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Short Communication

# Pseudohypophosphatemia associated with high-dose liposomal amphotericin B therapy

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ABSTRACT

*Background:* Hypophosphatemia is commonly observed in critically ill patients. Inorganic phosphorus is quantified by spectrophotometric measurement of a phosphomolybdate complex, a method with multiple documented interferents. Our clinical laboratory was contacted to investigate a case of asymptomatic hypophosphatemia in a patient receiving high-dose liposomal amphotericin B therapy (L-AMB).
*Methods:* In vitro experiments were performed by spiking L-AMB into residual plasma specimens. Phosphate was measured on the Beckman Coulter AU and Ortho Diagnostics Vitros instruments.
*Results:* When measured on the AU, phosphate in plasma with approximately 250 mcg/mL of L-AMB demonstrated a median negative bias of 3.45 mg/dL relative to unspiked samples. In contrast, Vitros phosphate measurements demonstrated excellent agreement for specimens with and without L-AMB (median bias − 0.2 mg/dL).
*Conclusions:* High L-AMB concentrations induced a significant negative bias on phosphate measured by the AU assay, but did not affect the Vitros assay. Laboratorians and clinicians should be aware of this phenomenon in patients receiving L-AMB who develop unexplained hypophosphatemia.

## 1. Introduction

The presence or absence of phosphate on molecules is crucial in the regulation of many metabolic processes; inorganic phosphate is therefore essential for normal metabolic function. Hypophosphatemia is a common finding in the critically ill, often occurring due to insufficient dietary intake, lack of intestinal absorption, increased urinary excretion, diarrhea, or medication side effect [1]. It can cause a variety of signs and symptoms that vary depending on both the chronicity and severity of phosphate depletion. These include skeletal and myocardial myopathies, metabolic encephalopathy, and derangements of renal reabsorption of calcium and magnesium that can produce a marked hypercalciuria. Symptomatic patients usually exhibit a plasma phosphate concentration below 1 mg/dL (0.32 mmol/L) [2].

Inorganic phosphate is typically measured on automated chemistry analyzers by reaction with acidic ammonium molybdate to form a phosphomolybdate complex, which is then measured spectrophotometrically in either an end-point or rate-based reaction. Most assays measure the phosphomolybdate complex directly at 340–365 nm while others reduce the complex with *p*-methylaminophenol sulfate prior to measurement at 670 nm. Hypergammaglobulinemia can lead to both pseudohyperphosphatemia [3–7] and pseudohypophosphatemia [8,9] using the direct phosphomolybdate method. Several other substances have been documented to interfere with a rate-based method (PHOSm), including bilirubin glucuronides, hemoglobin, nafcillin, and rifampin [10]. In addition, multiple reports describe pseudohyperphosphatemia in the context of high-dose liposomal amphotericin B therapy using PHOSm-based measurements [11–13].

Liposomal amphotericin-B (L-AMB) is a formulation of amphotericin B that has been embedded in a unilamellar phospholipid bilayer. This specific formulation is less toxic and has been approved for invasive *Aspergillus*, *Candida*, and *Cryptococcus* infections at dosages from 3 to 6 mg/kg/day [14]. While exposure to L-AMB is known to cause pseudohyperphosphatemia on PHOSm assays, to our knowledge there is no description in the literature of L-AMB-associated pseudohypophosphatemia. We report a case of spurious hypophosphatemia in a patient receiving high-dose liposomal amphotericin B therapy and characterize this previously unrecognized occurrence using in vitro investigations.

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## 2. Material and methods

### 2.1. Biological samples

All studies were performed using residual, anonymized plasma specimens without hemolysis. This study was performed as part of ongoing quality improvement studies at the University of Washington Department of Laboratory Medicine and was therefore not considered human subjects research.

### 2.2. Drugs

Drugs were purchased through the University of Washington Medical Center inpatient pharmacy. A single lyophilized vial of L-AMB (AmBisome®) containing 50 mg of amphotericin B was reconstituted with 12 mL of sterile water to yield a 4.16 mg/mL amphotericin B solution, as recommended by the manufacturer [14].

### 2.3. Measurement procedures

Two assays were utilized for inorganic phosphate measurement: AU 680/5812 (Beckman Coulter, Brea, CA) and Vitros 4600 (Ortho Clinical Diagnostics, Raritan, NJ) (Table 1).

The method used by the AU series of instruments is based on a modification of the method developed by Daly and Ertingshausen [15]. Briefly, inorganic phosphate reacts with molybdate to form a heteropolyacid complex in an acidic environment, with complex formation monitored in an end-point reaction by absorbance at primary and secondary wavelengths of 340 nm and 380 nm, respectively. Net absorbance is directly proportional to the inorganic phosphorus concentration in the sample.

The Vitros 4600 uses the same chemistry in a slide format to produce the heteropolyacid complex. This complex is then reduced by *p*-methylaminophenol sulfate to give a stable heteropolymolybdenum blue chromophore, which is measured by reflectance spectrophotometry at 670 nm and is directly proportional to the inorganic phosphorus concentration in the sample.

### 2.4. Interferences testing

For dose-relationship studies, residual plasma samples were combined to create low and high pools with phosphate concentrations of approximately 4 mg/dL and 6 mg/dL, respectively. Equal volumes of the reconstituted drug or water were spiked into 0.5 mL aliquots of pooled sample to generate various concentrations: 10, 20, 30, 40, and 50 mcL of L-AMB to generate approximate serum L-AMB concentrations of 83, 166, 250, 333, and 416 mcg/mL, respectively. These concentrations were chosen to test a range of therapeutically achievable concentrations, though prescribing information suggests that patients on high-dose L-AMB have maximum serum concentrations of 83 ± 35 mcg/mL [14].

**Table 1**
Analytical specifications for two commercial inorganic phosphorus assays.

| | Beckman AU 680/5812 | Ortho Vitros 4600 |
|---|---|---|
| Analytical measurement range (mg/dL) | 1.0–20.0 | 0.5–13.0 |
| Reportable range (mg/dL) | 1.0–60.0 | 0.5–26.0 |
| Adult reference range (mg/dL) | 2.5–4.5 | 2.8–4.6 |
| Low QC mean (% CV) | 2.0 (3.6%) | 3.5 (1.4%) |
| High QC mean (% CV) | 7.1 (1.0%) | 7.7 (1.6%) |
| Reaction type | End-point | End-point |
| Primary wavelength (nm) | 340 | 670 |
| Secondary wavelength (nm) | 380 | N/A |

For studies comparing the AU and Vitros phosphate methods, ten plasma specimens were selected to represent a range of phosphate concentrations (approximately 4 to 12 mg/dL) typically encountered in our laboratory and for which phosphate concentrations would still be measurable post-spike. Each of these samples (0.5 mL) was spiked with 30 mcL of reconstituted drug for an estimated drug concentration of 250 mcg/mL. This concentration was chosen based on results of the dose-relationship studies described above. Control specimens were diluted with an equivalent volume of water. Water- and drug-spiked specimens were stored at 4 °C until analysis, which occurred within 5 h of sample preparation.

## 3. Results and discussion

### 3.1. Case

A 58 year old woman with a medical history remarkable for IgA kappa-type multiple myeloma, recovering from a matched unrelated peripheral blood stem cell transplant one month prior, presented with treatment-related nausea and vomiting. Day 28 post-transplant bone marrow biopsy showed no evidence of disease, but her course had been complicated by an aspergillus pneumonia for which she was treated with L-AMB (5 mg/kg/day intravenous) and micafungin.

After administering L-AMB, the patient's laboratory values were consistently hypophosphatemic (Beckman Coulter AU) (Fig. 1A). However, she did not demonstrate symptoms of phosphate depletion, and there was no clear mechanism for the sudden onset of her hypophosphatemia. Despite increasing repletion, her plasma phosphate concentrations remained lower than expected, hovering around 2 mg/dL. The clinical team contacted the laboratory regarding possible interferences.

Plasma phosphate was quantified using an alternative method (Vitros 4600). Discrepant results between platforms supported the presence of interference (Fig. 1A, dashed line). Non-linear phosphate measurements in serially diluted specimens identified the AU method as erroneous (data not shown). For the remainder of the patient's hospitalization, phosphate concentrations were measured on the Vitros 4600. L-AMB was continued for an additional month after discharge, and the patient remained artificially hypophosphotemic to as low as 1.1 mg/dL when measured on Beckman AU analyzers. After drug discontinuation, the patient's hypophosphatemia corrected. Day 84 post-transplant monitoring showed no evidence of disease in her bone marrow, no evidence of gammopathy in SPEP and UPEP, and a consistent serum phosphate concentration between AU (4.9 mg/dL) and Vitros (5.2 mg/dL) measurements.

### 3.2. Interference characterization

#### 3.2.1. Exploration of dosage relationship to interference

We assessed the dosage-related effect of L-AMB on measured phosphate concentration by spiking 10 to 50 mcL of the reconstituted drug or water control into five 0.5 mL aliquots of pooled remnant plasma. The dose-dependent interference was characterized for two phosphate concentrations (Fig. 1B). As expected, increasing drug concentrations imposed a proportional negative bias on measured phosphate concentrations. Interestingly, at a drug concentration near the expected maximum concentration in patients receiving high-dose therapy, no effect on phosphate was observed. However, a considerable negative bias of > 3 mg/dL was present with a three-fold increase in drug concentration. The magnitude of this bias resembles the extent of pseudohypophosphatemia consistently observed in our index patient, supporting the notion that L-AMB concentrations high enough to induce this effect may be found in clinical specimens either as an accurate reflection of the physiologic concentration or resulting from a pre-analytic error (e.g., line contamination). A limitation of our study is that we were unable to confirm that the concentration of L-AMB in