


A case report of PTH elevation due to immunoassay interference

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Dear Editor,

Primary hyperparathyroidism (PHPT) is now commonly discovered before the onset of overt signs and symptoms related to the disease, in the form of an asymptomatic normocalcemic PHPT. In general, normocalcemic PHPT is consistent with the evidence of persistent (> 3 months) high-normal PTH with normal total albumin-adjusted and/or free-ionized calcium in patients with preserved renal function and adequate vitamin D intake, when all other secondary causes of PTH elevation have been excluded. Here, we report the case of a patient with an apparent asymptomatic normocalcemic PHPT, which turned out to be a case of PTH elevation due to immunoassay interference, reminding the fact that a diagnosis based only on laboratory analyses is inherently vulnerable to the limitations of PTH measurement.

A 66-year-old man was referred to our Endocrine Service because of repeatedly high PTH levels (> 400 pg/mL). PTH was measured with the Access[®] Intact PTH on the UniCel[®] DxI 800 (Beckman Coulter), which is a 2^o generation chemiluminescent microparticle sandwich immunoassay that we use for diagnostic purposes as well as for intraoperative use, in the setting of our routine core laboratory. The patient exhibited normal calcium and phosphorus (albumin-adjusted total calcium was 9.8 mg/dL), normal renal function and 25OH vitamin D levels were 23.6 ng/mL, for which he was taking 7500U of cholecalciferol weekly. He suffered from diffuse parenchymal lung disease, pulmonary hypertension and antiphospholipid syndrome, but had no history of fractures, malabsorption, nephrolithiasis, or renal

insufficiency. Physical examination revealed no abnormalities. We prescribed further exams showing that the patient had normal 24 h urine calcium excretion and the biomarkers for bone metabolism were within reference ranges. In particular, urine calcium was 124 mg/24 h; phosphatase alkaline was 92 U/L, bone phosphatase alkaline was 12.6 µg/L, and c-terminal telopeptide was 0.26 ng/mL. DEXA of the lumbar spine (L1-L4) showed a BMD of 1.4 g/cm², with *T*-score of 2.8 and *Z*-score of 3.6, and DEXA of the proximal femur (neck) showed a BMD of 0.8 g/cm², with *T*-score of -0.7 and *Z*-score of 0.3. Parathyroid imaging (dual-phase scintigraphy and ultrasound) did not show any abnormality. Given the discrepancy between the high PTH levels and a clinically unremarkable picture, in order to ascertain the cause of an apparent erroneously elevated PTH, we sent a sample to the Complaint Handling Unit of Beckman Coulter. In the sample provided, PTH was 338.4 pg/mL, which was consistent with our results. A subsequent dilution test indicated that there was a nonlinear pattern of PTH concentrations across multiple dilutions (Table 1), suggesting the presence of interferences. Then, PTH was re-measured after adding interference-eliminating proteins to the sample, whereby PTH values changed between -61 and 7.4% (Table 1), confirming the presence of interferences. In general, if there are no interferences, it is expected a percent change between -25 and +25%. Based on the type of blockers that were added to the sample, the high PTH levels of our case resulted in heterophile interference and interference related to alkaline phosphatase.

There are only a few reports of PTH elevation due to immunoassay interferences [1–4]. The most common cause accounting for it is represented by heterophile antibodies, which are found in 30–40% of serum samples, and lead to falsely elevated laboratory results in 0.5–3% of the cases. They are particularly common in rheumatologic conditions. In a study by Cavalier et al., among 743 patients with high PTH levels, 3.4% of the cases were due to heterophile antibodies, and 1.2% of the cases were due to rheumatoid factor (RF) [5]. Heterophile antibodies as well as RF interfere with immunoassays via the same mechanism. In sandwich

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Table 1 Dilution and interference testing

Dilution testing				
Description	Assay	Units	CHU results (corrected for dilution factor)	Percent change diluted/neat
S1 neat	PTH	pg/mL	338.4	N/A
S1, 1:2 dilution			1545.7	356.8
S1, 1:4 dilution			1314.7	288.4
S1, 1:8 dilution			326.9	− 3.4
S1, 1:16 dilution			163.8	− 51.6
Interference testing				
Description	Assay	Units	CHU results	Percent change
S1 neat	PTH	pg/mL	338.4	N/A
S1 + pool 1			243.3	− 28.1
S1 + pool 3			130.0	− 61.6
S1 + Goat IgG			363.3	7.4

CHU is for the complaint handling unit of Beckman coulter. Pool 1 is for blockers PolyMak33 and HBR-1, Pool 3 is for blockers AP Mutein and Scavenger ALP, Goat IgG is composed of animal derived antibodies

immunoassays, heterophile antibodies or RF can form a bridge between capture and labeled antibodies, resulting in false-positive interferences. Nevertheless, a 2021 update on immunoassay interferences indicates that there are several other molecules that can affect signal generation or quantification [6], such as alkaline phosphatase in alkaline phosphatase-conjugated immunoassays when it is not sufficiently removed during the washing step. In particular, alkaline phosphatase might bind to a conjugate antibody, act as a macromolecule and get trapped in the reaction mixture, causing an interference (7).

Our case reminds clinicians that a small subset of patients presenting with an apparent asymptomatic normocalcemic PHPT might have a PTH elevation due to immunoassay interferences. This occurrence should be suspected when PTH levels are unusually and extremely high as compared to calcium levels, and there is an unremarkable clinical picture. PTH elevation due to immunoassay interference should theoretically be considered also in cases of hypercalcemic PHPT. In any case, this condition can be diagnosed with the help of a laboratory physician.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Research involving human participants and/or animals This study does not contain any studies with animals performed by any of the authors.

Informed consent For this type of study, consent is not required.

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