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CASE REPORT

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Normocalcemia but still elevated parathyroid hormone levels after parathyroidectomy

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Key Clinical Message

Even when laboratory results at first match with clinical assessment, assay interference should still be on a clinician's mind when later results no longer fit with the patient.

KEYWORDS

calcium, hypercalcemia, laboratory interference, parathyroid hormone, parathyroidectomy, primary hyperparathyroidism

1 | INTRODUCTION

The most common cause for asymptomatic hypercalcemia is primary hyperparathyroidism (PHPT). For its diagnosis, hypercalcemia should be confirmed with a repeat sample and parathyroid hormone (PTH) should be elevated or inappropriately normal given the hypercalcemia.[1] The biochemical workup should further include assessment of renal function and vitamin D status.[2] When PTH is within the normal range or only minimally elevated it is recommended to evaluate for familial hypocalciuric hypercalcemia by a 24-hour urine measurement of calcium and creatinine. When PHPT is confirmed, parathyroidectomy is indicated for patients with symptomatology or a serum calcium level 0.25 mmol/L above the upper limit of normal.[2] We describe an ordinary case of primary hyperparathyroidism that became extraordinary when parathyroid hormone levels remained markedly elevated after surgical intervention.

2 | CASE REPORT

A 72-year-old man was referred to the endocrinologist because of hypercalcemia which was discovered incidentally by the cardiologist during evaluation of ventricular extrasystoles. The patient had no other complaints or symptoms. His medical history included radical prostatectomy, bullous emphysema and diabetes mellitus type 2. Physical examination revealed no abnormalities besides a slightly enlarged thyroid gland on the left side. Neck ultrasound showed a 1.8-cm solitary nodule in the inferior pole of the left lobe of the thyroid, close to the sternoclavicular joint. Serum total calcium level was 2.98 mmol/L (2.10-2.55 mmol/L), which was repeated and confirmed, with normal levels of albumin and total protein. Serum phosphate level was low at 0.50 mmol/L (0.74-1.20 mmol/L). Serum intact parathyroid hormone (PTH) level was 118 pmol/L (2.2-10.0 pmol/L) indicating hyperparathyroidism. Vitamin D (25-OH) level was suggestive of deficiency at 31 nmol/L (50-140 nmol/L), whereas calcitriol (1,25-$[OH]_2$-vitamin D) level was 171 pmol/L (65-225 pmol/L). Thyroid stimulating hormone (TSH), free thyroxine, prolactin, prostate-specific antigen, urine protein electrophoresis, erythrocyte sedimentation rate, basal hematological indices, serum electrolytes, renal and liver profile were all normal. Serum protein electrophoresis did reveal a monoclonal protein of the IgG-kappa type, but at an unquantifiable concentration. Four-dimensional computed tomography (4D-CT) scans of the neck showed a mass caudal of the left lobe of the thyroid gland suggestive of a parathyroid

adenoma (Figure 1). Additionally, a solitary mass with circular calcifications was found in the anterior mediastinum. The mediastinal mass had been identified on a CT scan 14 years earlier and compared to this scan showed minimal progression. Furthermore, ^{18}F-fludeoxyglucose (FDG) positron emission tomography (PET)-CT showed little ^{18}F-FDG uptake by the mediastinal mass, which was concluded to best fit with a remnant of a prior infection.

Based on the combination of serum calcium, phosphate, PTH, and 25-OH vitamin D levels a diagnosis of primary hyperparathyroidism with vitamin D deficiency was made. Given the strongly elevated PTH levels and the severe hypercalcemia it was considered unlikely that the patient might exhibit familial hypocalciuric hypercalcemia and an analysis of urinary calcium:creatinine ratio was therefore not performed. It was decided to first manage the hypercalcemia (total calcium progressed to 3.12 mmol/L) with cinacalcet 30 mg once daily, which was changed to twice daily 3 weeks later, with surgery planned 2.5 month later. Normocalcemia (based on both total and ionized calcium levels) was reached within 1.5 month of starting cinacalcet. Surgery was performed under general anesthesia with nerve integrity monitoring. As preoperative localization studies suggested unilateral pathology, minimally invasive selective parathyroidectomy was the procedure of choice. Caudal of the left lobe of the thyroid gland a 2-cm mass suggestive of a parathyroid adenoma was identified and resected. A frozen 2.5 × 2 cm fragment of the resected tissue was subjected to pathological analysis, that showed enlarged parathyroid tissue congruent with an adenoma. However, the PTH level of 138 pmol/L before surgery (10.20 AM) insufficiently decreased to 85 pmol/L 8.5 hours after resection of the parathyroidal tissue (8.00 PM). An intraoperative PTH measurement is not performed at our hospital. Ionized calcium levels 3 hours after resection and the following day indicated normocalcemia, after which the patient was discharged.

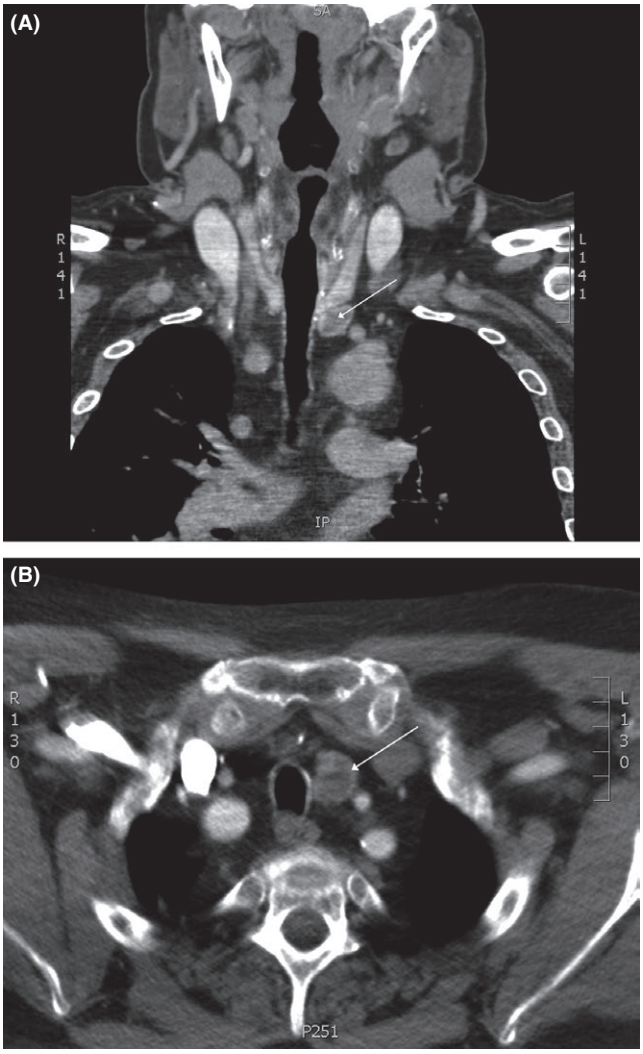

FIGURE 1 Four-dimensional computed tomography (4D-CT) scans of the neck showed a mass (denoted by the arrows) suggestive of a parathyroid adenoma.

Evaluation 3 weeks later revealed that cinacalcet had accidentally not been discontinued and that the patient displayed decreased total calcium levels (1.84 and 1.88 mmol/L on two separate occasions) with albumin at 30 g/L (mild corrected hypocalcemia). More surprisingly, PTH levels were found to be elevated at 125 pmol/L. Cinacalcet was discontinued and PTH was found to remain elevated for months despite normocalcemia and later also repleted vitamin D levels (Table 1). Altogether, a falsely elevated PTH was suspected which was put to the laboratory of clinical chemistry and hematology to investigate.

Firstly, we sent a sample to an external laboratory where a different intact PTH assay (Roche Elecsys) is used than at our center (Abbott Architect). The external laboratory reported a result within their reference range, which was confirmed by other intact PTH assays in use at other medical laboratories (Beckman Access, DiaSorin Liaison, Siemens ADVIA Centaur, Siemens Immulite). These results strongly suggested that the patient exhibited a falsely elevated PTH level because of a specific interference in the Abbott Architect intact PTH assay. Analysis of rheumatoid factor was negative and immunoglobulins were found to be within reference values. Concurrently, we had performed a serial dilution as well as polyethylene glycol (PEG) precipitation of the patient sample to indicate the presence of a heterophilic antibody [1] or a macroimmunecomplex [2,3] as causing the falsely elevated PTH measurements. The serial dilution with prescribed Abbott Multi-Assay Diluent suggested presence of a heterophilic antibody as linearity fell at a 1:64 dilution. In confirmation, application of heterophile blocking tubes (Scantibodies Laboratory Inc., Santee, CA, USA) also resulted in PTH measurements within reference range. The Abbott Architect intact PTH assay uses capture and detection antibodies that are both produced in goat. We therefore hypothesized that the interfering antibody could be directed to goat antigens and indeed addition of goat serum (1:1) to the patient sample ameliorated the observed interference. Furthermore, in

TABLE 1 Parathyroid hormone (PTH), total and ionized calcium, albumin and vitamin D levels of the patient (reference values in brackets)

Timeline/Analyte	Parathyroid hormone (2.2-10.0 pmol/L)	Total calcium (2.10-2.55 mmol/L)	Ionized calcium (1.15-1.33 mmol/L)	Albumin (35-50 g/L)	(25-OH)-Vitamin D (50-140 nmol/L)
Presentation cardiologist		2.98		37	
Evaluation endocrinologist	118	3.00			31
1.5 mo after start cinacalcet (30 → 60 mg daily)		2.50		35	
Before start of surgery	138		1.28		
After surgery	85		1.18		
3 wk after surgery, during cinacalcet use	125	1.88		31	
6 wk after surgery, cinacalcet discontinued	112	2.26		34	28
Start investigation interference (16 wk after surgery)	91	2.32		36	26
6 mo after surgery, with vitamin D suppletion	86	2.40		36	74
1 y after presentation	92	2.25		34	84

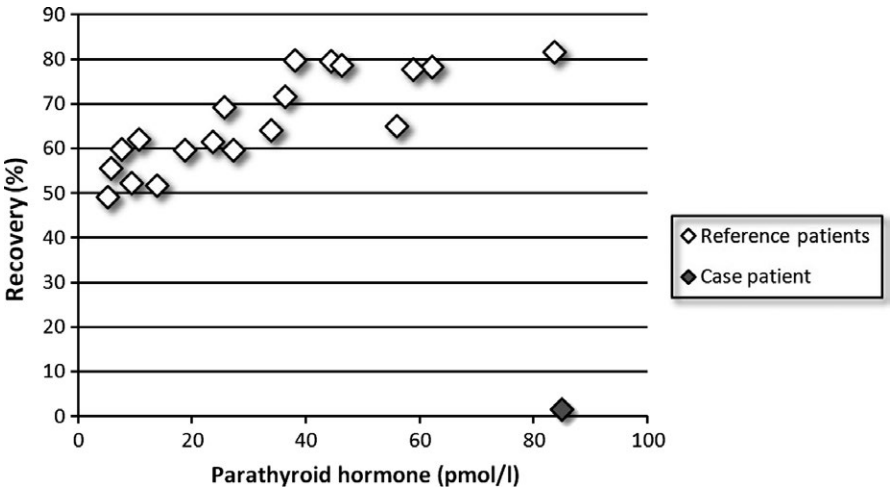

FIGURE 2 Polyethylene glycol (PEG) precipitation of 19 individual patient plasma samples and plasma from our case patient. Precipitation was performed by mixing 250 µl plasma with an equal volume of PEG 8000 (250 g/l in PBS). This mixture was incubated for 10 min at room temperature and then centrifuged at 5000 g for 10 min, after which parathyroid hormone (PTH) measurements were performed of the supernatant with the Architect intact PTH immunoassay.

random individual patient samples application of multiple assay diluent, heterophile blocking tubes or goat serum did not influence PTH measurements. In addition, we found that polyethylene glycol (PEG) precipitation of the patient's blood plasma also resulted in PTH measurements within the reference range. PEG precipitation did not affect random individual patient samples of elevated PTH levels (average 66% recovery) as compared to our case (1.7%) (Figure 2).

3 | DISCUSSION

Altogether it was concluded that the patient postoperatively exhibited normocalcemia and falsely elevated PTH levels. Of course, the patient most likely also exhibited falsely elevated PTH levels preoperatively. Unfortunately, these samples were no longer available when laboratory interference was suspected. The patient very likely exhibited less pronounced elevated PTH levels than measured because of the primary hyperparathyroidism and on top of that the laboratory interference resulting in strongly elevated PTH measurements exclusively in the Abbott Architect intact PTH assay. Most important, resection of the suspected adenoma did result in normocalcemia, which was the treatment goal. In follow-up, the patient was treated for his vitamin D deficiency and has now for over a year been without complaints after initial presentation and has consistently shown normocalcemia and PTH levels at 85-110 pmol/L.

The normalization of PTH upon application of sample dilution, goat serum, and heterophile blocking tubes all point toward interference by a heterophile antibody. PEG precipitation is routinely applied to indicate macroprolactinemia in clinical practice[3] and also applied in cases of suspected laboratory interference to indicate the presence of macrocomplexes of analyte and immunoglobulins.[4,5] PEG, however, not only precipitates macrocomplexes, but also soluble immunoglobulins[6,7] and therefore may have also precipitated heterophile antibodies in the current patient.[8]

The source of the heterophile interference remains elusive, although the effect of addition of goat serum suggests that the interference had its effect through anti-goat activity. The Abbott Architect intact PTH assay does not stand out from the other tested commercially available assays in this regard, however, as the DiaSorin Liaison intact PTH assay also utilizes capture and detection antibodies that are derived from goat. Furthermore, addition of nonimmune immunoglobulin can block heterophile interference irrespective of their origin.[9] On the other hand, assay components, most notably blocking agents, can infer differential sensitivity to antibody interference and therefore explain why a possible human anti-goat antibody caused positive interference in the Abbott Architect but not in the DiaSorin Liaison assay.[10]

Either way, the positive interference in the current case is likely a substance that can bring the capture and detection antibody together. Heterophile antibodies are the most common cause for interference in two-site immunoassays and can be found in all people. It is estimated that with modern two-site assays containing blocking agents heterophile interference occurs in less than 0.05% of analyses.[10] Relevant to our case, Cavalier et al[11] showed in a series of 2084 PTH samples a false-positive rate of 0.01% because of heterophile antibodies.

A possible source of the interference in our patient may be the identified monoclonal gammopathy of unknown significance. Gammopathy is a recognized albeit rare cause of interference in clinical chemistry assays.[12] Most commonly the mechanism of interference is the formation of turbidity and less frequently the binding to the analyte or one of the test components.[12] There have been case reports of association of monoclonal immunoglobulins with both positive and negative interference in immunoassays.[13-17] Positive interference in a two-site "sandwich" ELISA such as in the current case has been described less frequently, but Covinsky et al[15] could indeed demonstrate that a restricted anti-*E. coli* IgM clone displayed anti-mouse activity and positive interference in multiple sandwich immunoassays. We therefore cannot exclude that the monoclonal IgG-kappa identified in our patient at unquantifiable concentration is the cause of the persistently elevated PTH measurements due to anti-goat or indeed heterophile activity.

We have chosen not to further investigate the exact mechanism of interference as we judged it to be of no further clinical relevance and we did not want to impose further inconvenience on the patient.

4 | CONCLUSION

We present a patient diagnosed with primary hyperparathyroidism after an incidentally found hypercalcemia, for whom surgical intervention resulted in normocalcemia but persistently elevated PTH levels. Laboratory investigation revealed that assay interference caused falsely elevated PTH measurements. This case illustrates that even when elevated PTH results at first match with clinical assessment, assay interference should still be on a clinician's mind when later results don't fit with the patient anymore.

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CONFLICT OF INTEREST

None declared.

AUTHORSHIP

RD: has performed the laboratory experiments and written the manuscript. PN and RV: have treated the patient and cowritten the manuscripts. MJ: has codesigned the experiments and cowritten the manuscript.

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