Differential diagnosis of hematolymphoid tumors composed of medium-sized cells

Brian Skinnider
B.C. Cancer Agency,
Vancouver General Hospital
And now for something completely different...
Lymphoma classification

- Lymphoma diagnosis starts with morphologic differential diagnosis based on cell size and architecture
  - Small cells, diffuse or follicular
  - Medium-sized cells
  - Large cells
  - Large cells in a reactive background
Hematolymphoid tumors composed of medium-sized cells

- Burkitt lymphoma
- B cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL
- Lymphoblastic lymphoma
- Myeloid sarcoma
- Blastoid mantle cell lymphoma
- SLL/CLL with prominent proliferation centers
- T/NK cell lymphomas
Tumors with medium-sized cells

• These are uncommon diagnosis and may not be readily identified

• Diseases requiring immediate diagnosis and treatment:
  – Burkitt lymphoma
  – Double-hit lymphoma
  – Lymphoblastic lymphoma
  – Myeloid sarcoma
Cell size

• How do you decide cell size?

• WHO 2008 classification:
  – DLBCL: “…nuclear size equal to or exceeding normal macrophage nuclei…”
  – Burkitt lymphoma: “…medium-size cells (nuclei similar or smaller to those of histiocytes)…”
Nuclei of medium-sized cells have same size as histiocyte nuclei
Burkitt Lymphoma
Burkitt lymphoma

• Three clinical variants:
  – Endemic
  – Sporadic
  – Immunodeficiency-associated

• Sporadic Burkitt lymphoma
  – Predominantly in children/young adults
  – Rapidly growing bulky disease
  – Often extranodal (GI, ovaries, kidney, breast)
  – Lymph node involvement more common in adults
Burkitt lymphoma: Diagnosis

• There is no specific diagnostic marker for Burkitt lymphoma

• Diagnosis relies on combination of:
  – Morphology
  – Immunophenotype
  – Cytogenetics
Burkitt lymphoma
Burkitt lymphoma
Burkitt lymphoma: phenotype

- Mature B cell (CD20 with clonal surface light chain)
- Tdt negative
- CD10, Bcl-6 positive
- Bcl-2 negative or very weakly positive
- Mib-1 proliferation rate close to 100%
- EBV+ in ~40% of sporadic cases
Burkitt lymphoma: IHC

CD20

CD10

Bcl6
Burkitt lymphoma: IHC

Mib1

Bcl2

Tdt
Burkitt lymphoma: Cytogenetics

- Characterized by translocations involving MYC gene on chromosome 8q24
- Typically involves IG genes
- Can be detected by FISH
- MYC rearrangement is not specific for Burkitt lymphoma
Burkitt lymphoma may be negative for MYC rearrangement

- FISH probes may not cover all possible breakpoints
- May represent a true absence of MYC rearrangement
  - Recurrent 11q abnormalities associated with lymphomas with gene expression pattern of Burkitt lymphoma and absence of MYC rearrangement
    - Blood 2014;123:1187
- Diagnosis of BL can be made without MYC rearrangement, but classical morphology and immunophenotype must be present
Burkitt lymphoma in small biopsies

- Core biopsies, endoscopic GI biopsies
- May not be easily recognized:
  - Starry sky appearance not prominent
  - Biopsies often crushed
  - Often poor fixation
- Always consider Burkitt lymphoma in small biopsies that show an aggressive B cell lymphoma
- Use Bcl-2 and Mib-1 to help
Gastric mass, 21 yo male
Gastric mass, 21 yo male
Gastric mass, 21 yo male

FISH: positive for MYC gene rearrangement
Burkitt lymphoma

**Clinical**
- young age
- rapidly growing mass

**Morphology**
- uniform medium sized cells
- high mitotic rate
- starry sky appearance

**Phenotype**
- CD20 pos, CD10/Bcl6 pos
- Mib-1 ~100%
- Bcl-2 neg

**Genetics**
- MYC translocation
B cell lymphoma, unclassifiable, with features between DLBCL and BL

- Some lymphomas can show transitional features between Burkitt lymphoma and diffuse large B cell lymphoma
- Previously known as Burkitt-like lymphoma
- Heterogenous entity, not uniformly treated
- Clinically important to recognize “double hit” lymphomas (MYC rearrangement with BCL2 or BCL6 rearrangement)
- Proposed classification in upcoming WHO classification:
  - High grade B cell lymphoma:
    1) High grade B cell lymphoma with MYC and BCL2 or BCL6 rearrangements (double hit lymphoma)
    2) High grade B cell lymphoma, NOS
B cell lymphoma, unclassifiable, with features between DLBCL and BL

- Not a distinct morphologic or immunophenotypic entity
- Diagnosis should be made in:
  - lymphomas that have the immunophenotype of BL, but are too morphologically pleomorphic
  - lymphomas that have the morphology of BL, but one or more inconsistent findings such as:
    - strong BCL2 expression
    - proliferation rate significantly less than 100%
    - $BCL2$ or $BCL6$ gene rearrangements

- 30-50% will be “double hit” lymphoma
“Double hit” lymphomas

• 1999: Cytogenetics in Burkitt-like lymphomas identified subset of cases with MYC rearrangement and t(14;18)
• Very poor prognosis

JCO 1999;17:1558
Double hit lymphoma

- All B cell lymphomas, unclassifiable between DLBCL and BL should be examined for MYC, BCL2, BCL6 rearrangements by FISH

- Double hit lymphoma can also be seen in ~5% of diffuse large B cell lymphomas
B cell lymphoma, unclassified, DLBCL/BL
(Double hit lymphoma)
Double hit lymphoma, confirmed by FISH analysis

Bcl2

Mib1
Burkitt lymphoma

Intermediate between BL & DLBCL
Lymphoblastic lymphoma

- Lymphoblastic neoplasm presenting in tissue with less than 25% blasts in bone marrow
- Most are T cell
- Rare cases are B cell (typically present as lymphoblastic leukemia)
T-lymphoblastic lymphoma

- Highly aggressive disease
- Children, adolescents; M>F
- Mediastinal involvement common
- Any other nodal or extranodal site (skin, tonsil, CNS, testis)
- Morphology:
  - Lymphoblasts with high mitotic rate +/- starry sky appearance
  - May have a more mature appearance if not well-preserved
Immunophenotype

- **T cell markers:**
  - CD3 (usually cytoplasmic)
  - Polyclonal CD3 antibody is not T cell specific (detects zeta chain)
  - CD4, CD8 frequently coexpressed

- **Lymphoblastic markers:**
  - Usually Tdt positive (~95% of cases)
  - Other markers of immature cells (CD99, CD34, CD1a)

- Usually lack B-cell and myeloid markers
B cell lymphoblastic lymphoma

• Morphology
  – Lymphoblasts

• Immunophenotype
  – Tdt, CD10, CD19, CD22, CD79a, PAX5
  – PAX5 most sensitive and specific IHC marker for B cell lineage
  – May be negative for CD20

• Cytogenetics
  – Further classified based on cytogenetic abnormalities
  – Flow cytometry, cytogenetics recommended
  – FISH on paraffin tissue may be performed
2008 WHO Classification

**Precursor lymphoid neoplasms**  
B lymphoblastic leukaemia/lymphoma, NOS  
B lymphoblastic leukaemia/lymphoma  
with recurrent genetic abnormalities  
  B lymphoblastic leukaemia/lymphoma with  
  \( t(9;22)(q34;q11.2); BCR-ABL1 \)  
  B lymphoblastic leukaemia/lymphoma with  
  \( t(v;11q23); MLL \) rearranged  
  B lymphoblastic leukaemia/lymphoma with  
  \( t(12;21)(p13;q22); TEL-AML1(Etv6-Runx1) \)  
  B lymphoblastic leukaemia/lymphoma with  
  hyperdiploidy  
  B lymphoblastic leukaemia/lymphoma with  
  hypodiploidy (Hypodiploid ALL)  
  B lymphoblastic leukaemia/lymphoma with  
  \( t(5;14)(q31;q32); IL3-IGH \)  
  B lymphoblastic leukaemia/lymphoma with  
  \( t(1;19)(q23;p13.3); E2A-PBX1(Tcf3-Pbx1) \)
• Bone marrow negative
• No other tumor present following resection
• No material for cytogenetics
• Bone marrow involved at recurrence several months later
• Confirmed diagnosis of B cell lymphoblastic leukemia/lymphoma, NOS
Myeloid sarcoma

- Myeloid sarcoma (2008 WHO classification): tumor mass consisting of myeloid blasts with or without maturation, occurring outside the bone marrow
- Also known as extramedullary myeloid tumor, granulocytic sarcoma, chloroma
- “Sarcoma” designation may confuse some clinicians
- Represents tissue involvement by acute myeloid leukemia
Myeloid sarcoma

• Several clinical scenarios:
  – Concurrent diagnosis with AML involving bone marrow
  – Isolated primary diagnosis, without bone marrow involvement
  – Recurrent disease
  – Previous history of myeloproliferative or myelodysplastic disorder
Myeloid sarcoma

- Any site can be involved (skin, lymph node, GI tract, bone, testis)
- May rarely involve the female genital tract:
  - Am J Clin Pathol 2006;125:783
  - Report of 11 cases of myeloid sarcoma involving the gynecologic tract
  - 5 cases presented as isolated mass
  - Uterus was the most common site (5 in corpus; 3 in cervix)
  - Cytology ranged from immature to differentiated
Myeloid sarcoma

- Wide morphologic spectrum
- More likely to have myelomonocytic or monoblastic differentiation
- Can resemble lymphoblastic lymphoma
- Can mimic mature lymphomas
- Clues to the diagnosis:
  - Looks lymphoid, but:
    - Streaming
    - Amphophilic cytoplasm
    - Eosinophilic precursors
    - CD20, CD3 negative
AML, skin biopsy
Myeloid sarcoma: IHC

- Immunohistochemical markers in myeloid sarcoma, in decreasing frequency:
  - CD68 (KP1)
  - Myeloperoxidase
  - CD117
  - CD99
  - CD68 (PG-M1)
  - Lysozyme
  - CD34
  - Tdt
Myeloid sarcoma: IHC

- CD68 staining depends on antibody used:
  - KP1 identifies virtually all myeloid sarcomas and other malignancies
  - PG-M1 correlates with monocytic differentiation
- Myeloperoxidase is not positive in every case
- Several markers are nonspecific:
  - CD117, CD99, Tdt
- CD34 is positive in approximately 50% of cases
Myeloid sarcoma: IHC

- AML is not classified based on tissue biopsy
- AML best characterized by flow cytometry and cytogenetics/FISH
- Bone marrow aspirate and biopsy performed for definitive classification
- In cases without bone marrow involvement, consider FNA to obtain material for flow cytometry and cytogenetics
2008 WHO Classification

Acute myeloid leukaemia (AML) and related precursor neoplasms
AML with recurrent genetic abnormalities
- AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11
- Acute promyelocytic leukaemia with t(15;17)(q22;q12); PML-RARA
- AML with t(9;11)(p22;q23); MLL3-MLL
- AML with t(6;9)(p23;q34); DEK-NUP214
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2);
  RPN1-EVI1
- AML (megakaryoblastic) with t(1;22)(p13;q13);
  RBM15-MKL1
- AML with mutated NPM1
- AML with mutated CEBPA

AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
- Acute myeloid leukaemia, NOS
  - AML with minimal differentiation
  - AML without maturation
  - AML with maturation
- Acute myelomonocytic leukaemia
- Acute monoblastic and monocytic leukaemia
- Acute erythroid leukaemia
- Acute megakaryoblastic leukaemia
- Acute basophilic leukaemia
- Acute panmyelosis with myelofibrosis

Myeloid sarcoma
Myeloid proliferations related to Down syndrome
  - Transient abnormal myelopoesis
  - Myeloid leukaemia associated with Down syndrome

Blastic plasmacytoid dendritic cell neoplasm
myeloperoxidase
myeloperoxidase
Bone marrow: Acute myeloid leukemia with t(9;11)(p22;q23)
Bone marrow: AML with myelodysplasia-related changes
Immunophenotyping in lymphoblastic and myeloid tumors

- Flow cytometry is best method
- Bone marrow sample or FNA of extramedullary tumor
- IHC provides a more limited panel
- Tumors may have some overlap:
  - PAX5 in AML with t(8;21)
  - Single myeloid marker in B lymphoblastic leukemia
- Do wide IHC panel:
  - Myeloid (MPO, CD117), monocytic (lysozyme, CD68), B cell (PAX5), T cell (CD3), Tdt

Acute Leukemia Immunohistochemistry, Arch Pathol Lab Med 2008;132:462
Lymphomas with medium-sized cells

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Mantle cell lymphoma

- Typical mantle cell lymphoma is composed of small irregular lymphocytes
- May transform into “blastoid” mantle cell lymphoma, morphologically resembling lymphoblastic lymphoma
- Can occur in patients with a past history of mantle cell lymphoma, or occur as a de novo presentation
- CD20, CD5, cyclin D1, SOX11 positive
- High proliferation rate
- Associated with more aggressive disease
Typical mantle cell lymphoma
MCL, blastoid variant
# T-lymphoblastic lymphoma vs. Mantle cell lymphoma

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<tr>
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<th>T-lymphoblastic lymphoma</th>
<th>Mantle cell lymphoma</th>
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<tbody>
<tr>
<td>Age</td>
<td>Young</td>
<td>Older</td>
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<tr>
<td>CD3</td>
<td>+</td>
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<td>CD20</td>
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<td>CD5</td>
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<td>Cyclin D1, SOX11</td>
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SLL/CLL

• Diffuse infiltrate of small mature lymphocytes
• Most cases contain proliferation centers containing prolymphocytes and paraimmunoblasts, medium sized cells with single prominent nucleoli
• Proliferation centers may sheet out
• Should not be confused with transformation to diffuse large B cell lymphoma (Richter transformation)
SLL/CLL: Proliferation center

prolymphocytes and paraimmunoblasts
SLL/CLL with prominent proliferation centers
SLL/CLL with prominent proliferation centers
Survival from biopsy according to the histological patterns of “non-accelerated” CLL, “accelerated” CLL and DLBCL transformation: median survival 76 months, 34 months and 4.3 months, respectively (P<0.001).

Accelerated CLL:

Proliferation centers broader than a 20x field

OR

High proliferation rate:
>2.4 mitoses/proliferation center

OR Ki-67 >40% in proliferation center

T/NK lymphomas

- T/NK cell lymphomas can have a wide morphologic range, including a predominance of medium-sized cells.
- Diagnosis based on expression of T/NK cell markers.
- Exclude lymphoblastic lymphoma in monomorphous T cell lymphomas with high proliferation rate (may have a more mature morphology).
Extranodal NK/T cell lymphoma, nasal type
Summary

• Be aware of the differential diagnosis of medium-sized cells, especially diagnoses that require immediate treatment

• It is important to differentiate Burkitt lymphoma from B cell lymphoma, unclassified (BL/DLBCL), by immunophenotypic and FISH studies

• Consider myeloid sarcoma in tumors that have lymphoid morphology and are negative for CD20 and CD3
Summary

- Perform a broad IHC panel for myeloid and lymphoblastic tumors and be aware of exceptions, such as:
  - Some T-LBLs may be Tdt negative
  - Some myeloid sarcomas may be MPO negative
- Be aware of unusual morphologic variants of mantle cell lymphoma and SLL/CLL