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SHORT REPORTS

Multiple myeloma with bone marrow extracellular crystal deposition

Plasma cell dyscrasias are a heterogeneous group of disorders characterised by the expansion of a single clone of immunoglobulin secreting, terminally differentiated, end-stage B cells.¹ Many of their clinical and morphological features are the result of the production and accumulation of excessive amounts of monoclonal immunoglobulin. Intracellular inclusions, both intracytoplasmic (Russell bodies) and intranuclear (Dutcher bodies) are occasionally seen in bone marrow biopsy specimens from patients with plasma cell neoplasms, and less frequently in reactive states.^{2–3} Extracellular protein deposition also occurs, although less frequently. "Myeloma kidney" with tubular casts composed of a heterogeneous mixture of electron dense homogeneous material and Tamm–Horsfall protein, together with elongated crystals, some with a fibrillar or lattice substructure, is an important cause of myeloma-associated morbidity and mortality.^{4–15} The formation of extracellular crystalline structures within the cornea, skin, thyroid gland, intravascular space and bone marrow along with other sites has been described.^{5–9} However, this phenomenon is unusual and is limited to isolated reports. In our department, we see approximately 1200 bone marrow trephines per year, including about 300 cases of multiple myeloma, and they have not previously come to our notice.

Description of case

A 72-year-old man presented with back pain. A spinal x ray carried out six months later showed collapse of the first lumbar vertebra. Further investigations included haemoglobin 9.3 g/dL, creatinine 129 µmol/L, calcium 2.5 mmol/L, β₂-microglobulin 3.9 mg/L, IgG λ paraprotein 65 g/L and bone marrow aspirate showing 80% plasma cells.

The paraprotein decreased to 25 g/L after chemotherapy. Peripheral stem cell mobilisation and collection was carried out. A bone marrow trephine biopsy and aspirate were taken prior to peripheral blood stem cell transfer (PBSCT) with melphalan preconditioning. Six weeks after PBSCT, the paraprotein level was 16 g/L. Bone marrow trephine was repeated five months later.

The bone marrow trephines from before and after PBSCT and the corresponding bone marrow aspirates are the subject of this report.

The trephine biopsy specimens had been fixed in aceto-zinc formalin and decalcified in 10% formic acid, then routinely processed and paraffin-embedded. Sections (1 µm) were cut and stained with H&E, reticulin, Perls, Giemsa, toluidine blue and congo red stains. In the initial trephine, the cellularity was approximately 40%, with marked suppression of haematopoietic cell lines and replacement by sheets of plasma cells and plasmacytoid cells, which comprised approximately 80% of the marrow cells. There were numerous refractile rhomboidal and hexagonal eosinophilic crystals, predominantly in the interstitium. They were of variable length and had a diameter of 10–100 µm (fig 1A). There was not an associated giant cell reaction. Occasional intracellular inclusions, including Russell bodies, were also noted. There was no evidence of metaphase or of congo red positivity.

Immunohistochemical staining with CD79a (1:10; Dako, Cambridgeshire, UK), CD138 (clone BB4, 1:100; Serotec, Oxford, UK) and CD56 (1:100; Novocastra, Newcastle, UK) antisera was positive in the plasma cells. There was monotypic λ light chain expression. Staining with CD20 (1:200; Novocastra), EMA (1:100; Dako), cyclin D1 (1:50; Lab Vision, Newmarket, UK) and CD3 (1:50; Novocastra) was negative. The crystals showed weak, peripheral staining for λ light chain (1:10 000, Dako). Staining for κ light chain (1:10 000, Dako) was negative (fig 1B,C).

Unfortunately, tissue was not appropriately preserved for electron microscopy, which precluded ultrastructural examination.

The aspirate smears stained with May–Grunwald–Giemsa showed 23% of plasma cells, with easily identifiable extracellular crystals of similar appearance (fig 1D).

The second trephine contained 25% plasma cells with extracellular crystals, the proportion of which was similar with respect to the previous biopsy.

Discussion

The interstitial deposition of extensive extracellular material in the form of eosinophilic crystals is a highly unusual and interesting feature of this case, the precise aetiology of which is unclear. A previous description of a

Figure 1 (A) H&E stained bone marrow trephine biopsy showing neoplastic plasma cells and extracellular crystals of various sizes (×400). (B) Immunostaining for κ light chain. The neoplastic plasma cells and the crystals do not show any staining (×400). (C) Immunostaining for λ light chain. The neoplastic plasma cells show strong cytoplasmic staining and the crystals show peripheral staining of lesser intensity (×400). (D) Bone marrow aspirate stained with May–Grunwald–Giemsa showing neoplastic plasma cells and extracellular crystals of various sizes (×200).

case of monoclonal gammopathy of undetermined significance suggested that the crystalline appearance could be artefactual, due to precipitation during tissue fixation and processing.¹⁰ The observation that these structures were found in both the bone marrow aspirate and the trephine does not support this hypothesis, despite the lack of tissue reaction. Although immunohistochemistry for λ light chain is weak, these crystals are most likely to be composed of precipitated light chains. Keller *et al* have previously noted absence of staining for light chains in a case with renal crystalloid deposits.¹⁷ They have attributed this to conformational change of the protein with masking of epitopes during the process of crystallisation.

Colourless, refractile oxalate crystal deposition in the bone marrow interstitium occurs in oxalosis.¹⁶ Macrophages laden with cholesterol and cystine crystals may be identified in hyperlipidaemic states and in cystinosis respectively.¹⁶ All of these appear different to the eosinophilic crystals described in the current case; none has an association with myeloma.

Animal experiments on mice receiving intraperitoneal injections of myeloma-associated monoclonal protein have shown tubular casts, basement membrane precipitates or crystals in mice kidneys, histology comparable to that seen in patients from whom the protein was derived.⁴ Molecular analysis of the light chain sequences in patients with crystal storing histiocytosis and Fanconi's syndrome secondary to light chain gammopathy have shown unusual amino acid substitutions, altering light chain hydrophobicity.^{11,12} These data indicate that the physicochemical properties of the monoclonal protein determine its tendency to crystallise, in addition to the local concentration of the protein. Other local factors such as pH, temperature and concentration of other solutes may determine the precise location and pattern of deposition in each individual case. Of note, the potential of crystallisation does not appear to depend on the serum concentration of paraprotein, as seen in the current case.

Unfortunately we have been unable to examine the trephine biopsy taken prior to the institution of chemotherapy. The proportion of crystals did not change following PBSCT, which suggests that therapy has not exerted a significant effect. Mullen and Chahvardjian have previously considered that this may be a therapy-related phenomenon without proposing a potential mechanism.¹³ Crystals disappeared post-therapy in cases of cutaneous crystal deposition due to multiple myeloma and in cryoglobulinaemia.^{6,14}

The chief importance of these extracellular crystals is their morphological pointer towards the diagnosis of a clonal plasma cell proliferation. Renal extracellular crystal deposition has been associated with rapidly progressive disease¹⁸; whether these deposits in the bone marrow also herald clinically significant extracellular immunoglobulin deposition is as yet unknown.

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HDL-cholesterol and triglycerides: an overlooked issue?

Several prospective clinical trials and epidemiological studies have shown that a low concentration of high-density lipoprotein-cholesterol (HDL-C) is an independent risk factor for coronary heart disease (CHD).^{1,2} It is estimated that for every 0.0259 mmol/l decrease in HDL-C, the relative risk of CHD events increases by 2–3%.³ Accordingly, HDL-C determinations are included in CHD prevention programmes.⁴ Reliable and easy to perform HDL-C assays are therefore required for routine laboratory practice.

There is often an inverse relationship between HDL-C and triglyceride concentrations. Accordingly, specimens with a low HDL-C often have a raised triglyceride concentration. HDL-C assay manufacturers often recommend dilution when the triglyceride is above a particular concentration. We decided to evaluate our routine HDL-C method to assess what triglyceride cut-off we should routinely utilise before specimen dilution.

HDL-C concentrations were determined using an anti human- β -lipoprotein antibody that binds to non-HDL lipoproteins and allows the quantification of HDL-C by the presence of a cholesterol esterase and cholesterol oxidase/peroxidase enzyme chromogen system (Olympus Life & Material Science Europa GmbH (Irish Branch)) on an Olympus AU600 analyser. Triglyceride was determined with a glycerolphosphate oxidase phenol-aminophenazone method (Olympus Life & Material Science Europa) on an Olympus AU600 analyser. Both assays were performed according to the manufacturer's protocol. The manufacturer recommends that specimens are diluted with saline (no dilution factor stated) when the triglyceride concentration is >11.3 mmol/l. The setting of such a cut-off implies that such procedure is acceptable in routine clinical practice—but our concern related to the stated value of 11.3 mmol/l. Serum samples from 35 fasted subjects giving a triglyceride concentration range from 0.47 mmol/l to 18.60 mmol/l (8, 7, 10 and 10 specimens having triglyceride concentrations of <2.5, 2.50–5.00, 5.01–11.30 and >11.30 mmol/l respectively) were obtained. Each specimen had HDL-C measured using undiluted (range 0.51–2.57 mmol/l) and diluted serum. Specimens were manually diluted 1:3 with normal saline and the results were multiplied by 3. The percentage difference [(Neat – Diluted) $\times$ 100/Neat of Neat and Diluted] was assessed for each specimen. HDL-C percentage difference plots were plotted according to specimen number (fig 1A) and triglyceride concentration (fig 1B). The National Cholesterol Education Programme (NCEP) and European objective total error (TE) criteria for HDL-C of 8.9%⁵ and 11.1%⁶ respectively were included in these plots.

The plotting of the difference between the neat and the diluted specimens (fig 1B) suggests a proportional systematic error in HDL-C concentrations with increasing triglyceride concentrations, although the specimen with the triglyceride concentration of 18.6 mmol/l seems to be an outlier. Our data confirmed that the vast majority of specimens for HDL-C analysis with triglyceride concentrations >11.3 mmol/l require dilution according to both the NCEP and the European objective TE criteria (table 1). However, 28% and 24% of the 25 specimens with triglyceride concentrations <11.3 mmol/l had differences greater than the NCEP and the European objective