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Clinical Case Study

Unexpected Test Results in a Patient with Multiple Myeloma

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CASE DESCRIPTION

A 53-year-old male patient with an established diagnosis of IgG λ multiple myeloma was seen by a hematologist–oncologist in consultation from an outside hospital. He had previously received 1 cycle of chemotherapy treatment, but he was found to be intermittently noncompliant with his therapy. The patient reported occasional nosebleeds and fatigue. Except for a slightly cachectic appearance, the physical examination was unremarkable. Laboratory results are shown in Table 1.

Serum protein electrophoresis revealed monoclonal paraproteinemia in high abundance marked by an intense band in the γ region. Immunofixation electrophoresis was not ordered at that time, but it was previously performed at another institution and was positive for IgG monoclonal protein.

The attending pathologist noted the discrepancy between the presence of a monoclonal band by serum protein electrophoresis and the patient's quantitative immunoglobulin measurements. Several additional suspicious test results were also noted.

QUESTIONS TO CONSIDER

1. What are some expected laboratory results in a patient with multiple myeloma?
2. Which of the patient's laboratory test results are unexpected given his diagnosis of multiple myeloma?
3. What types of laboratory errors can occur in patients with multiple myeloma?

DISCUSSION

Multiple myeloma is a hematologic malignancy characterized by proliferation of a neoplastic plasma cell population that usually leads to abundant production of a monoclonal immunoglobulin, also called paraprotein or M-protein, as well as decreased concentrations of normal polyclonal immunoglobulins. Overwhelming expansion of plasma cells in the bone marrow with concomitant suppression of other normal blood cell lineages often results in thrombocytopenia and anemia, which are classically manifested as symptoms of bleeding and fatigue. Renal insufficiency, as evidenced by increased blood creatinine and blood urea nitrogen concentrations, also may occur and is likely due to filtration of associated monoclonal free light chains, which can cause tubular damage. Serum protein electrophoresis typically yields a discrete band in the γ region, and immunofixation demonstrates the presence of IgG monoclonal protein in over 50% of cases *(1)*.

Overproduction of plasma proteins in concentrations that exceed typical physiologic limits can be an important source of laboratory test interference. Paraproteins can adversely affect various instruments and methodologies. For example, a paraprotein-associated increase in blood viscosity can cause difficulty in sample aspiration by some instruments that results in assessments being performed on smaller-than-expected sample volumes and subsequent falsely decreased laboratory results *(2)*. In addition, M-proteins, especially those that are also cryoglobulins, can precipitate and cause erroneous measurements via several methods *(1)*. Other interferences such as the prozone effect and the volume displacement effect are discussed below.

IMMUNOGLOBULIN EXCESS AND THE PROZONE EFFECT

Quantitative immunoglobulin concentrations in this case were suspiciously low considering the marked increases in total protein, the large monoclonal band identified on serum electrophoresis, and the patient's anemia and thrombocytopenia, all of which suggested the presence of active disease.

Immunoglobulins IgA, IgM, and IgG were measured using polyethylene glycol-enhanced immunoturbidimetric methods in which antihuman immunoglobulin in the reagent binds to immunoglobulin in the patient sample to generate a precipitate, thereby increasing the turbidity of the solution *(3)*. The resulting absorbance is then used to calculate the immunoglob-

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Table 1. Chemistry and hematology laboratory results.

Laboratory test[a]	Reference interval	Result
IgG	700–1700 mg/dL	<140 mg/dL
	7.0–17.0 g/L	<1.4 g/L
IgA	70.0–450.0 mg/dL	<5.0 mg/dL
	0.7–4.5 g/L	<0.05 g/L
IgM	50–250 mg/dL	<21 mg/dL
	0.5–2.5 g/L	<0.21 g/L
Sodium	135–145 mmol/L	124 mmol/L
Potassium	3.6–5.1 mmol/L	5.2 mmol/L
Chloride	100–110 mmol/L	102 mmol/L
Carbon Dioxide	21–33 mmol/L	22 mmol/L
Creatinine	0.60–1.20 mg/dL	2.54 mg/dL
	53–106 μmol/L	224 μmol/L
Blood urea nitrogen	8–23 mg/dL	36 mg/dL
	2.9–8.2 mmol/L	12.9 mmol/L
Glucose	65–100 mg/dL	96 mg/dL
	3.61–5.55 mmol/L	5.33 mmol/L
Total calcium	8.9–10.7 mg/dL	9.2 mg/dL
	2.23–2.68 mmol/L	2.3 mmol/L
Total protein	6.4–8.5 g/dL	18.4 g/dL
	64.0–85.0 g/L	184 g/L
Inorganic phosphate	2.50–4.60 mg/dL	14.49 mg/dL
	0.81–1.49 mmol/L	4.68 mmol/L
White blood cell count	3.7–9.7 × 10^9/L	4.7 × 10^9/L
Hemoglobin	13.3–17.2 g/dL	8.2 g/dL
	133.0–172.0 g/L	82.0 g/L
Hematocrit	38.9%–50.9%	24.6%
Platelet count	179–373 × 10^9/L	104 × 10^9/L
Mean corpuscular volume	81.2–94.0 fL	91.6 fL

[a] Chemistry and hematology measurements were performed using the Siemens Healthcare Diagnostics ADIVA 1800 and ADVIA 2420 analyzers, respectively.

ulin concentration in the patient sample from the calibration curve.

Aberrant immunoglobulins expressed in monoclonal gammopathies may cause interference in routine immunoassay methods. Reagent immunoglobulins used in methods for immunoglobulin quantification are optimized to recognize the diverse constellation of circulating polyclonal immunoglobulins found in physiological states other than monoclonal gammopathy. The presence of an overabundance of a single clone has been shown to cause unexpected behavior in routine immunoassay methods, resulting in erroneously high or low immunoglobulin measurements *(4)*. It has been hypothesized that expression of atypical epitopes or large concentrations of single clones causes incomplete reaction of the reagent antibodies with the aberrant immunoglobulins, resulting in inaccurate measurements when using routine immunoassay methods.

Immunoturbidimetric and nephelometric methods are susceptible to an additional type of interference known as antigen excess or prozone effect. This phenomenon occurs when excessive quantities of antigen inhibit stable antigen–antibody complex formation. The subsequent decrease in detected absorbance leads to a falsely low measured concentration *(3, 4)*. Detection of the prozone effect requires dilution of the sample to remove the antigen excess and allow appropriate formation of the antigen–antibody complex, resulting in an accurate measurement *(3, 5)*. The prozone effect is more often observed when measurements are obtained using an immunoturbidimetric method; modern nephelometers are typically less susceptible to this effect because they employ an automatic dilution step to detect antigen excess *(5)*.

Testing was repeated with a 1:100 dilution (1 part serum to 99 parts normal saline) and an IgG concentration of 14 400 mg/dL (144.0 g/L) was obtained, which was consistent with the serum protein electrophoresis results and the patient's overall clinical presentation. Repeat measurements of IgA and IgM were not performed due to sample volume constraints.

IMMUNOGLOBULIN EXCESS AND THE VOLUME DISPLACEMENT EFFECT

The patient's initial serum sodium was 124 mmol/L, but he lacked symptoms of hyponatremia. The low sodium concentration was found to be a result of pseudohyponatremia, an artificially low measurement of sodium concentration produced by the presence of severe hyperproteinemia.

In healthy individuals, the plasma volume is comprised of approximately 93% water, which contains dissolved electrolytes and other compounds, whereas the remaining plasma volume consists of lipids and proteins *(2, 3)*. Substantial increases in plasma proteins and/or lipids can cause displacement of the aqueous component and its dissolved solutes in a phenomenon called volume displacement. Although the proportion of plasma water occupies a relatively smaller percentage of the total plasma volume, the solute concentrations within the plasma water are physiologically normal.

For measurement of serum electrolytes, many laboratories employ ion-selective electrodes (ISE) that utilize indirect potentiometry, in which patient samples are diluted before the measurement process. Prediluting the sample prevents excessive protein ex-

posure to the ISE membrane, thus minimizing electrode degradation and calibration drift *(6)*. Instruments that utilize a predilution step assume a normal ratio exists between the plasma water volume and the total plasma volume *(2)*. This assumption may be invalid when analyzing samples containing abnormal increases in proteins and/or lipids and can lead to falsely low measurements of solute, with sodium being most commonly affected. The volume displacement effect can be avoided in these samples by employing ion-selective electrodes that utilize direct potentiometry, in which electrolyte measurements are evaluated in an undiluted plasma sample *(6)*.

The original serum sodium result of 124 mmol/L, as measured by indirect potentiometry, was inconsistent with the patient's clinical presentation. In this case, the volume displacement effect was suspected as the most likely etiology of the pseudohyponatremia. The patient's serum sodium concentration was repeated utilizing direct potentiometry on the NOVA CCX analyzer, and a sodium concentration of 138.5 mmol/L, which is within the reference interval, was obtained.

IMMUNOGLOBULIN EXCESS AFFECTING MEASUREMENT OF PHOSPHOROUS

On initial measurement the patient's serum inorganic phosphate was markedly increased, but the calcium concentration was within the reference interval. Serum inorganic phosphate and calcium concentrations are maintained within a tight physiologic range via closely associated mechanisms regulated by the kidney, bone, and gut *(1)*. An interfering substance producing factitious hyperphosphatemia, or "pseudohyperphosphatemia," was suspected owing to the profoundly high inorganic phosphate concentrations in a relatively asymptomatic patient with calcium concentrations within the reference interval.

Spectrophotometric quantification of serum inorganic phosphate concentration is based on the reaction of phosphate ions with ammonium molybdate to form a phosphomolybdate complex *(1, 7)*. Absorbance of the unreduced phosphomolybdate complex is measured and indexed to a calibration curve to determine analyte concentration *(3, 7)*. The formation of the complex is pH dependent and uses an acid buffer *(3, 7)*. The low pH environment of the reaction mixture can lead to precipitation of immunoglobulins and result in increased turbidity and light scattering *(5, 7)*. Resolution of the interference is obtained by diluting the sample or removing the plasma proteins via various deproteinization methods *(5, 7)*.

The initial measurement of inorganic serum phosphate was 14.49 mg/dL (2.50–4.60 mg/dL) [4.68 mmol/L (0.81–1.49 mmol/L)], which exceeded the absorbance limit for the assay. A 1:5 dilution (1 part serum to 4 parts normal saline) was performed and a serum phosphate concentration within the reference interval [4.4 mg/dL (1.41 mmol/L)] was obtained.

POINTS TO REMEMBER

- Multiple myeloma is a hematologic malignancy characterized by proliferation of a neoplastic plasma cell population leading to increased production of a monoclonal immunoglobulin. It is often accompanied by thrombocytopenia, anemia, and renal failure.
- Immunoturbidimetric methods are subject to interference via the prozone effect, in which excess antigen inhibits stable antigen–antibody complex formation, resulting in decreased absorbance and a falsely low result. Detection of the prozone effect requires sample dilution.
- The volume displacement effect is caused by increased concentrations of protein or lipids which displace aqueous components of blood. This effect can be avoided by utilizing direct potentiometry in which electrolyte measurements are evaluated in an undiluted plasma sample.
- Spectrophotometric quantification of serum inorganic phosphate concentrations may give falsely increased results owing to immunoglobulin precipitation. Resolution of pseudohyperphosphatemia is obtained by diluting the sample, thereby reducing the quantity of plasma proteins.

SUMMARY

Multiple myeloma is a hematologic disease that often results in increased production of monoclonal immunoglobulins. As the paraprotein concentration rises, the potential for erroneous results increases. This case illustrates how unusually high concentrations of serum immunoglobulins can result in multiple laboratory interferences, including the prozone effect, the volume displacement effect, and precipitation interference. Laboratorians should remain attentive to potential sources of discrepant results in patients with high plasma protein concentrations and should take appropriate action to prevent the release of erroneous results.

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Commentary

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This case brings to mind the tale of Goldilocks and the 3 bears: we appreciate when monoclonal immunoglobulin concentrations are not too high and not too low, but just right. Most monoclonal gammopathies have concentrations that are easily detected and quantified by electrophoretic and/or nephelometric or turbidimetric methods. The extremes of low and high immunoglobulin concentrations, however, are part of the diversity of plasma cell proliferative diseases and may be orders of magnitude outside of ranges typically encountered. From experience with light chain myeloma and primary amyloidosis, we are most often concerned about low serum concentrations of monoclonal proteins. On rarer occasions high concentrations are problematic. Automated protein analyzers (unlike most chemistry analyzers) use various approaches to identify high-concentration prozone effects, including assessment of the initial rate of the light scatter reaction, addition of antigen at the completion of the reaction, and/or quantification at 2 dilutions.

The myeloma diagnosis in this case was known to the clinician, and laboratory results were consistent with myeloma-associated bone marrow suppression (hemoglobin, hematocrit, platelet count) and renal impairment (creatinine, urea nitrogen). The apparent absence of immunoglobulins might have suggested a nonsecretory or light chain myeloma except for the serum protein electrophoresis and dramatically increased serum total protein. Dilution and reanalysis of the serum yielded a quantitative IgG of 14 400 mg/dL, which was compatible with the serum M spike.

As Jelinek and Bachmann point out, there are other idiosyncratic analytic problems caused by monoclonal immunoglobulins that are unrelated to concentration. The most common of these are preanalytic and analytic cryoglobulin precipitates. The unique nature of each monoclonal gammopathy warrants our attention to the entire case, and evaluation of such cases frequently benefits from open lines of communication with clinicians.

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Commentary

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Multiple myeloma is named for the multiple lesions that form as malignant plasma cells appear in patients'