THE BONE MARROW IN PAGET'S DISEASE (OSTEITIS DEFORMANS) WITH SPECIAL EMPHASIS ON THE MEGAKARYOCYTES

BY

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In the course of a study of bone marrow in blood dyscrasias and other pathological states an increase of megakaryocytes was found to be rather frequent in idiopathic thrombocytopenic purpura, sickle cell anaemia, Hodgkin's disease, microcytic hypochromic anaemia, polycythaemia vera, and pregnancy. Simultaneously with this study, two patients with unexplained mild anaemia and no definite clinical diagnosis in whom sternal marrow examination showed an increase of megakaryocytes, and radiographs subsequently revealed Paget's disease of bone, were examined. A study was undertaken to investigate the sternal marrow of all available patients with monostotic and polyostotic forms of Paget's disease revealed radiologically.

Materials and Methods

Eight men at the Veterans Administration Hospital and two negro women from the Charity Hospital were studied. As controls, 10 patients were used who showed a megakaryocyte count in the marrow of 50 per c.mm. or above and had various blood dyscrasias. The sternal marrow (not more than 1 ml.) was aspirated with an 18 gauge spinal needle. A small quantity was used to make smears on slides and cover-slips. The remainder was added to 2 mg. of dry ammonium potassium oxalate (Heller and Paul, 1934). The oxalated marrow was diluted in a white cell pipette using Nile blue sulphate-aqueous propylene glycol (0.1% Nile blue in propylene glycol in equal parts of the glycol stain and distilled water at pH 6.5). The total nucleated elements were determined by means of a Levy-Neubauer chamber and the megakaryocytes were counted in a Fuch-Rosenthal chamber. With the Nile blue stain the megakaryocyte nucleus stained a deep blue and the cytoplasm, the periphery of which was often serrated, a clear or slightly granular blue. These cells measured from 20 to 50 μ . The other cells stained essentially the same, and the free fat stained a reddish orange.

The unused oxalated marrow was placed in a Wintrobe haematocrit tube and centrifuged to study the layer formation as fat, plasma, buffy coat, and red cell layer as recommended by Limarzi (1939). The buffy layer contained numerous megakaryocytes, and smears were prepared from this concentrated material. The smears were stained with Wright stain by the usual technique. It was found that the cover-slip gave a more uniform distribution of the giant cells as the slide method caused the accumulation of these cells in the feather-edge. One hundred megakaryocytes were counted and they

TABLE IA
PERIPHERAL BLOOD AND MARROW FINDINGS IN PAGET'S DISEASE

Case No.	Bones Involved	Age	Colour	Sex	Peripheral Blood			
					Red Cells (per c.mm. in Millions)	White Cells (per c.mm. in Thousands)	Alkaline Phosphatase	Acid Phosphatase
1	Lumbosacral vertebrae	56	C.	F.	4.9	9.6	16.9	0.51
2	Lumbosacral vertebrae, pelvis, femurs	57	C.	F.	4.1	7.8	7.0	*
3	Femur and pelvis	59	C.	M.	3.6	12.3	5.1	0.26
4	Parietal	75	W.	M.	5.2	6.5	*	*
5	Lumbar vertebrae, and ilium	66	W.	M.	2.6	6.8	5.1	0.1
6	Humerus	50	W.	M.	5.1	6.5	12.4	0.22
7	Parietal, scapula, thoracic vertebrae	61	W.	M.	3.1	5.0	5.6	0.55
	No. 7 (two months later)				4.5	6.0	*	*
8	Parietal, humerus, lumbosacral vertebrae, and ilium	58	W.	M.	4.6	5.0	4.9	0.69
9	Lumbosacral vertebrae, pelvis, and femurs	66	C.	M.	4.3	4.2	17.6	0.80
10	Lumbosacral vertebrae and pelvis	60	C.	M.	4.9	12.7	8.5	0.14

TABLE IB
PERIPHERAL BLOOD AND MARROW FINDINGS IN PAGET'S DISEASE

Case No.	Sternal Marrow														
	Differential														
	Marrow Count								Megakaryocyte Count						
	Fat (%)	Megakaryocytes (per c.mm.)	Nucleated Cells (per c.mm. in Thousands)	Granulocytes (%)	Erythroid (%)	Lymphoid (%)	Eosinophils (%)	Reticulo-endothelial Cells (%)	Megakaryoblast (%)	Promega-karyocyte (%)	Lymphoid Type (%)	Polykaryocyte (%)	Adult (%)	Degenerated Form (%)	Mitosis (%)
1	6	352	250	57.4	24.1	18.5	2.7	0.3	1	18	5	18	45	13	0
2	8	58	73	59.1	20.4	20.5	2.9	0.4	1	15	3	20	42	18	1
3	2	109	95	67.3	17.2	15.5	2.3	0.0	1	7	2	6	71	10	3
4	9	20	58	64.9	21.5	13.6	2.3	0.2	4	24	12	10	32	18	0
5	3	12	58	70.3	16.5	13.2	3.4	0.6	4	10	8	10	54	14	0
6	5	11	27	66.5	25.5	18.0	3.6	0.1	1	16	5	3	62	12	1
7	*	104	89	54.3	17.4	28.3	2.6	0.0	0	10	2	35	46	6	1
	3	53	50	57.5	15.1	27.4	5.3	0.4	2	6	2	9	65	16	0
8	9	59	112	59.7	22.8	17.5	1.5	0.0	3	14	8	7	51	16	1
9	7	66	48	47.6	29.4	23.0	1.9	0.0	0	9	4	9	66	11	1
10	6	40	84	63.0	23.7	13.3	2.3	0.0	3	7	9	10	55	15	1

* Not done.

were divided, with some modification, into the classification as recommended by Dameshek and Miller (1946) as megakaryoblast, lymphoid types, polykaryocytes, adult or multilobulated forms, degenerated varieties, and mitotic forms.

A differential count of 1,000 marrow cells on each patient with osteitis deformans was done, the cells being classified as granulocyte, erythroid, and lymphoid elements, eosinophils, and reticulo-endothelial cells (Table I). Erythrocyte and leukocyte counts of peripheral blood were recorded on patients with Paget's disease and on the control group. Serum acid and alkaline phosphatase estimations were performed on patients with Paget's disease and recorded in Shinowara-Jones-Reinhart units (normal, 0-1.1) and Bodansky units (normal, 2-9).

A differential megakaryocyte count was done on 10 patients with no purpuric or thrombotic tendencies as a control group; patients were selected who had megakaryocyte counts above 50 per c.mm. (Table II).

TABLE II
PERIPHERAL BLOOD AND MARROW FINDINGS IN PATIENTS WITH ELEVATED
MEGAKARYOCYTE VALUES

Disease	Colour	Sex	Age	Peripheral Blood		Sternal Marrow									
				Red Cells (per c.mm. in Millions)	White Cells (per c.mm. in Thousands)	Fat (%)	Megakaryocyte (per c.mm.)	Nucleated Cells (per c.mm. in Thousands)	Megakaryocyte Differential Count						
									Megakaryoblast (%)	Promegakaryocyte (%)	Lymphoid Type (%)	Polykaryocyte (%)	Adult (%)	Degenerated Form (%)	Mitosis (%)
Acute haemolytic anaemia	W.	M.	20	3.7	10.8	0	281	300	1	7	5	10	70	7	0
Carcinoma of stomach	W.	M.	61	3.2	19.6	10	156	303	2	10	5	8	55	19	1
Sickle cell anaemia ..	C.	F.	25	1.8	9.0	0	153	275	1	4	1	8	75	11	0
Polycythaemia vera ..	C.	M.	33	5.8	8.3	2	50	95	1	5	8	0	73	13	0
Thiouracil neutropenia ..	W.	M.	26	4.6	5.9	4	73	89	0	13	6	7	58	16	0
Macrocytic anaemia ..	W.	M.	64	4.2	8.5	9	73	190	3	11	5	11	45	24	1
Leukocytosis, unknown cause ..	W.	M.	50	5.1	15.1	*	75	106	1	7	14	6	59	12	1
Macrocytic anaemia ..	W.	M.	56	3.8	8.3	8	109	119	0	8	4	5	76	7	0
Hodgkin's disease	W.	M.	50	4.5	6.0	14	128	127	1	6	4	8	68	13	0
Multiple sclerosis	W.	M.	32	3.1	4.4	2	52	132	1	6	5	3	77	7	1

* Not done.

The possible relationship between the marrow megakaryocytes and the serum acid and alkaline phosphatase levels was studied on 11 patients with no obvious bone pathology (Table III).

TABLE III
MEGAKARYOCYTES AND SERUM ACID AND ALKALINE PHOSPHATASE LEVELS IN PATIENTS
WITH NO BONE PATHOLOGY

Megakaryocytes (per c.mm.)	Phosphatase	
	Acid	Alkaline
36	0.18	4.2
61	0.26	2.9
72	0.14	1.3
72	0.18	3.0
45	0.25	5.5
18	0.55	5.1
56	0.28	7.1
1	0.36	5.6
65	0.73	5.2
167	1.40	4.5
12	0.58	3.6

The diagnosis of osteitis deformans was made by radiographs of the bone showing the characteristic coarse trabeculations and cortical thickening. In one case of pathological fracture a biopsy of the fracture site at operation revealed Paget's disease (Case 6). As interpretation of radiological findings in the sternum was extremely difficult, no special technique was used in the detection of lesions in the sternum.

Comment

According to Jaffe (1933) the marrow in the typical picture of Paget's disease shows classical osteoclasts and osteoblasts with polymorphonuclear leukocytes, many of which are eosinophils, and varying numbers of haemosiderin-containing macrophages. There may be fatty marrow with islands of myeloid tissue. There may be areas of fibrous marrow which in the process of healing may become replaced by more lymphoid and fatty marrow. Since the Italian school of haematology, notably Di Guglielmo (1925), believes that the multinucleated megakaryocyte (polykaryocyte) is probably identical with the osteoclast and that the multi-lobulated forms may represent developmental stages in platelet formation, the increase of all forms of these giant cells may be significant in the pre-radiological evidence of osseous involvement. One feature in most of our cases was the increase of megakaryocytes out of proportion to the activity of the marrow. In a study of 10 normal individuals (Pizzolato, 1948) the average number of megakaryocytes was 13 per c.mm., with one individual having 22 per c.mm. as the upper limit. Three individuals with osteitis deformans had counts within the normal limits. One patient had a value of 352 per c.mm., the highest recorded in any condition. It is to be noted that the relative number of multinucleated forms was not increased; in only two cases were counts of 18% and 20% found recorded. These varieties may be more frequent, however, due to the fact that nuclei are often superimposed on each other, giving the impression of adult or multilobulated forms. Again, there are instances where platelets cover the nucleus. Nearly all megakaryocytes appear to have the capacity for platelet formation, and the platelets of the marrow and peripheral blood have normal morphological and chromatic characteristics; thrombotic phenomena do not occur.

In the control series an increase of megakaryocytes was associated with other activity in the bone marrow, such as anaemia or leukopenia. Only two individuals with Paget's disease showed hyperplasia of the marrow compared with the maximum normal nucleated elements of 105,000 per c.mm.

No correlation between the marrow megakaryocytes and the serum phosphatase levels was observed.

Eosinophils were not increased: the maximum was 5.2% compared with the classic study of Segerdahl (1935) on 18 persons above 66 years of age; her findings were 3.25%. Although various methods gave slightly different results, the values obtained did not suggest an eosinophilia (Pizzolato, 1949). The reticulo-endothelial cells or macrophages, as Jaffe refers to them, ranged from 0.0% to 0.6% and are not significantly different from Segerdahl's average of 0.09%.

Jaffe also frequently observed a fatty marrow. Fat, as encountered in the upper layer of the haematocrit, was increased from 5 to 9% in seven of the 10 instances compared with the normal of from 1% to 3%. The significance of this excess fat in the presence of a normal or slightly hyperplastic marrow we are not prepared to state.

Summary

In the presence of suspicious radiological findings in bone, a study of the bone marrow should be undertaken. Sternal marrow aspirated from 10 patients with osteitis deformans showed an increase in the number of megakaryocytes and in the fat as observed in the haematocrit tube. The remainder of the marrow was essentially normal.

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