Diagnosis of Lymphoma by Fine Needle Aspiration Biopsy

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Acknowledgements
Case examples from:
University of California San Francisco Cytology Study Set
Massachusetts General Hospital Cytology Study Set

I have nothing to disclose

Patient Evaluation

History
- Duration of lymphadenopathy
- Change in size, fluctuation
- History of cancer, lymphoma
- Recent travel
- Exposure to infectious agents
Patient Evaluation

Review of systems
- Recent viral illness, cold flu, sinusitis ...think back
- Dental procedures, problems
- B symptoms: Fever, night sweats, chills, weight loss, headache
- Rash, itching, skin changes
- Cough, back pain,
- Smoking...time to quit

Patient Evaluation

Physical exam:
- Size
- Characteristics:
  - Soft, firm, mobile, fixed, matted adjacent lymph nodes
  - Location
  - Amenable to biopsy
  - palpation
  - image guidance, US, CT

Lymph node FNAB

Establish clinical index of suspicion
- Clinically benign/reactive
- Possibly infectious
- Possibly metastatic
- Lymphoma
- Other
  - Surprise!!!

Lymph node FNAB

1st pass
- Reactive lymph node
- Acute or chronic lymphadenitis
- Infectious process

2nd pass and additional passes
- Reactive lymph node
- Acute or chronic lymphadenitis
- Infectious process and Lymphoma, non-Hodgkin
Lymph node FNAB

MAXIMIZE the diagnostic yield
Smears – save unstained for testing
Flow cytometry
Cell block, IHC, special stains,
Molecular testing
FISH
PCR
Sequencing

<table>
<thead>
<tr>
<th>Clinically benign/reactive</th>
<th>Smears, flow cytometry</th>
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</thead>
<tbody>
<tr>
<td>Infectious</td>
<td>Smears, culture, +/- cell block, +/- flow cytometry</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>Smears, flow cytometry, unstained, cell block, pcr, karyotyping</td>
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<tr>
<td>Possibly metastatic</td>
<td>Smears, cell block, +/- flow cytometry</td>
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<tr>
<td>Other/surprise</td>
<td>Adjust as needed</td>
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</table>
**Rapid Interpretation**

<table>
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<tr>
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<th>Desired Test</th>
<th>Processing</th>
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<tr>
<td>Benign/reactive</td>
<td>Smears</td>
<td>Diff-Quik, Pap, other</td>
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<tr>
<td></td>
<td>Flow cytometry</td>
<td>Saline or RPMI/media</td>
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<tr>
<td>Infectious</td>
<td>Smears</td>
<td>Diff-Quik, Pap, other</td>
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<tr>
<td></td>
<td>AFB, GMS, Gram stain</td>
<td>Unstained smear air-dry</td>
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<tr>
<td></td>
<td>Microbiology culture</td>
<td>Saline or culture media</td>
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<td></td>
<td>Cell block</td>
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<tr>
<td></td>
<td>FISH</td>
<td>Unstained smear air-dry</td>
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<td></td>
<td>Cell block for IPEX</td>
<td>Formalin needle rinse</td>
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<td></td>
<td>Karyotyping</td>
<td>Saline or RPMI/media</td>
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<td></td>
<td>PCR</td>
<td>Saline</td>
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<td>Metastasis</td>
<td>Smears</td>
<td>Diff-Quik, Pap, other</td>
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<td></td>
<td>Cell block for IPEX</td>
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<td></td>
<td>+/- Flow cytometry</td>
<td>Saline or RPMI/media</td>
</tr>
<tr>
<td>Other/surprise</td>
<td>Adjust as needed. Get extra samples to reserve for unanticipated tests.</td>
<td></td>
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**Lymph node FNAB**

**Lymphoma**

- **Morphology**
  - Monomorphic or heterogeneous? or both?

- **Cell size**—how to judge

- **Flow cytometry**
  - Assess flow results in the context of cell size
  - Assess cell size in the context of flow results

**FNAB approach**

**Assessing the first FNAB pass**

- **Heterogeneous**
  - Reactive, Infectious, Hodgkin’s
  - Smears
  - Unstained
  - Flow cytometry
  - Cell block

- **Monomorphic**
  - B-cell lymphoma
  - Smears
  - Flow cytometry
  - Unstained
  - +/- Cell block

- **Heterogeneous Malignant, lymphoid**
  - T-cell Lymphoma, DLBCL, Metastasis
  - Smears
  - Flow cytometry
  - PCR, cell block

**Lymph node FNAB**

**String of pearls**

- **Monomorphic**
- **Polymorphic**

**FNAB approach**

**Assessing the first FNAB pass**

- **Heterogeneous**
  - Reactive, Infectious, Hodgkin’s
  - Smears
  - Unstained
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  - Cell block

- **Monomorphic**
  - B-cell lymphoma
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  - +/- Cell block

- **Heterogeneous Malignant, lymphoid**
  - T-cell Lymphoma, DLBCL, Metastasis
  - Smears
  - Flow cytometry
  - PCR, cell block
Lymph node FNAB

When to use molecular cytogenetics and other ancillary testing

Discordance:
• Clinical impression
• Morphology
• Flow cytometry

Resolve with:
• FISH
• PCR
• Karyotyping
• IPEX

Lymph node FNAB

B- cell Lymphomas
Monomorphic- string of pearls
Flow confirmation
Cell size
  compared to macrophage nucleus
  small
  intermediate
  large

B- cell Lymphomas of small cells

Follicular lymphoma
Mantle cell lymphoma
Small lymphocytic lymphoma/CLL
Marginal zone lymphoma
Lymph node FNAB

Follicular lymphoma
20% of all lymphomas
Median age is in the sixth decade
Morphology:
  Monotonous population of small cells
  **Centrocytes**- small cells with cleaved irregular nuclear contours and inconspicuous nucleoli
  **Centroblasts**- larger cells with round to oval nuclear contours and prominent nucleoli

Follicular lymphoma immunophenotype
kappa or lambda light chain restriction
B cell markers: CD19+, CD20+, CD22+, CD79a and CD10+
also BCL2+, BCL6+
Negative: CD5, CD23
Grade 3 FL may lack CD10

Follicular lymphoma grading
Based on the proportion of centroblasts
Number of centroblasts/10 hpf in follicles
grade 1: 0-5 centroblasts/10 hpf
grade 2: 6-15 centroblasts/10 hpf
grade 3: >15 centroblasts/10 hpf

WHO recommends:
grade 1-2 or grade 3

Cytology
Requires **perfect** smears and samples
grade may be suggested:
Follicular lymphoma, favor grade 1-2
Follicular lymphoma, favor grade 3

Proliferation index on cell block
MIB-1 to support grade interpretation
Grade 1-2 proliferation fraction <20%
Grade 3 proliferation fraction >30%
Lymph node FNAB

Follicular lymphoma genetics
  t(14;18)(q32;q21)  90% of cases
  FISH is sensitive and specific for detection

  Immunoglobulin heavy and light chains are rearranged
  Nearly 100% can be detected by PCR

Case 1

45 year old woman who presents with axillary lymphadenopathy

ROS: She denies fever, chills, weight loss. No history of breast cancer.
  20 pack-year smoking history

PE: 3 firm mobile lymph nodes in the axilla, largest measures 2.0 cm
Follicular architecture

UCSF Cytology Study Set  Pap stain 2x

Follicles

UCSF Cytology Study Set  MGG 40x

Monomorphic pattern

UCSF Cytology Study Set  MGG 40x

Macrophage

UCSF Cytology Study Set  Pap stain 60x
Case 1

**Flow cytometry:**
Monoclonal population of lambda restricted B-cells expressing
CD19+, CD20+, CD22+, CD10+
Negative for: CD5, CD23,
**IPEX:** CD20+, CD10+, Ki-67 low proliferation,
Bcl-2 +
**FISH** t(14:18)
Follicular lymphoma, favor grade 1-2.
Mantle cell lymphoma
3-10% of all lymphomas
Median age of 60
Most patient present with stage III or IV
Lymphadenopathy, hepatosplenomegaly and bone marrow involvement
Peripheral blood involvement by flow cytometry

Aggressive clinical course

Mantle cell lymphoma
Cytomorphology:
- small to medium size lymphoid cells with irregular nuclear contours, may resemble centrocytes
- large cell transformation does not occur
- blastoid variant
- pleomorphic variant
- monomorphic population of small lymphoid cells with mitoses helps identify mantle cell lymphoma

Mantle cell lymphoma immunophenotype
Ig expression Kappa > Lambda (intense)
B cell markers: CD19+, CD20+, CD22+, CD79a and CD5
Negative CD10, CD23 (or weak)
Blastoid variant may lack CD5
Proliferation fraction: MIB-1 stain

Mantle cell lymphoma genetics
t(11;14)(q13;q32)
~100% of cases (rare cases negative).
cyclin D1 gene (CCND1)
rare variants t(2;12)(p12;p13)
Case 2

79 year old man presented with an inguinal mass.

ROS: He reports increasing fatigue no other B-symptoms.

PE: A 2.0 cm firm ovoid slightly mobile inguinal lymph node
Mitotic figures

MGH Cytology Study Set Pap stain 40x

MGH Cytology Study Set Cell block

Case 9
Cell block Ki67 40x

MGH Cytology Study Set Cyclin D1

MGH Cytology Study Set Mib-1
Case 2

Flow cytometry:
Monoclonal population of kappa-restricted B cells
Positive for: CD19+, CD20+, CD5+
Negative for: CD10-, CD23-, FISH:
CCND1 rearrangement.

Lymph node FNAB
Small lymphocytic lymphoma/CLL
Most common adult leukemia in Western countries
6.7% of non-Hodgkin lymphoma
Clinical presentation
May be subtle
Fatigue, anemia, infections, splenomegaly
Lymphadenopathy may be subtle
Asymptomatic

Lymph node FNAB
Small lymphocytic lymphoma/CLL morphology
- Small lymphocyte with clumped chromatin, round nucleus, rare small nucleolus
  (slightly larger than a normal lymphocyte)
- Larger forms:
  - Prolymphocytes- small to medium sized with clumped chromatin, small nucleoli
  - Paraimmunoblasts- larger cells with round to oval nuclei, prominent nucleoli
  - Richters transformation: large cell lymphoma, Hodgkin lymphoma
Lymph node FNAB

Small lymphocytic lymphoma/CLL
Immunophenotype
- Surface Ig kappa or lambda (usually dim)
- B cell markers: CD19+, CD20+ (dim), CD22+, CD79a
- and CD5 and CD23
- CD11c (weak) and CD43+

Negative: CD10
Prognostics: zap70

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Lymph node FNAB

Small lymphocytic lymphoma/CLL genetics
80% detectable by FISH
- del 13q14.3 (~50%)
- trisomy 12 (~20%)
- 11q del (~4%)
- 17p del (~3%)

FISH panel available – useful for prognosis.

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Case 3

69 year old man presents with bilateral inguinal lymphadenopathy

ROS: Reports increasing fatigue, bruising, no weight loss, fever, night sweats

PE: Bilateral inguinal lymph nodes, ranging from 0.5 to 1.0 cm, ovoid, mobile

UCSF Cytology Study Set Pap stain 2X
Case 3

**Flow cytometry:**
Gated on small cells
Monoclonal kappa restricted B-cells expressing
- CD19+, CD20+ CD5+, CD23+
- Negative for: CD10

FNA biopsy final diagnosis:
Small lymphocytic lymphoma

Recommend correlation with peripheral blood cell count with differential and flow cytometry
Lymph node FNAB

**Marginal zone lymphoma/MALT**

MALT 7-8% of B-cell lymphomas
Nodal marginal zone lymphoma (MZL) 1.5-1.8%
Median age in the 60s
Clinical history
- Hashimoto’s thyroiditis
- Chronic inflammation of mucosal tissue
- H. pylori in stomach
- 33% of patients have serum paraprotein
- Indolent low grade lymphoma

**Morphology**

Plasmacytic differentiation (33%)
Monocytoid cells
- Small cells with abundant pale cytoplasm and irregular nuclei, small nucleoli
- Centrocyte nucleus with more cytoplasm

**Immunophenotype**

- Light chain restriction Kappa or Lambda
- or loss of light chain restriction
- B cell markers: CD19+, CD20+, CD79α+
- Negative: CD10-, CD5-, CD23-
- No specific marker for MALT

**Genetics:**

- t(11;18)(q21;q21)
- t(14;18)(q32;q21)
- t(3;14)(p14.1;q32)
- t(1;14)(p22;q32)
- add +3
- add +18

Stomach, intestine, ocular, salivary gland, lung, skin, thyroid
Case 4

70 year old woman presents with a left cheek mass, present for 3 weeks

ROS: No fever, night sweats, weight loss, difficulty swallowing, dry mouth, facial pain

PE: A firm immobile 1.5 cm mass overlying the left parotid gland
Case 4

Flow cytometry:
Gated on small to intermediate size lymphocytes
Monoclonal population of B-cells, kappa light chain restricted and expressing
Positive for: CD19+, CD20+, CD22+
Negative for: CD5-, CD10-

FNA biopsy final diagnosis: MALT lymphoma

nuc ish(IGHx2)(5'IGH sep 3'IGHx1)[5/100],(MALT1x2)[100]

FISH evaluation for IGH rearrangement was performed on nuclei with the Vysis LSI IGH Dual Color, Break Apart Rearrangement Probe (Abbott Molecular) at 14q32 and is interpreted as ABNORMAL. Rearrangement was observed in 5/100 nuclei, which exceeds the normal range (up to 3%) established for this probe in the Cytogenetics Laboratory at BWH. An IGH rearrangement is a typical cytogenetic aberration in a subset of B-cell leukemias, lymphomas and myeloma.

FISH evaluation for MALT1 rearrangement was performed on nuclei with the Vysis LSI MALT1 Dual Color, Break Apart Rearrangement Probe (Abbott Molecular) at 18q21 and is interpreted as NORMAL. No rearrangement was observed in 100/100 nuclei. A normal MALT1 FISH finding can result from absence of MALT1 rearrangement, from a variant MALT1 rearrangement, or from an insufficient number of neoplastic cells in the specimen.
**B-cell lymphoma**

- Small

**Follicular lymphoma**
  - CD20+, CD10+
  - t(14;18)

**Mantle cell lymphoma**
  - CD20+, CD5+
  - t(11;14) cyclin D1

**Small lymphocytic/CLL**
  - CD20+, CD23+, CD5+
  - FISH panel

**Marginal zone/MALT**
  - CD20+, CD5-, CD23-

- MALT panel
  - t(11;18), t(14;18), t(3;14), t(1;14)

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**Lymph node FNAB**

**B- cell Lymphomas of intermediate size cells**

**Burkitt lymphoma**

**Lymphoblastic lymphoma**

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**Lymph node FNAB**

**Burkitt lymphoma**

**Endemic**
  - Most common childhood malignancy in equatorial Africa
  - Peak: Age 4-7

**Sporadic**
  - Throughout the world
  - Mostly children and young adults
  - 1-2% of lymphomas in USA and Western Europe

**Immune deficiency related**
  - HIV related

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**Lymph node FNAB**

**Burkitt lymphoma**

**Aggressive, high grade lymphoma**

**Patients usually present with bulky disease**

**Advanced stage**

**Treatment may result in tumor lysis syndrome**
Lymph node FNAB

Burkitt lymphoma
Cytomorphology
- Monomorphic appearance with sheets of malignant cells
- Starry sky, macrophages ingesting apoptotic debris
- Intermediate size cells with dense basophilic cytoplasm, small septate lipid vacuoles in cytoplasm, high nucleus to cytoplasm ratio, smooth nuclear contours, open chromatin, prominent nucleoli
- Mitotic figures, apoptotic debris

Burkitt lymphoma immunophenotype
Surface Ig kappa or lambda (moderate to strong)
B cell markers: CD19+, CD20+, CD22+
and CD10+
also BCL6+
Negative: BCL2-, CD5-, CD23-, TdT-
MIB-1 positive in nearly 100% of cells

Burkitt lymphoma genetics
MYC translocation in 90%
most common: t(8;14)(q24;q32)
less common: t(2;8)(p12;q24)
t(8;22)(q24;q11.2)
10% have other translocations
Lack MYC translocation by FISH
Karyotype- high mitotic rate allows growth

Case 5
38 year old man referred to the FNA clinic from the Emergency Department.
Initially presented to his PCP with a left cervical lymph node.
PCP did a “blood test” and told him he had no sign of infection or lymphoma.
The patient’s wife, a nurse referred him to the ER.
Case 5

ROS: He denies any weight loss, fever or night sweats.

PE: A 5.0 cm firm mobile smooth surfaced left level 2 cervical lymph node.

FNA biopsy was performed in 3 passes with 23 gauge needles.
Case 5

**Flow cytometry**

Monoclonal population of B-cells with bright kappa expression

Positive for: CD19+, CD20+, CD10+

Negative for: CD5-, CD23-

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Case 5

Flow cytometry

Monoclonal population of B-cells with bright kappa expression

Positive for: CD19+, CD20+, CD10+

Negative for: CD5-, CD23-

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Case 5

**FISH analysis:**

MYC rearrangement with 8q24 breakapart

Karyotype failed to grow.

Additional FISH requested on additional smears to exclude “double hit” DLBCL with myc translocation

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Case 5

FISH analysis:

- MYC rearrangement:
  - 8q24 break apart rearrangement
  - (26/30) nuclei

- IGH-BCL2 rearrangement:
  - no IGH-BCL2 rearrangement
  - trisomy/rearrangement of IGH (ch 14)
  - in 22/30 nuclei

- BCL6 rearrangement:
  - no rearrangement in 30/30 nuclei
Case 5

Final FNA diagnosis:
Left cervical lymph node, FNA biopsy:
- Burkitt Lymphoma.

The patient was treated on the basis of the FNA biopsy. Treatment was started 4 days following presentation to the ER.

Lymph node FNAB

Lymphoblastic lymphoma/leukemia
Lymph node involvement by acute lymphoblastic leukemia (ALL) without blood or bone marrow involvement
Childhood lymphoma 75% < 6 years old
10% are B-cell lineage, ~90% are T-cell lineage

Clinical presentation: bone marrow failure, anemia, thrombocytopenia, neutropenia

Lymph node FNAB

Lymphoblastic lymphoma/leukemia
Cytomorphology
Blasts
- small cells with high N:C ratio, inconspicuous nucleoli
- larger cells with a moderate amount of cytoplasm, open chromatin and prominent nucleoli
- cytoplasmic granules in 10%
- Mitotic figures, apoptosis and "starry sky" may mimic Burkitt lymphoma
Lymph node FNAB

Lymphoblastic lymphoma/leukemia

Immunophenotype for B-ALL/LBL

B cell markers CD19+, CD20+, CD79a+, cyto CD22+ and CD10 and TdT
also surface CD22, CD24, PAX5

Immunophenotype for T-ALL/LBL

T-cell markers variable:
CD1a, CD2, CD3, CD4, CD5, CD7, CD8 and TdT

Lymph node FNAB

B-cell lymphomas of large cells

Diffuse large B-cell lymphoma (DLBCL)

Variants:

- Immunoblastic diffuse large B-cell lymphoma
- T-cell rich B-cell lymphoma
- Follicular lymphoma grade 3/DLBCL
- Mediastinal
- Other

Lymph node FNAB

Diffuse large B-cell lymphoma, NOS

25-30% of non-Hodgkin lymphoma in West
Median age is in the 70s

with wide range including children, young adults

Immuo-deficient at higher risk

Clinical:

- Patients may be asymptomatic
- Rapidly enlarging mass lesions, nodal and extranodal
Diffuse large B-cell lymphoma, NOS

May emerge de-novo or through transformation from:
- follicular lymphoma
- small lymphocytic lymphoma/CLL
- marginal zone lymphoma
- nodular lymphocyte predominant Hodgkin lymphoma

Cytomorphology

Monomorphic proliferation of large lymphoid cells
Broad spectrum of cell types
- Centroblastic: large cells with scant cytoplasm, irregular nuclei and prominent nucleoli
- Immunoblastic: large cells with abundant basophilic cytoplasm and 1-2 macronucleoli
- Anaplastic: large R-S like cells with abundant cytoplasm and large hyperlobated nuclei, prominent nucleoli
- Other variants

Immunophenotype

Surface/cytoplasmic Ig in 50-75%
Positive for: CD19+, CD20+, CD22+, CD79a+ and CD10+ (30-60%), CD5+ (10%)

MIB-1 proliferation index >40% in most cases, >90% in some cases

Genetics

t(14;_): break-apart probe may not identify partner chromosome
t(14;18)
t(8;14) subset other...
Case 6

64 year old woman with a history of follicular lymphoma, treated years ago. She now presents with an enlarged cervical lymph node.

ROS: She reports increased bruising and weight loss. She denies fever and night sweats.

PE: A 2.0 cm ovoid firm, slightly mobile level 4 cervical lymph node.
Flow cytometry:
Kappa restricted population of B cells
Positive for: CD19+, CD22+, CD10+
Negative for: CD5-, CD23-

Surgical excision was performed.
Lymph node FNAB

Hodgkin lymphoma

**Classical Hodgkin Lymphoma**
- nodular sclerosing
- mixed cellularity
- lymphocyte rich
- lymphocyte depleted

Lymphocyte predominate

Lymph node FNAB

Classical Hodgkin Lymphoma
Immunophenotype
- CD30+ (~100%)
- CD15+ (~75-85%)
- Pax5 (~95%)
- CD3, CD20, CD79a

Use the full panel to distinguish from other lymphomas with CD30+ (DLBCL, ALCL)

Case 7

36 year old physician presents to the FNA clinic with an axillary lymph node, which has been present for 2 weeks. He reports severe pruritus of several months duration, treated with tri-cyclic antidepressants and analgesics.

ROS: No fever, chills, weight loss

MGH Cytology Study Set, Pap stain 2X
Case 7

FNA biopsy final diagnosis:
Hodgkin lymphoma, nodular sclerosis type

Patient declined excision due to the risk of lymphadema (3-5%).

Treated on the basis of FNA biopsy.

Lymph node FNAB

T-cell lymphomas
Clinical presentation:
when to suspect
Morphologic clues:
Heterogeneous but all malignant
Flow cytometry
PCR
Karyotyping
T-cell lymphomas

- Anaplastic large cell lymphoma
- Cutaneous T-cell lymphoma

Immunophenotype
- ALK positive (IHC or FISH)
- Loss of CD5, CD7, CD4/CD8 double positive

Genetics: PCR for T-cell receptor gene rearrangement

MGH Cytology Study Set

Lymph node FNAB

Surprise:
- Diplococci in clusters
- Granuloma
- GMS stain on direct smear

Penicillium marneffei

When should a lymph node be excised for diagnosis:

- ALWAYS
- SOMETIMES
- NEVER

Disclaimer:
Fine needle aspiration biopsy of a lymph node has a combined sampling and interpretive false negative rate of approximately 2-3% in the recognition of lymph node malignancy. Cytologic evaluation of a lymph node should be correlated with clinical history. Follow up in 2-3 months with re-evaluation if the lymph node enlarges is suggested, as clinically indicated.

Lymph node FNAB

- Use clinical presentation and morphology at the time of biopsy to anticipate need for ancillary testing of lymph node FNA
- Obtain samples for ancillary testing during the biopsy
- Sub-classify lymphoid lesions as much as possible with ancillary studies
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