

**MECHANISMS OF HUMAN DISEASE: LABORATORY SESSIONS
HEMATOPATHOLOGY**

January 25, 2012

FACULTY COPY

GOAL: Learn the appearance of normal peripheral blood elements and lymph nodes. Recognize abnormal peripheral blood and lymph nodes and learn some of the more common pathologic conditions affecting these organs.

OBJECTIVES:

1. Describe normal peripheral blood (WBCs, RBCs and platelets).
2. Describe normal lymph node architecture.
3. Describe the pathologic changes associated with common anemias.
4. Describe the pathologic changes associated with leukemias.
5. Describe the histologic appearance of Hodgkin disease.
6. Describe the histologic appearance of common non-Hodgkin lymphomas.

CLINICAL CASE/SLIDE:

Case 1

Normal peripheral blood smear

1. Describe the normal morphology of RBCs, WBCs, Platelets.
Estimate size, shape and hemoglobin concentration of the RBCs.

Normal RBCs are anucleate cells about 7 microns in size (about the size of a lymphocyte nucleus) and round with a central zone of pallor that is about one third the total diameter of the cell. WBCs are present in much smaller numbers compared to the RBCs (roughly 1-2 per high power field). Platelets are small cytoplasmic fragments with purple granules. About 10-20 are present in a high power field.

Case 2

A 45-year-old woman presents with perimenopausal menometrorrhagia

CBC findings:

WBC $9.7 \times 10^3/\mu\text{L}$

Hemoglobin 7.8g/dL

Hematocrit 23.5%

MCV 73 femtoliters (fL)

Red cell distribution width (RDW) 22.1

Platelets 187,000/ μL

The peripheral blood smear is reviewed.

1. Describe the morphology of the patient's RBCs (compare to normal peripheral smear)

RBCs are smaller than normal (microcytic) and display more variation in shape (poikilocytosis). The central pallor zone is larger than normal RBCs suggesting decreased hemoglobin content (hypochromic). The microcytosis and hypochromasia correlate with the CBC results. "Pencil cells" (elliptocytes) may also be seen.

2. What is your diagnosis?

In conjunction with the CBC findings, this morphology is consistent with iron deficiency anemia.

3. What symptoms and physical findings are associated with anemia in general?

**Pallor of skin, mucous membranes, conjunctiva and nail beds; fatigue, shortness of breath and decreased exercise tolerance; tachycardia and orthostasis.
Findings may be less prominent in long standing well-compensated anemias.**

Case 3

A 22-year-old African-American male presents with severe pain in several joints and diffuse abdominal pain. He denies fever or localizing signs of infection. He is active physically and participates in different sports several times a week.

CBC

WBC $13.2 \times 10^3/\mu\text{L}$

Hemoglobin 7.9g/dL

Hematocrit 22.8%

MCV 91.4 fL

RDW 24.1

Platelets 481,000/ μL .

The peripheral blood smear is reviewed.

1. Describe the morphology of the RBCs, WBCs and platelets.

Several sickle cells are seen. There is also general poikilocytosis including target cells (codocytes) and also polychromasia (suggesting increased RBC production). Note that the polychromatophilic RBCs are slightly larger than normal RBCs. Platelets appear increased and WBCs appear normal in morphology.

2. What are the most prominent abnormal findings on the smear related to the patient's symptoms?

Sickle cells and increased polychromasia are present, suggesting sickle cell anemia with hemolysis and increased RBC production. Toxic changes in the neutrophils suggesting infection do not appear to be present.

3. Diagnosis?

Sickle cell anemia

4. What conditions predispose towards sickle crisis?

Infection, dehydration. This patient likely became dehydrated from physical activity resulting in precipitation of crisis.

5. What are the most common genetic mutations associated with this disease?

Mutation in the b-globin gene - point mutation that leads to substitution of valine for glutamic acid in sixth position of the b-globin chain.

Case 4

A 60 year-old male presents with mild fatigue but no other complaints. Further questioning reveals a vague feeling of abdominal "fullness" and some occasional bruising. Physical examination is remarkable for splenomegaly to the level of the umbilicus.

CBC:

WBC $75.1 \times 10^3/\mu\text{L}$

Hemoglobin 8.5g/dL

Hematocrit 25.5%

MCV 88.4 fL

RDW 16.1

Platelets 56,000/ μL

The hematology analyzer has flagged the specimen for possible immature WBC forms. The peripheral blood smear is reviewed.

1. Describe the number and morphology of the WBCs, RBCs and platelets.

WBCs are markedly increased in number, predominantly cells of the neutrophil series. Many mature neutrophils are seen as well as earlier forms showing a) less nuclear segmentation (bands, myelocytes) and b) prominent primary granules without secondary fine pink granulation (promyelocytes). No blasts are seen. Basophils are easily found. RBCs appear decreased and several nucleated RBCs are seen. Platelets appear decreased in number but normal in morphology.

2. What do you suggest as the next step?

**While leukocytosis and neutrophilia can be reactive, for example to an infection, the markedly elevated WBC count (>30) and basophilia are not characteristic of an inflammatory process
A bone marrow biopsy is done.**

3. Diagnosis?

Chronic myelogenous leukemia

4. How do the history and physical examination findings relate to the differential diagnosis and to the CBC results?

Fatigue is likely related to the anemia. Bruising is related to the thrombocytopenia. The palpable spleen (abdominal fullness) in conjunction with the peripheral blood smear morphology and CBC results are consistent with chronic myelogenous leukemia.

5. What specific genetic mutation is seen in this disorder? What is the clinical significance?

Translocation between BCR gene on chromosome 9 and ABL gene on chromosome 22. BCR-ABL is a constitutively active tyrosine kinase. This deregulated tyrosine kinase is implicated in the development of CML

Tyrosine kinase inhibitors have been developed that block the action of the BCR-ABL pathway. Examples of two drugs are imatinib mesylate (Gleevec) and dasatinib (Sprycel). These drugs induce complete hematologic remissions in more than 90% of patients. Tyrosine kinase inhibitors are generally very well tolerated with only mild side effects and are the initial treatment of choice for almost all newly diagnosed patients with CML.

Case 5

Lymph node with reactive features

1. Observe the architecture of a lymph node with reactive features.

At low magnification, observe that the lymph node is surrounded by a thin capsule of pink connective tissue and fibroblasts. Outside of the node are adipose tissue and blood vessels. Beneath the capsule are several target-like secondary follicles (secondary because germinal centers are present). On higher magnification, the germinal centers are a mix of small and large lymphocytes, large lymphocytes, plasma cells and histiocytes. Sinuses can be seen as blood filled interconnected channels in the center of the node. Overall, we say this lymph node has “preserved architecture,” a feature favoring a benign process.

Case 6

A 28 year-old male is seen for an enlarged non-tender cervical lymph node. The node has shown progressive enlargement over the past 4 months. The patient denies other symptoms such as fevers, night sweats, weight loss or fatigue.

CBC:

WBC $7.2 \times 10^3/\mu\text{L}$

Hemoglobin 14.2g/dL

Hematocrit 43.3%

MCV 87.0 fL

RDW 14.1

Platelets 372,000/ μL .

An excisional biopsy of the lymph node was performed.

1. Describe the characteristic gross morphologic features.

Cross section of the lymph node shows a “fish-flesh” appearance.

2. Examine the biopsy of this patient’s lymph node in comparison to the reactive node.

At low magnification, the normal architecture is obliterated or “effaced” by broad bands of sclerosis/fibrosis dividing the tissue into discreet cellular nodules. Within the nodules, several isolated small areas of clearing (lacunae) can be seen even at low power. At higher magnification, the nodules are composed of predominantly small lymphocytes and eosinophils. Intermixed are extremely large cells, some with the clearing noted at low magnification. They have from one to several nuclei and

prominent eosinophilic nucleoli. Occasional binucleate “owl-eye” cells consistent with Reed-Sternberg cells can be seen.

3. Diagnosis?

Hodgkin Lymphoma, nodular sclerosis type

Other subtypes include:

Mixed Cellularity

Lymphocyte Predominant

Lymphocyte Depleted

Case 7

A 51 year-old male presents with fatigue, malaise and occasional low grade fevers. Physical examination reveals bilateral non-tender cervical lymphadenopathy and an enlarged inguinal lymph node.

CBC:

WBC $6.2 \times 10^3/\mu\text{L}$

Hemoglobin 11.8g/dL

Hematocrit 36.1%

MCV 92.0 fL

RDW 16.1

Platelets 180,000/ μL .

An excisional biopsy of a lymph node was performed.

1. Describe the low and high power appearance of the lymph node.

In contrast to the reactive node, in this biopsy, follicles are distributed throughout the nodal parenchyma. The follicles appear to have germinal centers. However, at higher magnification, the follicles are composed predominantly of small lymphocytes. Instead of round nuclei, these lymphocytes have angular, irregular or cleaved nuclear contours. Mitotic figures are not frequently seen.

2. Diagnosis:

Non-Hodgkins Lymphoma, follicular, grade I (presumed B-cell phenotype)

3. What specific genetic changes are seen in this type of lymphoma?

Translocation between *bcl-2* gene on chromosome 18 with the immunoglobulin heavy chain locus on chromosome 14 [t(14;18)].

Case 8

A 58 year-old male is seen by his primary care physician for a routine physical examination. Overall, he feels well without complaints.

Physical examination is unremarkable except for non-tender cervical and inguinal lymphadenopathy. The spleen is not enlarged.

CBC:

WBC $6.2 \times 10^3/\mu\text{L}$

Hemoglobin 14.5g/dL

Hematocrit 44.2%

MCV 89.0 fL

RDW 14.2,

Platelets 233,000/ μL

An excisional biopsy of a lymph node was performed.

1. Describe the low and high power appearance of the lymph node.

The architecture is effaced by a diffuse infiltrate of cells. Although in some areas the infiltrate appears vaguely nodular, discrete follicles are not seen. Lighter and darker staining areas may be appreciated on low power. At higher magnification, the infiltrate is predominantly small mature-appearing lymphocytes with generally round nuclei. Mitotic figures are infrequent. Lighter areas are seen on lower magnification that appear to have an increased number of larger lymphocytes that have prominent nucleoli in contrast to the smaller lymphocytes. Mitoses may be seen in these areas.

2. Diagnosis?

Small lymphocytic lymphoma

3. What is the difference between this entity and chronic lymphocytic leukemia (CLL) ?

CLL presents predominantly in the peripheral blood with an absolute lymphocytosis that may or may not have associated lymphadenopathy or splenomegaly.

Phenotypically, the malignant cells are considered identical.