

## MULTIPLE MYELOMA WITH BONE MARROW CRYSTAL DEPOSITION AND MARROW FIBROSIS

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Multiple myeloma (MM) is a malignant neoplasm usually involving plasma cells of the bone marrow. Through their proliferative activity and secretion of osteoclast-stimulating cytokines, they cause generalized osteopenia and erosions of skeletal bone that may eventually lead to pathological fractures, changes that may be detected by x-ray imaging techniques. Infrequently, instead of causing osteopenia, MM may be associated with significant bone sclerosis or bone marrow fibrosis.

Another feature of MM is the near universal (more than 99%) tendency of the malignant plasma cells to secrete monoclonal paraproteins<sup>1</sup> consisting of a single immunoglobulin (Ig), whose composition is restricted to a single light chain (LC). Such paraproteins, or M-proteins, are overwhelmingly maintained in a fluid state in the blood, although fragments of LC may be secreted in the urine where, under certain conditions, they may crystallize and be deposited in the renal tubules, contributing to the renal failure sometimes seen in MM. Less frequently, the immunoglobulin or its fragments may crystallize in the blood and other tissues, including the bone marrow itself.<sup>2-4</sup> Moreover, MM is occasionally associated with amyloid, which may be laid down as crystalline structures in various organs. In these circumstances, the amyloid is a derivative of LC fragments of the paraprotein, and is designated AL. However, it is highly unusual for either the deposited immunoglobulin crystals or amyloid derivatives to induce, or be associated with, a fibroblastic response in the bone marrow. In this report, we present a patient whose MM was associated with bone marrow crystal deposition, together with pronounced bone marrow fibrosis.

### Case Report

The patient was a 45-year-old woman who was referred with a history of intermittent epistaxis against a background of general malaise over a period of about one year,

with weight loss, left-sided abdominal pain and occasional fever. Examination confirmed epistaxis for which her nose had been recently packed. She was dehydrated and had a moderate hepatomegaly, but no manifest splenomegaly.

The hemoglobin was 56 g/L (normal 118-148 g/L), WBC  $5.1 \times 10^9/L$  ( $4-11 \times 10^9/L$ ), with 70% neutrophils, 12% bands, 10% monocytes, 4% lymphocytes and 4% atypical lymphocytes. The platelet count was  $25 \times 10^9/L$  ( $150-430 \times 10^9/L$ ). Blood urea nitrogen was 18.2 mmol/L (2-6 mmol/L), creatinine 159  $\mu\text{mol/L}$  (60-115  $\mu\text{mol/L}$ ), urate 469  $\mu\text{mol/L}$  (210-475  $\mu\text{mol/L}$ ), and serum calcium 2.7 mmol/L (2.10-2.60 mmol/L). Total protein was 86 g/L (65-81 g/L) and albumin 30 g/L (36-48 g/L). There was a monoclonal IgG kappa (k) of 12.1 g/L (6.9-16.2 g/L). Serum IgA was 0.76 g/L (0.5-5.8 g/L) and IgM was less than 0.2 g/L (0.6-2.6 g/L). The urine was negative for Bence Jones protein. Serum relative viscosity was 2.7 (1.4-1.8). A bone marrow aspiration biopsy produced a scanty yield, but was sufficient to demonstrate a scattering of malignant plasma cells. A skeletal survey showed diffuse osteolytic lesions with osteopenia. A bone scan showed no significant abnormalities.

The patient was rehydrated with intravenous fluids and given a single cycle of "M2," multiple chemotherapy

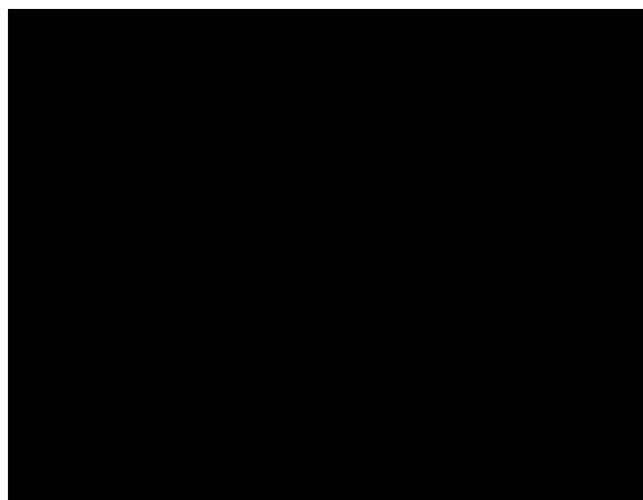


FIGURE 1. Cluster of malignant plasma cells (PC) together with crystals lying both extracellularly (arrow heads) and within histiocytes (arrows). (Wright-Giemsa stain, 400x).

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consisting of vincristine, cyclophosphamide, BCNU, melphalan and prednisone. She also received a course of plasma exchange to reduce the serum viscosity. About 4 weeks after admission, she developed staphylococcal septicemia with dissemination to the skin, left knee and both ankle joints. Despite vigorous treatment with antibiotics, the patient died on the 40th hospital day. An autopsy was not performed.

### Methods

Peripheral blood counts were performed on an automated cell counter (Coulter STKR). Peripheral blood smears were stained by Wright's stain and bone marrow smears by Wright-Giemsa stain. Histologic preparations of bone marrow trephine biopsies were fixed in B-5 fixative, then decalcified in diluted hydrochloric acid, and then washed and embedded in paraffin. Sections were cut at 2-3 microns and stained by a number of stains, including hematoxylin and eosin (H&E), Giemsa, periodic acid-Schiff (PAS), methyl green-pyronine (MGP), Congo red (CR), thioflavin T (TFT), von Kossa, silver impregnation for reticulin, and by immunoperoxidase methods, using goat anti-human kappa and anti-human lambda monoclonal antibodies conjugated with peroxidase. Bone marrow was prepared for transmission electron microscopy (TEM) by fixation in 2% glutaraldehyde and post-fixed in osmium tetroxide. The tissue was embedded in epon and thin sections cut at 0.7 microns. After staining with lead citrate and uranyl acetate, the sections were examined under a Phillips 301 electron microscope.

### Results

Light microscopy (LM) of the peripheral blood confirmed thrombocytopenia, but there was no evidence of leuko-erythroblastic changes. The bone marrow aspirate sample was scanty, and contained numerous scattered plasma cells with polymorphous plates and occasional needles of crystalline material in a background stained greyish-blue (Figure 1). In histologic sections, there was considerable fibroblastic proliferation, with fibroblasts intermingled with collagen fibers, histiocytes, plasma cells and crystals of various shapes and sizes (Figure 2). There was also a moderate increase in reticulin content. The plasma cells were monoclonal, as demonstrated by restriction of staining for kappa light chains.

#### *Light Microscopic Morphology of Crystals*

The crystals had numerous shapes and forms that were replicated in both smears and histologic sections. The most common form was rectangular, but square and needle forms were also commonly seen. A particularly interesting form was a square-shaped crystal, staining basophilic in smears and eosinophilic in H&E, bisected by an unstained stripe (Figure 3). Other shapes included irregular

polyhedra. There was also a barely discernible layering effect in the crystal structure, with an outer shell enclosing an inner core. The crystals manifested no birefringence when viewed by polarized microscopy.

#### *Fluorescence Microscopy*

The crystals, stained by a variety of methods, were examined by epi-fluorescence microscopy, the results of which are recorded in Table 1. There was moderate yellow-green fluorescence in crystals stained for H&E, von Kossa and MGP. With staining for reticulin, fluorescence microscopy gave the most brilliant effect, resulting in an apple-green color, whereas kappa, lambda and TFT staining resulted in moderate apple-green fluorescence. CR staining resulted in fluorescence, ranging from orange to yellow-green.

#### *Locations of the Crystals*

Most crystals had an extracellular location but very small elements were detected in the cytoplasm of plasma cells. In addition, many histiocytes contained large crystal deposits in bundles or stacks, sometimes resulting in distortion that conferred a "speeding rocket" profile to the affected cell (Figure 2).

#### *Ultrastructure*

TEM revealed a mixture of fibroblasts and histiocytes in a background of connective tissue composed of collagen fibers and fibrils. Crystals were demonstrable in both extracellular and intracellular locations. In the latter instance they appeared, when relatively small, to be located within the lysosomes of histiocytes. When large, they appeared unrelated to any cellular organelle, apparently lying outside the limits of any membrane structures. Bundles of collagen fibers appeared closely related to

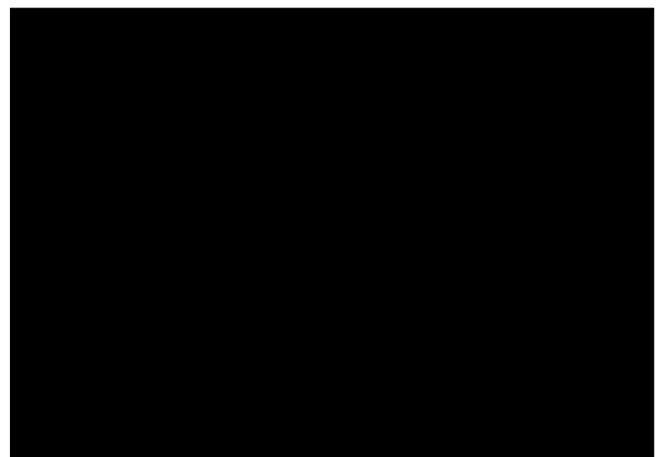


FIGURE 2. Section of trephine biopsy showing fibrotic bone marrow with increased fibroblasts and collagen. Numerous crystals, extracellular (arrow heads), as well as intracellular, are demonstrable. Histiocytes containing large crystals are distorted and manifest a "speeding rocket" appearance (arrows) (H&E, 400x).

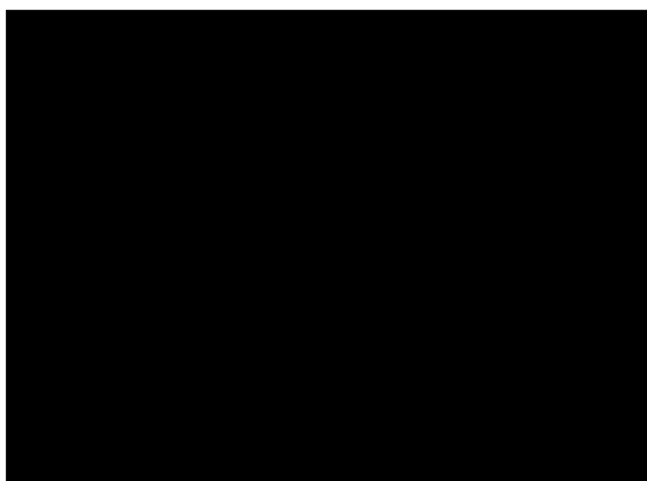


FIGURE 3. Single square-shaped crystal bisected by linear non-staining stripe (Wright-Giemsa, 400x).

TABLE 1. Staining and fluorescence characteristics of crystals.

Stain	Transmitted light	Fluorescence
Hematoxylin and eosin	Eosinophilic	Yellow-green
Giemsa	Pink/grey	None
Von-Kossa	Negative	Moderate yellow-green
Reticulin	Negative	Strong apple-green
Methyl-green pyronin	Negative	Moderate yellow-green
Kappa	Negative	Moderate apple-green
Lambda	Negative	Moderate apple-green
Congo red	Negative	Moderate yellow-orange to yellow-green <sup>†</sup>
Thioflavin-T	Negative	Moderate apple-green

<sup>†</sup>Strongest staining seen in long rectangular fibrillar crystals (see text).

intracellular crystals, but it was not clear whether these fibers were themselves intracellular. Crystals retained their varied forms, including rectangles, squares, needles and irregular polyhedra. Some forms, not at first apparent by LM, were clearly recognized by TEM. One such example was a fibrillar, elongated crystal, measuring approximately 3.9  $\mu$  in length, recognized by fluorescence in CR-stained sections. The layering effect observed in other crystals by LM was more clearly demonstrable by TEM, giving the impression that the material had been deposited in sequential waves (Figure 4). Furthermore, profiles of crystals with central linear clearings were apparent. At high magnification, crystals were mostly amorphous, but rare examples, when appropriately oriented, demonstrated an organized detailed structure similar to woven fabric (Figure 5).

### Discussion

We describe a unique patient presenting with MM and myelofibrosis together with both intracellular and extracellular BM crystal deposits. Fibroblastic myeloma is

uncommon. In 869 cases reviewed by Kyle from the Mayo Clinic, there was apparently no recorded case of fibrotic marrow,<sup>1</sup> although numerous single cases or small series have been reported by others.<sup>5-8</sup> Likewise, MM with crystal deposition is rare and apparently occurs with a frequency of about 1% in large series. Khalil et al.<sup>9</sup> encountered only one case (the present one) of crystal-associated MM among 92 cases. Crystal deposition and inclusions also occur in other types of lymphoproliferative disorders. Occasionally, the crystalline deposits have been widespread, occurring in extracellular locations, such as the glomerular capsular space, renal blood vessels, the myocardium, lungs and other organs.<sup>3,10-12</sup> Such crystals have usually consisted of immunoglobulin LC. Simultaneous occurrence of marrow fibrosis and crystal deposition is exceptional and, to our knowledge, has never been reported. Why such an association should be so rare is difficult to understand, since a fibroblastic reaction may be expected when there is tissue deposition of "foreign" bodies, such as crystals.

When bone marrow fibrosis has been reported in MM, it was not always clear whether the two conditions were consequences of each other.<sup>6</sup> Among the 11 patients described by Vandermolen et al.<sup>13</sup> in their clinico-pathological syndrome, "plasma cell dyscrasia with bone marrow fibrosis," six had MM with marked MF, but without leuko-erythroblastic blood changes or splenomegaly. The remaining five had the typical hematological and clinical changes of MF. On the other hand, Rondeau et al. reported that 9.7% of 67 patients with typical MF had a monoclonal paraprotein without overt myeloma.<sup>14</sup> Similarly, simple marrow fibrosis occurred with a frequency of 8.8% in 298 patients with MM. Among these, less than 1% had a leuko-erythroblastic blood picture.<sup>8</sup> In this series, the fibrosis was most prominent in areas infiltrated by plasma cells. Anti-MM therapy resulted in resolution of BM fibrosis in a number of instances, indicating that the fibrosis may have been secondary.<sup>6,15</sup> Another plasma cell dyscrasia syndrome, with the acronym POEMS, and associated with marrow fibrosis, has been described.<sup>16</sup> POEMS includes polyneuropathy, organomegaly, endocrinopathy, monoclonal protein and skin changes, but apart from the presence of monoclonal protein, hepatomegaly and fibrosis, none of the other facets of this syndrome were manifest in our patient.

The crystals in the present case were both intracellular and extracellular. They showed striking polymorphism with needle forms, rectangles, squares and irregular polyhedra. One peculiar form was a square-shaped crystal bisected by a non-staining linear stripe (Figure 3). The general outlines of the crystals were similar at both the LM and TEM level, the latter more clearly delineating aspects observed at LM. This was notable in the case of a layering effect, striking enough at the TEM level to suggest that the crystal material might have been deposited in waves (Figure 4). This phenomenon, to our knowledge, has not been previously reported. We also identified a long, rectan-



FIGURE 4. Layered structure of crystals of irregular shape together with other more solid crystals. Original magnification (Lead citrate & uranyl acetate, 4900x).



FIGURE 5. Detail of intracellular crystal. Note "woven fabric" appearance. TEM, original magnification (Lead citrate & uranyl acetate, 25,000x).

gular fibrillar variety at the ultrastructural level, corresponding to a similarly shaped structure easily detected by LM only by its bright fluorescence in sections stained by Congo red. An organized internal crystal structure was apparent at high magnification in a small proportion of crystals (Figure 5), apparently in those ideally orientated to the electron beam. In the majority of others, the internal crystal structure appeared featureless. The woven appearance of the crystals contrasted with other patterns noted in previous reports, which recorded linear,<sup>2,12,17</sup> cross-hatching,<sup>18,19</sup> or lattice periodicity<sup>10</sup> at high magnification.

The chemical nature of the crystals was not resolved. The staining and polarization characteristics appeared to exclude unaltered immunoglobulin LC or calcific entities (like calcium pyrophosphate), urate or cholesterol. Thioflavin T staining showed moderate apple-green fluorescence, and with Congo red, moderate orange-yellow-green, suggesting a possible AL amyloid origin. By TEM, the features simulated those reported for immunoglobulin LC.<sup>2-4,11</sup> However, AL amyloid, as demonstrated in other TEM reports,<sup>20</sup> could not be excluded.

The crystals, in some cases, were closely associated with the collagen fibers, suggesting that they may have played a part in inducing the fibrosis. However, because our patient died early in the course of therapy, it was not possible to show whether treatment might have reduced the fibrosis or crystallosis. But, however these pathological changes may have evolved under therapy, this patient stands unique among reported cases of MM.

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