case study [chemistry | hematology]

Pseudohyponatremia in a Myeloma Patient: Direct Electrode Potentiometry is a Method Worth its Salt

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Patient

49-year-old Caucasian male.

Chief Complaint

He presented to our cancer clinic complaining of malaise, tiredness, and bone pain.

Prior Medical History

The patient had a 4-year history of progressive multiple myeloma (IgG-lambda immunophenotype) with gradually increasing bone pain that was refractory to high dose chemotherapy and autologous stem cell transplantation. He had been treated recently with morphine, a bisphosphonate (pamidronate disodium), and radiation to the shoulders. During his recent visit, he received 2 units of packed red blood cells and 40 grams of intravenous immune globulin.

Recent Drug History

Morphine sulfate [MS Contin®], 220 mg bid; testosterone enanthate (Delatestryl®, to boost energy), 300 mg intramuscularly; pamidronate disodium, 90 mg intravenously; dexamethasone, 4 mg bid.

Principal Laboratory Findings

[**T1**]

Principal Laboratory Findings **T1**

Test	Patient's Result	"Normal" Reference Interval
Hematology		
WBC count	4.1	4.0-11.0 x 10^3/µL
Neutrophils	2.2	2.0-8.0 x 10^3/µL
Bands	0.1	0.0-1.3 x 10^3/µL
Lymphocytes	0.7	0.7-3.5 x 10^3/µL
Monocytes	1.1	0.0-1.0 x 10^3/µL
Eosinophils	0.0	0.0-0.7 x 10^3/µL
Polychromasia	1+	Negative
Rouleaux	3+	Negative
Nucleated RBCs/ 100 WBCs	1	0
Hemoglobin	8.6	13.7-18.0 g/dL
Hematocrit	25	40-54%
RBC count	2.4	4.5-6.0 x 10^6/µL
MCV	102	82-100 fL
MCHC	34.6	32.0-36.0 g/dL
Platelet count	181,000	150,000–400,000/µL
Chemistry		
Sodium	128	133-145 mmol/L
Potassium	4.4	3.5-5.0 mmol/L
Chloride	95	98-111 mmol/L
CO_2	28	21-31 mmol/L
Anion gap*	5	7-16 mmol/L
Creatinine	1.3	0.7-1.4 mg/dL
Calcium	10.9	8.4-10.2 mg/dL
Triglycerides	60	55 – 327 mg/dL
Total protein†	17.1	6.3-8.0 g/dL

**Anion gap = [sodium] – [chloride + CO2].*
†Using a diluted serum sample and correcting the result for the dilution factor.
WBC, white blood cell; RBC, red blood cell; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration

Results of Additional Diagnostic Procedure and Laboratory Tests

At the request of the laboratory director, the initial electrolyte measurements (sodium, potassium, and chloride) by an indirect ion-specific electrode (ISE) method were repeated using an alternate method/instrument (direct potentiometry/blood gas analyzer) [**T2**].

Repeat Electrolyte Results by Direct Potentiometry Method **T2**

Test	Patient's Result, mmol/L
Sodium	137
Potassium	4.8
Chloride	99
Anion gap*	10

Calculated using the equation and CO_2 value from* **T1.

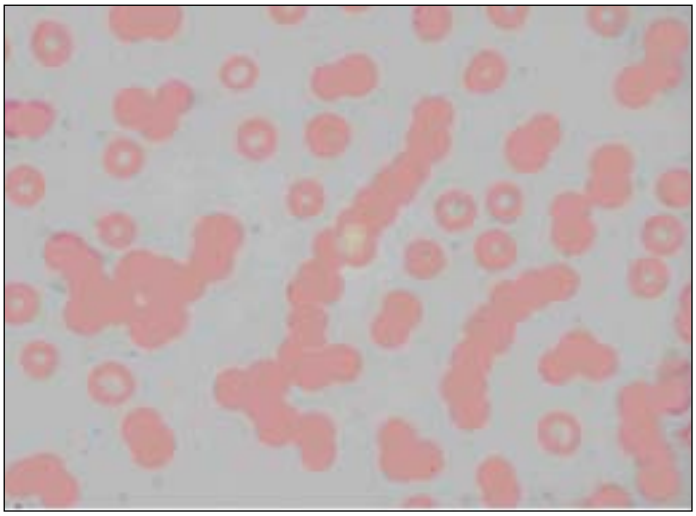
Questions:

1. What (is)are this patient's most striking laboratory result(s)?
2. How do you explain this patient's most striking laboratory result(s)?
3. Which additional test(s) should be performed? Why?
4. What is the most likely cause of the disparity between sodium values by indirect ISE versus direct potentiometry methods?
5. What is the most appropriate treatment for this patient and the most appropriate actions for the laboratory?

Possible Answers:

1. Markedly elevated serum total protein, hyponatremia, hypochloremia, Rouleaux formation (3+), and macrocytic anemia (increased MCV and decreased hemoglobin, hematocrit, and RBC count) with polychromasia and the presence of nucleated red blood cells (1 NRBC/100 WBCs) [**I1**].

2. The markedly elevated serum total protein concentration was caused by unregulated synthesis of a monoclonal immunoglobulin as occurs typically in patients with a plasma cell dyscrasia such as multiple myeloma or Waldenström's macroglobulinemia. The patient was known to have multiple myeloma and an IgG-lambda monoclonal serum component. The patient's hyponatremia and hypochloremia were not clinically anticipated. Rouleaux formation, the linear alignment of at least 4 RBCs in a thin area of a blood smear resembling a stack of coins, is caused by changes in the surface charge of the erythrocyte membrane when this membrane is coated with excessive amounts of protein such as globulins and fibrinogen. Other causes of Rouleaux formation include plasma expanders (dextran and hydroxyethyl starch) and radiographic contrast materials. The most common cause of Rouleaux formation, however, is paraproteinemia due to a monoclonal gammopathy. Rouleaux formation often increases the erythrocyte sedimentation rate as well. Moreover, hyperproteinemia may cause RBC aggregation leading to a spuriously high mean corpuscular volume (MCV). Another mechanism for an increased MCV in neoplastic diseases such as multiple myeloma is folate deficiency caused by increased folate utilization by the neoplasm leading to a macrocytic anemia. Elevated protein concentration may expand the plasma volume and displace some of the RBCs, thereby reducing the hemoglobin concentration disproportionately to the total RBC mass and exaggerating the anemia. It also may render a bluish background to a stained peripheral blood smear as in this case [**I1**].[1-5] Accelerated bone marrow release of reticulocytes in response to the marked anemia is the most likely explanation of the macrocytosis, polychromasia, and nucleated RBCs observed on this patient's stained peripheral blood smear.[1-2]



[**I1**] Rouleaux in a stained peripheral blood smear from our patient with multiple myeloma. The bluish-colored background is a consequence of the markedly increased protein concentration in this patient's serum.

3. Repeat sodium and chloride testing using an alternate method/instrument. Our patient's decreased sodium, chloride, and anion gap results, in conjunction with a markedly elevated serum total protein value, were brought to the attention of the laboratory director. We considered the possibility that protein precipitation during the quantitative measurement of total protein in our patient's serum sample may have resulted in a falsely increased total protein value; however, our patient was not prone to cryoglobulinemia and the total protein result was obtained using a diluted specimen. Once the serum total protein result was considered valid, we suspected that the sodium and chloride values might be falsely decreased due to protein interference with the indirect ISE method used in our chemistry analyzer (Hitachi 917, Roche Diagnostics **M**, Indianapolis, IN). Therefore, sodium and chloride (and potassium) testing on our patient's serum sample was repeated using the direct potentiometry method in a blood gas analyzer (ABL 725, Radiometer America **M**, Westlake, OH) with the results shown in **T2**.

4. The "volume exclusion effect" that occurs in sera with high protein concentrations. The initial low sodium concentration in our patient's serum sample by an indirect electrode method that corrected to a value within the reference range for sodium when this sample was re-tested for sodium using a direct electrode method is indicative of *pseudohyponatremia*. Pseudohyponatremia is associated with the "volume

exclusion effect" that occurs in lipemic serum samples and in sera with high concentrations of protein when these sera are assayed for sodium concentration using indirect electrode methods.[6] Direct and indirect ion-specific electrodes are both widely used in clinical laboratory instrumentation. As the name suggests, a direct ion-specific electrode method detects the activity of a specific ion in an undiluted specimen (ie, the specimen is analyzed "directly" with no intermediate specimen dilution step). Indirect ion-specific electrodes measure the activity of specific ions in a specimen following dilution of the specimen with a diluent of fixed composition [**F1**]. The advantages and disadvantages of each design have been reviewed elsewhere.[6] The typical water content of plasma is approximately 93% and indirect electrode methods for quantifying electrolyte concentrations assume that this proportion is constant. When plasma contains large concentrations of lipid or paraprotein, these extra components occupy volume and displace water, so that plasma contains less water per unit volume and less electrolytes per unit volume. Automated instruments with indirect electrodes pipette aliquots of plasma (eg, 10 µL of plasma contains 9.3 µL water) and add diluent (eg, 300 µL buffer) before determining the electrolyte concentration with an ion selective electrode. Lipemia and hyperproteinemia cause predictable decreases in the amount of water per unit volume of plasma (W_p) which can be estimated using Waugh's empirical equation [values for serum total protein concentrations (P_s) and serum triglyceride concentration (L_s) below were taken from **T2**][7]:

W_p (g/dL) = 99.1 - 0.73 x $\mathbf{P_s}$ (g/dL) - 1.03 x $\mathbf{L_s}$ (g/dL)
= 99.1 - 0.73 x 17.1 – 1.03 x 0.06
= 99.1 - 12.5 - 0.06
= 86.6 (or ~87%; % = g/100 mL = g/dL)

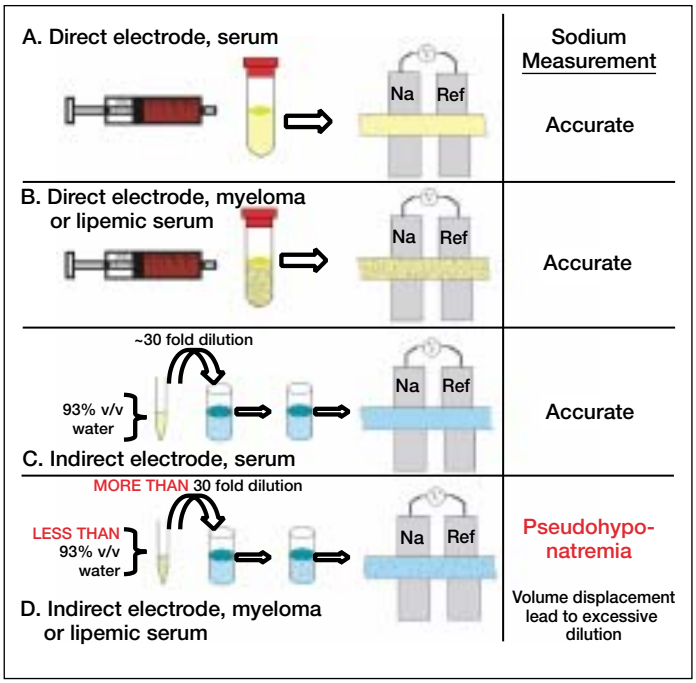
Therefore, the decrease in plasma water content = 100 – 86.6 = 13.4%.

Thus, a 10 µL aliquot of our patient's plasma contains 8.7 µL (ie, 87% of 10 µL) of water and the addition of a fixed volume of diluent (eg, 300 µL) will cause excess dilution (overdilution) of the water-phase of the aliquot and falsely low concentrations of electrolytes. Moreover, this effect will be directly proportional to the volume of water displaced by the lipid or paraprotein. Therefore, our patient's expected sodium concentration by an indirect electrode method can be estimated as follows:

$$\frac{8.7\ \mu L}{9.3\ \mu L} \times 137\ \text{mmol/L}\ Na^+_{\text{Direct Electrode}} = 128\ \text{mmol/L}\ Na^+_{\text{Indirect Electrode}}$$

Our patient's observed Na^+ concentration by an indirect electrode method was 128 mmol/L [**T1**].

Moreover, blood specimens from patients with multiple myeloma are prone to generating erroneous laboratory results through a variety of other mechanisms, including turbidity from precipitation of the paraprotein in cuvettes during the analysis, inaccurate pipetting due to the high viscosity of serum samples from patients with myeloma, and the inactivation of reagents by binding to the paraprotein. Monoclonal protein precipitates are responsible for the most obvious forms of cryoglobulins (type I and type II) that can erroneously lower measured values for the serum total protein concentration by creating flocculent precipitates that cause turbidity and optically interfere with many methods. Numerous examples of such interference have appeared as case reports and reviews, and monoclonal IgM is often responsible.[8-17] Due to the intermittent nature of the interference and the frequent reformulation of reagents by manufacturers, this type of laboratory error is difficult to anticipate, but it can be recognized within the laboratory by alert staff and brought to the attention of clinical colleagues to avoid misinterpretation. Some of the laboratory tests prone to interference by hyperparaproteinemia include: albumin, creatinine (Jaffe method), C-reactive protein, hemoglobin, MCH, MCHC, inorganic phosphate, thyroxine, urea nitrogen, uric acid, and sodium.



[**F1**] Schematic view of indirect and direct sodium electrodes depicting the susceptibility of serum samples from myeloma patients or lipemic serum samples to pseudohyponatremia when sodium is quantified by indirect electrode methods. The potentiometric measurement of sodium by direct electrode methods occurs on undiluted specimens (directly) using either anticoagulated whole blood, plasma, or serum, and the accuracy of these methods is not influenced by the "volume exclusion effect" (Panels A and B). Indirect electrode methods require an approximately 30-fold dilution of the specimen prior to the potentiometric measurement of sodium by an ion selective electrode, which renders sodium values by this method susceptible to a dilution error commonly referred to as pseudohyponatremia (Panels C and D).[6]

5. The patient should not be treated for hyponatremia. It is difficult for laboratory professionals to identify potentially erroneous laboratory test results due to hyperproteinemia; however, when marked hyperproteinemia is observed on any

serum specimen, the results of the tests identified above should be reviewed and electrolyte measurements repeated by a direct ISE method. Moreover, when pseudohyponatremia is confirmed, it is prudent to alert clinicians of this finding by providing this information on the laboratory report. Laboratories that serve cancer treatment clinics with large numbers of multiple myeloma patients may consider conducting all electrolyte tests on instruments with direct electrodes to avoid the repetitive uncertainty in the validity of the results.

Patient's Treatment and Course

The patient received 2 units of packed red cells and 40 grams of intravenous immune globulin. The laboratory report contained an interpretive comment about the likelihood of pseudohyponatremia, along with the serum sodium result from the direct electrode method. Treatment for hyponatremia was not initiated. The patient returned to the clinic 4 weeks later with hyperproteinemia, Rouleaux, and persistent pseudohyponatremia. "Any man is liable to err, only a fool persists in error." Marcus Tullius Cicero (106-43 B.C.), Roman orator.

Keywords: *pseudohyponatremia, ion-specific electrode, multiple myeloma, hyperproteinemia, paraproteinemia*

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