Pitfalls in the Diagnosis of Aggressive B-Cell Lymphoma: Borderlines, Variants and Mimics

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Objectives

• Recognize the morphologic and immunophenotypic spectrum of aggressive B-cell lymphomas

• Gain basic understanding of the approach to generating differential diagnoses and selecting ancillary studies

• Interpretation and limitation of ancillary studies in the workup of aggressive B-cell lymphomas
Mature B-Cell Lymphoma WHO 2008

1. Follicular lymphoma
2. Extranodal marginal zone lymphoma, MALT type
3. Nodal marginal zone lymphoma
4. Splenic marginal zone lymphoma
5. Lymphoplasmacytic lymphoma
6. Chronic lymphocytic leukemia/SLL
7. Mantle cell lymphoma
8. Primary cutaneous follicular
9. Splenic B-cell lymphoma/leukemia, unclassifiable
10. B-cell prolymphocytic leukemia
11. Hairy cell leukemia
12. Heavy chain diseases
13. Plasma cell neoplasms

1. Burkitt lymphoma
2. Diffuse large B-cell lymphoma, NOS
3. Primary mediastinal large B-cell lymphoma
4. Intravascular large B-cell lymphoma
5. ALK-positive large B-cell lymphoma
6. Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease
7. Plasmablastic lymphoma
8. Primary effusion lymphoma
9. DLBCL a/w chronic inflammation
10. Lymphomatoid granulomatosis
11. BCL unclassifiable: DLBCL/Burkitt
12. BCL unclassifiable: DLBCL/CHL
WHO 2008: DLBCL, NOS

Subtypes & Variants

Subtypes of DLBCL, NOS
1. T-cell/histiocyte rich large B-cell lymphoma
2. Primary DLBCL of the CNS
3. EBV-positive DLBCL of the elderly
4. Primary cutaneous DLBCL, leg type

Variants of DLBCL, NOS
1. Common variants: Centroblastic, Immunoblastic, Anaplastic
2. Rare morphologic variants
3. Molecular subgroups: GCB, ABC
4. Immunohistologic subgroups: GCB, Non-GCB, CD5+ DLBCL
WHO 2008: Borderline Categories

• **Not distinct entities**, but allow classification of cases not meeting criteria for distinct diagnostic categories
  - Whenever possible use pure categories
  - Be familiar with acceptable morphologic and immunophenotypic variation
  - May need broader IHC panels and additional ancillary studies such as FISH

• **Requires a multidisciplinary approach for patient management**
Cases 1 & 2

**Case 1**

11-year old boy with bilaterally enlarged tonsils

**Case 2**

8-year old boy with vomiting and weight loss with abdominal and pelvic masses and gastric outlet obstruction
Burkitt Lymphoma/Leukemia

- Frequently extranodal or leukemic (70% high stage)
- Ig/MYC translocations characteristic but not specific
- Aggressive but curable with high intensity short-duration chemotx
- Overall survival 80-90%, children > adults

  - **Endemic**
    - Equatorial Africa, Papua New Guinea (c/w endemic malaria)
    - Peak 4-7 years
    - EBV ~ 100%
    - Jaw, facial bones, orbit

  - **Sporadic**
    - Throughout world, median 30 years
    - 30-50% of childhood lymphomas
    - EBV < 30%
    - Abdominal masses

  - **Immunodeficiency**
    - HIV >>> allograft recipients & others
    - EBV 25-40%
Burkitt Lymphoma: IHC/Genetics

- Mature B & GC markers +
- Ki-67 ~ 100%
- Lacks CD5, BCL2, CD34, TdT
- 40% C-MYC as only defect
- Typically simple (~ 2) karyotype with IgH partner
- 15% light chains
- FISH is more sensitive
- Karyotype helpful to find other structural defects

**t(8;14) MYC/IGH@**

- MYC-negative Burkitt
- miRNA deregulation
DLBCL with C-MYC

• ~10% of DLBCL - aggressive with poor prognosis
• Non-Ig partners and complex karyotypes are common
• Even with Burkitt-like treatment no data to show improved outcome
  - Toxicity increases with advanced age
  - Performance status of patient
Case 3

• 65-year old man with enlarged abdominal and para-aortic lymph nodes

• Excisional biopsy of abdominal wall soft tissue was performed
Ki-67

Beware of necrosis!
Case 3: Summary

• Diffuse effacement by predominantly medium, some large sized cells
• Necrosis and high mitotic activity

• IHC: CD20+ CD79a+
• CD10+ BCL6+ BCL2+ BCL1-
• Ki67 ~80%
• EBV ISH-
• CD30-
• FISH: MYC+ BCL2+
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt Lymphoma
Diagnosis of Aggressive B-Cell Lymphomas
- An Algorithmic Approach -

Diffuse proliferation of medium – large lymphoid cells

Immunophenotyping
- Lineage: CD20, CD5
- Maturity: TdT
- Proliferation: Ki-67
- Other: BCL2, BCL1
- GC markers
- Transcription factors
- Light chains

Molecular Studies
- FISH
  - MYC
  - BCL2
  - BCL6

Karyotype
Step 1: Histology

Case

- Medium size, monotonous
- Cohesive growth
- Starry-sky
- High growth fraction
  - Favors Burkitt

- Medium–large
- Variable pleomorphism
- Variable proliferation
  - Favors BCLU

- Large size
- Pleomorphism
- Moderate proliferation
  - Favors DLBCL
Step 2: Immunophenotype

- CD20+
- CD5-
- TdT-
- BCL2-
- Ki-67 ~100%

Favors Burkitt

- CD5-/+
- BCL1-/+
- BCL2+/-
- Ki-67 variable

Favors BCLU

- BCL2+/-
- Ki-67 variable
- CD5-/+
- BCL1-/+

Favors DLBCL
Step 3: FISH

Case

• MYC
• BCL2
• BCL6

• MYC+
• BCL2-
• BCL6-

Favors Burkitt

• MYC+
• BCL2+
• BCL6+/-

Favors BCLU

• MYC +/-
• BCL2+/-
• BCL6+/-

Favors DLBCL
Step 4: FISH & Karyotype

- **MYC+ BCL2-**
  - Simple Karyotype
  - Ig/MYC
  - Burkitt

- **MYC+ BCL2+**
  - Complex Karyotype
  - Non-Ig/MYC
  - Burkitt histology
  - BCLU
  - DLBCL

Case
BCLU

Recommendations

• Aggressive mature B-cell lymphomas should be systematically studied using ancillary tests, in particular, FISH

• Features that are worrisome
  • GCB-immunophenotype with BCL2 expression
  • High growth fraction
  • BM or CNS involvement

• Proliferation index (Ki-67 IHC) is not a good indicator of aggressivity or double-hit cases
Case 4

• 64-year old woman with rapidly enlarging scalp nodule

• Then presented with an enlarged supraclavicular lymph node

• Staging bone marrow biopsy with flow cytometry
Case 4: Summary

- Scalp biopsy: diffuse infiltrate of medium sized cells, some with clefted outlines, others ‘blastic’
- Lymph node: low grade follicular lymphoma
- Bone marrow: flow cytometry +
- FISH: MYC+ BCL2+  
- DDX: B-ALL, Burkitt, Blastic mantle, Blastic follicular

Blastic transformation of follicular lymphoma
Case 5

• 21-year old man with chronic tonsillitis

• Bilateral tonsillectomy and adenoidectomy
Case 5: Summary

• DDX:
  • Large B-cell lymphoma
  • Hodgkin lymphoma
  • T-cell lymphoma
  • Infectious/reactive

• Ancillary studies and pitfalls
  • EBV EBER ISH
  • B & T-cell clonality
  • CD30+ immunoblastic proliferation

Necrotizing lymphadenitis consistent with Infectious mononucleosis
Case 6

- 19-year old man presented with intermittent high fever and unilateral cervical lymphadenopathy
- No known history of immunodeficiency
- Monospot test negative
- Cervical lymph node biopsy performed
Initial Diagnosis & Management

- EBV-positive lymphoproliferative disorder, consistent with infectious mononucleosis
- No clonality studies were performed
- The patient was followed without treatment
Biopsy 2

- Five months later, the patient presented with fever, prominent cervical lymphadenopathy and splenomegaly
- Repeat cervical lymph node biopsy
- Bone marrow biopsy with flow cytometry
Cytogenetics & Molecular Data

• Normal karyotype

• IGH and TCR gene rearrangements negative

• EBV clonality by PCR positive
Bone Marrow

Elevated ferritin
Elevated soluble IL-2 receptor
EBV viremia (11,700 copies/ml)
Flow cytometry negative

EBER
Diagnosis on Second Biopsy

- EBV-positive Diffuse Large B-Cell Lymphoma
- Complicated by hemophagocytic syndrome
- Treated with Rituximab and etoposide followed by R-CHOP
Clinical Course

• Two months later, patient returned with fever, neck swelling and septic shock from Klebsiella bacteremia
• CT-scan showed hypodense foci in liver & spleen
• Developed a massive gastrointestinal bleed
• Exploratory laparotomy showed a perforated jejunum with peritonitis
• Despite aggressive management, patient expired
Differential Diagnosis

- Chronic active EBV
- Hydroa vacciniforme-like lymphoma
- Systemic EBV+ T-cell LPD of childhood (Fatal IM)
- EBV+ DLBCL of the elderly
- Lymphoma - CHL, DLBCL, TCL
Case 6: Summary

- IM-like lesions that are not self-limiting may evolve into aggressive lymphomas
- Need for additional biopsies & molecular studies
- Evolution of aggressive histology over time
- Newer therapies with EBV-specific donor lymphocyte infusion are in development
Case 7

• 19-year old man with a 10 cm anterior mediastinal mass and superior vena cava syndrome

• No lymphadenopathy, hepatosplenicomegaly or other mass lesions on scans
Primary Mediastinal Large B-Cell Lymphoma

• Derived from thymic B-cells
• Young adults, median 35y, F:M=2:1
• Bulky ant sup mediastinal mass, SVC obstrn, dyspnea
• Low stage (Stage I-II in 66% at presentation)
• Sclerosis, clear cells
• Lack sIg, BCL2, BCL6, MYC
• CD30 ~70%, CD23 ~70%
• DDX: Classical Hodgkin lymphoma
• Aggressive but curable with multi-agent chemo + radiotherapy
• Long term remission correlates with initial stage
PMBL

- 38 yr old woman
- Thymic mass
- IHC
  - CD45+
  - CD20+
  - PAX5+strong
  - CD30+wk
  - CD15-
  - EBER-
- Stage 1A disease
- Complete remission with RCHOP
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL (primary mediastinal) and classical Hodgkin lymphoma
Case 8

• 61-year old man with a 5.0 cm mesenteric mass
**Case 8: Summary**

- Nodular and diffuse with CD20+ large cells in and outside nodules
- Background ranging from B-cell rich nodules to diffuse T-cell and histiocyte-rich areas
- NLPHL with progression to TCRLBCL/DLBCL
- Tendency for progression to an increasingly more diffuse pattern over time
- Pattern E predicts recurrence and progression to DLBCL
- IHC: CD20, CD3, CD21, PD-1

*Fan et al, AJSP 2003*
Past Medical History

• 1975: 33-year old with supraclavicular adenopathy
  • Imaging - multiple sites and spleen (Stage IIISA)
  • Intravenous pyelogram showed displacement of right kidney from presumed para-aortic disease
  • Biopsy diagnosed as cellular phase of classical Hodgkin lymphoma
• 1979 & 1988: Inguinal lymphadenopathy
  • Progressive transformation of germinal centers
CD20 on 1975 bx
TCRLBCL and NLPHL

Not a separate borderline category in WHO 2008
DLBCL/NLPHL

Summary

• Mixed nodular and diffuse nodal architecture
  - Look for the presence of atypical large cells and the company they keep
  - Look for an aggressive component
• A relatively short IHC panel is sufficient
  - First tier: CD20, CD30, CD15, PAX5
  - Second tier: EBV, CD45, OCT2, BOB1
  - FDC markers (CD21) useful to highlight pattern
  - Use caution in the interpretation of PD-1
Ancillary Studies in the Diagnosis of BCLU, DLBCL & Hodgkin

**DLBCL vs. Hodgkin**

**Immunophenotype**
- Lineage: CD20, CD5
- RS cells: CD30, CD15
- PAX5, OCT2, BOB1
- EBV-EBER
- LCA/CD45

**Molecular Studies**
- Only if sufficient numbers of large B-cells present

**FISH**
- Not needed

**Karyotype**
- Not needed
Case 9

- 74 year old woman underwent an exploratory laparotomy with cholecystectomy

- Gallbladder was thick and dense but no stones were found
Intravascular Large B-cell Lymphoma

• Rare subtype of large B-cell lymphoma predominantly in small to medium caliber vessels in adults
• Widely disseminated with fever and nonspecific symptoms
  - Western form: Sx related to organ - skin or CNS
  - Asian form: multiorgan failure, hepatosplenomegaly & hemophagocytic syndrome
• Rapidly fatal
Case 10

• 49-year old man with rapidly enlarging and painful mass in right neck, anorexia and an 80 lb weight loss in 2-3 m

• 10 cm firm, fixed, non-tender mass extended from angle of the jaw to the supraclavicular fossa, midline of the neck and to lateral occiput

• CT revealed a multi-locular mass representing a conglomeration of matted lymph nodes

• An excisional biopsy was performed

• Systemic workup revealed splenomegaly, but no pleural or pericardial effusions, ascites or other nodal or extranodal sites of disease
Immunophenotype

- Flow cytometry: negative, no CD45+ population detected
- Cytogenetics: normal male karyotype
- Immunohistochemistry:
  - Cytokeratin
  - CK5/6
  - EMA
  - MELAN-A
  - HMB45
  - S100
  - SALL4
  - CD68
  - CD45
  - CD20, CD79A
  - PAX5, OCT2
  - CD10, BCL6
  - BCL2
  - BCL1
  - CD5, CD3, CD43
  - CD138
  - CD10, BCL6
  - BCL2
  - BCL1
  - CD5, CD3, CD43
  - CD138
  - CD56
  - CD30
  - CD38
  - MUM1
  - Ki67
  - EBV-ISH
  - HHV8
Primary Effusion Lymphoma (PEL)

- Serous body cavity effusions (pleural, pericardial or peritoneal) without detectable tumor masses
- Most common in HIV+ patients
- Individual pleomorphic tumor cells may be present on random pleural biopsies
- Pleomorphic or immunoblastic morphology
- Survival <6 months regardless of treatment
PEL: IHC & Genetics

- CD45 +/- B- T-
- CD30+ CD38+ CD138+
- IgH /Igκ Rearranged
- TCR Germline
- HHV8 Positive
- EBV Positive/negative
Case 10: Summary

- Prompted by dx of **Extracavitary PEL**, HIV testing was performed
- Seropositive for HIV with a high serum viral load
- Marked anemia and neutropenia although bone marrow was not involved
- Six weeks after the diagnosis of EPEL, the patient was hospitalized for Salmonella bacteremia. HAART therapy was begun but he developed shortness of breath and mental status changes and expired
Case 11

- 38 year old man underwent right colectomy
- **Gross:** 1.3 cm mass protruding from mucosa
- **Histology:** nodular architecture, large cells with immunoblastic/plasmablastic features
- **IHC**
  - Positive: CD45RB, CD138, EMA, Kappa, ALK cyto, Ki67 75%
  - Negative: cytokeratins, CD30, CD20, CD3, CD2, lambda, PAX5
- **Molecular:** PCR+ for t(2;17)(p23;q23)
ALK+ Large B-cell Lymphoma

- Median age at diagnosis 43 years
- M:F = 5:1
- Hallmark
  - CLTC-ALK t(2:17)(p23;q23)
  - Granular cytoplasmic staining
- Advanced stage at diagnosis
  - Extremely aggressive
  - Median survival 12 months
  - Insensitive to Rituximab

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<td>HHV8</td>
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Laurent et al. JCO 2009
Case 12

• 70 year old woman presents with right cervical lymphadenopathy of 1 month duration

• 14-pound weight loss without fevers, chills or night sweats

• PET scan showed bilateral cervical, axillary, mediastinal, iliac and inguinal lymphadenopathy, bulky adenopathy of the lumbar retroperitoneum and focal involvement of spleen

• Staging bone marrow was negative
Case 12: Summary

- Nodular and diffuse with areas of large cells
- Interfollicular areas rich in T-cells and histiocytes
- DDX: NLPHL, TCRLBCL, PTCL, DLBCL
- Antecedent history 10 years previously of a lymph node biopsy showing follicular hyperplasia
- No additional workup & blocks unavailable
- Follicular lymphoma (grades 1-3B) with progression to diffuse large B-cell lymphoma (with unusual T-cell-rich interfollicular pattern)
Thank you!