CASE

A 56-year-old Caucasian man with a history of colon cancer status post resection and chemoradiotherapy presented to the emergency medicine department with unintentional weight loss and 6 months of dull right hypochondrial pain with no change in bowel habit. The abdominal pain turned sharp and severe on the day of admission. He was not jaundiced, and no abdominal mass was felt. Initial laboratory investigations revealed a macrocytic, normochromic anemia [hemoglobin, 71 g/L (reference interval, 126–169 g/L); mean corpuscular volume, 98.0 fl (reference interval, 80.1–96.7 fl); mean corpuscular hemoglobin concentration, 320 g/L (reference interval, 308–384 g/L)]. Serum creatinine, alanine aminotransferase, aspartate aminotransferase, and bilirubin values were within the reference limits. The total protein concentration was 113 g/L (reference interval, 65–82 g/L), and the albumin concentration was 33 g/L (reference interval, 38–48 g/L). The phosphate concentration was markedly increased at 4.84 mmol/L (reference interval, 0.85–1.45 mmol/L). The total and corrected calcium concentrations were 1.98 mmol/L and 2.20 mmol/L (reference interval for both, 2.15–2.55 mmol/L), respectively, and the magnesium concentration was 0.76 mmol/L (reference interval, 0.75–1.07 mmol/L). The analytical indices (lipemia, bilirubin, and hemolysis) were within acceptable limits. The only medication the patient was taking was atenolol for hypertension.

DISCUSSION

Phosphate, the most abundant intracellular anion, exists in organic and inorganic forms in the human body. Its multifaceted function involves providing structural support, regulation of intermediary metabolism, genetic coding, cellular signaling, and cell growth (1, 2). Nonpathologic homeostasis of phosphate is intimately related to calcium. It is maintained by bone, the kidneys, and the gut in response to changing concentrations of calcium and phosphate, which are mediated by vitamin D, calcitonin, and parathyroid hormone (1, 2). Only serum inorganic phosphate, which is <1% of the total body phosphate, is routinely measured (2).

Measurement of serum inorganic phosphate is commonly based on the reaction of phosphate ions with ammonium molybdate to form a phosphomolybdate complex, which is then measured with a spectrophotometer at 340 nm (1, 2). The phosphomolybdate complex can be further reduced to a molybdate complex, which is measured at 600–700 nm to avoid the positive interference of hemolysis, icterus, and lipemia associated with the 340-nm wavelength (1, 2). Other, less commonly used methods include the vanadate–molybdate and enzymatic methods (1, 2). The vanadate–molybdate method, which is carried out at an acidic pH, has a positive bias owing to the hydrolysis of organic phosphate esters, whereas enzymatic methods performed at neutral pH do not (1).

Hyperphosphatemia occurs when there is a decreased renal excretion of phosphate, an increased phosphate intake, or an increased extracellular phosphate load (1). Clinically, a decreased glomerular filtration rate (as in acute or chronic renal failure) leading to decreased renal phosphate excretion is the most common cause of hyperphosphatemia. In the absence of renal failure, increased tubular reabsorption, hypoparathyroidism, pseudohypoparathyroidism, and acromegaly should be suspected (1–3). Increased phosphate intake (frequently iatrogenic) may arise from excessive oral or intravenous phosphate administration or overuse of phosphate-containing laxatives or enemas. Respiratory or metabolic acidosis may hydro-
lyze intracellular organic phosphate-containing compounds and release them into the extracellular compartment (1). Cell lysis disorders, such as tumor lysis syndrome, hemolytic anemia, and rhabdomyolysis, may all give rise to hyperphosphatemia (1, 2).

Hyperphosphatemia is usually asymptomatic; however, an acute increase in the phosphate concentration may precipitate calcium and thereby lead to signs and symptoms of hypocalcemia, including paresthesia, tetany, seizure, Chvostek/Trousseau sign, and cardiovascular instability (3). Chronic hyperphosphatemia may lead to renal dystrophic calcification, secondary hyperparathyroidism, osteitis fibrosa, and metastatic calcification (3). Treatment is mainly targeted at the underlying cause but may involve hemodialysis, aggressive fluid hydration, administration of dextrose and insulin, or administration of acetazolamide acutely (3). Dietary restriction of phosphate and administration of phosphate-binding salts are useful for long-term management (1, 3).

**PSEUDOHYPERPHOSPHATEMIA**

Pseudohyperphosphatemia is a falsely increased phosphate concentration due to analytical or preanalytical errors in phosphate measurement. Clinical suspicion should be raised when a high phosphate concentration cannot be sufficiently explained by the patient’s pathophysiology. Hemolyzed, icteric, and lipemic samples are known to positively interfere with certain methods of phosphate measurement (2). Modern analyzers can detect most of these interferences as excessively high analytical indices. Additionally, a prolonged standing or clotting time for a sample may also raise the serum phosphate concentration because of a shift of phosphate from within erythrocytes and platelets to the serum (2). The use of liposomal amphotericin B is a lesser-known cause of a falsely increased phosphate concentration that has increasingly been reported with the rise in its use among immunocompromised patients (4). Two possible mechanisms have been postulated for this phenomenon. One suggestion is that biodegradation of the liposomal vehicle (for transporting the drug) may interfere with light scatter or precipitation, affecting the absorbance measurement. The second suggestion is that hydrolysis of the organic phosphate in liposome phospholipids is being measured by the assay. Another iatrogenic cause is heparin contamination of samples obtained from hemodialysis catheters (5). A simple discussion with the appropriate clinical staff will usually provide clues in the latter 2 scenarios.

Spurious hyperphosphatemia in patients with dysproteinemia is well documented (6, 7). Causes of dysproteinemia include multiple myeloma, Waldenström macroglobulinemia, and monoclonal gammopathy of undetermined significance (3). Frequently, persons with such conditions present with a very high serum phosphate concentration, a typical serum calcium concentration, and no symptoms related to hyperphosphatemia. Spurious hyperphosphatemia may be analytical (i.e., due to interference of paraproteins with the serum phosphate assay) or physiological (i.e., due to the presence of phosphate-binding proteins) (6–8). In one instance, hyperphosphatemia was actually thought to be physiologically active in a multiple myeloma patient with a depressed 1,25-dihydroxyvitamin D concentration (8).

Paraprotein interference in phosphate measurement may be suggested by a serum total protein concentration that is disproportionately higher than the serum albumin concentration, which may be typical or even low. Manual deproteinization of the sample by trichloroacetic/sulfosalicylic acid precipitation, dialysis, wet-ashing with nitric acid and perchloric acid, ultrafiltration, and extreme dilution have previously been described to achieve a more accurate measurement of serum phosphate (7, 9). Furthermore, the purine nucleoside phosphorylase–based enzymatic method has been suggested as an appropriate alternative assay for paraproteinemnic sera (2).

It is important to identify pseudohyperphosphatemia secondary to paraproteinemia because it not only eliminates unnecessary clinical interventions but also may reveal a major diagnosis. Use of dry-film technology, which removes proteins before phosphate analysis, reduces the likelihood of miscalling a dysproteinemic sample as hyperphosphatemic and avoids misleading clinicians (10). Ironically, the elimination of pseudohyperphosphatemia may in turn deprive clinicians of a valuable clue to the presence of these clinically important disorders if total protein is not measured. Not all patients with dysproteinemia and a high serum phosphate concentration will have pseudohy-

<table>
<thead>
<tr>
<th>Before deproteinization</th>
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<tr>
<td>Phosphate, mmol/L</td>
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<tr>
<td>Advia 2400</td>
<td>3.81</td>
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<tr>
<td>Vitros 5600</td>
<td>1.28</td>
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<tr>
<td>IgG, g/L</td>
<td></td>
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<tr>
<td>Integra 400 Plus</td>
<td>108.3</td>
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<tr>
<td>Total protein, g/L</td>
<td></td>
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<tr>
<td>Advia 2400</td>
<td>113</td>
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perphosphatemia; further workup may be required, depending on the clinical scenario. Good communication between clinical laboratory and clinical staff is key to identifying such unsuspected and rare cases of pseudohyperphosphatemia.

**RESOLUTION OF THE CASE**

Pseudohyperphosphatemia was suspected when the clinical presentation and initial laboratory investigations failed to explain the excessively high phosphate concentration, and the laboratory was consulted. In the absence of preanalytical causes such as abnormal analytical indices, medication, and prolonged processing of the patient’s sample, an analytical interference was regarded as the most likely source of the interference. Paraproteinemia was strongly considered in view of the discordant serum concentrations of total protein and albumin, and the patient was investigated for possible multiple myeloma.

The initial phosphate concentration was measured by means of the 1-step phosphomolybdate/UV principle (Advia 2400; Siemens Healthcare Diagnostics). We subsequently received a second request for a phosphate concentration, which was measured on both the Advia 2400 instrument and the Vitros 5600 platform (Ortho Clinical Diagnostics), which includes the additional step of converting the phosphomolybdate complex to heteropolyphosphate blue for measurement. The results were 3.81 mmol/L (Advia 2400) and 1.28 mmol/L (Vitros 5600). Immunoglobulin quantification revealed the following: IgA, 0.21 g/L (reference interval, 0.80–2.00 g/L); IgG, 108.30 g/L (reference interval, 5.00–15.00 g/L); and IgM, <0.13 g/L (reference interval, 0.80–2.00 g/L). The same sample was then subjected to ultrafiltration with a 10K Amicon Ultracel Centrifugal Filter device (Millipore), centrifuged at 1811 g for 30 min (Eppendorf centrifuge), and then measured again with the 2 analyzers. The phosphate

**POINTER TO REMEMBER**

1. Hyperphosphatemia can be caused by a decreased renal excretion of phosphate (most commonly due to renal failure), increased oral or intravenous phosphate intake (usually iatrogenic), respiratory or metabolic acidosis, or a major cellular lysis event, such as tumor lysis syndrome or intravascular hemolysis.

2. Pseudohyperphosphatemia is a falsely increased phosphate concentration that can be caused by preanalytical issues such as improper sampling, prolonged clotting, very high analytical indices, and, occasionally, medication. More importantly, it may be caused by dysproteinemia, especially in the presence of disproportionately high total protein concentration relative to the serum albumin concentration.

3. Chemical and physical deproteinization can be used to separate the interfering protein for a better measurement of serum inorganic phosphate, although an enzyme-based assay may work just as well.

4. Communication between the clinical laboratory and clinical staff is key to the early identification of potential pseudohyperphosphatemia.
concentration decreased noticeably to 1.15 mmol/L on the Advia 2400 and less so to 1.09 mmol/L on the Vitros 5600. The concentrations of IgG and total protein in the ultrafiltrate were also measured. The IgG concentration was <0.05 g/L with the Integra 400 Plus instrument (Roché Diagnostics), and the total protein concentration was 0 g/L with the Advia 2400. Table 1 summarizes the patient’s laboratory investigations.

The discrepancy between the phosphate results obtained with the Advia 2400 and Vitros 5600 was dramatically reduced with physical deproteinization of the blood sample. The ability of the Vitros 5600 instrument to measure the serum phosphate concentration in a dysproteinemic sample closer to its deproteinized state may be attributed to the effectiveness of the multilayered reaction slide used. The multilayered reaction slide is topped with a BaSO4 spreading layer, which is capable of filtering large molecules such as proteins, lipids, and hemoglobin (10). In this setting, the spreading layers appeared to be effective in removing IgG, which would otherwise interfere with the phosphate measurement. The eventual diagnosis for the patient was multiple myeloma, according to serum protein electrophoresis (Fig. 1) and hematologic studies, and he was treated accordingly.

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References


Commentary

Vivek Roy*

Laboratory tests are an important component of comprehensive medical evaluations. These tests provide helpful information that can complement or confirm clinical suspicion, and they can sometimes identify problems that are not clinically evident. On the other hand, tests may provide inaccurate or misleading information under certain circumstances.

Hyperphosphatemia is a relatively uncommon laboratory abnormality most often seen in patients with advanced renal failure, hypoparathyroidism, cellular lysis, or excessive phosphate intake (or iatrogenic administration). Hyperphosphatemia may occur artificially from a hemolysed, icteric, or lipemic sample; a prolonged delay between blood draw and analysis; or heparin contamination. Hyperglobulinemia (paraproteinemia) has long been recognized to cause spurious serum phosphate readings because of analytical interference. Paraproteinemia is also known to affect many other laboratory parameters, including blood urea nitrogen, creatinine, bilirubin, calcium, iron, sodium, and lipoproteins.

The precise frequency with which these artificial abnormalities are encountered in the clinic or laboratory is unknown, but the phenomenon is potentially substantially underrecognized. The frequency is likely to increase in the future. The prevalence of paraproteinemia increases with age, reaching >10% in persons >80 years of age. Older persons are also more likely to have multiple comorbidities and therefore require multiple laboratory tests. Thus, with the increasing mean age of the population, there are increasing opportunities for clinicians to...
encounter paraproteinemian related artifactual laboratory abnormalities. The importance of recognizing this phenomenon is self-evident: to avoid misinterpretation of data and unnecessary testing. This possibility should be considered when encountering a totally unexpected abnormality that does not “fit” in the clinical context. When paraproteinemian is suspected, further evaluation should be undertaken in consultation with a laboratory medicine specialist. Repeating the test after deproteination, further dilution of the sample, or use of an alternative testing methodology are some of the strategies available for further investigation.

**Commentary**

David B. Endres*

Loh et al. report the clinical case of a patient with pseudohyperphosphatemia owing to paraprotein (monoclonal immunoglobulin) interference and remind us of the importance of correlating laboratory results with clinical findings and of encouraging communication between the laboratory and physicians.

Paraproteins are common interferents in routine hematology assays. More than 70 published reports have documented paraprotein interference with a variety of analytes, including calcium, direct and total bilirubin, creatinine, glucose, HDL and LDL cholesterol, iron, phosphate, sodium, urea, uric acid, coagulation tests, and blood cell counts (1). The importance of paraprotein interference should not be underestimated, given the prevalence of paraproteinemian (3.2% and 5.3% for persons ≥50 and ≥70 years of age, respectively) (2).

Paraproteins cause interference via several mechanisms. The most common is the formation of turbidity due to the precipitation of paraproteins during the test reaction. Test results can be falsely low or high. Paraprotein interference is underrecognized and underreported. Systematic studies of sera from patients with monoclonal gammopathies have reported that interference is far more frequent than can be inferred from case reports (3).

Given the prevalence of paraproteins and their interference with analyte measurements, more approaches are needed for reducing and detecting this interference. Manufacturers of diagnostic reagents can optimize reagents (e.g., detergents, ionic strength) to reduce interference and can flag interference by monitoring reaction kinetics and sample consistency. Laboratories can use software to identify samples that may have paraproteins or paraprotein interference. Examples of the use of test results and interference indices to identify such samples include (a) samples with a low albumin concentration and an increased total protein concentration, (b) samples with discordant interference indices and test results (e.g., an increased icteric index and normal total or direct bilirubin values), (c) samples with discordant test results (e.g., a direct bilirubin value greater than the total bilirubin value), and (d) samples with negative or improbable undetectable results (e.g., HDL cholesterol).

**References**