BONE MARROW BIOPSY

Edited by

John R. Krause, M.D.

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John R. Krause, M.D.

Associate Professor of Pathology
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With Four Contributors

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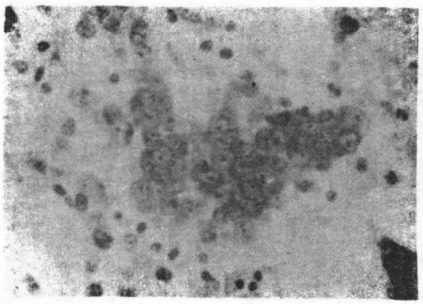
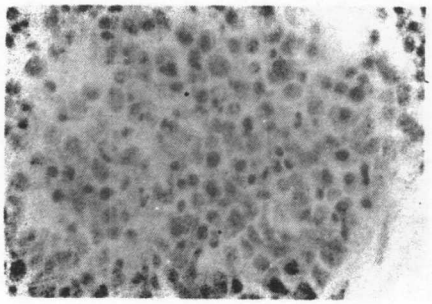
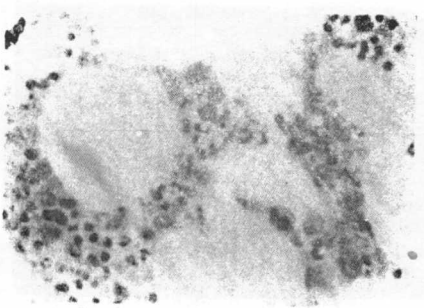
PLATE I



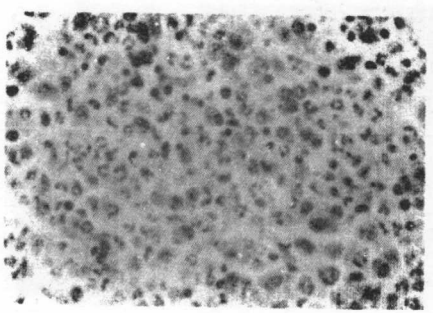
(A) Myeloblasts. The cells have nuclei containing one or more prominent nucleoli and a small amount of blue staining cytoplasm (Giemsa ×675).



(B) Chronic myelogenous leukemia. Most of these cells are myelocytes and metamyelocytes. In contrast to myeloblasts and promyelocytes, these cells have a pink cytoplasm. A few eosinophilic myelocytes (deep red cytoplasm) are also present (Giemsa ×675).

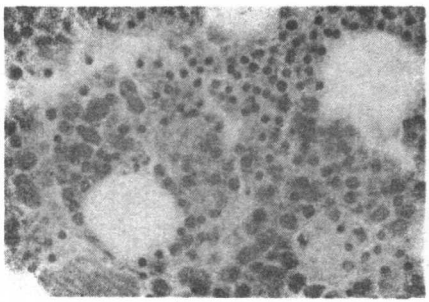


(C) Eosinophilia. Eosinophils have a deep red granular cytoplasm and stand out prominently with the Giemsa stain (Giemsa ×675).

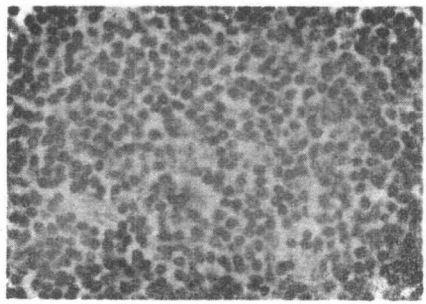


(D) Granulocytic hyperplasia. The predominant cells are the multilobed PMN's and horseshoe shaped band cells. These cells also have a pink cytoplasm (Giemsa ×675).

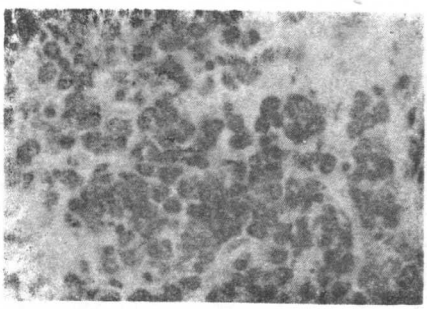
PLATE II



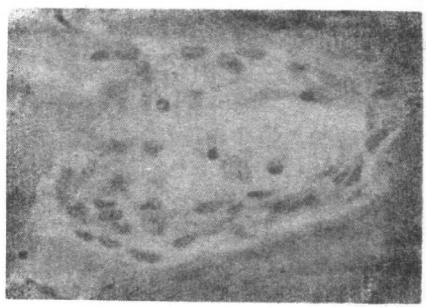
(A) Erythroid hyperplasia. Both late stage normoblasts (small dark nuclei) and early stage normoblasts (blue staining nuclei) are present. In normoblasts, the cytoplasmic borders are usually distinct (Giemsa ×675).



(B) Chronic lymphocytic leukemia. Lymphocytes have nuclear chromatin and very little cytoplasm. Cytoplasmic outlines are not prominent (Giemsa ×675).

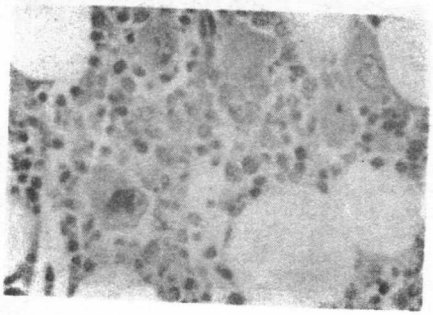


(C) Multiple myeloma. Cluster of plasma cells with eccentric nuclei (H&E ×675).

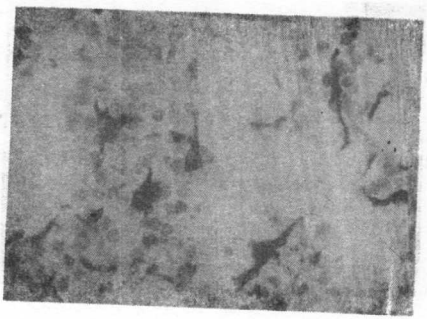


(D) Osteitis fibrosa cystica. There is extensive osteoblastic and osteoclastic activity involving this bony trabecula (H&E ×675).

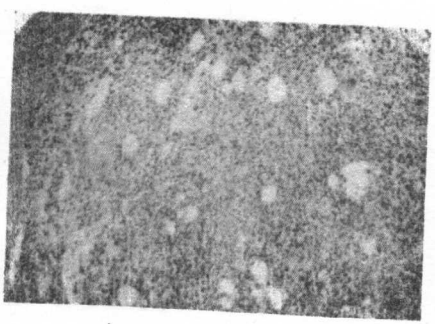
PLATE III



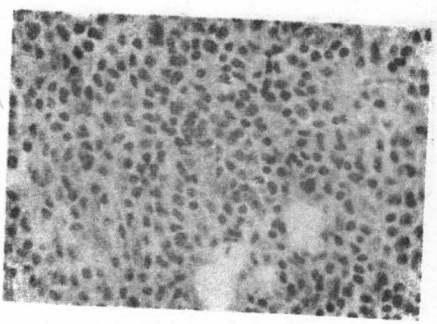
(A) Megakaryocytes. Megakaryocytes are the largest cells with abundant pink cytoplasm and multilobated nuclei (H&E ×675).



(B) Increased iron stores with a stellate pattern (Prussian blue ×675).

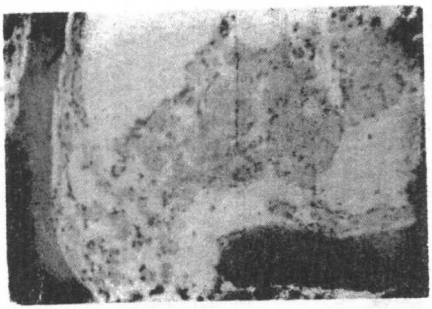


(C) Leukemic reticuloendotheliosis. Diffuse infiltrate of cells having a "lymphoid" appearance but having a light pink cytoplasm. Lymphocytes (see Plate II,B) do not have prominent cytoplasm. The needle biopsy is most important as the aspirate usually results in a dry tap due to increased reticulin fibers (H&E ×170).

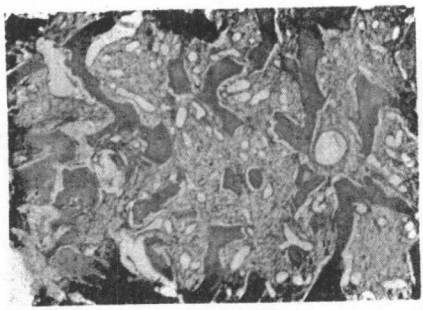


(D) Leukemic reticuloendotheliosis. Higher power demonstrates cells having pink cytoplasm and are "separate" from one another (H&E ×675).

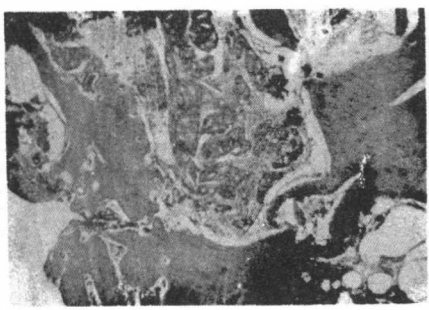
PLATE IV



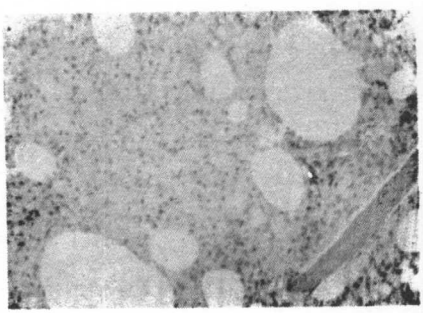
(A) Amyloid. In the biopsy, amyloid has a homogeneous, pink, somewhat glassy appearance. This pattern is the extravascular type (H&E ×675).



(B) Myelofibrosis. End stage myelofibrosis with diffuse fibrosis and osteosclerosis (H&E ×65).



(C) Metastatic carcinoma. The needle biopsy is extremely valuable for the detection of metastatic tumor in the marrow. This is a case of adenocarcinoma of the prostate (H&E ×170).



(D) Gaucher's disease. The marrow is infiltrated by a "foamy" histiocyte. In section, the cytoplasm will have a faint, fibrillar appearance (H&E ×170).

Contents

1

General Principles

Lawrence D. Ellis, M.D., Robert E. Lee, M.D., and John R. Krause, M.D.

INTRODUCTION

Needle biopsy of the bone marrow was not a commonly used diagnostic procedure until McFarland and Dameshek described a simple technique in 1958, using a Silverman needle. Subsequently, Westerman and Jensen modified the Silverman needle to allow more durability and to provide larger, more intact marrow specimens. Jamshidi then introduced a biopsy needle that was easier to use, and which provided similar, intact biopsy specimens. This text includes descriptions of the biopsy technique employing the Westerman-Jensen needle; biopsy specimens obtained through the proper use of such needles are larger than those obtained with other needles. In addition, marrow aspirates can be obtained at the same time, eliminating the need for an additional needle puncture.

Since 1959, when our first needle biopsy of the bone marrow provided a diagnosis of miliary tuberculosis, our 21-year experience at Presbyterian-University Hospital has included more than 15,000 bone marrow specimens. The diagnostic value of bone marrow needle biopsy has been consistently impressive in a variety of disorders; marrow biopsy section has established or confirmed a specific diagnosis in 18 percent of patients in our 21 years. From this experience, we conclude that needle biopsy of the bone marrow is indicated when there is a suspicion of one or more of the following disorders: disseminated granuloma, malignant lymphoma, myeloma including amyloidosis, myeloproliferative disorders, aplastic anemia, metastatic carcinoma, metabolic bone disease, storage diseases, and an alteration in marrow iron stores. It is also generally accepted that a bone mar-

row biopsy (or biopsies) is essential in the clinical staging of both Hodgkin's disease and the non-Hodgkin's lymphomas, and serial biopsies may be helpful in evaluating the efficacy of chemotherapy in acute leukemia, Hodgkin's disease, and the non-Hodgkin's lymphomas. Additionally, multiple or serial biopsies may be useful in assessing the total marrow space involved in aplasia, myelofibrosis, metastatic carcinoma, and malignant lymphoma.

There are virtually no contraindications to needle biopsy of the bone marrow; biopsies have been performed in patients with severe thrombocytopenia and in many other hemorrhagic disorders without any significant hemorrhage. Infection at the biopsy site has occurred only in rare patients with granulocytopenia, and in no instance has the biopsy been responsible for sepsis. No patients have complained of persistent pain or disability.

When properly obtained and processed, a bone marrow biopsy section will have its full diagnostic value only when assessed in conjunction with the clinical findings, peripheral blood smear, and marrow aspiration smears taken from each patient; in patients with hematologic disease, the combined evaluation of the marrow aspirate and biopsy specimens considerably enhances the diagnostic ability of the pathologist or hematologist as compared with an appraisal of either specimen individually. We hope that this text will demonstrate the spectrum of pathologic changes that can be observed when bone marrow tissue is properly prepared and studied.

OBTAINING A BONE MARROW ASPIRATE AND CORE BIOPSY

The sternum, anterior and posterior superior iliac crests, ribs, lumbar vertebrae, tibia, and spinous processes are the preferred sites for bone marrow sampling. There is no hard evidence that any one site is distinctly superior to another from the point of view of cellularity, and aspirate smears can therefore be made from any of the foregoing sites. However, because the pelvis is the safest area for performing a core biopsy, we utilize the posterior superior iliac spine as the site for both aspiration and core biopsy (Figure 1.1).

There are a number of needles available for marrow sampling; the most common and familiar include the Vim-Silverman (Becton, Dickinson & Co., Rutherford, NJ), Westerman-Jensen (Becton, Dickinson & Co.), Jamshidi (Kormed, Inc., Minneapolis, MN), and Johannah (Johannah Medical Services, Inc., Rochester, MN). These needles make it possible to obtain a core of bone and its enclosed marrow.

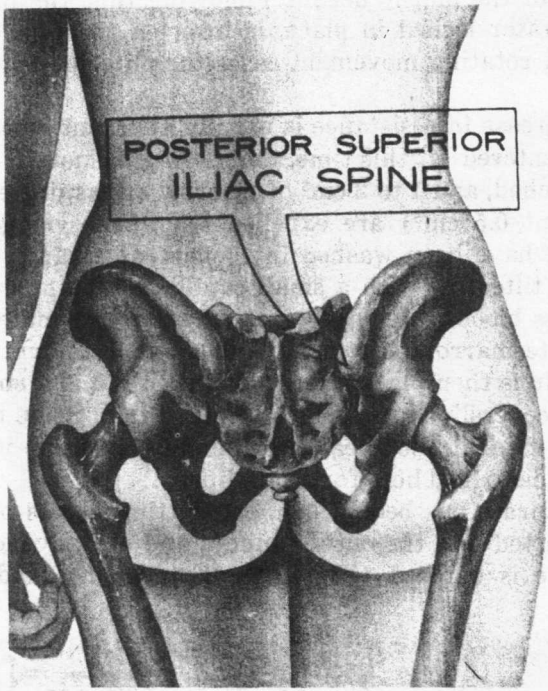


Fig. 1.1. Bone marrow biopsy site. The posterior, superior iliac spine is used as the site for both the aspirate smear and core biopsy. (Ellis, L.D., Jensen, W.N., & Westerman, M.P.: Needle biopsy of bone and marrow. Arch. Intern. Med., *114:* 213, 1964 with permission of authors and publisher).

A bone marrow aspirate can also be obtained through the Westerman-Jensen, Jamshidi and Johannah biopsy needles by means of an attached syringe; the procedure using a Westerman-Jensen needle is illustrated in Figure 1.2.

In the biopsy procedure, the patient is placed in the right or left lateral decubitus position. The skin area to be penetrated is then prepped and draped, and the dermis and subcutaneous tissue are infiltrated with 1 or 2 percent lidocaine (or novocaine) through a 25-gauge needle, producing a 5-mm to 1-cm papule. The 25-gauge needle is then replaced with a 21-gauge needle, which is inserted through the papule to the periosteum. With the point of the needle on the periosteum, approximately 2 ml of 2 percent lidocaine or novocaine are injected over a dime-sized area as the needle is rotated, after which the anesthesia needle is withdrawn. A 3-mm skin incision is next made over the biopsy site with a number-11 scalpel blade in order to facili-

tate insertion of the biopsy needle. Following this, the biopsy needle, with the obturator locked in place, is inserted through the skin and bony cortex. A rotating movement eases the anterior advancement of the needle.

A subtle decrease in resistance is usually apparent when the medullary cavity is entered; at this time, the stylet is removed, a 10 to 20-cc syringe is attached, and 1 to 2 cm^3 of marrow are aspirated. Aspirated particles (about 0.5 cm^3) are expelled from the syringe onto glass coverslips that have been washed in 70 percent ethanol. Each coverslip is quickly tilted or, with a small capillary tube having a suction bulb, the excess blood is removed from the marrow material, leaving pale, grey-white marrow fragments and a small amount of blood. A second coverslip is then placed on top of the first and a smear is made by pulling the coverslips apart in a sliding motion. Some practitioners prefer to use two alcohol-cleansed microscope slides in a manner similar to that described here for coverslips.

Once the aspirate has been taken, the cutting blades of the biopsy device are inserted into the outer cannula and are advanced until the medullary bone is entered (Fig. 1.2). The cutting blades are then

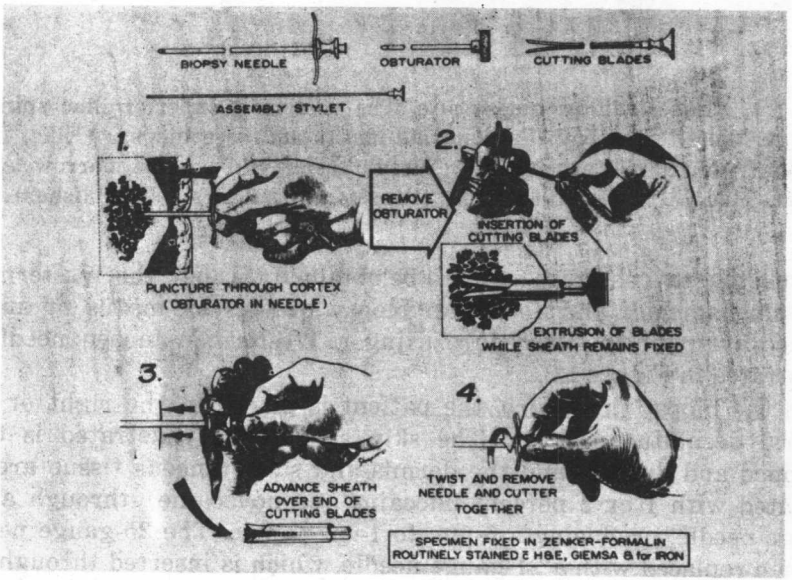


Fig. 1.2. Description of biopsy technique utilizing Westerman-Jensen needle. (Ellis, L.D., Jensen, W.N., & Westerman, M.P.: Needle biopsy of bone and marrow. Arch. Intern. Med., *114:* 213, 1964 with permission of authors and publisher).

Fig. 1.3. Representative bone marrow core biopsy specimen that may be routinely obtained (H&E × 8).

pressed into the medullary bone, with the outer cannula held firmly in a stationary position. Following this, the outer cannula is advanced over the cutting blades, with entrapment of the tissue, and the entire unit is withdrawn. The specimen is removed from the cutting blades by gently teasing it from the blade tips with a scalpel before withdrawing the blades through the cannula. Optimal specimens are of approximately ¾-inch length by 1/16 to ⅛-inch diameter, and have wet weights of about 150 mg (Fig. 1.3). Air-dried touch preps may be made from the biopsy specimen before it is put into Zenker's solution; cultures may either be taken at this time or may be obtained from the aspirated marrow.

Immediately following the biopsy the bone marrow specimen is put into Zenker's fixative; our procedure for fixing and processing the biopsy specimen is given in the Appendix, which includes preparation for electron microscopy (Fig. 1.4). In every case, we routinely cut six sections of bone marrow and prepare hematoxylin and eosin (H&E) and Giemsa and iron stains on each. The staining procedures that we use are also found in the Appendix. Postbiopsy care of the patient ordinarily consists of applying pressure over the posterior ilium for about 60 minutes, which is accomplished with a pressure dressing, and having the patient lie recumbent in bed. Patients with bleeding tendencies or other complications are carefully observed for longer periods. Analgesics are seldom necessary following the biopsy procedure.

CYTOLOGIC FEATURES OF MARROW CELLS

The value of the Giemsa procedure lies in its ability to differentially stain cells of the myeloid, lymphoid, and plasma cell series. The cytologic features of the various cells of the marrow are as follows:

Neutrophilic myelocytes and metamyelocytes are recognized in

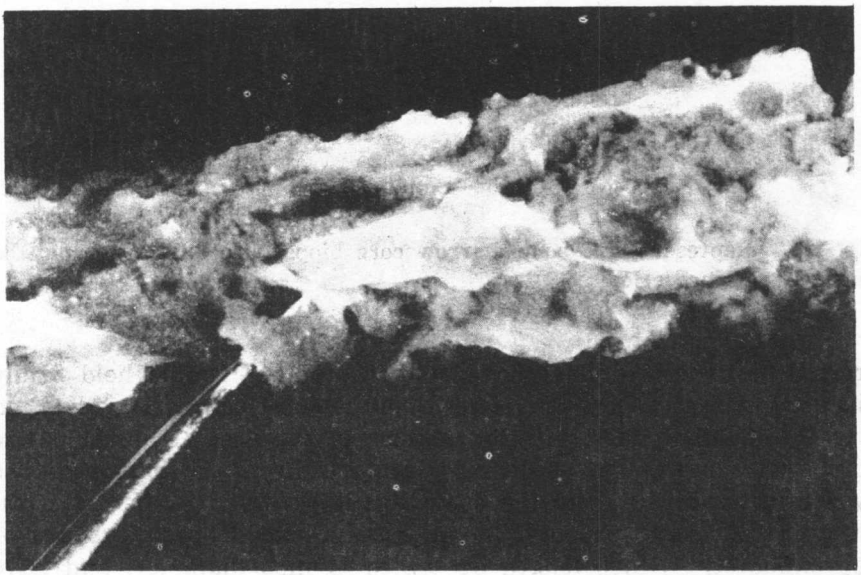


Fig. 1.4. For preparation for electron microscopy, samples of marrow may be teased from the bony trabeculae with a dissecting scope and dissecting needles. See appendix for details.

Giemsa-stained preparations by the light pink appearance of their cytoplasm (Plate I,B). The most mature neutrophils are recognized by their small size and darkly stained multiple nuclear lobes (Plate I,D); the cytoplasm of these most mature cells may not appear to stain, or may be a very light pink. Myeloblasts and promyelocytes have a cytoplasm that stains blue (Plate I,A), and cannot be separated from pronormoblasts other than through the tendency of the latter to cluster with the more mature normoblasts (Plate II,A). The cytoplasm of eosinophilic granulocytes characteristically has a more intense red staining, and these cells are easily recognized as the most brightly stained cells of the marrow (Plate I,B,C).

Older normoblasts can be identified through the intensity of their nuclear staining, this being so dark that light does not seem to penetrate the nuclei to any extent. The cytoplasm of these cells is not appreciably stained, but the plasma membrane margin can often be clearly discerned (Plate II,A). These cells often exist in groups or clusters, which can be recognized at medium power.

Lymphocytes are among the most difficult cells to recognize other than when they form clusters. In the latter case, the speckled nature

of the nuclear chromatin in a small, round nucleus, along with a slight amount of blue cytoplasm in Giemsa-stained sections, can be recognized as common features of small, mature lymphocytes (Plate II,B). More immature lymphocytes will have larger round or lobulated nuclei, but cytoplasm remains as a scant blue rim.

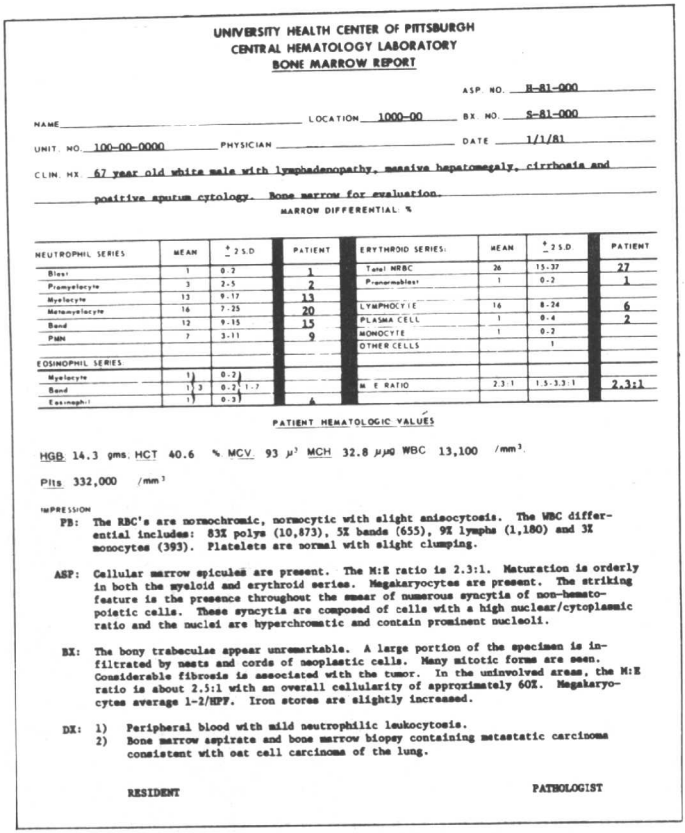
Plasma cells can be difficult to distinguish from myelocytes in hematoxylin-eosin stained marrow preparations, but are recognized with Giemsa staining as cells having eccentric, dark nuclei and blue cytoplasm with a pale, perinuclear Golgi zone located adjacent to the nucleus (Plate II,C). In contrast to the Golgi zone of the plasma cell, a similar zone in the osteoblast is round, and is not adjacent to the nucleus (Fig. 13.1). These pale zones are easily identified by virtue of an increased number of ribosomes in the surrounding cytoplasm, and it is these ribosomes that take on the methylene blue portion of the Romanovsky stains. Plasma cells are commonly located around blood vessels and osteoblasts surround the bony trabeculae. Osteoblasts tend to be more numerous when bone is being actively deposited, and under such circumstances may be increased, along with their multinucleated relatives, the osteoclasts (Plate II,D).

Megakaryocytes are easily recognized as the largest cells of the marrow, and have a quite characteristic multilobulated nucleus. The nucleus of older megakaryocytes becomes smaller and more darkly stained. With H&E staining, the cytoplasm varies from a light pink in younger cells to a lesser amount of a darker pink cytoplasm in older cells. A second variety of large cell is the osteoclast, which is multinucleated, rather than having the multilobulated nucleus of the megakaryocyte series. Golgi zones are not discernible in these cells.

BONE MARROW REPORTS

Our evaluation of any patient who has had a bone marrow biopsy consists of an examination of the peripheral blood and of the marrow aspirate smear, as well as of the core biopsy itself. All three of these facets of the evaluation are important in the total assessment of the individual. An example of our bone marrow report and of our reporting system is seen in Figure 1.5.

In evaluating each patient, a systematic approach is recommended, and we follow a definite pattern in our work-up. This is especially useful in the training of interns, residents, and fellows, since it emphasizes the need to examine all facets of the bone marrow, and not just to focus on a striking abnormality, thereby missing any associated or incidental findings.



UNIVERSITY HEALTH CENTER OF PITTSBURGH
CENTRAL HEMATOLOGY LABORATORY
BONE MARROW REPORT

ASP. NO. H-81-000

NAME _____ LOCATION 1000-00 BX NO. S-81-000

UNIT. NO. 100-00-0000 PHYSICIAN _____ DATE 1/1/81

CLIN. HX 67 year old white male with lymphadenopathy, massive hepatomegaly, cirrhosis and positive sputum cytology. Bone marrow for evaluation.

MARROW DIFFERENTIAL %

NEUTROPHIL SERIES	MEAN	± 2 S.D	PATIENT
Blast	1	0-2	1
Promyelocyte	3	2-5	2
Myelocyte	12	9-17	13
Metamyelocyte	16	7-25	20
Band	12	9-15	15
PMN	7	3-11	9
EOSINOPHIL SERIES			
Myelocyte	1	0-2	
Band	1 3	0-2 1-7	
Eosinophil	1	0-3	4

ERYTHROID SERIES:	MEAN	± 2 S.D	PATIENT
Total NRBC	26	15-37	27
Pronormoblast	1	0-2	1
LYMPHOCYTE	16	8-24	6
PLASMA CELL	1	0-4	2
MONOCYTE	1	0-2	
OTHER CELLS		1	
M E RATIO	2.3:1	1.5-3.3:1	2.3:1

PATIENT HEMATOLOGIC VALUES

HGB 14.3 gms. HCT 40.6 % MCV 93 μ^3 MCH 32.8 $\mu\mu g$ WBC 13,100 /mm^3.

Plts 332,000 /mm^3

IMPRESSION

PB: The RBC's are normochromic, normocytic with slight anisocytosis. The WBC differential includes: 83% polys (10,873), 5% bands (655), 9% lymphs (1,180) and 3% monocytes (393). Platelets are normal with slight clumping.

ASP: Cellular marrow spicules are present. The M:E ratio is 2.3:1. Maturation is orderly in both the myeloid and erythroid series. Megakaryocytes are present. The striking feature is the presence throughout the smear of numerous syncytia of non-hematopoietic cells. These syncytia are composed of cells with a high nuclear/cytoplasmic ratio and the nuclei are hyperchromatic and contain prominent nucleoli.

BX: The bony trabeculae appear unremarkable. A large portion of the specimen is infiltrated by nests and cords of neoplastic cells. Many mitotic forms are seen. Considerable fibrosis is associated with the tumor. In the uninvolved areas, the M:E ratio is about 2.5:1 with an overall cellularity of approximately 60%. Megakaryocytes average 1-2/HPF. Iron stores are slightly increased.

DX: 1) Peripheral blood with mild neutrophilic leukocytosis.
2) Bone marrow aspirate and bone marrow biopsy containing metastatic carcinoma consistent with oat cell carcinoma of the lung.

RESIDENT PATHOLOGIST

Fig. 1.5. Sample of bone marrow report.

The peripheral blood is examined first. The examination includes red cell morphology (size, shape, inclusions, and so forth), white cell numbers and morphology, and platelets (estimation of numbers and morphology). Examination of the peripheral blood will often help considerably in interpreting the bone marrow findings. The bone marrow aspirate or smear is examined next. Again, a differential count is done on each smear for teaching purposes, with recognition of the variability that may occur if only a small area of the aspirate is counted. Following the careful examination of each smear, a 300-cell differential count is done, for practical reasons, in a cellular area close to a bone spicule. The results of the differential count are then related to our impression of the smear as a whole, and then to the core biopsy. Finally, the core biopsy is examined. Because of the sam-