HIGH GRADE B-CELL LYMPHOMA

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OUTLINE

• High grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements
  • Patient presentation
  • 2008/2016 WHO classification
  • Epidemiology
  • Clinical features
  • Microscopic features
  • Genetic profile

• High grade B-cell lymphoma, NOS

• HGBL treatment
PATIENT PRESENTATION

• 54 year-old-man with hypertension and diabetes mellitus
  • Night sweats
  • Weight loss
  • Bilateral hip pain
• Primary care physician discovered pancytopenia
  • Anemia
  • Leukopenia
  • Severe thrombocytopenia
• Sent to Emergency Room
BONE MARROW BIOPSY

- 87% atypical cells
- 90% cellular
FLOW CYTOMETRY – MATURE B-CELL NEOPLASM, GERMINAL CENTER ORIGIN

<table>
<thead>
<tr>
<th>Side scatter</th>
<th>Cytoplasm complexity (granules)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45</td>
<td>Leukocyte common antigen</td>
</tr>
<tr>
<td>CD5</td>
<td>T-lymphocytes</td>
</tr>
<tr>
<td>CD19</td>
<td>All stages of B-lymphocytes</td>
</tr>
<tr>
<td>CD20</td>
<td>Mature B-lymphocytes</td>
</tr>
<tr>
<td>Kappa/Lambda</td>
<td>Mature B-lymphocytes; shows clonality</td>
</tr>
<tr>
<td>CD34</td>
<td>Immature lymphoid/myeloid</td>
</tr>
<tr>
<td>CD10</td>
<td>Germinal center B cells</td>
</tr>
<tr>
<td>TdT</td>
<td>Immature lymphoid cells</td>
</tr>
</tbody>
</table>

Mature (CD34- Tdt-), monoclonal (Kappa predominant) B-lymphocyte (CD19+, CD20+, CD22+) neoplasm, expressing CD10
IMMUNOHISTOCHEMISTRY (IHC)

CD20 +
BCL2 +
BCL6 +
cMYC +
MUM1 -
Ki67 100%
FISH - LYMPHOMA PANEL

IGH (14q32.3)
IGH 3' centromeric
IGH 5' telomeric
101g1f = Abnormal

LYMPHOMA PANEL

BCL6 (3q27)
BCL6 3' centromeric
BCL6 5' telomeric
101g1f = Abnormal

MALT1 (18q21.31)
MALT1 5' centromeric
MALT1 3' telomeric
2f = Normal

3q27 Region

LSI BCL6 Dual Color,
Break Apart Rearrangement Probe
HIGH GRADE B-CELL LYMPHOMA (HGBCL) WITH MYC AND BCL2 AND/OR BCL6 REARRANGEMENTS

nuc ish
(BCL6x2)(3’BCL6 sep 5’BCL6x1)[198/200],
(MYCx2)(5’MYC sep 3’MYCx1)[196/200],
(IGHx2)(3’IGH sep 5’IGHx1)[198/200],
(MALT1x2)[200/200],
(BCL2x2)(3’BCL2 sep 5’BCL2x1)[197/200]
# LYMPHOMA PANEL GENES

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene Name</th>
<th>Location</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGH</td>
<td>Immunoglobulin heavy locus</td>
<td>14q32.3</td>
<td>Antibody heavy chain</td>
</tr>
<tr>
<td>MYC</td>
<td>Cellular myelocytomatosis</td>
<td>8q24.1</td>
<td>Cell proliferation</td>
</tr>
<tr>
<td>BCL2</td>
<td>B cell lymphoma 2</td>
<td>18q21.3</td>
<td>Anti-apoptotic</td>
</tr>
<tr>
<td>BCL6</td>
<td>B cell lymphoma 6</td>
<td>3q27</td>
<td>Transcription repressor</td>
</tr>
<tr>
<td>MALT1</td>
<td>Mucosa-associated lymphoid tissue lymphoma translocation protein 1</td>
<td>18q21.3</td>
<td>Lymphocyte activator</td>
</tr>
</tbody>
</table>
ABNORMAL COMPLEX MALE KARYOTYPE

47, XY, t(2;18)(p11.2;q21.3), t(3;14)(q27.3;q32), t(8;22)(q24.2;q11.2), +12 [14] / 46, XY[6]

t(2;18)(p11.2;q21.3)  IGK + BCL2  →  BAD

t(3;14)(q27.3;q32)  IGH + BCL6  →  BAD

t(8;22)(q24.2;q11.2)  IGL + MYC  →  BAD

+ 12 (~1,000 genes)  →  Recurrent in double hit lymphomas. Uncertain significance

Wikipedia
PATIENT’S TREATMENT

- Used to treat various aggressive B-cell and T-cell non-Hodgkin lymphomas. ¹
- Shows a promising activity considering double/triple hit, double/triple expressing lymphoma-associated drug resistance. ²
- Seems to overcome drug resistance associated with \textit{BCL2/MYC/BCL6} overexpression, \textit{but not with TP53} deletion. ²

WHO 2016 NEW LYMPHOMA CATEGORY: HIGH GRADE B-CELL LYMPHOMA WITH MYC AND BCL2 AND/OR BCL6 REARRANGEMENTS

- So called ”triple hit” lymphoma

- Morphology should be given in the comment, since morphology may indicate behavior of the tumor
  - DLBCL – most cases
  - BL or DLBCL/BL ~50%
  - Blastoid – small portion

- Transformed from______
2008 WHO CLASSIFICATION OF TUMORS OF HEMATOPOIETIC AND LYMPHOID TISSUES

• “B-cell lymphoma, unclassifiable, with features in between Diffuse Large B-Cell Lymphoma (DLBCL) and Burkitt Lymphoma (BL)”
  • BCLU

• Included
  • Intermediate morphology between DLBCL and BL
  • Burkitt lymphoma with cytologic variation or BCL2 positive
  • ”Double hit” or “triple hit” lymphomas
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>BL</th>
<th>Intermediate BL/DLBCL</th>
<th>DLBCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only small/medium-size cells</td>
<td>Yes</td>
<td>Common</td>
<td>No</td>
</tr>
<tr>
<td>Only large cells</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Mixture</td>
<td>No</td>
<td>Sometimes</td>
<td>Rare</td>
</tr>
<tr>
<td>Proliferation (Ki67/MIB1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;90% and homogeneous</td>
<td>Yes</td>
<td>Common</td>
<td>Rare</td>
</tr>
<tr>
<td>&lt;90% or heterogeneous</td>
<td>No</td>
<td>Sometimes</td>
<td>Common</td>
</tr>
<tr>
<td>BCL2 expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative / weak</td>
<td>Yes</td>
<td>Sometimes</td>
<td>Sometimes</td>
</tr>
<tr>
<td>Strong</td>
<td>No</td>
<td>Sometimes</td>
<td>Sometimes</td>
</tr>
<tr>
<td>Genetic features</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MYC rearrangement</td>
<td>Yes*</td>
<td>Common</td>
<td>Rare</td>
</tr>
<tr>
<td>IG-MYC**</td>
<td>Yes</td>
<td>Sometimes</td>
<td>Rare</td>
</tr>
<tr>
<td>Non IG-MYC**</td>
<td>No</td>
<td>Sometimes</td>
<td>Rare</td>
</tr>
<tr>
<td>BCL2 but no MYC rearrangement</td>
<td>No</td>
<td>Rare</td>
<td>Sometimes</td>
</tr>
<tr>
<td>BCL6 but no MYC rearrangement</td>
<td>No</td>
<td>Rare</td>
<td>Sometimes</td>
</tr>
<tr>
<td>Double hit†</td>
<td>No</td>
<td>Sometimes</td>
<td>Rare</td>
</tr>
<tr>
<td>MYC-Simple karyotype***</td>
<td>Yes</td>
<td>Rare</td>
<td>Rare</td>
</tr>
<tr>
<td>MYC-Complex karyotype***</td>
<td>Rare</td>
<td>Common</td>
<td>Rare</td>
</tr>
</tbody>
</table>
BURKITT LYMPHOMA,

A) Typical BL
B) BL with variation in size and shape of cells
C) DLBCL/BL

Fig. 10.127 Three lymphomas with t(8;14). A Typical BL composed of medium-sized, monomorphic cells with round nuclei, multiple nucleoli, and a moderate amount of cytoplasm, which has a mosaic-like appearance. Prominent apoptosis is evidenced by the presence of macrophages engulfing nuclear debris, creating the "starry-sky" pattern. Nine of 11 experts who reviewed this case independently made a diagnosis of BL; 1 made a diagnosis of atypical BL. B Another case with similar overall appearance, but with slightly more variation in size and shape of the cells. Six reviewers called this BL and 5 called it atypical BL. C DLBCL with a t(8;14) has a prominent starry-sky pattern. The cells are larger and more pleomorphic than the Burkitt and atypical Burkitt cases. Nine experts called this DLBCL and 2 called it atypical BL. Reproduced from Harris NL and Horning SJ [896A].
"DOUBLE HIT" LYMPHOMA WITH FEATURES OF DLBCL AND BL

Fig. 10.128 Male patient, 61 years, with a rapidly growing cervical nodal mass. "Double hit" lymphoma with both 8q24/MYC and 18q21/BCL2 breakpoints. A Starry sky pattern. B Higher magnification showing a mixture of medium/large-sized nuclei with little pleomorphism but prominent nucleoli, absence of granular chromatin and many mitotic figures. C Strong BCL2 staining is very unusual for BL. D Ki67 staining was heterogeneous but elsewhere there were close to 100% positive cells.
DLBCL WITH MYC/BCL2 CO-EXPRESSISON (2013)
THIS SHOWS A DISTINCT GENE EXPRESSION SIGNATURE AND PROGNOSIS

Shimin Hu et al. Blood 2013;121:4021-4031
• 4-8% of DLBCL are double hit
• Usually in 5th and 6th decades of life
• Youngest cases approximately 30 years old
• Slightly more males than females
ETIOLOGY

• HGBL with \textit{BCL2} rearrangement is from germinal center B-cells (GCB)\textsuperscript{1}

• Rearrangement of \textit{MYC} might be secondary
  1. Progression from follicular lymphoma
     • BCL2 rearrangement $\rightarrow$ acquires \textit{MYC} rearrangement
  2. De novo disease
     • Some tumors with areas of both \textit{MYC} and \textit{BCL2} rearrangement, other areas only \textit{BCL2}

\textsuperscript{1} Scott DW, \textit{et al.} 2018. Blood. World Health Organization 2017 Classification of Tumours of Haematopoietic and Lymphoid Tissues
LOCALIZATION

- More than half of patients present with widespread disease, including outside of the lymph nodes.
- More than one extra-nodal site (30-88%)
- Bone marrow (59-94%)
- CNS (up to 45%)


World Health Organization 2017 Classification of Tumours of Haematopoietic and Lymphoid Tissues
CLINICAL FEATURES

• Most patients (70-100%) present with advanced disease (stage IV)

• High international prognostic index (IPI)
  • Age greater than 60 years
  • Stage III or IV disease
  • Elevated serum LDH
  • ECOG/Zubrod performance status of 2, 3, or 4 (bedridden)
  • More than 1 extranodal site
MICROSCOPIC FEATURES

- Most have morphology of DLBCL
- All cases with morphology of DLBCL should be tested for rearrangements
- Medium-size to large cells, abundant cytoplasm, irregular nuclear contours
MICROSCOPIC FEATURES

- ~50% have morphology of Burkitt Lymphoma or intermediate between DLBCL and BL
- Medium-size to large cells with large nuclei, monomorphic and with starry sky macrophages
- DLBCL/BL larger cells with less basophilic cytoplasm
MICROSCOPIC FEATURES

- Other morphology is blastoid: medium sized cells with high nucleus/cytoplasm ratio, small rim of cytoplasm and fine chromatin with inconspicuous nucleoli
  - Similar to centroblasts

- Tdt stain should be performed on every case to rule out a precursor neoplasm (B-lymphoblastic leukemia/lymphoma)
IMMUNOPHENOTYPE

• Mature B-cell lymphoma with
  • CD19
  • CD20
  • CD79a
  • PAX5
  • No TdT
  • No CD34
  • No Cyclin D1
  • Bright CD45

• Some lack surface Ig, possibly due to rearrangement involving Ig loci
  • Should not be interpreted as immature
PROLIFERATION

- Ki67 index can be variable
- Ki67 should NOT be used to screen for cases to perform molecular testing
GENETIC PROFILE – MYC AND BCL2 AND/OR BCL6

• Rearrangement in MYC
  • When paired with IG, this is more aggressive
• Also rearrangements involving BCL2 and/or BCL6
• MYC paired with other gene rearrangements (BCL3 or others) are not included in this diagnostic category
• BCL2 or BCL6 copy number increase or amplification not enough (must be rearrangement)
• Many other structural and numerical abnormalities
  • TP53 frequently mutated
  • MYD88 sometimes mutated
  • ID3 hemizygous mutations
  • Usually a complex karyotype
DIAGNOSTIC CATEGORIES

Morphology

Blastoid

B-LBL

HGBL, NOS

BL

HGBL, with MYC and BCL2 and/or BCL6R

DLBCL, NOS

Phenotype & cytogenetics

TdT+

TdT−, cyclin D1−

DLBCL

Diagnosis
MYC REARRANGEMENT PARTNER MATTERS

MYC-DH-IG

Overall survival according to MYC-DH partner gene including patients with no MYC rearrangement

With Number of Subjects at Risk and 95% Confidence Interval

Survival Probability

1: MYC non-IG
2: MYC-IG
3: No MYC rearrangement

OS (months)

1 2 3 4 5 6 7 8 9 10

19 18 17 16 15 14 13 12 11 10

123456789

1.0 0.8 0.6 0.4 0.2 0.0

High expressors:
HGBL-DH with IG-MYC
DE-DLBCL with iG-MYC

Intermediate expressors:
HGBL-DH with non-IG MYC
DE-DLBCL, no MYC translocation

Low expressors:
HGBL-DH without dual expression
other non-DE-DLBCL

BCL2 expression

Myc expression


Christiane Copie-Bergman et al. Blood 2015;126:2466-2474
DOUBLE/TRIPLE-HIT VS DOUBLE/TRIPLE-EXPRESSOR

- MYC + BCL2 expression is synergistic

- MYC rearrangement to IG worse than others

- Without rearrangements, cannot include in this diagnosis
  - Would be DLBCL with double expression

Alexandra Valera et al. Haematologica 2013;98:1554-1562

HIGH GRADE B-CELL LYMPHOMA, NOS

• Heterogeneous category
• Aggressive mature B-cell lymphomas that lack MYC plus BCL2 and/or BCL6 rearrangements
• Blastoid-appearing mature B-cell lymphomas (not mantle cell type)
• Rare, to be used only when truly unable to classify as DLBCL or BL
• Affects the elderly; males and females affected almost equally
• Poor outcome, though slightly better than those with double-hit HGBL
HGBL TREATMENT

- **R-CHOP** is inadequate induction therapy
- Future therapies may target **MYC** and **BCL2**

1. Dr. David Scott. 2015. Update on Molecular Classification of DLBCL. ASCO.
SUMMARY

• B-cell lymphoma, unclassifiable is now

  • High grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements (HG-DH/TH)
  • High grade B-cell lymphoma, NOS (HG-NOS)

• Treatment should be more aggressive