Interferences in the measurement of circulating phosphate: a literature review

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Abstract

Background
Inorganic phosphate in blood is currently determined by the reaction with molybdate. This report aims at reviewing conditions underlying spuriously altered levels of circulating inorganic phosphate.

Content
A systematic search without language restriction of the Excerpta Medica, the National Library Database and the Web of Science database was conducted from the earliest publication date available through January 31, 2020.

Summary
For the analysis, 80 reports published in English (n=77), French (N=1), German (n=1) and Spanish (n=1) were retained. Well documented pseudohyperphosphatemia was observed in individuals exposed to liposomal amphotericin, in patients affected by a gammopathy, in patients with hyperlipidemia and in patients with hyperbilirubinemia. An unexplained elevated inorganic phosphate level sometimes provided a clue to the diagnosis of a gammopathy. Well documented cases of pseudohypophosphatemia were observed in patients on large amounts of intravenous mannitol. Finally, pseudohypophosphatemia was occasionally observed on treatment with liposomal amphotericin and in patients with a gammopathy.

Outlook
In order to avoid unnecessary testing and treatment, the phenomenon of spuriously altered inorganic phosphate should be recognized. An unexplained hyperphosphatemia may provide a clue to the diagnosis of a gammopathy or a severe hyperlipidemia.
Introduction

Phosphate is present within the blood in organic (phospholipid, phosphoprotein) and inorganic forms, but it is the inorganic form that is determined routinely [1]. Hyperphosphatemia is relatively uncommon and is most often observed in patients with kidney injury, hypoparathyroidism, cellular lysis and excessive phosphate intake or administration [1]. On the other hand, hypophosphatemia results from decreased intestinal uptake, increased urinary excretion, or movement into cells [2]. Molybdate, which reacts with inorganic phosphate to produce a phosphomolybdate complex, is currently used for the determination of this ion in serum or plasma samples [3]. Narrative reports published in the seventies first insinuated the existence of drug-induced interferences [3]. The potential risk of a spuriously altered inorganic phosphate measurement is not inconsequential. The purpose of this report is to review the literature addressing the conditions and the mechanisms underlying spuriously altered levels of circulating phosphate.

Methods

Literature search strategy

This communication is presented in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses [4]. A systematic search without language restriction [5] of the Excerpta Medica, the National Library Database and the Web of Science database was conducted from the earliest publication date available through January 31, 2020. The following search terms were used: “Pseudohyperphosphatemia” OR “Spurious hyperphosphatemia” OR “Factitious hyperphosphatemia” OR “Pseudohypophosphatemia” OR “Spurious hypophosphatemia” OR “Factitious hypophosphatemia”. Following elimination of duplicates, titles and abstracts of the search results were
screened for relevance by two authors (V.M. and M.G.B.). The texts of the remaining results were independently assessed by the mentioned authors for inclusion. The list of ultimately included publications was decided on by discussion, with agreement required for inclusion. The references of included reports were screened for additional studies. No disagreements required resolution by a third author.

**Selection criteria**

Articles and letters detailing subjects with spuriously altered inorganic phosphate values were eligible. Reports were included uniquely if the falsely altered laboratory values normalized after eliminating the underlying interference or if the mechanism underlying the interference was properly documented by in vitro studies.

**Results**

**Search Results**

The literature search process is summarized in Figure 1. The chance-adjusted agreement between the two investigators on the application of inclusion and exclusion criteria was 0.88. For the final analysis, we retained 80 reports [6-85] published between 1981 and 2020 in English (N=77), French (N=1), German (N=1) and Spanish (N=1). They had been reported from the following continents: 35 from America (United States, N= 32; Argentina, N=1; Brazil, N=1; Canada, N=1), 29 from Europe (United Kingdom, N=6; France, N= 5; Netherlands, N=5; Turkey, N=4; Greece, N=2; Switzerland, N=2; Belgium, N=1; Finland, N=1; Italy, N=1; Spain, N=1; Sweden, N=1), 12 from Asia (India, N=3; Israel, N=3; People's Republic of China, N=2; Islamic Republic of Iran, N=1; South Korea, N=1, Singapore, N=1, Thailand, N=1), two from Afrika (Morocco, N=1; Tunisia, N=1) and two from Oceania (Australia, N= 2).
Spurious hyperphosphatemia

Exogenous causes

- Liposomal Amphotericin B

Six clinical reports [6, 7, 9-12] containing each from one to 80 cases, analyzed 196 individuals exposed to liposomal amphotericin, who were surprisingly found to have elevated phosphate levels.

To support the hypothesis of spurious hyperphosphatemia, Bohm [11] compared the prevalence of hyperphosphatemia in 80 adults managed with liposomal amphotericin and in patients managed with triazole antifungals. The prevalence of hyperphosphatemia was found to be significantly higher in liposomal amphotericin patients both with normal (approximately 40% versus 10%) or poor renal function (approximately 60% versus 20%). Knoderer [9] found that 21 of 64 oncology children managed with liposomal amphotericin had new onset hyperphosphatemia. The diagnosis of spurious hyperphosphatemia induced by liposomal amphotericin was made for two reasons. First, the prevalence of high phosphate level was more common in children treated with liposomal (18 out of 40 cases) as compared with those treated with lipid complex amphotericin (3 out of 24 cases; P<0.0001). Second, none of the children had a poor kidney function. Miller [10] found 36 children with hyperphosphatemia while on treatment with liposomal amphotericin. He made the presumptive diagnosis of spurious hyperphosphatemia because none of them presented conditions known to predispose to hyperphosphatemia such as poor renal function or co-medication with drugs known to alter the concentration of this ion. In 5 cases [6, 7, 12] of hyperphosphatemia, the diagnosis of spurious hyperphosphatemia caused by liposomal amphotericin was made because circulating inorganic phosphate normalized after removing amphotericin from the blood sample by ultrafiltration. Finally, an in vitro study [8] demonstrated that liposomal amphotericin interferes with the determination of inorganic phosphate.
• Other medicinal products

Both the fibrinolytic agent alteplase and heparin contain excipients rich in inorganic phosphate. Unsurprisingly, therefore, 11 case reports documented the existence of spurious hyperphosphatemia in blood samples contaminated with these agents. Alteplase was associated with spurious hyperphosphatemia in one child [15] and eight adults [16, 17]. On the other hand, heparin caused pseudohyperphosphatemia in each one child [14] and one adult [18]. Finally, spurious hyperphosphatemia was noted in a 55-year-old man on long-term hemodialysis because a saline solution containing inorganic phosphate was used to dilute the patient’s serum sample in the laboratory [13].

Endogenous causes

• Hyperlipidemia

Spurious hyperphosphatemia was documented in 18 patients with cloudy serum secondary to hyperlipidemia [19, 20]. In these patients, the tendency to hyperphosphatemia disappeared after removing lipids by ultrafiltration.

• Hyperbilirubinemia

Spurious hyperphosphatemia was documented in 6 patients [21] with severe hyperbilirubinemia (>330 µmol/L). This tendency was confirmed in 4 reports examining the interference from bilirubin on the determination of this ion [22-25].

• Gammopathy

Forty-one reports published between 1986 and 2020 [26-33, 35, 36, 39-57, 59-64, 66-69] documented 258 cases of monoclonal gammopathy associated with spurious hyperphosphatemia: 150 cases affected by multiple myeloma, 4 by Waldenström’s macroglobulinemia and 104 by further forms of monoclonal gammopathy. In many cases, phosphate concentration was found normal after removing proteins from serum or plasma [26-28, 30, 32, 33, 36, 39, 41-43, 45, 47, 49-54, 55, 56, 64, 66, 68, 71].
Spurious hyperphosphatemia was also documented in 12 patients found to have a polyclonal gammopathy: chronic liver disease (N=9; 32, 65], low grade splenic marginal zone lymphoma [N=1; 58], AIDS related syndrome [N=1; 32, 37] and colon carcinoma (N=1; 32].

Finally, two reports addressed the interaction between circulating paraprotein concentration and the increase in phosphate concentration. Bowles et al. [33, 38] investigated 35 patients with paraproteinemia secondary to multiple myeloma, Waldenström's macroglobulinemia or monoclonal gammopathy of uncertain significance. These authors found that the spurious elevation in phosphate concentration positively correlates with the paraprotein concentration. Duly E et al. [29, 34] investigated 15 patients with multiple myeloma and found that only paraproteins at levels ≥3 g/L interfere with the determination of inorganic phosphate.

In 17 cases, an unexplained elevated phosphate level provided a clue to the diagnosis of a gammopathy [28, 29, 32, 35, 40, 48, 49, 51, 53, 64, 56, 59, 61, 62, 67, 70].

Finally, artfactually elevated potassium and phosphate serum values were observed in a patient with very severe thrombocytosis (2,700 x 10⁹/L) after splenectomy. Concurrent determination of potassium and phosphorus in serum and plasma revealed that the patient actually had normal potassium and phosphorus levels [72].

**Spurious hypophosphatemia**

Factitious hypophosphatemia was observed [73, 75] in three female patients (10, 45 and 72 years of age) on large amounts of intravenous mannitol (resulting in mannitol levels of approximately 20-25 mmol/L). The underlying mechanism is an interference in the reaction of molybdate with inorganic phosphate that decreases the rate of phosphomolybdate formation [74].

Spurious hypophosphatemia was also documented in a 58-year old female managed with high-dose liposomal amphotericin B [79] and
in 8 patients (6 males and 2 females aged from 60 to 85, median 69 years) affected by a monoclonal gammopathy [63, 76, 77, 79, 80, 82, 83]: multiple myeloma (N=6), monoclonal gammopathy of undetermined significance (N=1) and Waldenström's macroglobulinemia (N=1). In the mentioned 8 patients, circulating phosphorus concentration normalized when determined in deproteinated serum. Spurious hypophosphatemia was also observed in a patient with a polyclonal gammopathy [74]. Finally, a 40-year old female affected by acute myeloid leukemia [81], spuriously low phosphorus and potassium levels were associated with extreme hyperleukocytosis (310 x 10⁹/L), a recognized cause of cellular uptake of these ions.

Discussion

Laboratory tests are a more and more important component of medical evaluation. The results of this review on spuriously altered levels of circulating phosphate complement an elegant report published 25 years ago [41] and can be summarized as follows. First, drug therapy with liposomal amphotericin B tends to cause spurious hyperphosphatemia and, by far less frequently, hypophosphatemia. Second, spurious hyperphosphatemia occurs in patients with severe hyperlipidemia, severe hyperbilirubinemia or a gammopathy. Like in the case of drug therapy with liposomal amphotericin B, spurious hypophosphatemia has also been documented, albeit exceptionally, in the context of a gammopathy. Third, limited but well documented evidence points out that pseudohypophosphatemia may occur on treatment with intravenous mannitol. Finally, spurious hyperphosphatemia may unsurprisingly occur if blood for the determination of phosphate is drawn from a vascular catheter locked with phosphorus containing heparin or alteplase or in the presence of severe thrombocytosis. The frequency and clinical relevance of these conditions are suggested in Table 1.
Significant effort has been devoted to the determination of phosphate using new strategies including phosphate-selective electrodes, biosensors and vanadate [1]. Nonetheless, 100 years after the initial report [2], almost all techniques used to determine phosphate are based on the reaction of phosphate with molybdate to form a colourless phosphomolybdate complex. A voluminous literature exists on the analytical strategy. The colourless phosphomolybdate complex may be detected directly at 340 nm or reduced to molybdenum blue and measured at 600 to 700 nm. An acidic pH is required for the formation of molybdenum blue, but it must be controlled because both complex formation and reduction of molybdate are pH-dependent. A less acidic pH can result in spontaneous reduction of molybdate. The rate of complex formation is also influenced by protein concentration. Solubilizing agents are also sometimes used to prevent protein precipitation. Assessment of unreduced complexes has advantages, including simplicity, speed and reagent stability. Disadvantages of this technique include greater interference by hemolysis, hyperlipidemia and hyperbilirubinemia when determination is made at 340 nm. Various reducing agents have been used in producing the molybdenum blue complex, including aminonaphtholsulfonic acid, ascorbic acid, ferrous ammonium sulfate, methyl-p-aminophenol, semidine hydrochloride and stannous chloride [1]. Currently there are no guidelines or agreement among laboratories on a suitable analytical method without interference.

In everyday clinical practice, the identification of spuriously altered levels of inorganic phosphate is challenging and is made in three steps (table 2). Misdiagnosing this laboratory error may result in redundant investigations and unnecessary interventions aiming at treating this in vitro phenomenon.

Three communications point out that hyperphosphatemia is highly prevalent in nephrotic syndrome [82-84 86-88]. Since this kidney disease is usually associated with severe hyperlipidemia and the issue of spurious hyperphosphatemia was not discussed, we
speculate that hyperphosphatemia was spurious in these studies [89].

There are limitations and strengths that should be considered when reading this communication. The major limitation results from the somewhat small number of published cases of spuriously altered levels of circulating inorganic phosphate and their low-grade evidence (case reports and case series, sometimes poorly documented). A further limitation results from the fact that the final proof of spuriously altered concentration of inorganic phosphate induced by liposomal amphotericin, high dose mannitol and hyperlipidemia is problematic in everyday practice. Finally, we were not able to identify the mechanisms underlying the interferences observed in the literature. The most relevant strength relates to the exhaustive literature search, which aimed at surveying the entire literature.

In conclusion, clinicians and clinical biochemists need to be aware of the phenomenon of spuriously altered inorganic phosphate levels [1, 41] to avoid confusion and unnecessary testing or treatment (including among others medication with phosphate binders). In addition, an unexplained elevated inorganic phosphate level may provide a clue to the diagnosis of a gammopathy or a severe hyperlipidemia. Finally, we encourage communication between clinicians and biochemists and further optimization of laboratory techniques [90].

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**Figure 1 - Legend**

Spuriously altered circulating inorganic phosphate. Flowchart of the literature search process.