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

Figure 1 (A) Cumulative high-density lipoprotein-cholesterol (HDL-C) percentage difference plot shows that of 35 specimens investigated, 7 had a lower 1:3 dilution compared to the neat as per the National Cholesterol Education Programme (NCEP) total error requirements; 7 had a lower 1:3 dilution compared to the neat and 6 specimens had a higher 1:3 dilution compared to the neat as per the European total error requirements. (B) The HDL-C percentage difference plot with [triglycerides] confirms that there is a significant difference as judged by the NCEP and European total error requirements when [triglyceride] >11.3 mmol/l. However, approximately 1 out of every 4 specimens with [triglyceride] <11.3 mmol/l failed the objective quality requirements.

TE criteria respectively. In particular, 25% of the small number of specimens investigated (ie, 2 of 8) with a triglyceride concentration <2.5 mmol/l had TE greater than the criteria set down. All the undiluted specimens with triglyceride concentrations <11.3 mmol/l and significantly different HDL-C concentrations on dilution with respect to the TE limits had lower values on dilution. More specifically, both specimens with triglycerides <2.5 mmol/l had differences greater than 20%.

Knowledge of the HDL-C concentration plays an important role in CHD risk estimation. Minimising false negatives is important in screening, and thus the TE criteria must be adhered to. As clinicians treat individuals, it is important that the TE criteria apply to the vast majority of patients. This is especially the case for those patients who are at increased risk of

developing CHD—those with chronic kidney disease, diabetes mellitus, insulin resistance and abdominal obesity. In view of the heterogeneous nature of high density lipoproteins, it would not be unexpected if the specimen with the triglyceride of 18.6 mmol/l came from one of these patient groups. Not only may differences result in the underestimation of individual cardiovascular risk; the degree of the underestimation may be very significant as every 0.0259 mmol/l increase in HDL-C is associated with a decrease in the relative risk for CHD events of 2–3%.³ Of the specimens with triglyceride concentration <2.5 mmol/l, two had differences greater than 20%, with the respective absolute differences being 0.46 mmol/l and 0.63 mmol/l. Thus, the use of neat specimens in those two examples may either be associated with underestimation of

their relative risk for CHD events of the order of 36–54% and 48–72% respectively, or the use of diluted specimens overestimates the relative risk by a similar amount. Random non-fasting specimens are advocated for screening purposes in the first instance in the United Kingdom.⁴ Accordingly, such random specimens are likely to have higher triglyceride concentrations compared to fasting and may be more prone to a negative HDL-C bias.

It may be argued that pre-dilution of the specimens invalidates the results. However, this procedure is in line with the European Union In Vitro Diagnostics Directive and CE (Conformité Européenne) marking as per the manufacturers' instructions when the triglyceride concentration is >11.3 mmol/l. Furthermore, the specimen is diluted in any case with reagents in the reaction cuvette. The basis for the cut-off point of 11.3 mmol/l is unclear. As all diluted sera had HDL-C concentrations well above the assay limit of detection of 0.002 mmol/l provided by Olympus, imprecision is unlikely to be a major contributory factor to our results.

While confirming that dilution is essential when the triglyceride concentration is >11.3 mmol/l measured by our HDL-C method, our small study also raises the possibility that in specimens with triglyceride concentrations <2.5 mmol/l there may be a significant difference between neat and diluted serum specimens for some patients. This raises questions about the robustness of HDL-C for CHD estimation in a significant minority of patients. We believe that further investigation is required to confirm these findings, as the numbers in our study are small using all currently employed HDL-C methodologies.

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Table 1 Percentage of specimens that were outside the National Cholesterol Education Programme (NCEP) and European total error (TE) limits according to triglyceride concentration

mmol/l	No of specimens	Percentage outside NCEP TE limit	Percentage outside European TE limit
<2.5	8	25	25
2.5–5.0	7	29	14
5.1–11.3	10	30	30
>11.3	10	80	70
Total	35	46	40