Is this Lymphocyte
Normal
Reactive
Malignant?

Steve Marionneaux
September 16, 2017
From CAP Survey

Lymphocyte correctly identified by 96.8% of participants.
<table>
<thead>
<tr>
<th>Number of Responses</th>
<th>Percent of Labs</th>
<th>Cell type or finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>228</td>
<td>63.0%</td>
<td>Plasma cell</td>
</tr>
<tr>
<td>74</td>
<td>20.4%</td>
<td>Hairy cell</td>
</tr>
<tr>
<td>29</td>
<td>8.0%</td>
<td>Nucleated red cell</td>
</tr>
<tr>
<td>22</td>
<td>6.1%</td>
<td>Reactive/Atypical lymphocyte</td>
</tr>
<tr>
<td>7</td>
<td>1.9%</td>
<td>Normal lymphocyte</td>
</tr>
<tr>
<td>Number of Responses</td>
<td>Percent of Laboratories</td>
<td>Cell Identified</td>
</tr>
<tr>
<td>---------------------</td>
<td>-------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>295</td>
<td>73.8%</td>
<td>Lymphoma/Sézary cell</td>
</tr>
<tr>
<td>67</td>
<td>16.8%</td>
<td>Atypical lymphocyte</td>
</tr>
<tr>
<td>22</td>
<td>5.5%</td>
<td>Monocyte</td>
</tr>
<tr>
<td>9</td>
<td>2.3%</td>
<td>Segmented neutrophil</td>
</tr>
<tr>
<td>4</td>
<td>1.0%</td>
<td>Normal lymphocyte</td>
</tr>
</tbody>
</table>
Study Which Evaluated Consistency with Morphologic Evaluation of Lymphocytes

157 hospitals were sent a set of 56 photomicrographs from a 3 year old orthopedic patient.

As 114 labs responded with 671 individuals participating, the technologists were asked to differentiate between normal lymphocytes, atypical, plasma cells, prolymphocytes, and blasts.

For 7 cells (normal), there was >90% agreement. No agreement was seen with the other 49 images.

The Divergent Morphological Classification of Variant Lymphocytes in Blood Smears

Summary of author’s conclusions:

- Morphology of lymphocytes is complex; unfortunately a uniform definition of abnormal lymphocytes is lacking
- Confusing terminology is used: variant, atypical, etc
- Proving clinical information at bench should lead to a better interpretation of morphology
- Recognition of abnormal lymphs can contribute to rapid diagnosis of various conditions

Today’s talk will address these issues and hopefully provide useful information on how to recognize and classify the various forms of lymphoid cells that can be encountered on peripheral blood smears

Consider sample age

Storage artifacts in lymphocytes

• Hyperchromatic cells

• Vacuolated

• Smudged or necrobiotic

• Nuclear membrane breakage, nucleoplasm leaks mimicking
  – Cytoplasmic inclusions
  – Nuclear lobes

• Cytoplasmic blebbing or projections
Storage artifacts
Normal Lymphocyte

Can be T or B (cannot distinguish morphologically)

Small, round to ovoid cell, 7 to 15 μm, high N:C ratio.

Chromatin is diffusely dense or coarse and clumped

Nucleoli, if present, are small and inconspicuous.

Some normal lymphs are medium-sized due to an increased amount of cytoplasm
Normal Medium Sized Lymphocyte
Large Granular Lymphocytes

- Larger lymphocytes: LGLs
- Dense chromatin; pale blue cytoplasm
- Distinct red-purple azurophilic granules
- They are NOT reactive, atypical, or variant

Normal range: Up to 15% of circulating lymphs (<600/uL).
Should comment if appear increased
Lymphocytosis: Absolute vs Relative

Should lymphocytosis be defined in terms of absolute or relative numbers?

Consider this patient

- WBC = 2000/uL (5000-10000/uL)
- Neutrophils = 20% (50-70)
- Lymphocytes = 80% (20-40) – Relatively increased but……

- Calculate absolute numbers
  - Neutrophils: 2000/uL x .20 = 400/uL (2000–7000/uL)
  - Lymphocytes: 2000/uL x .80 = 1600/uL (1000–4000/uL)

- Absolute neutropenia. The lymphocyte number is actually normal.

**Assessment should always be based on absolute number (>4,000/uL)**
<table>
<thead>
<tr>
<th>Reactive Lymphs</th>
<th>Malignant Lymphs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterogeneous population</td>
<td>Homogeneous population</td>
</tr>
<tr>
<td>Variation in N:C ratio</td>
<td>High N:C ratio</td>
</tr>
<tr>
<td>Variable nuclear shapes</td>
<td>Variably clumped chromatin</td>
</tr>
<tr>
<td>Chromatin smooth - clumped</td>
<td>Morphology consistent with type of neoplasm</td>
</tr>
<tr>
<td>Nucleoli may be visible</td>
<td>• Frayed cytoplasm</td>
</tr>
<tr>
<td>Appearance of Cytoplasm</td>
<td>• Single prominent nucleolus</td>
</tr>
<tr>
<td>• Increased amount</td>
<td>• Cerebriform nuclei</td>
</tr>
<tr>
<td>• Variable deep basophilia</td>
<td>• Irregular nuclear contours; bizarre shapes; clefting</td>
</tr>
<tr>
<td>• May be indented by surrounding cells</td>
<td></td>
</tr>
</tbody>
</table>
# Patient Case

19 year old male feeling awful for 1 week

- Sore throat and fatigue
- Fever 100.7°F
- Right cervical lymphadenopathy

<table>
<thead>
<tr>
<th>LABS</th>
<th>PATIENT</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>16,500 /uL</td>
<td>5000 – 10,000 /uL</td>
</tr>
<tr>
<td>Absolute Lymphocytes</td>
<td>12,375 /uL</td>
<td>1000 – 4000 uL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>13.6 g/dL</td>
<td>13.5 – 16.0 g/dL</td>
</tr>
<tr>
<td>Platelets</td>
<td>209,000 /uL</td>
<td>150,000 – 400,000/uL</td>
</tr>
<tr>
<td>ALT</td>
<td>303</td>
<td>5 – 37 U/L</td>
</tr>
<tr>
<td>AST</td>
<td>276</td>
<td>10 – 37 U/L</td>
</tr>
</tbody>
</table>

Throat culture negative for β-Hemolytic Streptococci
Presence of Atypical Lymph flag triggered review

<table>
<thead>
<tr>
<th>Manual Diff</th>
<th>%</th>
<th>Cells/uL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil</td>
<td>20</td>
<td>3,300</td>
</tr>
<tr>
<td>Variant Lymph</td>
<td>60</td>
<td>9,900</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>15</td>
<td>2,475</td>
</tr>
<tr>
<td>Monocyte</td>
<td>5</td>
<td>825</td>
</tr>
</tbody>
</table>
Infectious Mononucleosis

Epstein-Barr virus (EBV) infection

Adolescents and young adults

Self-limiting

Clinical

• Fever, malaise, pharyngitis, cervical lymphadenopathy, splenomegaly

Pathology

• Reactive absolute lymphocytosis (T-cells)
• Infects B lymphocytes and oropharyngeal epithelium. Both share CD 21 receptor
Infectious Mononucleosis

3 criteria for laboratory confirmation

- Absolute lymphocytosis
- Variant (Atypical? Reactive?) lymphocytes on peripheral smear
- A positive serologic test for Epstein-Barr virus (EBV)
Differential Diagnosis

- Rubella
- Diphtheria
- Cytomegalovirus Infection (CMV)
- Hepatitis B
- β-Hemolytic Streptococci
- Toxoplasmosis
Reactive Lymphocytosis: Other Causes

- Toxoplasmosis
- Idiosyncratic drug reactions
  - Phenytoin
  - Other
- Listeria
- Mycoplasma
- Post vaccination
- Autoimmune disease
- Sudden onset of stress from myocardial infarction

Foucar K, Reichard K, Czuchlewski D. Bone Marrow Pathology. 3rd ed. ASCP Press; 2010
Reactive Lymphocytes

Monocyte ?

Kyoto Univ.
Plasmacytoid Lymphocyte

Form of reactive lymphocyte

Has some plasma cell features

- Prominent chromatin clumping
- Perinuclear halo
- Eccentric nucleus

Often seen mixed in a heterogeneous population of reactive lymphocytes

Not plasma cells

image.bloodline.net
LGL or Reactive Lymphocyte?
http://www.bloodline.net/external/image-atlas.html
WHO Classification of Hematopoietic Neoplasms

Stratifies hematopoietic neoplasms according to lineage:

• Myeloid
• Lymphoid
• Histiocytic/dendritic

Precursor neoplasms are considered separately from mature

Classification of lymphoid neoplasms is based on pathologic, immunophenotypic, genetic and clinical features
Lymphoid Neoplasms: Introduction

B cell and T/NK neoplasms are clonal tumors of immature and mature B cells, T cells or natural killer (NK) cells at various stages of maturation.

B and T cell neoplasms often mimic stages of normal differentiation, so to some extent are classified according to the normal stage.

- Not always: hairy cell leukemia

Leukemia/lymphomas share malignant cell types:

- Leukemia = mostly marrow (>20%) and blood involvement
- Lymphoma = predominate tissue infiltration with <25% marrow involvement

Leukemia and lymphoma differ only in where disease presents:
Lymphoid Neoplasms: Classification

Lymphoid neoplasms are classified mostly using morphology and immunophenotype.

No one marker (CD) is specific for any neoplasm.

Combination of morphologic features and a panel of antigenic markers is necessary for accurate diagnosis.

Within any given disease entity, variations in antigenic make up (CD markers) can be seen.

Morphology and immunophenotype can change over time as a result of additional genetic mutations and clonal evolution.
T Lymphocytes

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Relative Frequency of Lymphoid Malignancies

- Non-Hodgkin’s lymphoma: 62.4%
- Plasma cell disorders: 16%
- Hodgkin’s disease: 8.2%
- ALL: 3.8%
- Follicular lymphoma: 22%
- Diffuse large B cell lymphoma: 31%
- MALT lymphoma: 7.6%
- Mature T cell lymphoma: 7.6%
- Small lymphocytic lymphoma: 6.7%
- Mantle cell lymphoma: 6%
- Mediastinal large B cell lymphoma: 2.4%
- Anaplastic large cell lymphoma: 2.4%
- Burkitt’s lymphoma: 2.4%
- Nodal marginal zone lymphoma: 1.8%
- Precursor T lymphoblastic lymphoma: 1.7%
- Lymphoplasmacytic lymphoma: 1.2%
- Others: 7.4%


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CLL: CD19+, CD20+ (CD19>CD20), CD5+, CD23+, CD200+, surface immunoglobulin (dim), CD10-, FMC7 dim or -, CD79b dim or –

PLL: CD19+, CD22+ (bright), surface immunoglobulin (bright), FMC7 +, CD5 +/-, CD10 –T cell: CD4+ or CD4+/CD8+ (dual), TCL1 +, CD2+, CD3+, CD7+, CD52+, Tdt -

HCL: CD20+ (bright), CD25+, CD103+, CD11c+, surface immunoglobulin (bright), CD5 + (dim) or -CD10-

LGL: T cell: CD8+, CD3+, CD57+, CD16+, CD2+, CD5+(dim), CD7 + (dim) or - NK cell: CD56+, CD3-, CD16+, CD2 + (dim) or -, CD7+ (dim) or -, CD57+(dim) or -

ATLL: CD2+, CD5+, CD3+, CD7-, CD4+, CD25+(bright), CD30+ , CCR4+, FOXP3+
BL: CD19+, CD20+, CD22+, CD10+, surface immunoglobulin (bright), Ki67+, Tdt –

FL: CD19+, CD20+, CD10+, surface immunoglobulin (bright) CD5-, CD23-

DLBCL: CD19+, CD20+, CD79b+, CD22+(bright), CD10 +/-

MZL: CD19+, CD20+, CD22+, CD79b+, surface immunoglobulin (bright), FMC7+, CD200-, CD22+, CD5-, CD10-, CD23-

MF/ SS: CD2+, CD3+, CD5+, CD7-, CD4+, CD26-

MM: CD45 dim or -, CD38+, CD138+, CD56+, CD117+, cytoplasmic light chain
MATURE B-CELL NEOPLASMS
Chronic lymphocytic leukemia / small lymphocytic lymphoma
Monoclonal B-cell lymphocytosis*
B-cell prolymphocytic leukemia
Splenic marginal zone lymphoma
Hairy cell leukemia
Splenic B-cell lymphoma/leukemia, unclassifiable
  Splenic diffuse red pulp small B-cell lymphoma
  Hairy cell leukemia-variant
Lymphoplasmacytic lymphoma
  Waldenström macroglobulinemia
Monoclonal gammopathy of undetermined significance (MGUS), IgM*
Mu heavy chain disease
Gamma heavy chain disease
Alpha heavy chain disease
Monoclonal gammopathy of undetermined significance (MGUS), IgG/A*
Plasma cell myeloma
Solitary plasmacytoma of bone
Extraosseous plasmacytoma
Monoclonal immunoglobulin deposition diseases*
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
Nodal marginal zone lymphoma
  Pediatric nodal marginal zone lymphoma
Follicular lymphoma
    In situ follicular neoplasia*
    Duodenal-type follicular lymphoma*
Pediatric-type follicular lymphoma*
Large B-cell lymphoma with IRF4 rearrangement*
Primary cutaneous follicle center lymphoma
Mantle cell lymphoma
    In situ mantle cell neoplasia*
Diffuse large B-cell lymphoma (DLBCL), NOS
    Germinal center B-cell type*
    Activated B-cell type*
T cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the CNS
Primary cutaneous DLBCL, leg type
EBV positive DLBCL, NOS*
EBV+ Mucocutaneous ulcer*
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma

World Health Organization (WHO) classification of lymphoid neoplasms

Swerdlow, S. H. The 2016 revision of the World Health Organization (WHO) classification of lymphoid neoplasms Blood 2016 :blood-2016-01-643569; doi: https://doi.org/10.1182/blood-2016-01-643569
Intravascular large B-cell lymphoma
ALK positive large B-cell lymphoma
Plasmablastic lymphoma
Primary effusion lymphoma
HHV8 positive DLBCL, NOS*
Burkitt lymphoma
Burkitt-like lymphoma with 11q aberration*
High grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements*
High grade B-cell lymphoma, NOS*
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

MATURE T-AND NK-NEOPLASMS
T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Chronic lymphoproliferative disorder of NK cells
Aggressive NK cell leukemia
Systemic EBV+ T-cell Lymphoma of childhood*
Hydroa vacciniforme-like lymphoproliferative disorder*
Adult T-cell leukemia/lymphoma
Extranodal NK/T-cell lymphoma, nasal type
Enteropathy-associated T-cell lymphoma
Monomorphic epitheliotropic intestinal T-cell lymphoma*
Indolent T-cell lymphoproliferative disorder of the GI tract *
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma

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Swerdlow, S. H. The 2016 revision of the World Health Organization (WHO) classification of lymphoid neoplasms
Blood 2016 :blood-2016-01-643569; doi: https://doi.org/10.1182/blood-2016-01-643569
MATURE T-AND NK-NEOPLASMS

Mycosis fungoides
Sézary syndrome
Primary cutaneous CD30 positive T-cell lymphoproliferative disorders
   Lymphomatoid papulosis
   Primary cutaneous anaplastic large cell lymphoma
Primary cutaneous gamma-delta T-cell lymphoma
Primary cutaneous CD8 positive aggressive epidermotropic cytotoxic T-cell lymphoma
Primary cutaneous acral CD8+ T-cell lymphoma*
Primary cutaneous CD4 positive small/medium T-cell lymphoproliferative disorder*
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma
Follicular T-cell lymphoma*
Nodal peripheral T-cell lymphoma with TFH phenotype*
Anaplastic large cell lymphoma, ALK positive
Anaplastic large cell lymphoma, ALK negative *
Breast implant-associated anaplastic large cell lymphoma*
Mature Lymphoid Neoplasms

Chronic lymphoproliferative neoplasms are clonal proliferations of morphologically & immunophenotypically mature B or T cells characterized by

- Low proliferation rate (most cases)
- Prolonged cell survival due to failed apoptosis

Leukemia vs Lymphoma: what’s the difference?

- Leukemias: primary manifestation in bone marrow and blood; sometimes also involve extramedullary sites
- Lymphoma: presents in lymphoid organs and other tissues; may also involve marrow and blood
  - Hodgkin Lymphoma: not covered today. Reed-Sternberg cell not found in circulation
  - Non-Hodgkin Lymphoma – cells can be found in circulation
72 year old male

Annual physical
Feels great
No lymphadenopathy
No splenomegaly

CBC
• WBC: 82,000/uL
• HGB: 14.2 g/dL
• Platelets: 231,000/uL
• Manual Diff
  • 95% Lymphs
  • 5% Neutrophils
  • Smudge cells

Is the patient neutropenic? 82000 x .05 = 4100/uL
Chronic Lymphocytic Leukemia

Accumulation of mature B-Lymphocytes in bone marrow, blood, lymph nodes, spleen and liver

Most common leukemia in the US
- 25% of all leukemia cases diagnosed annually
- 15,500 new cases per year and 4390 death

Older adults, with a higher incidence in males

Family history of CLL is the most important risk factor for the development of CLL; 8-10% of patients with CLL have a family history
Chronic Lymphocytic Leukemia: Clinical

Symptoms - depends on disease progression

• Most patients are asymptomatic at diagnosis; some are detected during a routine physical exam or pre-surgical testing
• Lymphadenopathy in some
• Neutropenia, anemia and thrombocytopenia usually later in disease
• When patients have symptoms, they are non-specific:
  • Weakness
  • fever
  • frequent infections
  • Bleeding
  • night sweats
  • weight loss.
CLL: Diagnostic Criteria

Sustained lymphocytosis > 5000/uL

Immunophenotype

• Specific Pattern of Reactivity
  • Expression of > 1 B cell antigen (CD19, CD20, CD23)
  • Expression of T cell antigen CD5 (without expression of other pan-T cell antigens)
• Weak or negative expression of sIg (weak/neg)
• Clonal light chain restriction (only one type of light chain)
CLL Lymphocyte Morphology

Most lymphocytes have scant cytoplasm – high N:C ratio

Chromatin pattern is atypically dense with clumps of dark chromatin separated by narrow pale spaces - “soccer ball”

- Larger cell with single prominent nucleolus
- Less condensed chromatin
- Pale blue cytoplasm

Prolymphocytes are frequently seen but represent less than 10% of the total lymphocyte population

Smudged lymphocytes
Characteristic Chromatin Clumping pattern
Question: What terminology does your laboratory use to identify these cells?

• Normal lymphocytes? (are they?)
• Atypical lymphocytes?
• Variant lymphocytes?
• Other?
Atypical CLL (15% of cases)

>15% large lymphoplasmacytoid cells and/or cleaved cells.

CLL/PL (prolymphocytes)

- 10% - 55% circulating prolymphocytes with the rest being small CLL cells.
- May present at initial diagnosis or develop as a transformation following a chronic phase.
- More aggressive disease
- Higher lymphocyte count
- Marked splenomegaly.
Atypical CLL – Cleaved Forms
Atypical CLL – Lymphoplasmacytoid Cells
Atypical CLL – Lymphoplasmacytoid Cells
Smudge cells in CLL

- Falsely decrease count of CLL lymphocytes
- Falsely increase other cell %

Albumin + EDTA blood decreases smudging

Another option: count smudge cells as lymphs in the manual differential (ONLY in CLL patients)

(New guidelines from ICSH)

Reference needed
76 y/o man in good health with testicular swelling

CT scan and ultrasound showed significant splenomegaly

Flow cytometry: CD19+, CD20+, CD22+, CD79b+, CD23-, kappa clonal excess

WBC-415,000/uL; HGB-12.9 g/dL; PLT-181,000
Prolymphocytic Leukemia

- Rare, most patients >60y
- Prolymphocytes are >55% of lymphoid cells
- Marked leukocytosis
- Splenomegaly
- Aggressive disease
- Survival: 30-50 months

Genetic testing
- 17p deletion in 50% - assoc with TP53 gene mutation – likely underlies aggressive course & treatment resistance
- Prolymphocyte
  - Intermediate stage of lymphocyte maturation
  - Pale blue cytoplasm
  - Moderately clumped chromatin
  - Prominent nucleolus
Bone Marrow Aspirate: Myeloma

Mature Plasma Cells
Bone Marrow Aspirate: Myeloma

Immature Plasma Cells
Myeloma plasma cells: morphology

• Broad morphologic spectrum of plasma cells in myeloma
  – Normal
  – Small cell/lymphoid: lymphoplasmacytoid
  – Cleaved/lobulated forms
  – Multinucleated
  – Immature:
    • Proplasmacytes
    • Plasmablasts
  – Flame cells
  – Mott cells
brake
48 year old male

Presented with headaches, abdominal pain, weakness and fatigue

Physical examination found marked splenomegaly

HGB = 8.6 g/dL, PLT = 110,000/uL, WBC = 4000/uL

Bone marrow aspiration for flow cytometry was difficult due to marrow fibrosis
FLOW Cytometry

- Light chain restricted
- CD19
- CD20
- CD22
- CD11c
- CD25
- CD103
Courtesy of Peter Maslak
 Courtesy of Peter Maslak
Hairy Cell Leukemia

- Indolent mature B-cell neoplasm
- Rare, <5% of leukemias
- Middle age – elderly, usually men (4:1)
- Splenomegaly
  - Including profound monocytopenia
  - May be masked with auto-differentials that classify hairy cells as monocytes
- Pancytopenia
Cytoplasmic border may be serrated, frayed, or wind-blown

Nuclei usually round – oval but may appear as spindled, reniform, horseshoe-shaped, or bi-lobed
Hairy Cell Morphology

- The cytoplasm stains pale gray-blue with a textured look.
- Chromatin is partially clumped and granular appearing - intermediate between a mature lymphocyte and a blast.
- Nucleoli usually not visible.
Hairy Cell Morphology

Hairy cells sometimes difficult to find (Look at thicker area and feathered edge of smear)

Many don’t have “textbook” features – no villous projections

A careful search usually reveals some with typical morphology

“Non-textbook” forms become easier to identify
Hairy Cell Morphology

On smear review, sometimes called

- Monocytes (profound monocytopenia in Hairy cell leukemia)
- Variant lymphocytes
- Large granular lymphocytes
- Normal lymphs
Splenic Marginal Zone Lymphoma

- Low grade small B-cell lymphoma
- Age at diagnosis: 22-79y; median: 68y
- Most patients present with moderate–massive splenomegaly
- Lymphadenopathy: rare
- Anemia (64%); Thrombocytopenia (15%)
- Absolute lymphocytosis in 75% - malignant cells usually found in peripheral blood
Splenic Marginal Zone Lymphoma

Immunophenotype
• Surface IgM ++, CD 19+, CD20+, CD79b+
• CD5-, CD10-, CD23-, Bcl-6 neg & cyclin D1 neg.

Cytogenetics
• Complex abnormalities in 80% of cases
• Gains in 3q and 12q
• 7q deletion

Histology
• Spleen & lymph nodes: micronodular lymphoid infiltrate

• Round-oval nucleus
• Condensed chromatin
• Basophilic cytoplasm with short villi which may be polar
• Can see morphologic heterogeneity
• Lymphoid cells without villous features
• Lymphoplasmacytoid cells
• Resemble prolymphocytes
• Monocytoid cells
Marginal Zone Lymphoma

Courtesy of Peter Maslak
41 year old HIV patient

41 yo male presented with ptosis of the left eyelid and right testicular swelling.

Severe headaches, weakness, and weight loss of approximately 50 to 60 pounds in the previous 5 to 6 months

Diagnosed with HIV 2 months ago

- CD4 = 58/uL (600 - 1400/uL)
- HIV viral load = 500,000 copies/mL

On physical examination, the patient was afebrile and cachectic appearing

Infectious Diseases in Clinical Practice: March 2007 - Volume 15 - Issue 2 - pp 116-118
No lymphadenopathy was appreciated in inguinal, cervical, supraclavicular, or axillary areas.

Labs: WBC = $3.2 \times 10^3/\mu L$ with 7% atypical lymphocytes, HGB = 9.1 g/dL, HCT = 28%

After 2½ weeks of HAART therapy, CD4+ = 166/\mu L; viral load = 5600 copies/mL.

Ultrasound revealed enlarged, hyperemic testicles with a diffuse echo texture suggestive of a metastatic neoplasm

MRI of brain showed small mass
Lumbar puncture did not reveal any malignant cells.

Bone marrow specimen showed atypical, blast-like cells (next slide).

Flow cytometry was positive for CD19, CD20, CD22, CD10, BCL6, CD38, **CD77 and CD43**. Kappa light chain restricted. **TdT negative**

FISH detected t(8;14)

Patient refused chemotherapy for personal reasons and died 4 months later.
• Intermediate sized cells with deeply basophilic cytoplasm and vacuoles.
• Pleomorphic nucleus.
• Relatively immature chromatin pattern with nucleoli.

Should these cells have been labeled “atypical?”
Will “atypical” adequately alert the clinician?”
Burkitt Lymphoma

Aggressive B cell neoplasm. Three types:

- Endemic type - tropical Africa, most common malignancy in children
- Sporadic type – worldwide; children & young adults
- Immunodeficiency associated

Previously classified as ALL L3 (leukemic phase)

EBV and malaria association

t(8;14) in >90%

Incidence of marrow and blood involvement varies but found more in immunodeficiency related
- IG heavy chain genes located on chromosome 14
- Protooncogene \textit{c-myc} moves from chromosome 8 to 14 close to IG gene
- \textit{c-myc} is now in a region of active gene transcription
- Overproduction of the \textit{c-myc} product (a transcription factor essential for cell division) stimulates lymphocyte mitosis activity and disease!
Follicular lymphoma

Follicular lymphoma accounts for 20% of all lymphomas

Mostly adults; median 6th decade

Most patients have widespread disease at diagnosis

• Peripheral and central (abdominal, thoracic) lymphadenopathy and splenomegaly
• Otherwise asymptomatic
• Marrow involved in 40-70%

“Indolent lymphoma” but not curable (some exceptions)

Biopsy lymph node – cell suspension for flow analysis

• Monoclonal surface Immunoglobulin, pan B-cell antigens
Follicular Lymphoma

<table>
<thead>
<tr>
<th>B CELLS</th>
<th>Slg+</th>
<th>CD20+</th>
<th>CD79a+</th>
<th>BCL6+</th>
<th>CD5-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small-Medium Sized</td>
<td>CD19+</td>
<td>CD22+</td>
<td>BCL2+</td>
<td>CD10+</td>
<td>CD43-</td>
</tr>
</tbody>
</table>

LYMPHOCYTE MORPHOLOGY

**Small – Medium Sized**
- Angulated, elongated, twisted or cleaved nuclei
- Inconspicuous nuclei
- Scant pale cytoplasm

**Large Cells**
- 3X size of lymphocyte
- Round/oval occasionally dented or multilobulated nuclei
- Vesicular chromatin
- 1-3 nucleoli
- Scant cytoplasm

**SIg+**
**CD20+**
**CD79a+**
**BCL6+**
**CD10+**
**CD43-**
Follicular Lymphoma
• $t(14;18)$ present in >80% of cases
• BCL-2 gene (from chr 18) juxtaposed with Ig heavy chain gene (chr 14)
• Overexpression of BCL-2 protein
• Inhibition of apoptosis
Follicular
Next Patient

- WBC: 2,600/uL (1800 lymphs)
- HGB: 8.2 g/dL
- PLT: 43,000
NRBCs and teardrop cells suggest_______?

• WBC: 2,600/uL (1800 lymphs)
• HGB: 8.2 g/dL
• PLT: 43,000
Mantle Cell Lymphoma

<table>
<thead>
<tr>
<th>Description</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>6% of non-Hodgkin lymphoma diagnosed annually in USA</td>
<td></td>
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<tr>
<td>M/F = 4:1; median = 60y</td>
<td></td>
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<tr>
<td>70% present with widespread disease at DX</td>
<td></td>
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<tr>
<td>Lymphadenopathy (90%), Splenomegaly (60%)</td>
<td></td>
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<tr>
<td>Cytopenias related to marrow involvement</td>
<td></td>
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<tr>
<td>Absolute lymphocytosis associated with circulating malignant cells</td>
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</tbody>
</table>

http://emedicine.medscape.com/article/203085
Mantle Cell Lymphoma

May be challenging to diagnose

Overlapping immunophenotypic markers

Highly variable morphology

• Blast like resembling ALL
• Mod-large amount cytoplasm mimicking splenic marginal zone or hairy cell
• Prominent nucleoli – resembles prolymphocytic leukemia
• Morphology consistent with CLL
Mantle Cell Lymphoma

- t(11;14) results in over expression of the PRAD1/CCND1 gene, encoding the cyclin D1 protein

- Cyclin D1 plays a key role in cell-cycle regulation, activating cyclin-dependent kinases, propelling cells through the G1 checkpoint into the S phase of the cell cycle.

- Cyclin D immunohistochemical stain important for diagnosis
Next.....53 year old female

July 2006: Presented with rash over right thumb, index finger, abdomen, posterior thighs, breast, arms and neck

Bone marrow: 9% small T-cells (CD2, CD3, CD4, CD5)

Punch biopsy of rash: atypical dense lymphoid infiltrate consistent with Adult T-cell lymphoma

Oct 2006: PET/CT revealed hypermetabolic activity in abdomen
53 year old female

Underwent chemotherapy - clinical trial; no standard of care for initial treatment

Multiple hospitalizations: hypercalcemia

Oct 2007: patient extremely frail and seen at clinic

July 2007: PET showed splenic involvement and positive axilla, subpectoral, periaortic, pelvic wall, and inguinal lymph nodes
- WBC: 44,100/μL
- HGB: 12.1 g/dL
- PLT: 194,000/μL
- Leukemic phase
T Cell Lymphoma (HTLV-1 Related)

Monocytes, Reactive Lymphocytes or Lymphoma Cells??
• Somewhat heterogeneous
• Appear reactive?
Adult T-Cell lymphoma/Leukemia

- HTLV-1 positive: 2.5 % of carriers will develop lymphoma after long latency
- Very aggressive multisystem disease
- Prominent extramedullary disease
- Skin and bone lesions
- Hypercalcemia
- Short survival times
Acute Adult T-Cell lymphoma/Leukemia

- Striking leukocytosis with abnormal lymphoid cells:
  - Heterogeneity of size and nuclear configuration
  - Pronounced nuclear irregularities:
    - coarse lobulation
    - cloverleaf forms
  - Variably clumped chromatin, variable amount cytoplasm
Adult T-cell Lymphoma/Leukemia

• Immunophenotype
  ➢ TdT –
  ➢ CD2 +
  ➢ CD3 +
  ➢ CD5 +
  ➢ Most cases are CD4 helper phenotype

• Molecular
  ➢ Clonal T cell receptor gene rearrangement
Next T-Cell Disease: Sezary Syndrome

- Closely related to mycosis fungoides
- Generalized exfoliative erythroderma with characteristic blood involvement
- Mostly males >60 y
- Generalized lymphadenopathy
- Aggressive disease; 5 year survival 10-20%
Mycosis Fungoides
Sezary Syndrome

Clonal T-cells in skin, lymph nodes and blood

Demonstrate Sezary cells (>1000/uL) in blood by immunophenotype and morphology

- Immunophenotype: mature T helper cell phenotype, CD4/CD8 ratio >10
- Abnormal lymphoid cells in circulation:

<table>
<thead>
<tr>
<th>Variable/phenotype</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable Size</td>
<td>Absent/inconspicuous nucleoli</td>
</tr>
<tr>
<td>Cerebriform nuclei</td>
<td>High N:C ratio</td>
</tr>
<tr>
<td>Variable/coarse chromatin</td>
<td>Cytoplasm: basophilic &amp; agranular</td>
</tr>
</tbody>
</table>
Sezary Cells

• Large (15-25um)
• Pale blue cytoplasm
• Convoluted cerebriform nuclei
• Coarse chromatin
Sezary cells

Courtesy of Peter Maslak
Courtesy of Peter Maslak
Diffuse Large B-cell Lymphoma

• most common form of aggressive non-Hodgkin lymphoma
• usually symptomatic
• extranodal involvement is common
• cell of origin: germinal center B-cell
• curable in ~ 40%
Diffuse Large B-Cell Lymphoma

- Large lymphoid cells with variable nuclear features
- May mimic acute leukemia
  - Should they be called BLASTS or LYMPHOMA CELLS or SOMETHING ELSE?
Large lymphoid cells with condensed chromatin
Diffuse large B cell lymphoma

Courtesy of Peter Maslak
my suggestions

terminology and reporting methods

recommended
• Instrument diff was already released
• Smear is reviewed for RBC morph, confirmation of auto diff or other reason
• Variant lymphoid cells (appear reactive or malignant/abnormal) are seen on smear

As long as the smear confirms the instrument lymphocyte count, use a qualitative statement to describe the abnormal population of lymphs such as:

- Lymphocytes with reactive features features seen on smear
- Abnormal lymphoid cells with blast-like features seen on smear
- Abnormal lymphoid cells with irregular nuclear contours seen on smear
- Abnormal lymphoid cells with folded/clefted nuclei seen on smear
Abnormal lymphoid cells with villous cytoplasmic projections seen on smear (when Hairy cells are suspected)

Abnormal lymphoid cells with cerebriform nuclei and moderately condensed chromatin seen on smear (when Sezary cells are suspected)

Abnormal lymphoid cells with prominent nucleoli seen on smear (Reserve for cells resembling prolymphocytes)

Increased number of prolymphocytes / large atypical / lymphoid cells with cleaved nuclei seen on smear (Use in CLL patients with >10% of these cells)

Some of the lymphocytes appear variant (or reactive/abnormal)
Variant lymphoid cells (appear reactive or malignant/abnormal) are encountered while performing a manual differential and they cannot be separated from the normal lymphocyte population.

use a qualitative statement to describe the abnormal population of lymphs such as “the lymphocyte population includes a population of

- Lymphocytes with reactive features
- Abnormal lymphoid cells with blast-like features
- Abnormal lymphoid cells with irregular nuclear contours
- Abnormal lymphoid cells with folded/clefted nuclei
Abnormal lymphoid cells with villous cytoplasmic projections (when Hairy cells are suspected)

Abnormal lymphoid cells with cerebriform nuclei and moderately condensed chromatin (when Sezary cells are suspected)

Abnormal lymphoid cells seen on smear (CLL cases where there is an absolute lymphocytosis and the number of prolymphocytes or atypical forms is <10%)

Increased number of prolymphocytes / large atypical / lymphoid cells with cleaved nuclei seen on smear (Use in CLL patients with >10% of these cells)

Cells with variant (or reactive/abnormal) morphology
Scenario: automated differential was already reported and smear is being reviewed for morphology, confirmation of automated counts, or other reason

And more than 5% variant lymphocytes (appear reactive) are seen,

Confirm that the automated lymphocyte count (%) reflects the total lymphocyte population

If yes, then report “Variant lymphocytes seen on smear”

If no, replace automated with manual differential
And more than 15% of the lymphocytes appear to be large granular lymphocytes

Confirm that the automated lymphocyte count (%) reflects the total lymphocyte population

If yes, report “increased number of large granular lymphocytes seen.”

If no, replace automated with manual differential

Scenario: automated differential was already reported and smear is being reviewed for morphology, confirmation of automated counts, or other reason
Scenario: More than 15% of lymphocytes appear to be large granular lymphocytes (LGL) on manual differential

Count the LGLs as lymphocytes

and add comment: “Increased number of large granular lymphocytes seen”
Digital images of normal leukocytes
<table>
<thead>
<tr>
<th>WBC</th>
<th>HGB</th>
<th>MCV</th>
<th>PLT</th>
<th>NRBC</th>
<th>Ne</th>
<th>Ly</th>
<th>Mo</th>
<th>Eo</th>
<th>Ba</th>
<th>NRBC</th>
<th>VLYM</th>
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<tbody>
<tr>
<td>15.25</td>
<td>14.6</td>
<td>96.1</td>
<td>148</td>
<td>0.1</td>
<td>46.3</td>
<td>4.4</td>
<td>7.4</td>
<td>3.4</td>
<td>1.5</td>
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Cell images with annotations.
<table>
<thead>
<tr>
<th>WBC</th>
<th>HGB</th>
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<th>PLTi</th>
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<th>Ly</th>
<th>Mo</th>
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<th>Ba</th>
<th>NRBC</th>
<th>VLYM</th>
<th>Meta</th>
<th>Myelo</th>
<th>Pro</th>
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<tr>
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<td>8.9</td>
<td>69.50</td>
<td>377</td>
<td>417</td>
<td>17.8</td>
<td>79.7</td>
<td>2</td>
<td>0.5</td>
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<td>342</td>
<td>44.8</td>
<td>28.4</td>
<td>8.5</td>
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Cell images correspond to the indicated values.
<table>
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<tr>
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<th>Mo</th>
<th>Eo</th>
<th>Ba</th>
<th>NRBC</th>
<th>Comments</th>
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<tbody>
<tr>
<td>5.9</td>
<td>9.0</td>
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<td>4</td>
<td>11</td>
<td>4.2</td>
<td>95.3</td>
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<td></td>
<td>9.8</td>
<td>Large number of the lymphocytes are abnormal, many with blast like features; 43.5% smudge cells</td>
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<tr>
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<td>1</td>
<td>21.3</td>
</tr>
</tbody>
</table>

Images 1-21 of blood smears, with annotations indicating specific cells or regions of interest.
Lymphoma or blasts?
Thank you

stevenmarionneaux@gmail.com