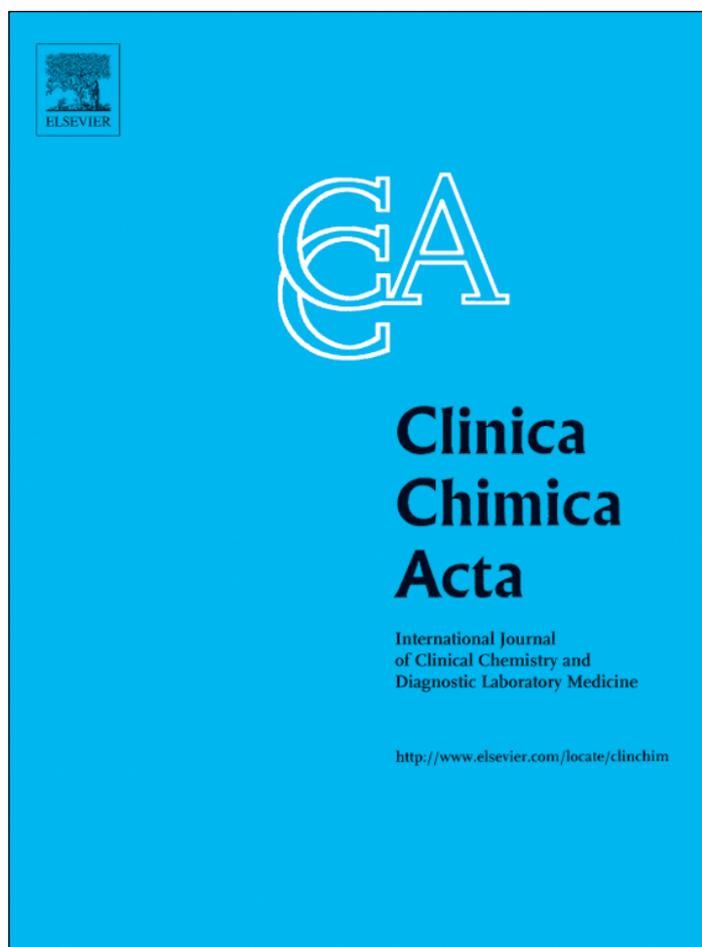


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Hypercalcemia and elevated concentration of vitamin D: A case report too easy to be true



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ABSTRACT

Background: Endogenous (heterophile, human *anti*-animal ...) antibodies are a known cause of interference in immunoassays.

Case report: A patient with hypercalcemia and low PTH levels was investigated. The serum 25OH vitamin D (25OHD) concentration was above the analytical range of the automated analyser (>150 ng/mL) but serum dilutions were not linear. A myeloma-related monoclonal peak of immunoglobulin G (30 g/L) was found.

Results: Alternative 25OHD assays (RIA, automated analysers, mass spectrometry) all found concentrations <25 ng/mL. Nab™ columns (Thermo Scientific) eliminated the endogenous immunoglobulin from the serum thus allowing the initial analyser to provide correct results.

Discussion and conclusion: The potentially misleading point was that the apparent very high 25OHD levels were concomitant with hypercalcemia and low PTH levels thus mimicking vitamin D intoxication. Identifying assay interferences requires clinical awareness but, when suspected, one should be aware that technical tools or alternate assays are available to correct some interferences, including monoclonal immunoglobulins.

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A 61 yr old male patient was investigated in the university hospital of Bordeaux for asthenia, nausea, anorexia. He complained about intense thirst and was clinically dehydrated. He has lost 8 kg during the last 3 wk. Biological examination revealed hypercalcemia (2.9; ref [2.2;2.6] mmol/L) with acute kidney failure. PTH was low (12; ref [12;88] pg/mL, DxI analyser, Beckman-Coulter) excluding the diagnosis of primary hyperparathyroidism. The patient denied any oral vitamin D intake but 25OH vitamin D (25OHD) concentration was above the analytical range of the automated analyser (>150 ng/mL; deficiency < 10 ng/mL, insufficiency 10–30 ng/mL, sufficiency 30–100 ng/mL, toxicity > 100 ng/mL) (Liaison XL, Diasorin) suggesting the diagnosis of vitamin D intoxication. However, dilutions of the patient's serum showed non-linear concentrations of 25OHD: dilution 1:2 > 150 ng/mL, 1:5 40 ng/mL, 1:10 40 ng/mL. Similar dilutions of another serum sample from this patient gave the same results. This was highly evocative of the existence of an analytical interference. We then attempted to eliminate hypothetical heterophilic antibodies

using heterophilic blocking tubes (HBT, Scantibodies, Santee, CA, USA). No linearity of the concentrations was obtained after dilution of this treated serum: no dilution > 150 ng/mL, 1:21 19 ng/mL, 1:4 40 ng/mL. The results of serum protein analysis suggested another possible cause of interference. Indeed, protein electrophoresis – requested because of an elevated serum C reactive protein level – showed a monoclonal peak of immunoglobulin G (29.8; ref [6.7;12.8] g/L) with elevated free lambda chains (734; ref [8.3;27.0] mg/L) that was subsequently attributed to a myeloma. We used a Nab™ column (Thermo Scientific) to eliminate the endogenous immunoglobulins from the patients' serum and to subsequently assay 25OHD. 25OHD concentration in this “cleaned” serum sample was then found dramatically reduced: 18.0 ng/mL. To further characterise this interference evidenced when using the Liaison assay four alternative 25OHD assays were conducted. The results of the latter – in untreated serum – were consistent: 16.0 ng/mL (25-hydroxyvitamin D RIA, Diasorin, Sallugia, Italy), 10.3 ng/mL (Cobas, Roche, Meylan, France), 22.0 ng/mL (iSYS, IDS, Bolton, UK), 14.0 ng/mL (LC-MSMS, tandem mass spectrometry [1]).

Taken together, we present here a case of analytical interference in 25OHD automated assays likely caused by a peak of immunoglobulin G which was suppressed by eliminating the immunoglobulin using a

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commercially available device. This interference was not obvious in the alternative assays that were conducted although we cannot totally exclude that it had some influence. Indeed, the difference between the 25OHD concentration measured with the Cobas and the iSYS platform in this patient (more than twice higher with the iSYS than with the Cobas analyser) may be considered higher than what can be expected from the results of the DEQAS proficiency testing where the inter-method variability of these two assays is around 20% for this range of concentrations. Anyway, even if this interference had some effect with one or several of the alternative assays that we performed, it was much weaker than with the Liaison XL and had no consequence on the clinical interpretation.

Interferences caused by abnormal immunoglobulin concentrations have been reported when using immunoassays. For instance, various monoclonal increases of serum immunoglobulins caused interferences in nephelometric assays [2], in radioimmunoassays for beta-endorphin [3] or tri-iodothyronine [4] and in chemiluminescent assays [5]. Interestingly, we encountered a case very similar to the first one: 25OHD concentration above the analytical range of the automated analyser (>160 ng/mL) (Architect C8200, Abbott Diagnostics, Abbott France, Rungis). No linearity of the concentrations was obtained after dilution. When assayed by the same LC–MSMS than for the first case, the 25OHD concentration was 25.2 ng/mL. Protein electrophoresis showed a monoclonal peak of immunoglobulin A (76 g/L). Although no further investigation could then be performed this immunoglobulin was likely involved in the interference. Automated assays dissociate 25OHD from vitamin D-binding protein (VDBP) before performing a competitive assay [6]. The Liaison and iSYS use proprietary agents in buffers with ethanol. The Architect assay uses 8-anilino-1 naphthalene sulfonic acid in triethanolamine methanol buffer. The Cobas uses, after denaturing endogenous VDBP, ruthenylated VDBP to bind 25OHD. The Diasorin RIA is preceded by an acetonitrile extraction step. From incomplete data about proprietary buffers it is impossible to further speculate on successes and failures of these assays in the present case. To ascertain assay interference one can perform four different investigations, each with merits and some pitfalls [7–11]. These investigations include serum dilution, use of different assays, or extracting the interfering analytes and identifying them. Firstly, performing serial dilutions of the serum showed here a non-linear distribution of the 25OHD concentrations. Note that, such a procedure is usually inappropriate with free hormone assays. Secondly, analysing the sample with different assays gave us critical information, as the results were clearly not similar to our initial automated assays. This is a frequent finding for various reasons: the specificities of antibodies, the species they originate from, and the means developed by each manufacturer to minimize antibody-related interference. Thirdly, an investigation of plausible suspects identified a potential culprit, a monoclonal immunoglobulin G. Fourthly, eliminating this potentially interfering immunoglobulin was successful in our hands when we used a Nab™ column. Various Thermo Scientific NAb Spin Columns are available for the purification of antibodies. They consist in immobilized forms of different proteins with various affinities for immunoglobulins and may be used to

eliminate the latter from serum. Here, although a likely culprit the monoclonal peak of immunoglobulin cannot be uncontroversially proven guilty as the columns may have suppressed other interfering immunoglobulins.

In conclusion, close interactions between clinical and biological departments reduce both the risks of errors and the delays to correct analytical interferences. Here, the potentially misleading point was that these apparent very high 25OHD levels were concomitant with hypercalcemia and low PTH levels thus mimicking vitamin D intoxication. Although well known, assay interferences often remain difficult to detect in routine processing. Identifying them requires clinical awareness but, when suspected, one should be aware that technical tools or alternate assays are available to correct some interferences.

Disclosure treatment

The authors have nothing to disclose.

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