Low-Grade B-Cell Lymphomas in WHO Classification

<table>
<thead>
<tr>
<th>Lymphoma Type</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular lymphoma</td>
<td>22.1 %</td>
</tr>
<tr>
<td>Extranodal MALT-lymphoma</td>
<td>7.6 %</td>
</tr>
<tr>
<td>Small lymphocytic lymphoma/ CLL</td>
<td>6.7 %</td>
</tr>
<tr>
<td>Nodal marginal zone lymphoma</td>
<td>1.8 %</td>
</tr>
<tr>
<td>Lymphoplasmacytic lymphoma/ WM</td>
<td>1.2 %</td>
</tr>
<tr>
<td>Splenic marginal zone lymphoma</td>
<td>&lt;1 %</td>
</tr>
</tbody>
</table>


Follicular Lymphoma
Definition

“A neoplasm composed of follicle center B-cells (typically both centrocytes and centroblasts) which usually has at least a partially follicular pattern... Lymphomas composed of centrocytes and centroblasts with an entirely diffuse pattern in the sampled tissue may be included in the category.”

Definition is cytology + GCB immunophenotype

bcl-2 expression or t(14;18)/IGH-BCL2 are not required to establish the diagnosis

2017, WHO book p. 266

Follicular Lymphoma
Clinical Features

Age (median): 60 yrs
M-to-F ratio: 1 to 1.7
Lymph nodes: 80%
Splenomegaly: 60%
Hepatomegaly: 50%
Bone marrow: 40%
Leukemic phase: 5%
Stage: 80% III-IV; 20% I-II
Lab abnormalities: 10-15% (anemia #1)
Median survival: ~ 10 yrs

**Follicular Lymphoma**

**Morphologic Findings**

- Many follicles
- Monotonous
- Cortex and medulla

**Follicular Lymphoma**

**Grading**

Count number of centroblasts in 10 neoplastic follicles and divide by 10

- **Grade 1**: 0-5 centroblasts / hpf
- **Grade 2**: 6-15 centroblasts / hpf
- **Grade 3**: > 15 centroblasts / hpf

- **3A**: centrocytes present
- **3B**: solid sheets of centroblasts

Counts based on hpf = 0.159 mm² (1x ocular with 18 mm field of view and 40X objective)

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**Do we need grades 1 vs. 2?**

“As recommended in the 2008 WHO classification, the combined designation of grade 1-2 is preferred, due to the lack of clinically significant differences between grades 1 and 2, the considerable interobserver variation in grading, and variations in grade within a given biopsy.”

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**Follicular Lymphoma Grade 3**
FOLLICULAR LYMPHOMA GRADE 3B
A Follicular Variant of DLBCL?

Some Evidence in Favor
- Often aggressive clinically; +/- extranodal
- Looks like DLBCL (except for follicles)
- Often MUM1/IRF4+
- Less often CD10+ or bcl-2+
- Lower frequency of t(14;18)
- Higher frequency of BCL6 translocation
- Gene expression profile more like DLBCL?

Follicular Lymphoma
Role of Ki-67

Ki-67 correlates with number of centroblasts
Helpful, but exceptions occur

Low Histologic Grade Follicular Lymphoma With High Proliferation Index
Morphologic and Clinical Features
Sa A. Wang, MD, * Lan Wang, MD, † Ephraim P. Hochberg, MD; ‡ Alona Mathias, MS, § Nancy Lee Harris, MD, * and Robert P. Basserjian, MD*

24 of 142 (18%) of FL were low-grade but had high proliferation (Ki-67)
Patients with these tumors had lower disease-specific survival more like grade 3 FL

Follicular Lymphoma Grading
Potential Drawbacks of System

Other information of value is not included
- Large cleaved cells
- Mitotic figures
- Proliferation indices (e.g. Ki-67)

Counting cells in general
- Where do you count?
- Many FL cases show a cytologic spectrum of follicles

Follicular Lymphoma

Immunophenotype
- Surface Ig, pan B-cell antigens
- CD10, BCL-6, LMO2, GCET2/HGAL

New antibodies: MEF2B, STMN1/ stathmin

Karyotype
- t(14;18)(q32;q21) 80%
- del(1p36) 25%
- Trisomy 7 20%
- Trisomy 18 20%
- t(3q27; variable) 15%
- del(6q23-26) 15%
- 17p abnormalities 15%

Follicular Lymphoma

Genome-wide Copy Number Alterations

106 cases of FL; 92% with alterations

71 alterations in >10% of FL cases

3 Common Abnormalities
- del(1p36) ~25% (TNFRSF14)
- del(6q13.3) ~15% (CASP8AP2)
- del(6q23.3) ~15% (TNFAIP3)

Blood 113: 137, 2009

Follicular Lymphoma

Gene Mutations

Chromatin remodeling (epigenetic) genes are often mutated
- KMT2D
- EZH2
- CREBBP
- EP300
- ARID1A
- BCL2 ~75%
- EP2A ~70%
- CREBBP ~50%
- EZH2 ~20%
- MEF2B ~15%
- ARID1A ~15%
- EP300 ~15%
- CARD11 ~10%
- STAT6 ~10%
- TNFAIP3/A20 5-25%
- FOXO1 5-10%
- FAS 5-10%
- TP53 ~5%
Transformation of Follicular Lymphoma

Risk of histologic transformation 2-3% per year

Clinical Features
- Rapidly enlarging mass
- B symptoms
- Elevated serum LDH
- High serum calcium
- Low hemoglobin
- High SUV on PET scan

Transformation of Follicular Lymphoma

Histologic Variants
- Diffuse large B-cell lymphoma
- High-grade B-cell lymphoma (Burkitt-like)
- TdT-negative small blastoid lymphoma
- TdT-positive lymphoblastic transformation
- Plasmablastic lymphoma
- Hodgkin-like CD30+ transformation

Follicular Lymphoma Transformed to DLBCL

Lymphoblastic Transformation of FL

2009

2015

TdT
FL and transformed FL arise by divergent (branched) evolution from a common precursor cell.

FL characterized by mutations in Chromatin remodeling genes KMT2D/MLL2, CREBBP, EZH2

Transformed FL is characterized by Dysregulation of cell cycle Altered DNA damage response CDKN2A/B, MYC, TP53

Cell Reports 6:130, 2014

Follicular Lymphoma Double Hit – MYC and BCL2

Morphologic Features
Pattern: 6 follicular, 1 follicular and diffuse
Grade: 5 grade 1-2, 2 grade 3
Ki-67: 4 10-20%, 2 10-50%, 1 90%

Hum Pathol 58: 72, 2016

FISH showed:
MYC RIGH-BCL2
CD10
BCL-2
C-MYC

MIP Assay (Oncoscan)
Profile similar to other cases of FL
Gains
2p25.3-p11.1
7p22.3-q36.3
12q11-q24.33
Losses
18q21.32-q23

Fewer CNAs than high-grade B-cell lymphomas with MYC and BCL2 and/or BCL6 rearrangement

Mod Pathol 2017 [Epub]
**Follicular Lymphoma**

**Double Hit - MYC and BCL2 or BCL6**

WHO classification recommendation

Designate these cases as follicular lymphoma
Pattern and stage

Do not classify as high-grade B-cell lymphoma
i.e. as double hit lymphoma

2017 WHO book, p. 335

I still think the term “double hit follicular lymphoma” is OK

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**Follicular Lymphoma**

**Variants or More than One Disease?**

~80% of cases of FL are systemic
Arise in adults
Nodal-based
t(14;18)(q32;q21)/IGH-BCL2
Usually bcl-2 positive

~20% of cases of FL diffuse
Younger age
Often extranodal
Often negative for bcl-2 by IHC
Often negative for t(14;18)/IGH-BCL2

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**Follicular Lymphoma**

**Pediatric Type**

Age: 15-18y
10 M to 1 F
No diffuse areas
No B symptoms
No dissemination
Excision OK

CD10+
BCL6+
BCL2-
MUM1-

BCL2 G
BCL6 G

Blood 120: 2395, 2012

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In retrospect, many of these cases are pediatric type FL (verbal communication)
Whole exome sequencing
*MAP2K1* mutations in 9 of 21 (43%) cases
Missense activating mutations in regulatory/catalytic domains

Few mutations in chromatin remodeling genes (unlike adult FL)
*KMT2D, MLL2, CREBBP, EZH2*, etc.

**Follicular Lymphoma**
*IRF4* Translocation

- Median age: 12 y
- Range: up to 70 y
- M=F
- Head/neck LNs
- Waldeyer ring
- Need Chemo/Rx

**Follicular Lymphoma**
Testis

- Rare; more common in children
- Low stage; often grade 3A
- Excision often adequate Rx

**Follicular Lymphoma**
Duodenal-type

- Adults; M=F
- 2nd portion of duodenum
- Distal small intestine ~75%
- Stage I; clinically indolent

**Follicular Lymphoma**

**Follicular Lymphoma**
Blood 118:139, 2011
Primary Cutaneous Follicle Center Lymphoma

#1 B-cell lymphoma of skin
Median age ~50 years; M-to-F = 1.5
Usually localized; head and neck, trunk
Nodules or plaques most often
bcl-6+, CD10 -/+ , bcl-2 -/+ , MUM1/IRF4 -
Low frequency of t(14;18)/IGH-BCL2
Good prognosis (5 yr survival > 95% )
Do not grade or provide pattern

Follicular Lymphoma

Age: adults
M<F
Inguinal
Diffuse
Low stage
Low grade
Often need Rx

CD10 +/- , bcl-6 + , bcl-2 + , CD23 +
t(14;18)/IGH-BCL2 ~15%
Del(1q36)/TNFRSF14 mutations
STAT6 mutations ~85%

Blood 113: 1053, 2009
Mod Pathol 29:570, 2016

Characterization of a variant of t(14;18)
negative nodal diffuse follicular lymphoma
with CD3 expression, 1q36/TNFRSF14
abnormalities, and STAT6 mutations

Mod Pathol 29: 570, 2016
**Follicular Lymphoma CD5+**

- CD5+ in ~ 3% of FL cases
- Patients with CD5+ FL have higher:
  - FLIPI scores
  - Frequency of DLBCL (concurrent or subsequently)
  - Poorer progression-free survival

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**EBV+ Follicular Lymphoma**

- EBER+ in 2.6% of FL cases
- Older age
- M=F
- Grades 3A or 3B
- CD30+
- Subsequent DLBCL (5/10)

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**Nodal Marginal Zone B-cell Lymphoma**

**Definition**

A primary nodal B-cell neoplasm that morphologically resembles lymph nodes involved by MZL of extranodal or splenic types but without evidence of extranodal or splenic disease

- Old name: monocytoid B-cell lymphoma
What do normal MZ lymphocytes do?

First line of defense against foreign organisms
  - T-cell independent
  - Bulk of primary antibody response

Short-lived antibody production
No memory B-cells are generated

Nodal Marginal Zone Lymphoma
Clinical Findings

<table>
<thead>
<tr>
<th></th>
<th>NMZL</th>
<th>FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>60 yrs</td>
<td>59 yrs</td>
</tr>
<tr>
<td>Male</td>
<td>50 %</td>
<td>42 %</td>
</tr>
<tr>
<td>BM+</td>
<td>33 %</td>
<td>42 %</td>
</tr>
<tr>
<td>B symptoms</td>
<td>20 %</td>
<td>28 %</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>60 %</td>
<td>33 %</td>
</tr>
<tr>
<td>III-IV</td>
<td>40 %</td>
<td>67 %</td>
</tr>
</tbody>
</table>

2017 WHO book, p. 263
Blood 89: 3909, 1997
Nodal Marginal Zone Lymphoma

Cytologic Spectrum

Monocytoid

Plasmacytoid

Histologic Features

Marginal zone distribution
Abundant cytoplasm
Often pale at low power
Plasmacytoid differentiation +/-
Follicular colonization +/-
Residual GC cells

Immunophenotype

I pox CD19, CD20, PAX5, BCL2
Flow CD11c +/-, CD23+/-, CD25+/-, FMC7+/-, CD5-, CD10-

Molecular Features

No characteristic translocations

Molecular Features

No characteristic translocations

Diagnosis | MNDA Positive
----------|----------------
NMZL      | 16 / 24 (66.7%)
EMZL      | 27 / 44 (61.4%)
SMZL      | 5 / 21 (23.8%)
CLL/ SLL  | 4 / 31 (12.9%)
MCL       | 9 / 140 (6.4%)
LPL       | 2 / 8 (25.0%)
FL        | 6 / 110 (5.5%)
DLBCL     | 2 / 61 (3.3%)

Hum Pathol 45: 1730, 2014
**Immunoglobulin superfamily receptor translocation-associated 1**

IRMZL 307/329 (93%)

NMZL 154/210 (73%)

SMZL 0/21 (0%)

DLBCL 69/256 (27%)

All other B- and T-NHLs are negative for IRTA1

Histopathology 61: 930, 2012

**Differential Diagnosis of Nodal MZL**

**Other types of MZL**

Usually not too difficult because there are extranodal sites or spleen

Lymphoplasmacytic lymphoma/ Waldenstrom macroglobulinemia

Follicular lymphoma

**The genetics of nodal marginal zone lymphoma**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>KMT2D</td>
<td>34%</td>
</tr>
<tr>
<td>PTPRD</td>
<td>20%</td>
</tr>
<tr>
<td>NOTCH1/2</td>
<td>20%</td>
</tr>
<tr>
<td>KLF2</td>
<td>17%</td>
</tr>
</tbody>
</table>

**PTPRD**

Receptor type protein tyrosine phosphatase-delta

Mutations unique to nodal MZL


**Lymphoplasmacytic Lymphoma/ Waldenstrom Macroglobulinemia**
### Nodal MZL versus LPL/WM

<table>
<thead>
<tr>
<th>Feature</th>
<th>Nodal MZL</th>
<th>LPL/WM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IgM</td>
<td>~ 5-10% pts</td>
<td>100% of WM</td>
</tr>
<tr>
<td>Bone marrow +</td>
<td>30-40% of pts</td>
<td>100%</td>
</tr>
<tr>
<td>Distribution</td>
<td>Cortical</td>
<td>Medullary</td>
</tr>
<tr>
<td>Sinuses</td>
<td>Often effaced</td>
<td>Usually patent</td>
</tr>
<tr>
<td>Follicles</td>
<td>Large, reactive</td>
<td>Usually small</td>
</tr>
<tr>
<td>Cell cytoplasm</td>
<td>Pale (monocytoid)</td>
<td>Darker (purple)</td>
</tr>
<tr>
<td>Dutcher bodies</td>
<td>Uncommon / few</td>
<td>Common / often many</td>
</tr>
<tr>
<td><strong>IRTA1, MNDA</strong></td>
<td>+/-</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>MYD88 L265P</strong></td>
<td>Very rare</td>
<td>&gt;90%</td>
</tr>
<tr>
<td><strong>PTPRD mutation</strong></td>
<td>15%</td>
<td>Negative</td>
</tr>
</tbody>
</table>

### Nodal MZL with Follicular Colonization versus FL

<table>
<thead>
<tr>
<th>Feature</th>
<th>Nodal MZL</th>
<th>Follicular Lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution of follicles</td>
<td>Confined to lymph node</td>
<td>Extend into perinodal fat</td>
</tr>
<tr>
<td>Growth pattern</td>
<td>Tumor begins outside follicle and grows in</td>
<td>Tumor begins in follicle and grows out</td>
</tr>
<tr>
<td>Cytology</td>
<td>Often rounder cells +/- plasm. differentiation</td>
<td>Centrocytes/centroblasts Plasm. diff is rare</td>
</tr>
<tr>
<td>Cell cytoplasm</td>
<td>Pale (pink)</td>
<td>Darker (blue)</td>
</tr>
<tr>
<td>CD10/ BCL6</td>
<td>Germinal centers+/-</td>
<td>Tumor cells-</td>
</tr>
<tr>
<td>bcl-2</td>
<td>Germinal centers-/ Tumor cells+/-</td>
<td></td>
</tr>
<tr>
<td>Ki-67</td>
<td>Germinal centers high Tumor low</td>
<td>Usually low (unless grade 3 FL)</td>
</tr>
<tr>
<td><strong>IGH-BCL2</strong></td>
<td>Absent</td>
<td>Present</td>
</tr>
</tbody>
</table>

### Follicular Lymphoma vs Nodal MZL

- **IRTA1**
- **MNDA**
- **CD10**
- **BCL-6**
- **LMO2**
- **HGAL**

Brand and Van Krieken, Haematologica 100: e359, 2015
Nodal MZL versus Follicular Lymphoma
Does It Matter Clinically?

Patients with FL have
- Higher stage
- More frequent BM +
- More abdominal LNs +
- More frequent transformation to DLBCL

FL usually transforms to DLBCL of GCB type
NMZL usually transforms to DLBCL of non-GCB type

May not matter much for patient at diagnosis
Few therapeutic implications
Seems to have prognostic importance

Leuk Lymphoma 57: 1649, 2016
FL grade IIIB cases cluster with other cases of FL rather than GCB-DLBCL.

Haematologica 93: 1033, 2008

“This method has been used by one of us (CWB) for over 15 years both in clinicopathologic studies and in daily teaching of fellows and residents.... We have chosen to actually count the number of large cells observed within a field of the neoplastic nodule at high dry magnification (10X ocular, 40X objective). At least twenty fields are counted and an average is taken.”

Large cells = vesicular nuclei with nucleoli

Fl Transformed to High-grade B-cell Lymphoma

Clues to Diagnosis of Nodal MZL

No extranodal sites of disease
Adundant pale cytoplasm
Marginal zone distribution
Dutcher bodies (less than LPL or MALT lymphoma)
Appropriate B-cell immunophenotype

CD20+, CD5-, CD10-, BCL6-, cyclin D1-
IRTA1+ and MDNA+

NMZL is often a diagnosis of exclusion

There is still overlap with LPL/WM (in LNs)

It is OK to not be sure - ask for workup
11/7/2017

22 patients
Age: 10-64 years
Sex: 19 male, 3 female
Location: 21/22 head and neck

“11 cases misdiagnosed as lymphoma”

Am J Clin Pathol 78:493, 1982

Criteria for large cell transformation in MZL?

Sheets of large cells are best and perhaps only criterion
Otherwise be conservative
Ki67 and p53 can be helpful

16 months later

Primary Cutaneous Follicle Center Lymphoma

Looks low-grade

Nodal Marginal Zone Lymphoma
Colonizing Follicles

NMZL
DLBCL

BCL-2
The genetics of nodal marginal zone lymphoma

PTPRD mutations close to specific but not sensitive for NMZL