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Bone Marrow Examination: Adventures in Diagnostic Hematology

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The professional responsibilities of a clinical pathologist include a continuous search for improving the laboratory diagnosis of clinical disorders. To meet the challenges of these responsibilities, we have studied over the past 25 years diagnostic features of the bone marrow in a variety of hematologic and nonhematologic conditions, such as anemias of different types, leukemias, lymphomas, plasma cell disorders, metastatic neoplasms, and metabolic and endocrine diseases as well as inflammatory and reactive changes of the marrow.

Highlights of these studies include:

1. Comparative value of various preparations, including methacrylate sections
2. Dry tap in marrow aspiration
3. Bone and marrow changes in chronic renal failure
4. Lymphoid follicles, lipid granuloma and plasmacytosis of the bone marrow
5. Bone marrow iron stores
6. Epithelioid granulomas of the bone marrow in non-Hodgkin's lymphoproliferative malignancies

1. Comparative values of various preparations including methacrylate sections

Techniques in the sampling, processing and staining of bone marrow specimens and methods of evaluating the preparations are of considerable importance in the diagnosis of a variety of hematologic and nonhematologic disorders. However, these techniques vary greatly in different institutions and different parts of the world. A critical comparison of the value and limitations of various types of bone marrow preparations, such as films, sections of aspirates, imprints (touch preparations) and sections of the needle biopsy has been adequately reviewed (Table 1).

Table 1. Bone marrow examination: types of preparations

1. Aspiration
   - A. Smears (thin spread, squash, cover slip)
   - B. Paraffin sections
   - C. Plastic (methacrylate) sections (direct, paraffin to plastic)
   - D. Electron microscopy (glutaldehyde)
   - E. Cultures, cytogenetic studies, etc.
2. Biopsy
   - A. Imprints (touch preparations)
   - B. Sections (paraffin, plastic or paraffin to plastic)
   - C. Electron microscopy

Methacrylate (plastic) embedding of bone marrow specimens has become popular in recent years. Methacrylate (plastic) sections generally show superior morphologic detail allowing improved evaluation of nuclear and cytoplasmic structures and of enzyme histochemical reactions. The specimen may be embedded directly in methacrylate with retention of enzymes for superior histochemical reactions. It is also possible to use paraffin-embedded material for plastic embedding. Such a technique is extremely valuable, particularly when the quality of the paraffin sections is poor.

2. Dry tap in marrow aspiration

Dry tap in aspirating the marrow is an unpleasant experience which is sometimes encountered even by the most skilled. Our definition of dry tap includes both the cases where the marrow is unobtainable and where the marrow blood is easily obtained but no units are found in films or sections.

Table 2-6 illustrate our studies on "dry tap." As shown in Table 7, we strongly recommend the routine use of biopsies and aspirations together (and if indicated bilateral aspirations and biopsies) in order to maximize the opportunity for positive findings. Our motto is "the more tissue you have, the more likely

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Table 2. Bone marrow aspiration: dry tap-causes

1. Faulty technics
2. Myeloproliferative disorders with myelofibrosis/osteosclerosis, often
3. Secondary myelofibrosis/osteosclerosis due to tumors (metastatic, often; primary, rare)
4. Compactly cellular marrow (acute leukemias, lymphomas, plasmacytic dyscrasias, etc.)
5. Leukemic reticuloendotheliosis, often
6. Granulomas, infrequent

Table 3. Bone marrow examination: incidence of dry tap

Total number (January 1973-July 1976)	1,357
Aspiration only	97
Biopsy only	10
Aspiration and biopsy	1,250*

* biopsy repeated in 5

Table 4. Incidence of dry tap in 1,250 concomitant aspirations and biopsies

Dry tap	85 (6.8%)
No material aspirated	32
Blood aspirated (no units)	53

Table 5. Biopsy diagnosis in 85 patients with dry tap

Leukemias		22
AGL	12	
CGL, MF, blast crisis	1	
ALL	4	
CLL	3	
Hairy cell	2	
Myelofibrosis, primary		6
Myelofibrosis in PV		3
Lymphomas		8
Lymphocytic	6	
Histiocytic	2	
Myeloma		1
Metastatic carcinomas*		10
Total		50

* 8 of 10 with myelofibrosis/osteoscelerosis

Table 6. Biopsy diagnosis in 85 patients with dry tap (con't)

Normal	7	Eosinophilia	2
Iron deficiency	7	plasmacytosis	2
Hypoplasia	5	AHA	1
Hemosiderosis	4	PA	1
Granulocytic hyperplasia	3	Erythroid hyperplasia	1
Granulomas (sarcoid)	2	Total	35

Table 7. Bone marrow aspiration: dry tap recommendations

1. If technic is at fault: repeat aspiration by an experienced person
2. If myelofibrosis/osteosclerosis or leukemic reticuloendotheliosis is suspected: biopsy (with imprints)
3. If compactly cellular marrow/granuloma is suspected: repeat aspiration
4. Why not do biopsies and aspirations together routinely?

you will be able to detect pathologic alterations."

3. Bone and marrow changes in chronic renal failure

The recent introduction of bone marrow biopsy in addition to routine aspiration for the evaluation of hematologic and nonhematologic disorders has made it possible to study morphologic alterations of the bone including osteosclerosis, osteoporosis, osteomalacia, Paget's disease and primary and secondary (mostly renal) osteodystrophy. In a study of 2,004 consecutive bone marrow examinations performed in our institution covering a 5 year period, we encountered 72 instances of renal osteodystrophy in 70 patients (Table 8-11). Renal osteodystrophy was diagnosed in 3.9 percent of 1,851 consecutive marrow biopsies but not in the marrow aspirates of the same. Routine use of the bone marrow biopsy, facilitated by the availability of the Jamshidi needle, has been found most useful in detecting both clinically

Table 8. Renal osteodystrophy in BM biopsy: pathology

1. Bone resorption by osteoclasts.
2. Progressive thinning of cancellous and cortical bone, with fibrosis of marrow.
3. Osteoblastic activity with new bone formation.
4. Cyst formation or brown tumor as seen in advanced cases not observed.

apparent and clinically unsuspected cases of renal osteodystrophy. The use of the marrow biopsy is often superior to the study of well established biochemical parameters in the early diagnosis of renal osteodystropy.

Table 9. Renal osteodystrophy in bone marrow biopsies
(5 year Period: 1973-1978)

Specimens	No/ specimens	Renal Osteodystrophy
Biopsy and aspiration	1972	72 (all in biopsies)
Aspiration only	153	0
Biopsy only	59	0
Total	2004	72

Incidence: Biopsy specimens: 72/1851 (3.9%)
Aspiration sections: 0/1945 (0%)

72 marrows with renal osteodystrophy in 70 patients, 28 males/42 females (M:F=1:1.5)

Table 10. Renal osteodystrophy in bone marrow biopsies: associated clinical conditions (70 patients)

Clinical Conditions	No.	%
ASCV disease	24	34.3
with hypertension	(18)	(25.7)
without hypertension	(6)	(8.6)
Diabetes mellitus	17	24.3
Chronic pyelonephritis	8	11.4
Polycystic kidneys	3	4.3
Postnephrectomy for calculi	3	4.3
Chronic glomerulonephritis	2	2.9
Anemia of unknown cause	2	2.9
Miscellaneous	11	16.6
Total	70	100.0%

Table 11. Renal osteodystrophy in bone marrow biopsies

Diagnosis of renal disease	Number (%)
Before admission	41 (58.6%)
During admission	29 (41.4%)
Suspected before BM Bx	17 (24.3%)
Unsuspected until BM Bx	12 (17.1%)
Total number if patients	70 (100%)

4. Lymphoid follicles, lipid granuloma and plasmacytosis of the bone marrow

Morphologic evaluation of the bone marrow is still probably the most important step in the differentiation between reactive and neoplastic plasmacytic proliferations. A consecutive series of 1,000 bone marrow aspirates was analyzed for percentage of plasma cells, incidence of plasmacytic satelli osis, associated clinical disease states, lymphoid follicles, lipid granulomas, hemosiderin content and various combinations thereof. Plasmacytosis, a common finding, tended to parallel the presence of lymphoid follicles, lipid granulomas and plasmacytic satellitosis. The latter is emphasized as a normal phenomenon, may reflect morphologically a physiologic response of the B cell

Table 12. Normal plasma cell percentage in bone marrow

Steiner & Pearson (1966)	0-0.6%
Berman (1949)	1.0%
Diggs (1948)	0-1.0%
Whithy & Britton (1975)	0-1.0%
Custer (1974)	0-1.2%
Leitner (1945)	1.2%
Vaughan & Brockmyre (1947)	0-1.5%
Miale (1972)	0.1-1.5%
Hyun & Ashton (1976)	0-2.0%
Israels (1955)	0-2.0%
Wintrobe (1974)	0-3.5%
McDonald et al (1970)	0.1-3.5%
Lucia & Hunt (1947)	0-4.1%

Hyun BH, et al: Reactive plasmacytic lesions of the bone marrow. Am J Clin Pathol 64:921, 1976

Table 13. Plasmacytosis vs plasmacytic satellitosis

No. Aspirates	Plasmacytosis	Plasmacytic Satellitosis
1000	276 (27.6%)	128 (46%)

Table 14. Plasmacytosis: plasmacytic satellitosis

No. Aspirates	Plasmacytes (%)	Plasmacytic Satellitosis
689	Normal, <2%	69 (10.0%)
239	2-4%	111 (46.4%)
37	>5%	17 (45.9%)

system to antigenic stimulation, and is conspicuously absent in plasmacytic neoplasia. Analyses made in this study are illustrated in Tables 12-16.

Lipid granuloma is the most common form of granulomas of the bone marrow characterized by lipid vacuoles, fibroblasts/histiocytes, eosinophils, lymphocytes, plasmacytes (Russell bodies etc.) and occasional foreign body giant cells. Lipid granulomas, lymphoid follicles, plasmacytosis, plasmacytic satellitosis are seemingly unrelated nonspecific lesions of the bone marrow, but they may well be interrelated as they may in part be the result of chronic antigenic stimulation.

5. Bone marrow iron stores

The hemosiderin content and particle pattern in the marrow are of great diagnostic value and are evaluated routinely (Tables 17, 18).

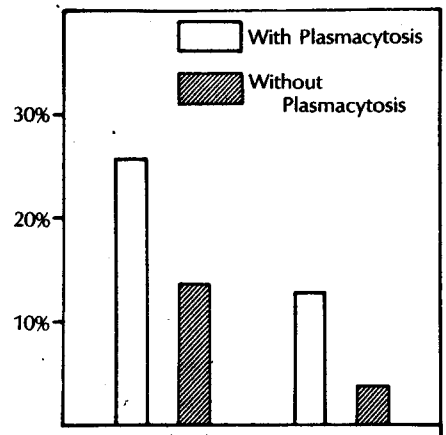
An analysis of 500 consecutive marrow aspirates from various hematologic and nonhematologic disorders in our laboratory revealed the following:

a. The absence of iron stores in the bone marrow is the sine qua non of iron deficiency anemia. However, the absence of iron may also be found in acute hemolytic crisis, in early phase of therapy for pernicious anemia, polycythemia vera, nutritional iron deficiency in infancy or in pregnancy and

Table 15. Plasmacytic lesions of bone marrow

Clinical Situations	No. of cases	Percent 0 10 20 30 40 50 60 70
A. Neoplastic diseases	296	
Carcinoma	91	
Leukemia/lymphoma (exc. Hodgkin's)	151	
Hodgkin's disease	24	
Myeloproliferative disorders (exc. PV, CGL)	21	
Polycythemia vera	9	
B. Nonneoplastic hematologic diseases	221	
Iron deficiency anemia	113	
Megaloblastic anemia	39	
Marrow hypoplasia	21	
Hemolytic anemia	12	
Thalassemia and sideroblastic anemia	11	
Idiopathic thrombocytopenic purpura	25	
Infectious and inflammatory conditions	309	
Fever of unknown origin	32	
Viral infections	17	
Bacterial infections	37	
Cirrhosis	57	
Granulomatous diseases	30	
Collagen disorders	26	
Nonspecific inflammatory conditions	110	
D. Diabetes mellitus	18	
E. Cardiovascular diseases	14	
F. Miscellaneous conditions	107	
G. Plasma cell neoplasia	35	
Total	1000	
PLASMACYTOSIS		PLASMACYTIC SATELLITOSIS

Table 16.



	276 with plasmacytosis	689 without plasmacytosis
Lymphoid follicies	71 (25.7%)	94 (13.6%)
Lipid graniomas	34 (12.3%)	25 (3.6%)

Table 17. Grading of bone marrow hemosiderin content

	Absent	Decreased	Normal	Slight Increase	Moderate Increase	Marked Increase
Units on films	0	±−1+	1+	2+	3+	4+
Units in sections	0	0−±	1+	2+	3+	4+

Table 18. Bone marrow hemosiderin: pattern

The pattern (the particle size) of the hemosiderin has also been found useful in the proper interpretation of marrow iron.

Bone Marrow: Hemosiderin Particle Size

	Small	Medium	Large
Films	0.5-2μm	2-5μm	>5μm
Sections	0.5-2μm	2-5μm	>5μm

acute blood loss.

b. In malignant disorders and inflammatory diseases the iron content is variable, tends to be increased and to show a large particle pattern. An increase in hemosiderin parallels the incidence of reactive plasmacytosis and plasmacytic satellitosis of the bone marrow.
c. Hemosiderin is usually normal or increased in secondary polycythemias, a feature of differential diagnostic significance.
d. In hemolytic anemias the hemosiderin content is variable but tends to be increased and to show a small particle pattern. The presence of ring sideroblasts is of paramount importance in the diagnosis of a variety of sideroblastic anemias.

Table 19. Bone marrow granulomas in lymphoproliferative malignancies: literature survey

Authors	Hodgkin's Disease	Non-Hodgkin's Lymphoproliferative Malignancies	Total Examined
Pease, 1952, 1956	8	7 2 (multiple myeloma)	221
Kadin and associates, 1970	1	–	145 (HD)
Kim, Dorfman, 1974	–	0	75 (non-HL)
Sacks and co-workers, 1978	2	–	608 (HD)
Te Velde & colleagues, 1978	2	–	80 (HD)
Yu, Rywlin 1982	–	9	?
Choe, Hyun, et al, 1984*	–	10	372

* Am J Clin Pathol 81:19, 1984

Table 20. Bm granulomas in non-Hodgkin's lymphoreticular malignancies (Muhlenberg Hospital: 1964-1980)

	No. of Cases	BM Granulomas	%
Lymphoma	149	6	4.0%
ALL	55	1	1.8%
CLL	85	0	0.0%
MM	83	3	3.6%
Total	372	10	2.7%

6. Epithelioid granulomas of the bone marrow in non-Hodgkin's lymphoproliferative malignancies

Both Hodgkin's disease and non-Hodgkin's lymphomas are known to be associated with epithelioid granulomas of the bone marrow (Table 19). Ten cases of such granulomas in the bone marrow of patients with various non-Hodgkin's lymphoproliferative malignancies have been encountered in our institution (Table 20). It is of note that 3 cases were in patients with multiple myeloma and 1 in a patient with acute lymphocytic leukemia. The latter to our knowledge is the first such case reported in the literature.

CONCLUSION

A variety of interesting and useful studies can be carried out on routine bone marrow aspirates and biopsies, and such systematic studies have proved of practical diagnostic value in a variety of clinical situations.

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