Multiple Myeloma and Hypercalcemia?

Editors: Jack H. Ladenson and Jay M. McDonald
Presenter: James Aguanno
Discussants: James Aguanno, Marshall Goren, and Jack H. Ladenson

We present a case of multiple myeloma with high values for total calcium. The case discussion focuses on the role of the laboratory in diagnosis and monitoring of multiple myeloma and on the causes of the associated hypercalcemia. We present evidence for calcium binding to the paraprotein as a cause for the "hypercalcemia" in this case and we document a difference in the results for total calcium among various methods when this patient's serum was analyzed.

Presentation of the Case

A 59-year-old black man was admitted to Barnes Hospital for evaluation, having shown a total calcium (CaT) value of 3.60 mmol/L (normal, 2.25–2.58), total protein of 124 g/L (normal, 65–85), and hemoglobin of 112 g/L (normal, 140–180) at the time of a routine checkup just before admission.

His weight decreased by 5.4 kg during the month before admission, which he attributed to dieting at his physician's suggestion. He had smoked 40 cigarettes a day for at least the last 20 years. He had experienced increasing weakness during the last year with some shortness of breath upon exertion and a constant dull pain in the right arm during the last six to eight months. A recent infection of the upper respiratory tract was treated with ampicillin. He had had malaria at age 14, and three episodes of "thyroiditis" during the last five years, which were treated with drugs.

The patient was 180 cm tall and weighed 94.5 kg. His blood pressure was 130/90 mmHg, his pulse rate 72/min and regular, and his temperature 36.8 °C. The only aspect of the physical examination that was remarkable was a large, multinodular, firm thyroid gland.

The radiological examination of the chest at the time of admission revealed osteopenia. Roentgenographic films of the skull showed multiple discrete lytic ("punched out") lesions, those of the spine showed diffuse osteopenia, but those of the right arm showed nothing abnormal.

Abnormal laboratory test results at admission included a creatinine value of 22 mg/L (normal, 8–13), CaT of 2.95 mmol/L, serum total protein of 120 g/L, serum albumin of 27 g/L (normal, 35–48), 1+ proteinuria, and a blood-hemoglobin concentration of 103 g/L. Subsequent studies showed that 2 to 4 g of protein was being excreted per 24 h and immunoglobulin light chains (Bence Jones protein) were present in the urine. Protein electrophoresis on cellulose acetate revealed the presence of a paraprotein in both serum and urine, which migrated between the beta- and gamma-globulin regions. Immunoelectrophoresis showed this to be IgG with lambda light chains. The erythrocyte sedimentation rate was 142 mm/h (normal, 0–9). The creatinine clearance was 69, 36, and 55 mL/min, based on three successive 24-h urine collections.

Examination of the bone marrow showed 40% large plasma cells with abnormal nuclei and prominent nucleoli, and relative erythroid hypoplasia. A diagnosis of multiple myeloma was made. The patient was treated with isotonic saline and furosemide for his hypercalcemia and with a four-day course of melphalan (20 mg orally) and prednisone (120 mg orally) for the multiple myeloma. He was discharged after six days, his last CaT being 2.73 mmol/L. During this hospital admission the CaT value varied between 2.73 and 3.75 mmol/L.

The patient was readmitted two weeks later because of continued above-normal CaT. At admission his CaT was 3.45 mmol/L, creatinine 17 mg/L, total protein 120 g/L, albumin 29 g/L, thyroxine 87.5 mmol/L (normal, 64–141.5), and hemoglobin 85 g/L. Urinalysis was unremarkable. The hypercalcemia was aggressively treated with large amounts of fluids, high Na intake (18 g/day), furosemide, and prednisone. The treatment resulted in a diuresis of 6–15 L per day during the 14 days of hospitalization. Despite this therapy, the CaT values fluctuated between 2.33 and 3.65 mmol/L (Figure 1). Because of these fluctuating CaT values, an oral clinical chemistry consultation was obtained on the 7th hospital day, during which it was noted that the high CaT values were obtained by either continuous-flow analysis (SMA 12/60; Technicon Corp., Tarrytown, NY 10491) or by atomic absorption spectrophotometry, whereas the lower values were consistently obtained with the aca (DuPont). These data suggested possible methodological problems, which were investigated further and are

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Clinical Chemistry Case Conferences of the Division of Laboratory Medicine, Departments of Pathology and Medicine, Washington University School of Medicine, Barnes Hospital, St. Louis, MO 63110. Correspondence and reprint requests should be addressed to the conference editors.

1 Nonstandard abbreviations used: CaT, total calcium; CaF, free calcium; AAS, atomic absorption spectroscopy; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetate.
presented in the Discussion. A CaT value obtained on the 10th hospital day was slightly below the normal range (1.08 mmol/L; normal, 1.13–1.25). Nevertheless, the “hypercalcemia” was further treated with mithramycin (1.25 mg intravenously during 6 h) on day 11.

The patient was discharged 16 days after admission, still being treated with prednisone, high salt intake, and furosemide; he received a second course of melphalan five weeks later as an outpatient. He was readmitted to the hospital six weeks later with streptococcal pneumonia and bacteremia, which responded to therapy with antibiotics. During this admission the CaT values generally were above normal, ranging from 2.78 to 3.03 mmol/L; they were normal on four occasions, ranging from 1.14–1.24 mmol/L. During this admission a written clinical chemistry consultation explained the methodological problems with CaT measurements and the significance of the CaT values (see Discussion). The patient was discharged in good condition 12 days after admission, his calcium status to be monitored with CaT measurements.

Discussion

Multiple myeloma (also called plasmacytic myeloma, plasma cell myeloma, or myelomatosis) is a malignant disease of plasma cells, characterized by an uncontrolled proliferation of immature atypical plasma cells. Many soft tumors of various size are observed throughout the bone marrow and sometimes in other tissues, thus the term multiple myeloma. It is the most common tumor of bone, the incidence rate being 1.6 to 3.4 per 100 000 population (1). The incidence of the disease increases with age, markedly so after 60 years.

Multiple myeloma is diagnosed from a combination of cytotologic, laboratory, and radiological criteria. The criteria recommended by the National Cancer Institute (2) are shown in Table 1. The patient presented here clearly fulfilled these criteria, having demonstrable paraprotein in both serum and urine, marrow plasmacytosis (40%), and multiple osteolytic lesions.

In the more common benign condition (benign monoclonal gammopathy), found in 1 to 3% of the population, paraprotein usually is present in serum but none of the other manifestations of multiple myeloma are seen. Patients with this condition should be examined periodically because 7.4% reportedly develop multiple myeloma within five years (3).

The various methods used to detect and characterize suspected paraproteins have been reviewed (4). Immunoelectrophoresis of serum or urine, or both, is of the most value in patients with suspected multiple myeloma. This technique can confirm the presence of a paraprotein suspected because of a high value for total protein or the pattern seen on cellulose acetate electrophoresis, or both. Moreover, it can detect the presence of paraproteins that are not identified by cellulose acetate electrophoresis. In a series of 869 patients with multiple myeloma (1, 5) a normal pattern for serum protein electrophoresis was found in 15% and a polyclonal peak in 1% (generally ascribable to IgA monoclonal proteins, which tend to polymerize). In 8% of these patients a monoclonal protein in the serum was demonstrated by immunoelectrophoresis, and in 81% electrophoresis or immunoelectrophoresis of urine demonstrated a paraprotein. In only three of the 869 patients was a monoclonal protein not detected by serum or urine studies. This low incidence of “non-secretory” myeloma has also been found by others (2), but monoclonal protein has been demonstrated within the plasma cell in some of these cases.

Immunoglobulin light chains are found in the urine of 80% of patients with multiple myeloma. These light chains are responsible for the phenomenon investigated by Bence Jones in the 19th century: a protein is present that reversibly precipitates at 56 °C but redissolves at 100 °C. When used clinically, this heat test can have both false positives and false negatives, and it is recommended (1, 4) that immunoelectrophoresis of a concentrated urine sample be used to detect immunoglobulin light chains.

Immunoelectrophoresis is also of value in determining the immunoglobulin type of a suspected paraprotein. The demonstration of IgM is particularly important because it can indicate Waldenström’s macroglobulinemia, which is generally considered to be an entity distinct from multiple myeloma (6). Patients with multiple myeloma are often classified as to immunoglobulin heavy chains (IgG, IgA, IgD, IgE) or light chains (kappa or lambda). Group differences in survival time and complications have been demonstrated for the various classifications by some workers (7, 8), but such classification

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**Table 1. Diagnostic Criteria for Multiple Myeloma**

(2)

I. Patients with a paraprotein in serum or urine plus one or more of A, B, C, D.

A. Marrow plasmacytosis (more than 5% plasma cells).

Patients satisfying these criteria who also have rheumatoid arthritis, a chronic infection, lymphoma, or leukemia should be studied separately unless other features make the diagnosis of myeloma clear.

B. Biopsy tissue demonstrating replacement and distortion of normal tissue by plasma cells.

C. More than 500 plasma cells per mm³ in the blood.

D. Osteolytic lesions unexplained by other causes.

II. Patients without a paraprotein in serum or urine require A or B plus one or more of C, D.*

A. Osteolytic lesions unexplained by other causes.

B. Palpable tumors

C. Marrow plasmacytosis of more than 20% when 200 cells are counted in aspirates or biopsy specimens from two sites, in the absence of another disease capable of causing a reactive plasmacytosis.

D. Tissue biopsy specimens (bone, lymph node, nasopharynx) demonstrating replacement and distortion of normal tissue by plasma cells.

* Fewer than 1% of patients with multiple myeloma have no demonstrable paraprotein.
has no value in predicting the course of the disease or response to therapy in a given patient (1, 7). Several sequelae that occur in patients with multiple myeloma can often be monitored with laboratory tests. Hematopoietic suppression, the mechanism of which is unestablished, leads to a normocytic, normochromic anemia in virtually all patients. The hemoglobin value is initially \(<120\) g/L in 62% of patients (1, 5), as it was in our patient. Leukopenias and thrombocytopenias occur less frequently. Leukocyte counts of \(<4000/mm^3\) are present in 18% of patients when they are first seen, and platelet counts \(<100\ 000/mm^3\) in 12%. In our patient the leukocyte count was below normal only during the third hospital admission (after therapy), and the platelet count was never below normal.

Renal insufficiency is eventually found in 50% of patients with multiple myeloma (9). Proteinuria is present in 88% of patients, and 26–32% have initial serum creatinine values above 20 mg/L. The first-measured values for both urinary protein and serum creatinine were above normal in our patient. The renal failure may be caused by hypercalcemia, obstruction of the tubules by large laminated casts ("myeloma kidney"), amyloid deposition, hyperuricemia (not found in our patient, but present in 39% of males and 61% of females (1)), or pyelonephritis, or it may be secondary to administration of nephrotoxic drugs. However, in some patients the exact etiology is obscure (1, 9).

Electrolyte values may be abnormal. Low sodium values without any symptoms of hyponatremia have been reported. This pseudohyponatremia is thought to be due to a decrease in plasma water caused by the increased total protein concentration (10, 11). The decrease in plasma water leads to a proportional decrease in substances contained in the plasma water when measured by using a dilution of the sample, regardless of whether the detector is a flame photometer or ion-sensitive electrode (12). Presumably, methods based on direct (without dilution) potentiometry will not have this error (12, 13). In our patient, sodium values determined by flame photometry (SMA 6/60) ranged from 130 to 136 mmol/L when the values for total protein ranged from 100 to 120 g/L. A subnormal anion gap in patients with multiple myeloma has also been reported (14, 15) and attributed to the cationic nature of the paraproteins (14). In our patient slightly low anion gaps were observed (2–13 mmol/L).

Hypercalcemia is found initially (as in our patient) in 22–30% of patients with multiple myeloma (5, 8), the exact cause of which is not known. Factors postulated to be responsible for the hypercalcemia associated with malignancies, in general, are direct pressure effects of the tumors and the release, either locally or systemically, of chemical or hormonal substances by the tumor (16). Various chemical or hormonal factors have been implicated in the hypercalcemia associated with malignancy: parathyroid, vitamin-D-like sterols, prostaglandins, vasoactive intestinal peptide, and osteoclast activating factor (16–19).

In patients with multiple myeloma the role of osteoclast activating factor has received much attention. It increases osteoclast number and activity and causes ultrastructural changes consistent with enhanced bone resorption. This substance is produced by normal leukocytes when they are activated by phytohemagglutinin or an antigen to which the lymphocytes have been previously exposed. Cultured cells taken from bone-marrow aspirates of patients with multiple myeloma produce a factor with chemical and biological characteristics similar to osteoclast activating factor, as do cultured tumor cells from various lymphomas (17, 19). It has been recently shown (20) that production of this factor by bone-marrow cells from patients with multiple myeloma is related to the extent of bone disease and tumor mass but not to serum calcium concentration. More work in this area is required to elucidate the exact mechanisms responsible for the hypercalcemia associated with multiple myeloma.

Another important and relatively unappreciated cause for a high \(Ca\)= in patients with multiple myeloma is the possibility of abnormally high calcium binding to the paraprotein. Patients with multiple myeloma and supernormal total calcium but normal free or diffusible calcium have been described (18, 21–26), and the myeloma protein from one such patient was clearly shown to bind more calcium than did normal immunoglobulins (24).

We measured free calcium (\(Ca^+\)) in our patient during the second and third admissions, after the laboratory was consulted about the fluctuating total calcium values. The results (Table 2) indicate that the patient had a normal \(Ca\)= value, which was stable with time and an increased total calcium value, which appeared to parallel the amount of total protein. Although the data in Table 2 are best explained by a large binding of calcium by the myeloma protein, the fluctuating calcium values were still puzzling. Further investigation suggested a method–dependent phenomenon, because the calcium values measured with the DuPont \(aca\) (used for "stat" requests) tended to be lower than those obtained by other methods (Figure 1). This was systematically evaluated (Table 3). The values for total calcium were similar by the three non-\(aca\) methods but were distinctly different for assays done with the \(aca\). Although the chemical basis for measuring calcium differs among the non-\(aca\) methods, this was not a likely source of the discrepancies because in both the \(aca\) and the SMA 12/60 cresolphthalein complexone is used to detect calcium.

Thus the most likely explanation for the method discrepancies is the difference in the way calcium is released from the proteins. In the SMA 12/60, acid is added to release the calcium, which is then dialyzed out and reacted with cresolphthalein complexone under the required basic conditions. In the \(aca\) the calcium is reacted directly with the cresolphthalein complexone in basic solution. We surmise that the acid release and dialysis step released the calcium from the protein in the serum of our patient, but not all the calcium was

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**Table 2. Some Clinical Laboratory Values for the Patient**

<table>
<thead>
<tr>
<th>Admission</th>
<th>(Ca^+), mmol/L</th>
<th>Albumin, g/L</th>
<th>(\text{Total protein, g/}\text{L})</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd</td>
<td>Orion SS-20</td>
<td>Nova-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.13</td>
<td>1.16</td>
<td>3.30</td>
<td>107</td>
<td>7.40</td>
</tr>
<tr>
<td>3rd</td>
<td>1.14</td>
<td>2.78</td>
<td>74</td>
<td>7.40</td>
</tr>
</tbody>
</table>

\(^a\) Values given are the median of triplicate measurements.

\(^b\) \(Ca^+\) was measured by atomic absorption spectrophotometry (reference range, 2.25–2.58 mmol/L), \(pH\) at 37 °C by microelectrode (Radiometer E5021), total protein by the biuret reaction (DuPont aca), and albumin by reaction with brom cresol green (DuPont aca).

\(^c\) The sample from the 2nd admission was measured with both the instruments shown. The electrodes in use on the Orion SS-20 at this time were of the older design, which reportedly give values lower by 0.045 mmol/L than do the currently available electrodes (27). We also find this to be true, and also that the Orion SS-20 with the current electrodes and the Nova-2 give similar results for serum or whole-blood samples. The reference range for the Nova-2 and current Orion SS-20 is 1.12 to 1.25 mmol/L.
available for reaction when measured by aca. Presumably the problem of low values for total calcium obtained in our patient with the aca would also occur with other methods based on use of cresolphthalein complexone directly, but we did not specifically test this idea. The EGTA titration method also involves basic conditions, but the EGTA used to titrate the sample is presumably a stronger chelating agent than cresolphthalein complexone and can remove all the calcium from the paraprotein. Before this patient, our experience in comparing calcium results from the aca and AAS had been excellent, as reported by others (29).

To see if the artifactually low values for total calcium would be found in other patients with a calcium-binding paraprotein, we obtained sera from another such patient (28). Sera from this patient gave similar values (Table 3) for total calcium, whether measured with AAS or the aca. This indicates that such methodological discrepancies for CaT cannot be expected for every patient with abnormally high calcium binding to a paraprotein. This phenomenon is unpredictable, but the laboratory must be alert to its existence.

Of more serious consequence is the high total calcium and normal free calcium concentrations found in patients such as the one discussed here. High calcium binding to the paraprotein in patients with multiple myeloma is presumed to be rare; normal CaF with above-normal CaT was found in only one of 15 patients in one study (24) and in one of 61 sera screened in another study (28). We have not systematically examined all patients with multiple myeloma and hypercalcemia, but in a small study of 21 consecutive outpatients with multiple myeloma we found that 18 had normal values for both CaF and CaT, two had increased values for both, and one had a normal CaF and increased CaT. This last patient had IgG kappa myeloma; the patient presented here had IgG lambda. All but one of the patients reported with this phenomenon have had an IgG paraprotein, although the light-chain type has varied, and in one case IgA paraprotein was present (23). Such patients can have typical osteolytic lesions, as was the case in our patient.

A calcium-binding paraprotein as a cause of hypercalcemia (increased CaT) can often be unappreciated, as was the case with our patient. Our oral explanation of the calcium data during the second admission proved insufficient and a chart note was written on the third admission, which resulted in a series of CaT measurements, which we believe to be the proper test for monitoring such patients (presumably a measure of ultrafiltrable calcium would also suffice). This and other similar experiences indicate to us that the laboratory should write explanatory notes when it performs special studies on patients, to avoid confusion in interpretation.

The practical consequences of the calcium-binding protein in this case were a prolonged hospitalization to treat an asymptomatic hypercalcemia and the use of a potentially toxic drug, mithramycin, because of laboratory values that we believe did not indicate such treatment. The use of this drug could have been responsible for the patient’s third admission, for pneumonia, but this is difficult to prove. Infections of the lung and kidney are common complications of multiple myeloma because antibody response is impaired (1), so that the infection certainly could have been the result of the disease or of therapy with melphalan and prednisone. On the other hand, mithramycin, which is believed to act by inhibiting DNA-directed RNA synthesis, can have many side effects, although they are more commonly observed when it is given in higher dosages than were used here (16, 18). Because bone-marrow depression, particularly thrombocytopenia and leukopenia, have been observed (31) it is not inconceivable that the administration of this drug contributed to the development of the infection which caused the third hospitalization.

Conclusions

Electrophoretic measurement of proteins has a major role in the diagnosis of multiple myeloma. Renal function, uric acid, blood counts, and electrolytes also must be monitored. Electrolyte abnormalities in patients with multiple myeloma are common. Some of these abnormalities may be analytical artifacts or clinically misleading and should not require specific treatment. These include an analytical artifact causing low sodium values because of a decrease in plasma water resulting from high protein concentration, and high values for total calcium caused by calcium binding to the paraprotein. Direct potentiometric methods for sodium and calcium should not give these misleading values and so should be of special value for monitoring patients with multiple myeloma. As shown by our patient, total calcium values may also be in error, depending on the method utilized. It is important for the laboratory to be cognizant of analytical artifacts and the interrelationships of physiological and methodological phenomena in order better to advise the clinical staff on how to interpret laboratory data.

References