Lymphoma work-up: Basics, Do’s and Dont’s!

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Outline

• Lymph node
  • Anatomy
  • Lymph node grossing for lymphoma workup
  • Ancillary studies overview
  • Specimen triaging guidelines
  • Do’s and Dont’s
  • Case review

• Spleen
  • Anatomy
  • Splenic lymphomas
  • Case review
Lymph node, Anatomy

Images: www.pathpedia.com
Lymph node, Histology

- **Paracortical zone** (T-cell rich)
- **Germinal centers** (B-cell rich)
- **Mantle zone** (B-cell rich)

Lymphoproliferative disorders

- Clinical presentations
  - Lymphadenopathy: Isolated, contiguous or generalized
  - Organomegaly: Hepatosplenomegaly
  - Extranodal mass lesions: Retroperitoneum, nasal, mediastinal, bone
  - B-symptoms: Fever, night sweats, weight loss (classical Hodgkin lymphoma)
  - Unexplained cytopenia(s): Bone marrow involvement
  - Peripheral blood leukocytosis: Leukemic presentation (Chronic lymphocytic leukemia)
  - Skin rash/papule/plaque: cutaneous lymphoma
Lymphoma work-up

• Surgeon responsibilities
  • Send specimen in fresh or in normal saline
  • Pathology should be notified immediately
  • Surgeon should warn pathologist of hazards
    • Eg. Tuberculosis
Lymph node grossing

• Identify lymph nodes and measure in 3 dimensions
• Serially section at 0.2 cm intervals perpendicular to the long axis
• Describe the cut surface, including color, nodularity, hemorrhage and necrosis
Specimen triaging

• Touch imprints, especially useful in smaller biopsies
  • Stained – for diagnosis and triaging decisions
  • Unstained – for potential FISH studies

• Well prepared touch imprints provide architectural and cytologic details

• Blot/dry blood/tissue fluid from cut surface of tissue

• Gently roll glass slide from one surface of the slide from one end to the other

• Air dry for Romanowsky stains (Diff-Quik, Wright-Giemsa)
  • Better staining for cellular granules

• Fix in alcohol for H&E stains
Touch preparation technique

Touch preparation on lymph nodes (Figures 1-4): Gently roll a clean glass slide over the tissue from one end to the other, in a constant speed, without pulling at the end. Sideways shear can disrupt fragile cells. Alternately, gently press the tissue against the glass slide.

Scrape preparation

- Surface of lymph node scraped with sharp scalpel blade
- Semifluid material spread on glass slides
- Especially useful if tumor cells embedded in fibrotic stroma
- Always do both touch and scrape preps
Touch preparation

- Reactive lymphoid hyperplasia
  - Polymorphous population
  - Tingible body macrophages
Touch preparation

• Predominantly small lymphocytes

  • Small B-cell lymphoma
    • Follicular lymphoma
    • Small lymphocytic lymphoma
    • Marginal zone lymphoma

  ❖ Immunophenotype is key
    • Morphology/IHC
    • Flow cytometry
      • Monotypia (Kappa or Lambda restriction)
      • Immunophenotype

• T-cell lymphoma
Touch preparation

• Predominantly intermediate sized lymphocytes

  • Monomorphemic, cytoplasmic vacuoles
    • Burkitt lymphoma
      ✤ Immunophenotype by IHC
      ✤ FISH for MYC rearrangement t(8;14).

  • Blastoid morphology
    • Myeloid sarcoma
      ✤ Immunophenotype by IHC
      ✤ Flow cytometry for blast and myeloid markers (usually negative by IHC)
      ✤ Unstained touch prep for cytochemical stains

  • Lymphoblastic lymphoma
    ✤ Immunophenotype by IHC
Touch preparation

• Predominantly large lymphocytes

• Diffuse large B-cell lymphoma
  ❖ Immunophenotype for prognosis & subtype
    • Germinal center/non-germinal center phenotype by immunohistochemistry
    • EBV by EBER ISH
    • BCL2/MYC co-expression by immunohistochemistry
  ❖ FISH for prognosis & subtype
    • Double/triple hit lymphoma
    • FISH for MYC/BCL6/BCL2 rearrangements
  ❖ Flow cytometry and molecular studies less important

• T-cell lymphoma
  • Anaplastic large cell lymphoma
  • Peripheral T-cell lymphoma-NOS
  ❖ Immunophenotype is key
    • CD30, ALK1, T-cell markers
Touch preparation

• "Hodgkin-like" large lymphocytes

• Classical Hodgkin lymphoma
  • Large "Reed-Sternberg" like-cells may be few in a polymorphous background
    ▶ Immunophenotype is key
    ▶ Flow cytometry/FISH not useful

• Anaplastic large cell lymphoma
  ▶ Immunophenotype
    ▶ CD30, ALK1, T-cell markers
## Lymphoma Workup on Small Samples

<table>
<thead>
<tr>
<th>Cytology of Touch Prep</th>
<th>Most Common DDX</th>
<th>Triaging priorities</th>
</tr>
</thead>
</table>
| Predominantly small                 | • Small B-cell lymphoma  
                                        • Reactive lymphadenopathy           | Morphology/IHC  
                                        Flow cytometry                      |
| Mixed small and medium              | • Small B-cell Lymphoma  
                                        • Diffuse large B-cell lymphoma  
                                        • Reactive lymphadenopathy          | Morphology/IHC  
                                        Flow Cytometry                      |
| Predominantly medium with vacuoles  | • Burkitt Lymphoma                                    | Morphology/IHC  
                                        Cytogenetics/FISH                    |
| Large “Hodgkin-large”               | • Hodgkin lymphoma                                    | Morphology/IHC                        |
| Few                                 |                                                     |                                      |
| Many                                | • Hodgkin lymphoma                                    | Morphology/IHC                        |
|                                     | • Anaplastic large cell lymphoma                      |                                      |
|                                     | • Diffuse large B-cell lymphoma                       |                                      |
Fixatives

• Neutral buffered formalin—most common
  • Morphology, IHC, FISH, molecular studies can be performed

• B5 fixative
  • Superior cytologic detail
  • Contains mercuric chloride (carcinogenic, environmental hazard)
  • Not compatible for molecular and FISH studies
  • If using, select 1-2 representative sections, while fixing rest in formalin

Why do we use alternate fixative

- Improves cellular morphology
- Improves diagnostic confidence
- Prevents diagnostic delay or rebiopsy
Alternative fixatives

- Acetic-Zinc-Formalin
- B-Fix
- B-Plus Fix
- Buffered Zinc Formalin

- Some have demonstrated compatibility with IHC and molecular studies
  - B-Plus Fix (University Hospital/UT Health SA, Unpublished)

Discarded with formalin waste
Importance of fixation

- Poorly/under fixed tissue leads to poor morphology, immunohistochemistry and erroneous diagnoses
- Fixative to tissue ratio at least 10-15:1
- Fixative penetrates thinner section better, ~1mm/hr (slices 2-3mm thick)
- Needs fixation of at least 6 hrs for good morphology and immunohistochemistry
- Poorly fixed tissue leads to poor/thicker sections on microtome

RPMI medium

- Developed at Roswell Park Memorial Institute in 1966 for the growth of human leukemia cells in monolayer or suspension cultures.
- Good culture medium for lymphocytes (contains phosphate, amino acids, vitamins, bicarbonate).
- Tissue collected in RPMI for flow cytometry/cytogenetic/molecular studies
- At least 0.2-0.3 cm viable tissue in ~15 ml RPMI and store in fridge for flow/cytogenetics
- Check expiration date (normal: peach/salmon, expired: bright pink)

Images: www.sigmaaldrich.com/Area_of_Interest/Life_Science/Cell_Culture/Product_Lines/Classic_Media_Salts/RPMI_Media.html
Flow cytometry

• Technology to measure and analyze physical characteristic of cells in a fluid stream through a beam of light
• Cells in fluid stream intercepted by laser
• Cell bound antibodies tagged with fluorochromes emit fluorescent signals, that are captured by detectors, and converted to electronic signals
• Data analyzed on scatter plots

Flow cytometry

- Each cell represented by an event/dot on scatterplot and groups of cellular events identified based on immunophenotypic characteristics
- Morphology not visualized
Flow cytometry

- Immunophenotype – multiple antibodies similar to IHC, co-expressions of antigen seen better

- **Monotypia in B-cells (superior to IHC)**
  - Reactive B-cells carry a mixture of surface Ig Kappa and Lambda light chains
  - Majority of neoplastic B-cells are monotypic for kappa or lambda
  - Important in small B-cell lymphomas and reactive lymph nodes
Flow cytometry

- T-cell lymphomas
  - CD4: CD8 ratio (abnormally high or low)
  - Loss of T-cell antigens seen (suggestive of abnormal T-cells)
  - No monotypia can be determined by flow cytometry
  - Immunophenotype (most done by immunohistochemistry)
Flow cytometry

- Blasts
  - Immature antigens
  - Myeloid vs. lymphoid antigens
    - Myeloid/blast markers may be negative by immunohistochemistry in myeloid sarcoma
Fluorescence in-situ hybridization

- Desired cells are fixed to glass slides and hybridized to nucleic acid probe
- This anneals to complementary sequence in specimen DNA and labelled with reporter molecule attached to fluorochrome
- Identify gene rearrangements in lymphoma

Fluorescence in-situ hybridization

- Fusion probes – detect 2 genes involved in reciprocal translocations
  - Eg. t(14;18) IgH/BCL2 fusion in follicular lymphoma
  - Red+green = yellow

- Break-apart probes – detect gene rearrangement, does not identify partner gene
  - Eg. MYC rearrangement in Burkitt lymphoma
  - Yellow = Red + green
Fluorescence in-situ hybridization

• Common FISH tests
  • *BCL6*, *MYC* and *BCL2* gene rearrangements in DLBCL work up to rule out double/triple hit lymphoma

  • t(14;18)*BCL2/IgH* – Follicular lymphoma
  • t(8;14)*IgH/MYC* – Burkitt lymphoma
  • t(11;14)*IgH/CCND1* – Mantle cell lymphoma

• Can be performed on unstained slides, fresh tissue in RPMI or paraffin embedded tissue
Molecular/PCR studies

• IgH/TCR clonality for B-cells and T-cells (fresh, frozen or FFPE tissue)
  • Each B-cell has a unique antigen receptor molecule on its membrane
  • Chance of multiple lymphocytes to coincidentally bear the same receptor is very low
  • Clonal peaks identified by real time PCR and interpreted based on guidelines
  • Common scenarios:
    • IgH clonality in MALT lymphoma of stomach (immunophenotype not definitive, flow cytometry for monotypia often not available as tissue is formalin fixed)
    • T-cell lymphoma with no definitive immunophenotype
    • Aberrant immunophenotypes to determine lineage

Molecular/PCR studies

Specimen triaging

• Excisional biopsies
  • **Morphology/Paraffin embedd**ed tissue is always first priority!
    • H&E stain and immunohistochemistry
    • FISH on FFPE tissue
    • Molecular studies on FFPE tissue

• Flow cytometry 2\textsuperscript{nd} priority – collect viable tissue in RPMI and refrigerate
  • Immunophenotype with 5-25 antibodies
  • Most useful in low grade lymphoma and acute leukemia

• Snap frozen tissue, if adequate, for molecular studies.
• Tissue in RPMI for cytogenetics (more important in acute leukemia than lymphoma)
Specimen triaging

• Core biopsies
  • Morphology/Paraffin embedded tissue is always first priority!
  • Immunohistochemistry on FFPE tissue for immunophenotype
  • FISH can be performed on FFPE tissue
  • Embed 1-2 cores per block
  • If possible, provide instructions to cut additional unstained slides during grossing to avoid refacing block by histotech

• Additional tissue should be requested if intraoperatively lymphoma suspected on touch imprints
Special considerations

• **Viability of tissue in RPMI**

  • Processing for flow cytometry/cytogenetics immediately is best

  • Low-grade/small cell lymphomas – low cell turnover – survive longer (process within 48-72 hrs, lab policy)

  • High grade lymphomas – high cell turnover (large cell, necrosis, apoptosis) – process within 24 hrs
Special considerations

• **Decalcification of bony specimens**

  • If lymphoma suspected in bony tissue, do not dunk whole specimen in decal solution!!

  • Separate fleshy/non-bony tissue and submitting sections before decalcification

  • Decalcification permanently denatures DNA and is not recommended for FISH/molecular studies (EDTA better than formic acid)

  • Immunohistochemistry optimal in non-decalcified tissue

Special considerations

• If infection suspected
  • Wear personal protective equipment including fit-tested N95 mask
  • Grossly lymph nodes appear white and necrotic
  • Touch preparations show abundant necrosis, neutrophils or granulomas
  • DO NOT FREEZE
  • Communicate to clinician to send sterile specimen for culture
  • Decontaminate/disinfect surfaces and equipment

Special considerations

- **Emergent scenarios**
  - Mass compressing airway
  - Superior vena cava syndrome
  - Risk of GI perforation

- Information need to be communicated to pathologist immediately and specimen needs expedited processing
Noteworthy clinical settings associated with lymphoma

- H. pylori gastritis, Hashimoto’s thyroiditis, Sjogren’s syndrome – MALT lymphoma
- Lymphomatous polyposis – mantle cell lymphoma
- Post chemoradiation for solid tumor – acute leukemia/myeloid sarcoma
- Lung – lymphomatoid granulomatous
- Sinonasal mass – Extranodal T/NK-cell lymphoma
- Splenomegaly – Hairy cell leukemia, Hepatosplenic T-cell lymphoma, splenic marginal zone lymphoma
Do’s:

✓ Adequate clinical history, including h/o prior lymphoproliferative disorders, infectious or high risk conditions
✓ Communication with pathologist handling the case
✓ Well prepared touch/scrape imprints to make triaging decisions on morphology
✓ Tissue needs to be well fixed before processing
✓ Morphology/FFPE always first priority
✓ Judicious triaging for flow cytometry
  • Low grade lymphomas, acute leukemia
Common errors to avoid:

- Beware of infectious risk – PPE/decontamination
- DOCUMENT how specimen was triaged – unstained touch imprints, tissue saved in RPMI/snap frozen
- If using alternate fixatives, limit to 1 or 2 blocks and fix the remaining tissue in formalin
- Sample any heterogenous appearing areas
- Separate soft tissue/non-bony lesional tissue without decalcifying entire specimen
- Weekend effect: Tissue in RPMI need to processed immediately, especially if higher grade lymphoma. Communicate with pathologist/appropriate lab
Case 1

• 40 year-old man with mediastinal mass and supraclavicular lymph node

• Supraclavicular lymph node excised
  • 1 cm in greatest diameter (small)
Case 1: Triage

Touch imprint

Scrape preparation
Case 1: Classical Hodgkin Lymphoma
Summary Case 1

• Due to fibrosis, scrape preparation was better than touch imprint (Do both routinely!)

• Small lymph node
  • Triage allowed conservation of tissue for morphology
  • Flow cytometry not sent
  • Cytogenetics not sent
  • Tissue submitted for histology and IHC
Case 2

• 23 year-old woman with mesenteric/bowel wall mass
• 3.5 cm lymph node
Case 2: Triage of Touch Imprints

Air dried touch imprints (Diff Quik) artificially increases the size of large cells.
Case 2: Flow Cytometry

- Flow Cytometry
  - No abnormal lymphoid population
  - B-cells (red) express polytypic kappa and lambda light chain
Case 2: Benign Lymph Node

**Follicular Hyperplasia**

**Progressive Transformation of Germinal Centers (PAX5)**

Likely responsible for the larger nodules seen grossly
Summary Case 2

• Benign lymph node
  • Difficult case
  • Diagnosis aided by negative flow and cytogenetics
• Plenty of tissue to do all studies
  • Don’t miss out on an opportunity (Pathologists will thank you)
  • Or if you are uncertain, do set aside tissue in RPMI
Case 3

- 67 year-old female with small bowel mass
- Sent fresh for evaluation
Due to aggressive appearance, cytogenetics and FISH were expedited
Cytogenetics

46,XX,t(8;14)(q24;q32),del(13)(q12;q14),t(14;18)(q32;q21)[18]

FISH for 8q24/C-MYC Rearrangement Using Break-apart Probes

Leukemia & Lymphoma 2008;48(1):75-80
Summary Case 3

• High grade B-cell lymphoma with MYC and BCL2 rearrangements
  • “Double hit lymphoma” does respond to standard therapy for DLBCL

• Cytogenetics can aid diagnosis and guide therapy*

*Leukemia & Lymphoma 2008;48(1):75-80
Immature Hematolymphoid Neoplasms

- Myeloid Sarcoma (AKA chloroma or granulocytic sarcoma)
- Lymphoblastic Lymphoma
- Do Treat like “lymphoma workup”

**Clues to the diagnosis**
- History of
  - Acute myeloid leukemia (AML)
  - Acute lymphoblastic leukemia (ALL)
- Green** color suggests myeloid sarcoma

**Cancer Research 1954;14(3):157-163
Normal Spleen

• Normal spleen
  • 150-250 grams

• White pulp (5-22%)
  • Children +/- germinal centers
  • Adults no germinal centers
  • Marginal zones are seen

• Red pulp (75%)
  • Macrophages (CD4+)
  • sinuses (CD8+)

https://library.med.utah.edu/WebPath/HISTHTML/NORMAL/NORM137.html
Normal Spleen
Patterns of lymphoid neoplasms in spleen

**Red Pulp Expansion**
- "Small B-cell lymphomas"
  - Small lymphocytic lymphoma
  - Follicular lymphoma
  - Marginal zone lymphoma
  - Mantle cell lymphoma

**White Pulp Expansion**
- Leukemias (blood and bone marrow based)
  - Chronic lymphocytic leukemia
  - Chronic myelogenous leukemia
  - Acute leukemia
  - Mastocytosis

**Mass Lesion**
- Aggressive lymphomas and Hodgkin
  - Diffuse large B-cell lymphoma
  - Hodgkin lymphoma
  - Anaplastic large cell lymphoma
Example White Pulp Expansion

• Mantle cell lymphoma
Examples of Red Pulp Expansion

**Hairy cell leukemia**

![Image of Hairy cell leukemia]


**Splenic diffuse red pulp B-cell leukemia**

![Image of Splenic diffuse red pulp B-cell leukemia]

Case 4

- 31 year-old man with abdominal pain
- Can’t tie his shoes
- Touch imprint showed mixed small and large lymphocytes
Splenic Marginal Zone Lymphoma
Summary Case 4

• Increased white pulp narrows the differential diagnosis
  • “low grade lymphomas”

• Flow cytometry was extremely helpful to establish clonality

• **Do** treat suspicious spleens as lymphoma workups
Questions?