Falsely elevated levels of 25(OH) vitamin D measured on alinity: two case reports

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ABSTRACT

Background: Conventional biochemistry parameters employ the use of photometry and enzymatic reactions which reduce the possibility of analytical errors. On contrary, tests performed with immunoassay are known to be more prone to analytical interferences and may subsequently yield incorrect values.

Case presentation: We present two cases investigated due to very high levels of vitamin D in the serum that exceeded the measurable limit of the analyzer. Protein electrophoresis showed a monoclonal peak, revealing elevated immunoglobulin G and elevated immunoglobulin M respectively. Gold standard analysis of vitamin D using liquid chromatography-mass spectrometry revealed normal vitamin D concentrations in both cases. Further, bone marrow puncture revealed a diagnosis consistent with multiple myeloma and Waldenströms macroglobulinemia.

Conclusion: Falsely elevated levels of vitamin D exceeding the maximum measurable limit is highly suspicious. Although analytical interference in immunoassay is limited, we should keep in mind that results obtained by this method are more prone to analytical errors.

Keywords: Immunoassay interference, 25(OH) vitamin D, falsely elevated, paraprotein, case report.

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Background

Immunologically based tests, such as immunoassays, are more prone to analytical errors than routine laboratory tests that use enzymatic and photometric methods, which are on the contrary more prone to pre-analytical or administrative errors [1]. Most analytical errors in immunologically based tests occur due to interference from other substances in the sample, leading to incorrect results. According to Ismail et al. [2] the cited incidence of interference in immunoassay varies from about 0.4% to 4%, and it is mainly caused by an inappropriate "cross-binding reaction" [3]. The antigen-antibody binding reaction is primarily dependent on the shape of the molecule and the electron at the binding site, therefore molecules with similar shapes can compete and bind with the receptor site, which can affect the monospecificity in immunoassay. The level of interference depends on the concentration of the interfering substance, and on its affinity/avidity to the binding site. The interferences in immunoassay mostly occur due to the presence of other substances that usually lead to falsely elevated results or rarely falsely low results. We recently encountered two cases of falsely elevated levels of a total of 25-hydroxy vitamin D [25 (OH) vitamin D] with immunoassay, measured with Alinity ci-series (Abbott, Chicago, IL).

Case Presentation

The first case is a 78-year-old woman who was admitted due to deterioration of her general condition, fatigue, and repeated episodes of falling. This patient had hypothyroidism from a previous hemithyroidectomy which prompted the intake of L-thyroxine 50 mcg once daily. Thyroid parameters were normal Thyroid stimulating hormone 0.55 mU/l (0.35-4.94), fT4 14.7 pmol/l (9.0-17.0), biological examination revealed mild hypercalcemia 3.34 mmol/l (2.2-2.57) with acute kidney failure in addition to chronic kidney failure. Parathyroid hormone was low at 11.8 ng/l (15.0-68.3), excluding the possibility of primary hyperparathyroidism. The patient was receiving vitamin D supplementation of D-cure® 25,000 IU once a month (recommended dose 25,000 IU once a week) with a measured total 25 (OH) vitamin D concentration above the analytical limit of the analyzer >384.38 nmol/l (hypervitaminosis >250 nmol/l, intoxication >375 nmol/l) [4].

The second case is a 55-year-old woman who came for a follow-up after a breast reconstruction surgery and had complaints of blurred vision, headache, and low blood pressure. There was no relief of symptoms after rest and pain medication, and the patient was referred to internal medicine for investigation. The patient had a history of

Table 1. *Results of serial dilutions of the samples (calculations by alinity-ci).*

TOTAL 25 (OH) VITAMIN D NMOL/L	WITHOUT DILUTION	½ DILUTION	⅓ DILUTION	¼ DILUTION	⅛ DILUTION
Patient 1	>384.38	253.84	122.55	46.43	/
Patient 2	>384.38	89.86	/	17.97	<8.74

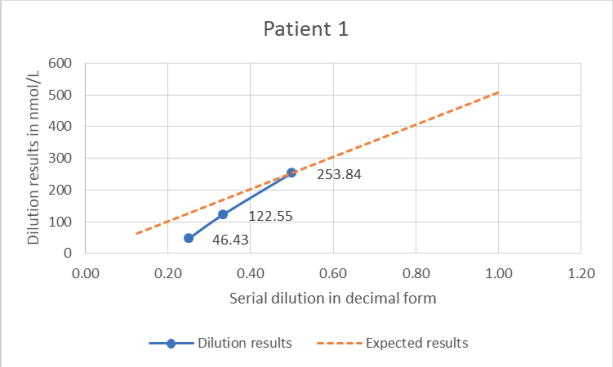

Figure 1. *Linear plot of serial dilutions in patient 1.*

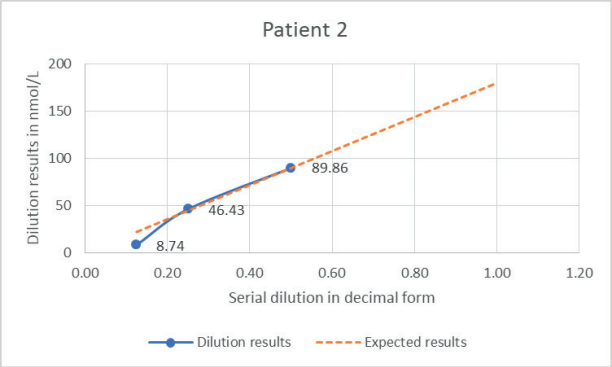

Figure 2. *Linear plot of serial dilutions in patient 2.*

Crohn's disease, osteoporosis, fibromyalgia, and esophageal spasm, and has a Breast cancer gene mutation which prompted the bilateral mastectomy. Laboratory results showed macrocytic normochromic anemia, hemoglobin 72 g/l (117-151), hematocrit 23.0% (35.4-46.1), Mean corpuscular volume 103 fl (83-99), slightly elevated vitamin B12 739.5 ng/l (130-651.65), and an elevated protein level 123 g/l (64-83). The patient was taking D-cure® vitamin D supplement 25,000 IU once a week and the measured total 25 (OH) vitamin D level was, identical to the aforementioned case, above the analytical limit of the analyzer >384.38 nmol/l.

Discussion

Vitamin D is a fat-soluble prohormone comprising two relevant forms – vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). Both forms can be absorbed from food, but vitamin D2 mainly comes from an artificial source such as supplements. Approximately only 10%-20% is supplied from nutritional intake, which implies the requirement for supplementation. Vitamin D from food and supplements needs to be converted to its active form through two hydroxylation reactions. The first hydroxylation occurs in the liver, producing 25 (OH) vitamin D, which is the major storage form. The second hydroxylation occurs in the kidneys which converts the 25 (OH) vitamin D into the biologically active form 1,25-dihydroxy vitamin D (1,25 $(OH)_2$ vitamin D), also known as calcitriol. The 25 (OH) vitamin D2 and 25 (OH) vitamin D3 are present in the blood in much higher concentrations than the biologically active form and have a longer half-life of 2-3 weeks versus 4 hours. Hence, a total of 25 (OH) vitamin D (D2 and D3) is a better analyte for the determination of vitamin D status.

The elevated values of a total of 25 (OH) vitamin D in both patients suggested vitamin D intoxication, which usually manifests with symptoms secondary to increased calcium levels or hypercalcemia. Symptoms would include confusion, polydipsia, polyuria, vomiting, anorexia, and muscle weakness; however, these symptoms were not compatible with the clinical status of both patients. Additionally, they were no suspicion of overdosage with vitamin D supplementation, which is the most frequent cause of vitamin D intoxication.

Serial dilutions of the serums were made to obtain samples with decreasing concentrations which can subsequently provide measurable values of a total of 25 (OH) vitamin D. The results of these serial dilutions showed non-linearity and are listed in Table 1. The linear plot of serial dilutions in patients 1 and 2 are shown in Figures 1 and 2, respectively. These linear plots were based on the serial dilution results of the samples compared to the expected reference values per measured dilution factor.

According to the manufacturer, analytical interferences can occur from the effects of high concentrations of triglycerides (>500 mg/dl), which were not elevated for both patients. However, case reports published for analytical interferences that occurred in vitamin D automated immunoassay were caused mainly by elevated immunoglobulin levels [5,6]. Hence, to check for interference, immunoglobulin levels were measured, and serum protein electrophoresis and serum immunotyping was carried out. The results of the serum protein analysis suggested the possible cause of interference in both cases. The first patient had normal IgA 0.84 g/l (0.69-5.17) and IgM 0.20 g/l (0.33-2.93) levels with elevated IgG 68.14 g/l (5.52-16.31). Protein electrophoresis

Table 2. *Summary results of vitamin D with different analytical platforms.*

TOTAL 25 (OH) VITAMIN D NMOL/L	ALINITY CI-SERIES, ABBOTT (CLIA)A	COBAS 6000, ROCHE (ECLIA)B	ATELLICA, SIEMENS (CLIA)C	SCIEX 5500 QTRAP, (LC-MS/MS)D
1	>384.38	104.33	154.75	102.84
2	>384.38	70.64	97.34	53.17

[a]Abbott Laboratories, IL. [b]Roche Diagnostics, Basel, Switzerland. [c]Siemens Healthineers AG, Erlangen, Germany. [d]Sciex, Framingham, MA.

showed a monoclonal peak in the gamma fraction and consequent immunofixation revealed a paraprotein IgG lambda (Sebia Hydrasys-2, Paris, France) and elevated lambda free chains 582.1 mg/l (5.7-26.3; Optilite Binding site, Birmingham, United Kingdom). The second patient had an elevated IgM >65.0 g/l, decreased IgG <1.08 g/l, and normal IgA 0.35 g/l levels. Protein electrophoresis showed a monoclonal peak in the gamma fraction and immunofixation revealed a strong paraprotein IgM kappa and minimal paraprotein IgG lambda.

The two serum samples were sent to three other laboratories for the measurement of vitamin D on different analytical platforms. Two platforms utilized chemiluminescence immunoassay (CLIA) from different manufacturers, measuring the total 25 (OH) vitamin D, while the remaining platform used a liquid chromatography-mass spectrometry (LC-MS/MS), which is considered the gold standard for vitamin D quantification, measuring 1,25 $(OH)_2$ vitamin D. The LC-MS/MS machine is a chromatography system from Shimadzu (Kyoto, Japan) tandem with a mass spectrometer from Sciex 5,500 QTRAP (Framingham, MA). The results are shown in Table 2. Variability in the results of vitamin D measurement on the different platforms is due to the lack of standardization for quantification, which was demonstrated in a recent study conducted by the vitamin D External Quality Assessment Scheme [6]. The values obtained with these platforms were two to five times lower compared to the original results obtained with Alinity. The internal quality control utilized in Alinity is from an external body, BioRad laboratories (California), performed once every 24 hours with 2 levels, while external quality control is from Sciensano (government quality management system organization of Belgium), performed 3-4 times yearly.

After detection of the analytical interferences and confirmation of normal 1,25 $(OH)_2$ vitamin D levels, bone marrow puncture was advised for both patients. In the first patient, this revealed an image consistent with multiple myeloma or Kahler's disease with a displacement of the erythroblastic and megakaryocytic series by a population of moderately atypical plasma cells (36%). Bone marrow puncture in the second patient revealed 57% lymphoplasmacytic cells, an image compatible with Waldenströms macroglobulinemia. The patient was admitted and immediately received plasmapheresis for persisting hyperviscosity symptoms and was discharged after 6 days of hospitalization.

When a sample is suspected to contain interfering substances, several steps or techniques can be carried out. These investigations include serum dilution, use of commercially available heterophilic antibody blocking tubes, identification of interfering substances, and use of different assay platforms [9]. However, despite advances in technology and understanding of the mechanisms of immunoassay interferences, there is no single procedure that can rule out all possible interferences. Analytical interferences are well known but remain difficult to detect in routine processes and laboratory staff needs to be aware of these possibilities. Lastly, good communication between the clinical setting and the laboratory staff is necessary to reduce the risks of errors and avoid unnecessary investigations and inappropriate treatments.

The findings in our cases are partially comparable to a case report by Whittle et al. [7] which described a patient with artefactually elevated total 25 (OH) vitamin D caused by an interference with IgM kappa paraprotein in the setting of an undiagnosed Waldenstrom's macroglobulinemia. Similarities of these cases include the use of the same platform but different models (Alinity-ci series *vs.* Abbott Architect) for the quantification of a total of 25 (OH) vitamin D. In addition, assessment of linearity with serial dilutions showed non-linearity in both our cases in contrary to the case report by Whittle et al. [7].

Limitations of this case report include the lack of heterophilic antibody blocking tubes which can inhibit the occurrence of interference and the determination of linearity which was only performed once per patient.

Conclusion

In conclusion, interferences in immunoassays are a known phenomenon, and results obtained with this method are more prone to analytical errors than conventional biochemistry tests. [7,8]. This report illustrates two cases of falsely elevated total 25 (OH) vitamin D in the serum due to elevated paraprotein causing analytical interference with total 25 (OH) vitamin D measurement in the Abbott Alinity ci-series.

What is new?

Interferences are a rare phenomenon in routine biochemical analysis, however, tests performed with immunoassay are known to be more prone to analytical interferences. The authors encountered two cases in which hematological malignancies caused falsely elevated levels of Vitamin D, mimicking Vitamin D intoxication. This is the first report with a series of two cases of this rare phenomenon.

List of Abbreviations

25 (OH) vitamin D	25-hydroxy vitamin D
1,25 $(OH)_2$ vitamin D	1,25-dihydroxy vitamin D
CLIA	Chemiluminescence immunoassay
LC-MSMS	Liquid chromatography - Mass spectrometry.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this Case Report.

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Consent for publication

Written consent was obtained from all the patients (parents/guardians of the patient).

Ethical approval

Ethical approval is not required at our institution to publish an anonymous case report.

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Summary of the case

1	Patient (gender, age)	78-year-old female, 55-year-old female
2	Final diagnosis	Multiple myeloma and waldenströms macroglobulinemia
3	Symptoms	First case: General condition, fatigue, and repeated episodes of falling. Second case: follow up after a breast reconstruction surgery and had complaints of blurred vision, headache, and low blood pressure.
4	Medications	Maintenance medications, pain relievers, and chemotherapy.
5	Clinical procedure	Bone marrow puncture, plasmapheresis, and chemotherapy.
6	Specialty	Clinical biology – laboratory medicine.