UMMC
Department of Pathology

HEMATOPATHOLOGY ROTATION
DIVISION OF CLINICAL PATHOLOGY -- LABORATORY MEDICINE

Welcome to the University of Mississippi Medical Center and the Hematopathology Rotation. You will be an integral member of the team with a number of responsibilities. The more responsibility you can assume in performing daily tasks, the more time we can spend learning about the various aspects of hematology.

Daily duties and assigned tasks:

Coverage of Hematology lab in Clinical Pathology:
Review peripheral blood smears and cytocentrifuge preps of body fluids as required by the College of American Pathologists.

a) After morning arrival, review slides, and sign out with attending about 1000 hours.
b) In cases where it is necessary to inform the clinician of results, call the appropriate **physician** and **note** their **name** and **date and time** at which you spoke to him or her. **Enter** this into the EPIC information system, **alongside the abnormal value,** which was flagged for review. Do not ender your name, and do not verify this entry. Instead, wait for your attending to review and sign-out.
c) Arrange a flexible schedule to stop by the slides-for-review box periodically to review any slides present. Notify appropriate attending of any emergency cases such as organisms in a CSF, or blasts in an undiagnosed acute leukemia.

PANIC VALUES GENERALLY REQUIRE **NOTIFICATION** OF THE PATIENT'S PHYSICIAN AND **DOCUMENTATION** OF THIS IN THE COMPUTER AT THE TIME OF VERIFICATION.

d) Notify the clinical lab medical technologists how to reach you in an emergency.

Preparation for sign-out:

1. Obtain marrow aspirate, peripheral blood film, and CBC results from Clinical Pathology Hematology Lab. Clinical lab couriers deliver marrow aspirate smears from the Cancer Institute Laboratory.

2. Collate with peripheral smear and Wright's stained marrow aspirate. That means put them in order together in a folder or **on a large slide board if there are too many slides for a folder.** Do not put slides on top of each other in the folder. On cases where there is **pertinent previous material**, as in acute leukemia, for example, pull **representative diagnostic** slides, representative slides from any **important intervening cases**, and representative slides from the **last previous case.**

3. Obtain pertinent history such as presentation, meds, prior history and previous surgical pathology specimens.

4. Pull up the surgical pathology report for the case in the computer **(Copath). Enter the CBC data after** the brief clinical history under Clinical Data or engage the aid of one of the secretaries to help you do this (See Quick Text CBC shell under Bone Marrow). Coverslip the peripheral smear. **Label** the peripheral smear and marrow aspirate with the surg. path. no. and **do not obscure** the patient's handwritten name.

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5. Depending upon your level of expertise, screen aspirates/tissue sections in the morning, with the attending or independently, for high grade lesions and for lesions requiring additional studies such as immunoperoxidase, cytochemical stains, special stains for organisms, reticulin, Leder, Giemsa, Kinyoun, Gram, Toluidine blue stains, for example, and submit request in Histology lab so that these stains will be ready in p.m. for signout. **LABEL** THE MARROW **ASPIRATE** AND **BLOOD FILM** WITH THE **SURG. PATH. ACC. NO. Order Kinyoun and GMS stains in HIV + marrows. Order a reticulin stain on the marrow biopsy in chronic myeloproliferative neoplasms and hairy cell leukemia. Order recuts on blocks with sections not cut deep enough.**

6. **Write up** the cases by hand until you have developed the diagnostic expertise to submit them to be typed into the computer. **Set time** to sign out with attending.
   a) Hand write microscopic evaluation, commenting upon the maturation of each of the hematopoietic lines, other pertinent features, and diagnosis
   b) After sign-out, set aside representative slide(s) for the monthly Hematopathology conference and file the rest -- after making sure **all** slides are **properly labeled** with the surgical path no.

Continuing Education

1. Present case(s) at monthly Hematopathology conference (second Thursday) at 1pm in the Multiheaded Microscope Room.
   a) Print a list of hemepath cases since the last conference and select cases with attending.
   b) Select one or two cases of interest from the past two weeks to present yourself.
   c) Print a copy of each final report and put with each case.

2. To promote resident education, including that of your colleagues, the enhancement of the Hemepath study set is recommended, so that you will have pertinent cases to review as you read basic texts. These cases could include:

   Acute and chronic leukemias
   Peripheral smear positive for yeast or bacteria, or extraordinarily good pseudo-Pelger-Huet cell
   Extraordinarily good example of cryptococcus in the CSF
   Pseudo-Gaucher cell, Gaucher cell, sea-blue histiocyte, Mycobacterium or yeast on marrow aspirate smear (Extra slides may be requested from the bone marrow lab.)

NOTE: The slides that the medical technologists leave for review must be returned to the Hematology lab for filing. Slides for study sets must be additional slides made for that purpose.

Hematopathology Rotation Study Guide: First Hematopathology Rotation
Assumption: Resident has already read Robbins Basic Pathology, 9th ed., , Chapter 11 (Hematopoietic and Lymphoid Systems).

Skill Level I

Objectives:

I. Patient care

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1. Participate in bone marrow aspirations/biopsies and keep a log of those attended—find the pediatric or adult Heme-Onc fellow on service and notify him/her that you would like to attend marrow procedures.
2. Demonstrate the ability to assess gross specimens, perform well-stained touch imprint, apportion tissue for appropriate tests such as flow cytometric immunophenotyping, cytogenetics, and cultures, and dictate pertinent findings (Ex.: spleen, lymph node, and bone marrow)
   a) Pertinent positives/negatives—Ex.: spleen: presence of hilar node, infarcts, granulomas, other focal lesions, intact capsule
   b) Ability to evaluate stains and adjust Diff-Quik on touch imprints
3. Demonstrate the ability to evaluate HPLC for hemoglobinopathies.
4. Gather relevant data for bone marrow evaluation, lymph node, spleen, and other types of tissue evaluations.
5. Provide consultation with clinician regarding appropriateness of tests—Ex.: FISH for Philadelphia chromosome.

II. Medical knowledge—First Hematopathology Rotation

1. The resident/student will show a basic understanding of the principles of automated cell counting.
2. The resident/student will identify white blood cells, platelets/megakaryocytes, and red blood cells including abnormal forms on a peripheral blood film and marrow aspirate smear.
3. The resident/student will perform a manual differential on a peripheral blood film.
4. The resident/student will discuss the underlying principles of the erythrocyte sedimentation rate, sickle cell prep, reticulocyte staining and the significance of abnormal results.
5. The resident/student will describe normal findings on HPLC as well as results expected with hemoglobin S, C and E traits, and homozygous S, C, and E.
6. The resident/student will describe findings associated with alpha and beta thalassemias and sickle cell trait with regard to red blood cell indices, morphologic changes, and results of hemoglobin electrophoresis.
7. The resident/student will diagnose chronic myeloid leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, and acute lymphoblastic leukemia on a peripheral blood film or marrow smear.
8. The resident/student will describe cytogenetic abnormalities, immunophenotypes, typical results of cytochemical stains in CML, CLL, AML, and ALL.
9. The resident/student will compare/contrast myelodysplastic syndromes and chronic myeloproliferative neoplasms.

First Rotation Schedule:

First week: Read Henry, 22st ed. Pp. 509-535 (Determining the Conc. Of Hemoglobin, Hematocrit, RBC Indices, and impedance principle

Lab: Make a blood smear, review it, perform a manual differential count on it and another one on a body fluid using a hemocytometer; discuss the Coulter histograms, flags, and red cell indices.

Second week: Read Foucar Chapter 1 (Basic examination of blood and marrow).

Review: Glassy, E.F., Color Atlas of Hematology (updated 1/18/2012), CAP Press, 1998.

Lab: Perform erythrocyte sedimentation rate, sickle cell prep, reticulocyte count (manual and flow).

Third week: Read Henry Chapter 32, Erythrocytic Disorders)

Lab: Perform hemoglobin HPLC for hemoglobin A, S, F, and A2.

Fourth week: Read Henry Chapter 33, Leukocytic Disorders.

Lab: Discuss the Sudan black B stain, specific and non-specific esterase stains, and PAS with the pathologist. Review smears in Pediatric Hematology Laboratory with attending, when smears are available.

Basic Background Reading:

Glassy, E.F., Color Atlas of Hematology (updated 1/18/2012), CAP Press, 1998.

McPherson, Richard A, Henry's Clinical Diagnosis and Management by Laboratory Methods, 22st ed, Saunders, 2011, Chapters 4 (Principles of Instrumentation), 10(Quality Control), 28 (Basic Examination of Urine), 29 (Cerebrospinal, Synovial, and Serous Body Fluids), 30 (Basic Examination of Blood and Bone Marrow), 31 (Hematopoiesis), 32 (Erythrocyte Disorders), and 33 (Leukocytic Disorders).

Galagan, Katherine A., Color Atlas of Body Fluids, 2006, College of American Pathologists.

Robbins Basic Pathology, Kumar, Abbas, and Aster,, 9th ed, Elsevier Saunders, 2013, Hematopoietic and Lymphoid Systems (Chapter 11)

Rosai, Juan, Rosai and Ackerman's Surgical Pathology, 9th ed, Mosby, 2004, Chapter 21 (Lymph nodes), pp. 1878-1917 (Normal Anatomy, Lymph Node Evaluation, Primary Immunodeficiencies, Patterns of Hyperplasia, and Inflammatory/Hyperplastic Diseases).

II. Medical Knowledge—Second and Third Hematopathology Rotations
Writing up a Bone Marrow and Introduction to Lymphoid Neoplasms Study Guide

Skill Level II

Objectives:

Second Rotation Objectives

1. Must demonstrate ability to assess bone marrow aspirate, particle preparation and biopsy and lymph node biopsies.
   a.) Cellularity, numbers of each lineage, myeloid to erythroid ratio
   b.) Maturation of each hematopoietic line [complete, orderly, nuclear maturation delay (megaloblastoid, dyspoietic), nuclear blebs in normoblasts (dyspoietic), widely-separated nuclear lobes in megakaryocytes (dyspoietic)],
   c.) Focal lesions (blast and paratrabecular or non-paratrabecular lymphoid aggregates,

granulomas, metastatic tumor)
d.) Normal bony trabeculae vs. evidence of remodeling/resorption
e.) Storage Iron; must recognize ring sideroblasts on iron stain and EM
f.) Practice detecting Mycobacterium, yeast, when opportunity presents itself

2. Must be able to recognize follicular lymphoma and differentiate from reactive follicular hyperplasia, small lymphocytic lymphoma and give complete differential diagnosis of of subtypes, large cell malignant neoplasm and give differential diagnosis.

3. The resident/student will demonstrate basic understanding of the concept of lymphoid neoplasms as clonal processes.

4. The resident/student will describe and demonstrate on slides the differences between reactive lymphocytes, neoplastic small, round, mature-appearing lymphocytes, cleaved and transformed lymphocytes, and the nodular and diffuse patterns of lymphoma.

5. The resident/student will classify lymphomas into low, intermediate, and high grades.
6. The resident/student will discuss the immunophenotypes of selected lymphoid neoplasms including small lymphocytic lymphoma/chronic lymphocytic leukemia, mantle cell lymphoma, follicular center cell lymphoma, hairy cell leukemia, prolymphocytic leukemia, marginal zone B cell lymphoma, lymphoma of mucosa-associated lymphoid tissue (MALT), mycosis fungoides (cutaneous T cell lymphoma), adult T cell leukemia/lymphoma.

7. The resident/student will list cytogenetic abnormalities associated with various lymphoid neoplasms (especially those noted above), and molecular diagnostic tests which may be helpful in diagnosis.

8. The resident/student will differentiate between Hodgkin and non-Hodgkin lymphoma (especially T cell rich B cell lymphoma).

9. The resident/student will compare and contrast the immunophenotypes of nodular lymphocyte predominant Hodgkin disease and classical Hodgkin lymphoma.

10. The resident/student will list lymphoid neoplasms associated with infectious organisms.

11. The resident/student will discuss plasma cell neoplasms (clinical presentation, morphology, immunophenotype, and prognosis).

Suggested reading:

Swerdlow, S.H., et. al., World Health Organization Classification of Tumours, Tumours of Haematopoietic and Lymphoid Tissues, IARC Press, Lyon, 2008.

Foucar, Kathryn, Bone Marrow Pathology, 3nd ed, ASCP Press, 2010.

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Rosai, Juan, Rosai and Ackerman's Surgical Pathology, 9th ed, Mosby, 2004, Chapter 21 pp. 1917-1979 (Lymph Nodes, Malignant Lymphoma), Chapter 22 (Spleen), and Chapter 23 (Bone Marrow)

II. Medical Knowledge—Fourth Hematopathology Rotation

Skill Level III (final rotation)

Continuation and solidification of previous objectives. After having demonstrated proficiency in previous rotations, in addition to daily duties, the resident may select specific areas for more intensive study. The resident may briefly present a case illustrating a current interesting or controversial topic in Hematopathology at one of the monthly conferences.

Must demonstrate knowledge of typical immunophenotypes, cytochemical results where applicable, associated proto-oncogenes, translocations or other genetic anomalies associated with various neoplasms and hematogones, including:

a.) Follicular lymphoma, small lymphocytic lymphoma of the chronic lymphocytic leukemia type, mantle cell lymphoma, marginal zone lymphoma, anaplastic large cell lymphoma
b.) Acute promyelocytic leukemia t(15;17)(q22;q12), PML/RAR alpha; acute myeloid leukemia with t(8;21) (q22;q22), AML1/ETO; acute myelomonocytic leukemia with eosinophilia and inv(16))p13q22), or t(16;16)(p13;q22), CBF beta/MYH11; acute myeloid leukemia with 11q23 (MLL) abnormalities.
c.) Burkitt lymphoma (common translocations), lymphoblastic lymphoma, anaplastic large cell lymphoma (ALK-1+), diffuse large cell lymphoma (follicular center origin vs. activated B-cell phenotype)
d.) Lymphomas with a primarily reactive background such as Hodgkin disease and T-cell rich B-cell lymphoma
e.) Acute precursor B-cell lymphoblastic leukemias, "mature" B-cell leukemia (Burkitt-associated), T-cell leukemias including prolymphocytic leukemia, adult T-cell leukemia/lymphoma, large granular lymphocyte leukemia, NK/T-cell lymphoma/leukemia, Sezary syndrome
f.) Hematogones (pattern on flow cytometric immunophenotyping of marrow)
g.) Chronic lymphocytic leukemia (genetics, immunophenotype, prognosis of immunophenotypic subtypes)

Peruse the World Health Organization Classification of Tumours: Tumours of Haematopoietic and Lymphoid Tissues, ed. Jaffe, et. al., IARC Press, Lyon, 2008, to augment knowledge about the above-listed "basic" neoplasms and other topics of interest.

READ ABOUT YOUR CASES!

Recommended Reference Texts:

Swerdlow, S.H., et. al., World Health Organization Classification of Tumours, Tumours of Haematopoietic and Lymphoid Tissues, IARC Press, Lyon, 2008.

McPherson, Richard A, Henry's Clinical Diagnosis and Management by Laboratory Methods, 22st ed, Saunders, 2011,

Foucar, Kathryn, Bone Marrow Pathology, 3nd ed, ASCP Press, 2010.

Iaochim, H.L, and Medieros, L.J., Ioachim's Lymph Node Pathology, 4th ed., 2009, Lippincott Williams & Wilkins.

Kumar, Vinay, Robbins and Cotran Pathologic Basis of Disease, 9th ed., 2013, Elsevier.

Glassy, E.F., Color Atlas of Hematology (updated 1/18/2012), CAP Press, 1998.

Kjeldsberg, C.R., Practical Diagnosis of Hematologic Disorders, 4th ed., 2006 ASCP Press.

Rosai, Juan, Rosai and Ackerman's Surgical Pathology, 9th ed, Mosby, 2004.
J.L. Carey, J.P. McCoy, and D.F. Keren, Flow Cytometry in Clinical diagnosis, 4th ed., ASCP Press, Chicago, 2007.

Kjeldsberg, Carl, Body Fluids, 3rd ed., ASCP Press, Chicago, 1993.

The following are based on the Residency Review Committee Guidelines:

III. Practice-Based Learning and Improvement

1. Quality assurance—the resident will review new malignant diagnoses with at least two hematopathologists.
2. Resident will research his/her cases utilizing appropriate journals, reference books, and web-based sources.
3. After the first Hematopathology rotation, the resident will conduct/participate in at least one regulatory inspection or mock inspection of the Clinical Pathology Hematology lab (CAP checklist).

IV. Interpersonal and Communication Skills

1. The resident will demonstrate the ability to communicate effectively to clinicians, pathologists, and medical technologists.
2. The resident will present a case at the End-of-the-Month Clinical Pathology Conference to demonstrate the ability to communicate with peers/staff.
3. The resident will include pertinent data on written reports.

V. Professionalism

1. The resident will demonstrate integrity, respect, compassion, and willingness to learn in interactions with staff and patients.

2. The resident will demonstrate commitment to ethical principles (confidentiality, informed consent).
3. The resident will attend required conferences (Grand Rounds, Autopsy Conference, Clinical Pathology Conference, Hematopathology Conf., special surgical pathology conferences (such as Dermatopathology Conference).
4. The resident will assume responsibility for his/her cases.
5. The resident will demonstrate the ability to organize the daily workload and prioritize tasks.
6. The resident will show a commitment to the learning of hematopathology and to the improvement of spoken and written communication skills.
7. When in contact with patients, the resident will show sensitivity to culture, age, gender, and disabilities.
8. The resident will act in the best interests of the patient.

VI. **System-Based practice**

1. The resident will try to gain an understanding of how her/his practice affects other systems and society.
2. The resident will reflect upon and attempt to utilize cost-effective lab practices that do not compromise patient care. (Choice of tissue to sample in grossing different types of surgical specimens and number of blocks to submit.)
3. When possible, the resident will participate in instrument purchase.
4. The resident will work with management to improve and advocate for patient care.
5. The resident will assist clinicians and other health professionals dealing with computer informatics and system complexities.
6. The resident will develop an awareness of business, reimbursement, and legal issues in hematopathology.

APPENDIX

Bone Marrow Preparation

Sampling
- Larger size increases likelihood of finding focal lesions
- Unilateral vs. bilateral

Specimen Type
- Core (trephine biopsy)
- Pattern of cellularity
- In situ appearance of hematopoietic cells
- Immature myeloids adjacent to trabeculae
- Abnormal localization of immature precursors
- Location of focal lesions
- Paratrabecular
- Interstitial
- Enables examination of inaspirable marrow

Especially important in cases of agnogenic myeloid metaplasia,
Hairy cell leukemia, Hodgkin's disease, for example
Evaluation of bone
Paget's disease
Renal osteodystrophy
Remodeling due to neoplasia (multiple myeloma, carcinoma)
Decalcification may leach iron as well as calcium
Adds two hours to preparation for overnight processing

Fixation
Same concerns as with lymph node
Despite the decalcification procedure, usually antigens are preserved well
Enough for good results with current innumoperoxidase techniques

Particle preparation or clot preparation
Lacks bony trabeculae
Less processing involved (no decalcification step, so some prefer this
For evaluation of iron stores)
Good sample reveals pattern of cellularity
Interstitial vs. paratrabecular infiltrates may not be as obvious

Fresh aspirates
Sterile: Cytogenetics
To Microbiology for culture
Non-sterile: Flow cytometry

Touch imprints and marrow aspirate smears
Touch imprints of biopsy made in each case may be helpful, but may be
Crucial in the case of an inaspirable marrow

Cytochemical stains (Sudan black, PAS, specific and non-specific esterases, acid phosphatase)

LYMPH NODE WORK-UP FOR SUSPECTED HEMATOPOIETIC/LYMPHOID NEOPLASM (LYMPHOMA/LEUKEMIA)

1. Check surgery schedule daily to anticipate lymph node biopsies. Contact gross room staff to ensure that they call you immediately whenever they receive a fresh lymph node.

2. When the specimen arrives in the Histology laboratory, keep the tissue on the sterile field sent with it or another sterile surface, and, using sterile blades, slice off a pole and place in a sterile cup prior to making touch imprints. If touch imprints suggest cultures are needed, utilize this sterile sample for Cytogenetics and Microbiological cultures. Carry to Micro lab and request **miscellaneous, Mycobacterium (typical and atypical), and fungal CULTURES**. Optimally, gross lesions such as granulomas should be sent. When lymphoma and "small blue cell tumor of childhood" are in the differential diagnosis, send **sterile** tissue to **Cytogenetics** for **karyotyping.**

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3. Serially section node PERPENDICULAR TO LONG AXIS. Sections should be 2-4 mm--no thicker than a nickel. Examine cut surfaces.

4. Make several, aid-dried touch imprints/scrape for Diff-Quik stain, and quickly place one or two in alcohol for H&E stain. Examine touch imprints to determine the adequacy of the tissue for evaluation of lymphoma/leukemia. Decide whether flow cytometry is desirable and whether there is sufficient tissue for diagnosis. Call the surgeons and inform them whether the tissue is sufficient.

5. If the tissue appears to be involved by a hematopoietic/lymphoid neoplasm, do the following:
    a) Save a couple of unstained touch imprints (2-4) until the case is signed out. (Could be used for fluorescent in-situ hybridization, cytochemical stains or immunophenotyping).
    b) If flow cytometry is desired, deliver fresh sample in RPMI growth media to the Flow Cytometry Laboratory(0.5-1.0 cm2 should be plenty, if touch imprints are cellular).
    c) If the tissue is held overnight, place it in RPMI and save it in the refrigerator. **For holding overnight,** the Immunopathology techs recommend that you do **not** **mince** the sample because that will release proteolytic enzymes. Request the antibody panel desired, depending upon your differential diagnosis.
    d) Snap-freeze several small pieces to store at -70 C for possible future studies, which might include PCR, genetic probe, frozen section immunoperoxidase.
    e) Put several tiny (0.1 - 0.2 cm) pieces in EM fixative, when there is a very undifferentiated-appearing tumor.
    f) Put several thin sections of the tissue in a white cassette to fix in formalin. Ideally, sections should fix for at least four hours, preferably longer. Very small or thin specimens may only need an hour or two of fixation
    g) Include the disposition of the tissue in your gross dictation, for example: a piece is put in EM fixative, a piece is snap frozen, and a gross photograph is made.

Specimen Preparation (Banks, Peter in Jaffe, Surg. Path. of LN, 1995)

Optimal Sampling (Clinician, Surgeon, Pathologist before biopsy)
Intraoperative Evaluation of Tissue
    Frozen section vs. touch imprints or scrape
        Imprints--superior cytologic detail, minimize hazard from infectious processes—Mycobacterium avium complex and yeasts such as Histoplasma, Blastomyces, Coccidiodomycosis may be diagnosed on Diff-Quik or Wright's stained touch imprints of lymph nodes

    Anticipation of need for ancillary studies to aid diagnostic work-up
        Sterile
        Cytogenetic studies
        Culture
        Non-sterile
        Banking of frozen tissue (-20-70C)
        Frozen section immunoperoxidase
        Genetic probe
        Tissue for research
        Fresh tissue for flow cytometry

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Tissue fixed for possible electron microscopy
Unstained touch imprints
Cytochemical stains (Sudan black, PAS, acid
Phosphatase, specific/non-specific esterases, Oil Red O)
Fluorescent in-situ hybridization (FISH)

Tissue transport, from O.R. in saline-soaked gauze pad or towel to prevent irreversible edge artifact

Blocking: 2-4 mm sections, good cross-section of LN offers better assessment of architecture

Fixation for permanent sections:

Formalin (10% aqueous solution buffered to neutral or slightly alkaline pH;
Causes bridges between apposed hydroxyl and amino groups) : At least one block should be placed in formalin for PCR, other molecular studies, Warthin-Starry stain, EM
Formalin artifact: swelling of cells, bubbly artifact, loss of antigenicity for some antibodies (esp. after 24 hours of fixation)

Alcohol-based (Dehydrational denaturation may "burn" small specimens: shrinkage of cells with loss of cytologic detail, but good preservation of antigenicity.