuse large B cell lymphomas

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<tr>
<th></th>
<th>8q24</th>
<th>q21</th>
<th>12p</th>
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<td>MYC</td>
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Sietse M et al Blood 2010, prepubished online Nov 30
Timing and synergy of translocation in DH lymphomas

- BCL2 and CCND1 breakpoints are most likely mediated by RAG1/2 in precursor cells.
- Most MYC breakpoints are likely mediated by AICDA in mature B cells (erroneous somatic hypermutation or class switch).
- 5% of FL with BCL2 breakpoints will acquire MYC breakpoint during the course of disease.
- Sporadic cases of lymphomas with two clones with different breakpoints have been reported.
- Secondary MYC breakpoints affect the Ig light chain whereas primary MYC breakpoints (BL) affect the Ig heavy chain.
- MYC breakpoint is most likely a secondary event in the case of BCL2 or CCND1 breakpoint.

How can the aggressive course of DH lymphomas be explained?

- MYC and BCL2 translocation may be detected in normal B cells.
- Adult BL have much more favorable prognosis than DH lymphomas.
- DH lymphomas often have a very complex karyotype.

1. DLBCL specified by site
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3. DLBCL associated with EBV or HHV8 infection
4. DLBCL, NOS
5. Lymphoma non-classifiable

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 (other mechanisms for MYC activation).
- BL should be accepted only after exclusion of DH lymphoma (30-50% of unclassifiable cases have non-IG-MYC translocation and 15% have BCL2 translocation).
Proposed algorithm for highly aggressive lymphomas


Useful diagnostic markers for identifying MYC translocated lymphomas

- Rodig SJ et al. The pre-B-cell receptor associated protein VpreB3 is a useful diagnostic marker for identifying c-MYC translocated lymphomas. Hematologica 2010;95:2056

Plasmablastic lymphoma

- Initially characterized as an aggressive lymphoma arising in the jaw and oral mucosa of HIV-infected patients (oral PBL)
- Recognized as a distinct category of aggressive lymphoma in the WHO classification
- EBV associated in 15-80% of the cases
- Extraoral PBLs tend to occur in patients with underlying non-HIV-related immunosuppression and demonstrate plasmacytic differentiation (distinct entity?)
- Immunophenotype: CD138+, MUM1+, CD20-, CD79a +/-, K/lambda +/-, ki67 >90%
- 50% associated with Ig/MYC rearrangements without double hit but with karyotype complexity

WHO classification: selection of unresolved issues

- DLBCL and intermediate category DLBCL-BL
  - Role of immunohistochemical algorithm to characterize DLBCL
  - Role of MYC-R in aggressive lymphoma
  - Role of DH lymphomas (DLBCL, CLL, FL, SL, MCL, PBL)
- Lymphoma with plasmablastic/plasmacytic differentiation (DLBCL immunoblastic, PBL, large B-cell lymphoma arising in HHV8 multicentric Castleman disease, primary effusion lymphoma, MALT with plasmacytic differentiation, extraskleletic myeloma, splenic ML, plasmacytic lymphoma)
- Characterization of indolent lymphomas (CLL, FL, MCL, MZL)