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The Eosinophilic Fibrohistiocytic Lesion of the Bone Marrow

A Mastocellular Lesion in Bone Disease

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Summary. Seven patients are described, six with severe osteoporosis and the seventh with osteogenesis imperfecta with moderate osteoporosis. The iliac bone marrow trephine biopsy specimens of all seven showed peculiar infiltrates consisting of elongated mast cells, eosinophils, plasma cells, and varying numbers of lymphocytes. Only one patient exhibited signs of allergy with urticaria pigmentosa; the other six patients had no abnormalities that could be related to a known mast cell disease. The lesions described here are the same as those described in five patients by Rywlin as "eosinophilic fibrohistiocytic lesion in the bone marrow". However, in our methacrylate sections the fibrohistiocytes are shown to be mast cells. Although a relationship with drug hypersensitivity is disputed, the presence of the mast cells and eosinophils suggests an allergic condition.

Key words: Eosinophils – Mast cells – Osteoporosis – Allergy – Bone marrow histology.

Introduction

In 1972, Rywlin et al. described a peculiar lesion in the bone marrow of five patients, showing agglomerates of eosinophils, lymphocytes, and elongated "fibrohistiocytes". The authors suggested that the lesion was associated with drug hypersensitivity, because they saw a change in or absence of the lesions in bone marrow aspirate sections prepared one month after withdrawal of the drug incriminated.

In our files of methyl-methacrylate embedded trephine biopsy specimens we found seven patients with peculiar mastocellular eosinophilic infiltrates containing plasma cells and lymphocytes, differing distinctly from the picture seen

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in mast cell reticulosis. In retrospect, these lesions correspond completely with the descriptions of Rywlin's fibrohistiocytic lesions, except for the staining properties of the elongated "fibrohistiocytes" all of which proved to be mast cells in our cases. No relationship to drug hypersensitivity could be established in our patients, but another feature was present in that all patients suffered from a severe bone disease, mainly osteoporosis.

Materials and Methods

Biopsy Specimens

Two files of undecalcified, methyl-methacrylate embedded trephine biopsies taken from the iliac bone were available for study.

In the Pathology Department of the Leiden University Hospital 1500 bone marrow investigations had been performed by the Burkhardt technique during the last 5 years. The specimens had been fixed in buffered isotonic methanol-formaldehyde (2:1), dehydrated in methanol, and embedded in methyl-methacrylate (te Velde, 1977). Sections, cut 2 micron thick, were routinely stained with Giemsa after Gallamine etching, with PAS, Gomori's reticulin, Turnbull's iron with Trevan's methyl-green-pyronin as counterstain, and Goldner's variant of Masson's trichrome for osteoid.

In some cases the naphtol-ASD-chloracetate esterase reaction was performed for myeloid and mast cells (Hennekheuser, 1972).

By far the greater part of the trephine biopsy specimens in this file had been obtained from patients with primary haematological disorders. Only about 10% of the biopsies had been performed to document a generalized bone disease, including 35 cases of osteoporosis. Because all cases with abnormal mast cell infiltrates had been indexed since the establishment of the file, we are convinced that not more than 4 patients (ns. 2,4,5 and 7, Table 1) in this file suffered from the lesions discussed here.

In the Department of Endocrinology trephine biopsy specimens from patients with metabolic bone disease only had been embedded in methyl-methacrylate, giving a total of about 1000 specimens over the last 5 years. Sections had been stained only with Goldner's trichrome for osteoid. Duplicate sections had been sent to the Pathology Department for examination of the bone marrow. If any haematological abnormality was suspected, additional marrow sections were made and stained as described above.

This screening method yielded three patients (nos. 1,3, and 6, Table 1) with the lesions discussed here. Recently, we reviewed the 140 specimens from patients with osteoporosis in this file and found no additional cases not previously identified.

Patients

The clinical features of our seven patients are summarized in Table 1. Physical examination and routine biochemical tests revealed no abnormalities except findings consistent with the bone disease. None of the patients showed haematological disturbances.

Several of the patients were also investigated for auto-immune or collagen diseases. The Coombs tests and tests for ANF, rheumatoid factors, LE cell phenomena, and cold agglutinins were negative. Serum proteins, complement, and immune electrophoresis for IgA, G, and M showed no anomalies. In one patient (no. 2) a muscle biopsy showed no vasculitis or other abnormality. Only patient 7 differed from the others in having urticaria pigmentosa and allergy, with consistent findings.

Results

Histological investigation of the trephine biopsies all showed lesions identical to those described by Rywlin et al. (1972, 1976), with some additional features.

Table 1. Relevant clinical data in the present series

Patient no.	Sex	Age	Main complaints	Biopsy at age	Drugs taken at time of biopsy	Allergy
1.	f	33	vertebral fracture in pregnancy, due to osteoporosis at age 27	28[a] 29	oestradiol 10 mg weekly oestradiol 10 mg weekly	none none
2.	f	52	hypertension; low back pain due to severe osteoporosis	51 52	calcium gluconate propanolol calcium gluconate Na-fluoride Vit D propanolol prazonine	none none
3.	m	53	ulcus ventriculi at age 48; vertebral fracture due to generalized osteoporosis at age 48	51	calcium gluconate Na-fluoride 25 mg daily	none
4.	m	57	since 33 years gastritis; vertebral fractures due to osteoporosis at age 53	57	antacids containing bismuth, bicarbonate, and magnese (Hygroton until 1 month before biopsy)	none
5.	m	62	vertebral fracture due to osteoporosis at age 54	54[b] 62	androgens calcium lactate/gluconate androgens calcium lactate/gluconate	none none
6.	f	77	severe osteoarthrosis, osteoporosis, and kyphoscoliosis since age 61	74	none	not known
7.	m	31	since age 2 multiple fractures, blue sclerae: osteogenesis imperfecta (also in his son), moderate osteoporosis; urticaria pigmentosa since age 28	30	none	penicillin dust

[a] Half of the biopsy cylinder decalcified and embedded in Paraplast
[b] Only decalcified Paraplast-embedded core biopsy specimen

Infiltrates varying from 0.1 to 2 mm diameter were present centrally in the marrow, or around arterioles, or in a cresentic form along sinuses. These infiltrates were composed of eosinophils, lymphocytes, plasma cells, and large numbers of elongated cells with round to oval, or bean-shaped nuclei (Fig. 1). These elongated cells showed a varying number of metachromatically stained granules (Figs. 3–5) which were PAS positive and which reacted positively in the esterase reaction for myeloid and mast cells which had been performed in three cases. In the lymphoid follicles some reticulum cells were present.

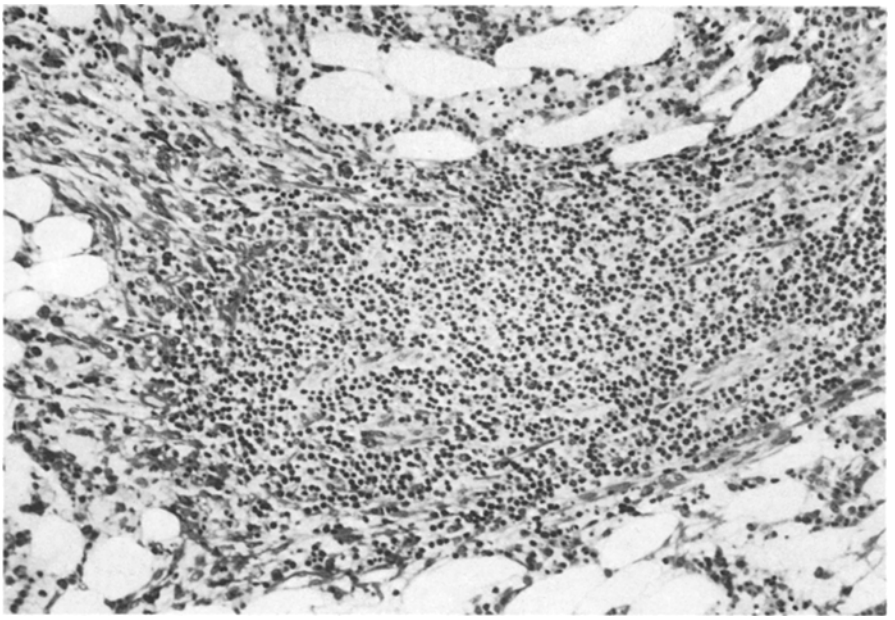

Fig. 1. Central lesion with lymphoid centre and surrounded by a rim of elongated mast cells with eosinophils and plasma cells. Patient 3, methacrylate, Gallamine-Giemsa, original magnification: $\times 100$

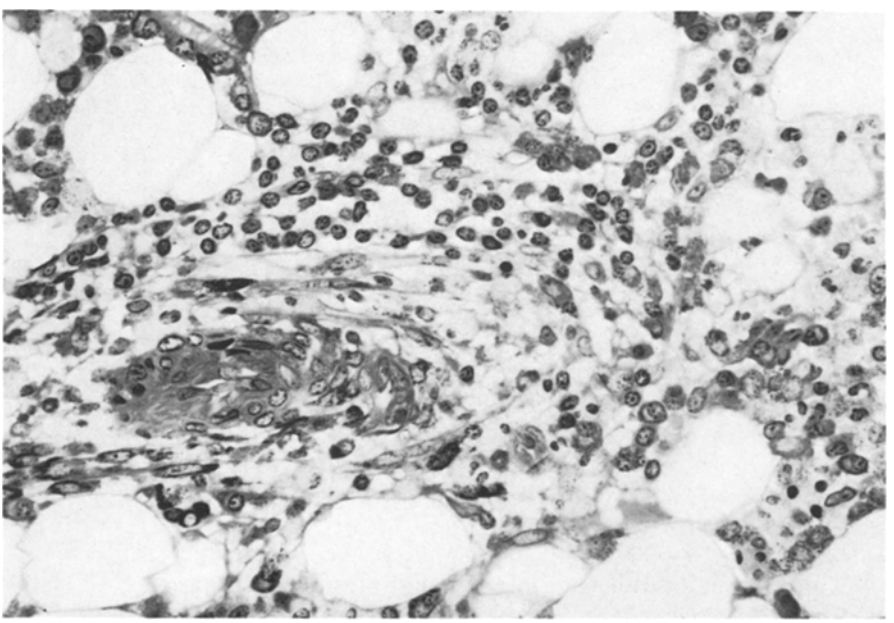

Fig. 2. Lesion around contracted arteriole. The mast cell granules are black, the eosinophil granules are very faint. Patient 2, methacrylate, Gallamine-Giemsa, original magnification: $\times 450$

Fig. 3. Perisinusoidal lesion. Sea-green-tinged serum in the sinus, bordering an area with predominently mast cells and lymphoids. Note the different staining intensity of the mast cells. Patient 4, methacrylate, Gallamine-Giemsa, original magnification: $\times 180$

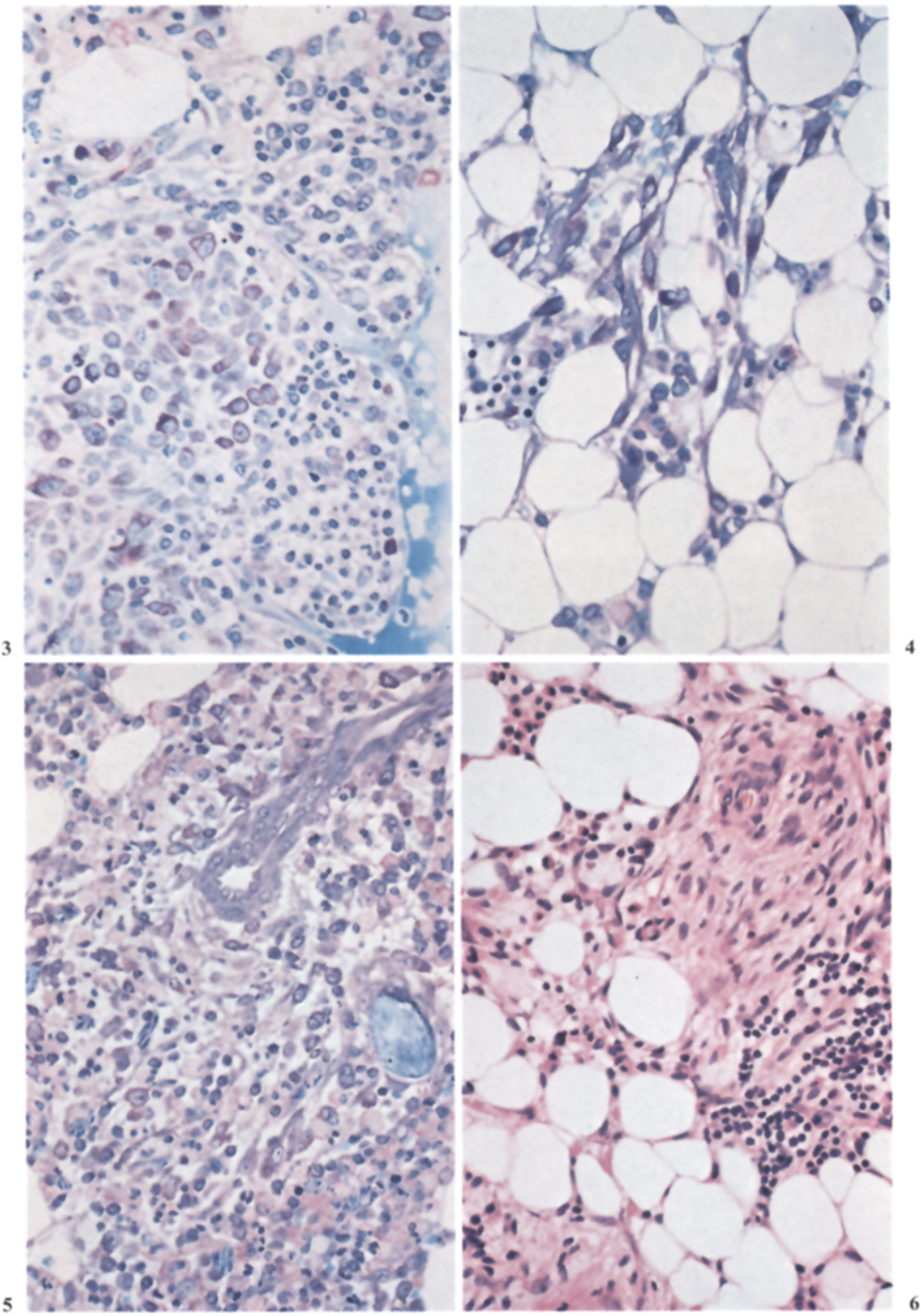

Fig. 4. Elongated mast cells in the marrow outside the lesions. Patient 5, methacrylate, Gallamine-Giemsa, original magnification: ×600

Fig. 5. Periarteriolar lesion. The eosinophils are stained light orange; the purple, metachromatic mast cells vary considerably in staining intensity. Charcot-Leyden crystals are sea-green. In the lesion on osteoporotic bone trabeculum can be seen. Patient 7, methacrylate, Gallmaine-Giemsa, original magnification: ×180

Fig. 6. Same patient as in Figure 4: H & E stained, decalcified specimen, taken 8 years earlier. Original magnification: ×180

Table 2. Histological findings (methacrylate sections)

Patient no.	1		2		3	4	5	6	7
	1st	2nd	1st	2nd					
Surface of bone and bone marrow section (mm^2)	75	15	40	65	60	50	35	50	45
Central lesion, lymphocytes centrally (nr)	1	3	1	–	3	2	–	1	2
Central lesion, lymphocytes peripherally (nr)	1	–	–	–	–	2	–	–	–
Perivascular localization (nr)	8	1	9	6	1	8	3	–	6
Perisinusoidal localization (nr)	–	–	–	1	–	3	2	2	–
Eosinophils in the lesions	+	++	+	++	++	++	+	+	++
Lymphoids in the lesions	+	+	+	+	++	++	+	+	++
Mast cells in the lesions	+	+	+	+	+	++	++	+	++
Charcot-Leyden crystals	+	–	+	++	++	–	+	–	+
Mast cells outside the lesions:									
in de marrow	++	+	+	+	–	++	++	+	+
at the endosteal surface	+	+	+	–	+	++	++	–	+

The occasional histiocytic, foamy macrophages did not contain granules. The eosinophils sometimes formed compact monomorphic infiltrates, called eosinophilic abcesses by Rywlin. In the border areas of the lesions Charcot-Leyden crystals were seen, sometimes in large quantities, among eosinophils and mast cells (Fig. 5). Rywlin distinguished between three types of lesions occurring in the same patients and composed of the same cells, but with a different localization, which he called the lymphofollicular type – with centrally or peripherally small or large lymphoid follicles –, the peri-arteriolar type, and the perisinusoidal type. This distinction could also be made in our patients in whom the perivascular type was the most frequent (Figs. 1–3 and 5, Table 2).

Mast cells were also present in large numbers outside the lesions in most sections, some being round and lodged against the bone trabeculae, others, often elongated with oval nuclei (Fig. 4), were distributed diffusely in the marrow.

The bone marrow outside the lesions also showed a variety of minor changes without any consistent pattern. In most cases a diffuse increase of plasma cells was noted, as well as an increase or decrease of the number of megakaryocytes.

The bone tissue from the iliac crest did not show signs of osteoporosis in most of the patients, despite the severe porosis in the vertebral column with vertebral fractures. Generally, an increased number of osteoblasts was found without signs of abnormal mineralization.

In one patient (no. 5) we were able to review a Paraplast-embedded, decalcified biopsy specimen taken 8 years before (Fig. 6). In the pathology report the peculiar "granuloma-like fibrotic foci" had been described as containing few mast cells. Review of these sections showed only very faint metachromatic mast cell granules, but these could be seen in almost every "fibrocyte". The same discrepancy between Paraplast- and methacrylate- embedded sections was

noted in the biopsy specimen of patient 1, half of which had been decalcified and routinely processed in Paraplast, the other half embedded in plastic. In the Paraplast sections the granules showed only very faint staining and generally only the familiar round mast cells with abundant amounts of granules were recognized easily as mast cells.

Discussion

Mast cells are still enigmatic elements in the inflammatory reaction. Little is known about their origin, fate, or regulation. After contact with IgE, they release and activate several substances as heparin, histamine, and such factors as the eosinophilic chemotactic factor of anaphylaxis, which points to a role in allergic diseases such as bronchial asthma (Austen, 1975; Wasserman, 1977).

In bone disease only incidental observations have been made on the mast cell. An increase in the number of mast cells has been reported in such metabolic bone diseases as osteoporosis (Frame, 1968; Kruse, 1973; Peart, 1975) and this was confirmed by some of our biopsy specimens from patients with a metabolic bone disease. Heparin is said to induce osteoporosis. However, in mast cell reticulosis the mast cells are found embedded in masses of atypical, even calcifying osteoid, and systemic mastocytosis is characterized predominantly by osteosclerosis (Kruse 1973; Mutter, 1963; Udoji, 1975). This has led to two theories: mast cells induce porosis by releasing heparin (Frame, 1968) or, alternatively, mast cells are present as a secondary phenomenon, opposing the porosis (Kruse, 1973). However, all current theories are based on limited and circumstantial evidence.

The lesions described here differ distinctly not only from the diffuse increase of mast cells seen in metabolic bone disease in general, but also from the monomorphic infiltrates with osteosclerosis and eosinophilia in the surrounding hyperplastic marrow seen in mast cell reticulosis. These lesions contain peculiar mixed infiltrates of mast cells, eosinophils, lymphoids, and plasma cells. Our findings confirm Rywlin's conclusion that this is a distinctive histological lesion (Rywlin, 1972 and 1976). The similarities between our mastocellular and Rywlin's fibro-histiocytic lesion are obvious: the same localization, the same relationship to eosinophils and eosinophilic abcesses (Rywlin) and with lymph follicles, and the abundance of elongated cells with nuclei measuring 12–14 by 4–6 μ and indistinct amphophilic cytoplasm in the H&E sections. The demonstration of specific mast cell granules in all of these elongated cells in our sections may be ascribed to the differences in fixation and embeddings procedures. The histotechnique used here gives excellent preservation of cellular detail (te Velde, 1977), which probably also accounts for the higher number of plasma cells seen in our cases.

Rywlin has suggested an association of these lesions with drug hypersensitivity. His arguments are, however, rather weak in that he links three commonly used drugs with these rare lesions in five patients, each of whom was taking many different drugs. The haematological improvements seen after withdrawal of the incriminated drugs are dubious (e.g. Hb values changing from 9.2 to 11, or from 13 to 15.2 g/l in 2 of the 4 patients). His strongest argument is probably the change in the lesions seen in follow-up specimens taken a month after with-

drawal of the drug. But since Rywlin's biopsy material derived from aspirates, this variation could very well be due to sampling differences. Our trephine biopsy sections showed considerable variation in the number of mast cells between infiltrates in the same section, and even in lesions with the same localization relative to the vessels. We found the same lesions in 2 patients at intervals of one year, and even in a Paraplast embedded trephine biopsy specimen taken 8 years before in a third patient (Table 1). These observations make it unlikely that the effect of withdrawal of a drug can be evaluated on the basis of a histological "change" of disappearance in follow-up aspiration specimens. Moreover, none of the drugs mentioned by Rywlin was being taken by any of our patients at the time of or shortly before biopsy.

The coincidence of the lesions with calcium therapy in our patients (3/7) is best explained by the fact that they all suffered from the same disease: in 6 of the 7 patients the biopsy had been performed to investigate osteoporosis. Rywlin's series also included a patient with osteoporosis.

This relationship with osteoporosis is even more striking because the 1500 bone marrow investigations in the Pathology Department's file cover predominantly haematological disorders, including cases of drug hypersensitivity. Less than 10% of the specimens belonged to patients with generalized metabolic bone disease and there were only 35 cases of osteoporosis, including 3 of the 4 cases in the present series (no's. 2,4, and 5, Table 1). The fourth patient (no. 7, Table 1) had been biopsied to investigate the presence of mast cells in the marrow, because he had developed urticaria pigmentosa concurrently with osteogenesis imperfecta with moderate osteoporosis. We have had no further experience with the histological picture presented by the bone marrow in urticaria pigmentosa, and the literature has been of little help in this respect. An increase in the number of mast cells has been described in urticaria pigmentosa (systemic mast cell disease), but we have found no detailed descriptions matching the lesions discussed here.

Two children with osteogenesis imperfecta in our files did not show increased numbers of mast cells in the marrow biopsy specimens.

Since mast cell disorders have been linked to hypersensitivity diseases, we searched for relevant clinical and biochemical evidence in our patients, but found none except in patient 7. As in Rywlin's cases, tests for autoimmune or collagen diseases were negative. No haematological abnormalities like those described by Rywlin were present in our patients. Interest in the mast cell is increasing, however, and knowledge concerning the role of this cell in such diseases as bronchial asthma is accumulating. A large number of components released or activated by mast cells have been described and investigated (Austen, 1975 and Wasserman, 1977)—for instance cAMP, bradykinin, and prostaglandins, which are also being studied in relation to metabolic bone disease. Several of these substances have a pronounced effect on the circulation, and osteoporosis is viewed by some as a sequel to diminished blood flow through the marrow (Burkhardt, 1973). The obviously contracted state of the arterioles in the lesions in our sections (Fig. 5), seems to suggest a vasoconstrictive process, but it is still too soon to formulate a pathophysiological theory giving the mast cell a special place in the evolution of osteoporosis. Further investigations in patients with lesions like those described here might, however, provide some clues

concerning mastocellular lesions outside the skin and the respiratory or digestive tract in man, or even define a still unknown form of osteoporosis, for which a specific drug therapy might be indicated. For the moment, the eosinophilic mastocellular lesion of the bone marrow can only be said to form a distinctive histopathological entity associated with bone disease, especially osteoporosis.

Conclusions

The seven patients described here showed the same bone marrow lesion as that described by Rywlin et al. in five patients, which he called the eosinophilic fibro-histiocytic lesion and thought was probably related to drug hypersensitivity. The fibro-histiocytes are, however, mast cells, whose granules stain much less intensely in Paraplast-embedded specimens than in methacrylate-embedded specimens. A relationship of this lesion with drug hypersensitivity is less probable, but the coincidence with osteoporosis is striking.

Since the pathophysiological role of these lesions in osteoporosis is not yet clear, the eosinophilic mastocellular lesion in bone disease can only be called a distinctive histopathological entity whose significance is not known.

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