Chapter 5
Parathyroid Hormone Measurement Considerations in Primary Hyperparathyroidism

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Case Presentation

A 48 year-old female was referred by oncology to endocrinology for evaluation of hypercalcemia. She was premenopausal at the time of her diagnosis of stage 1 (T1c, N0, M0) invasive ductal carcinoma breast cancer 5 years ago. The tumor cells were estrogen receptor negative, progesterone receptor positive, and HER2/neu negative. Treatment had consisted of wide local excision after initial breast conservation surgery which showed high-grade ductal carcinoma in situ (DCIS) at one of the margins with a single sentinel lymph node negative for metastatic disease, followed by radiation, adjuvant chemotherapy, and

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subsequent tamoxifen therapy. Due to side effects with tamoxifen combined with menopausal transition, she was switched to aromatase inhibitor therapy 2 years after her initial diagnosis. She had remained without evidence of breast cancer recurrence based on her mammogram, laboratory testing, and oncology history and physical examination. However, hypercalcemia was identified a year prior to referral, with a serum calcium of 10.3 mg/dL (nl, 8.9–10.1). Her maximum serum calcium level was 11.1 mg/dL which led to her referral. Her repeat laboratory tests were as follows: serum calcium, 10.4 mg/dL; PTH, 30 pg/mL (nl, 15–50 pg/mL); phosphorus, 4 mg/dL (nl, 2.5–4.5); albumin, 4.0 g/dL (nl, 3.5–5.0); 25-hydroxyvitamin D, 31 ng/mL; and 24 h urine calcium, 152 mg (20–275 mg/specimen). All of the following labs were normal: TSH, complete blood count, creatinine, AST, and alkaline phosphatase.

She denied history of nephrolithiasis, fragility fractures, polyuria, polydipsia, abdominal pain, fatigue, and constipation. Due to a declining dual-energy X-ray absorptiometry (DXA) bone mineral density (BMD) since initiating aromatase inhibitor therapy (femur neck T-score of −0.5 and lumbar spine T-score of −0.9), she was started on risedronate 1 year prior to her referral. Her family history was devoid of hypercalcemia or any other familial endocrine syndromes, and she denied prior head or neck radiation. Her dietary calcium intake was estimated at 600–800 mg/day, and she did not take calcium or vitamin D supplements and had never used lithium or hydrochlorothiazide. Her medications at referral were metoprolol tartrate, venlafaxine, anastrozole, levothyroxine, and risedronate. Her physical examination was unremarkable.

Assessment and Diagnosis

Primary hyperparathyroidism (PHPT) is defined by hypercalcemia associated with an elevated or non-suppressed serum

PTH measured by a two-site immunometric assay with high specificity for intact PTH (full-length PTH, PTH(1-84)). Such an assay is superior to C-terminal and mid-molecule PTH assays in distinguishing PHPT from non-PTH-mediated causes of hypercalcemia. Hypercalcemia of malignancy and other non-PTH-mediated hypercalcemic processes typically present with a low-serum intact PTH concentration [1], whereas intact PTH levels are reported to be elevated in approximately 80 % of patients with PHPT [2]. In hypercalcemic patients with PTH concentrations that are not elevated above the healthy population reference interval, levels in the upper one-half to one-third of the reference interval are considered inappropriately "normal" and have been used as a cutoff for PHPT [3]. However, PTH levels in the lower third of the reference interval have also been observed in an estimated 3 % of cases of pathologically confirmed cases of PHPT [4–7].

Lower than expected PTH measurements can be caused by a several biological factors. PTH in PHPT is influenced by the dynamic nature of its secretion [8], its circadian rhythm [9], the serum calcium level [10], vitamin D stores [11], and calcium intake [12]. Hemoconcentration, immobilization, and pH-dependent changes in protein-bound calcium can also influence both PTH and calcium measurements [13].

Other considerations in case of unexpectedly low PTH measurements center on assay-specific problems. Extremely high serum PTH concentrations can result in a hook effect in single-step, sandwich-type immunometric assays. In this type of assay, the assay's capture and detection antibodies are present at the same time to interact with the patient sample. When the PTH concentration in a sample exceeds the combined molar concentration of detection and capture antibody, each of these antibodies will be individually saturated with PTH, and very few actual sandwiches of AB-PTH-AB can be formed. This results in a false-low PTH measurement [7]. Serial sample dilution is required to obtain the true PTH concentration. Atypical, but bioactive, forms of PTH might also be encountered and are

often not detected by PTH assays [7]. The presence of such variants might be suspected, if serial sample dilutions deviate significantly from linearity [7].

Immunometric assays in general, including PTH assays, might also be vulnerable to false low interference that is exerted by chemicals or biomolecules that interfere with the assay's chemistry, analyte capture, or signal detection. General interferences of this nature include an extremely high lipid or protein content of a sample, both of which can hinder binding of the analyte to the assay antibodies, or high concentrations of optically active substances, like bilirubin or hemoglobin, which can interfere with signal detection. In addition, depending on an assay's precise configuration, there may be other interferences that can cause false low results in one or another (but not every) assay system. Examples of these include high biotin levels or anti-streptavidin antibodies in a patient serum [14–16]. Many assays use biotin-streptavidin binding to capture the antibody onto a solid support before signal readout from the detection antibody. Excess biotin or anti-streptavidin antibodies in a patient sample will prevent this reaction, leading to false low interference in assays using biotin-streptavidin capture. Antibodies or chemicals in a patient's serum that interfere with components of the signal generation system can similarly cause false low results. Individually, the rates of biological interferences, PTH concentration/fragment-dependent interferences, and assay-related interferences are very low, but collectively, these problems occur with appreciable, though still relatively low, frequency.

In our case, none of these interferences appeared to be present, and given the lower than expected PTH and the patient's history of breast cancer, further evaluation of non-PTH-mediated causes of PHPT was performed, all the while bearing in mind that the most likely diagnosis was still PHPT. Indeed, PHPT has been described when coexistent causes of hypercalcemia, including malignancy, have also been present [17]. Further testing in this patient revealed the following: 1,25-dihydroxyvitamin D at 21 pg/mL (nl, 22–67) and PTH-related peptide (PTHrP) at 110 pmol/L (nl, <2). Assessment for complications related to PHPT was also

performed. Her kidney-ureter-bladder (KUB) plain film radiograph with tomography was negative for kidney stones, and her one-third radius DXA BMD T-score was 0.7. Because of the unexpected PTHrP result, the test was repeated and the laboratory was called. The repeat PTHrP was 95 pmol/L and the specimen had linear dilution. Subsequently, a bone scan and fluorodeoxyglucose (FDG) positron emission tomography (PET) scan were performed. Both were unremarkable.

Management

A further repeat PTHrP was performed at a different reference laboratory and was undetectable. Both PTHrP assays used goat antibodies but had different binding sites. Subsequently, the specimen was reanalyzed at the initial laboratory after pretreatment of the sample with heterophile-blocking reagents, with no change in the elevated result. However, a repeat dilution series was nonlinear, consistent with interference from either a heterophile antibody or the presence of PTHrP fragments or variants.

Based on the patient's relatively stable and mild hypercalcemia, heterophile (from the Greek words heteros meaning the other, and philos meaning the friend – friend of the other) antibodies (HAB) were felt to be the most likely culprit. HAB are endogenous antibodies found in serum or plasma of patients that can bind to immunoglobulins of other species, such as the antibodies used as reagents for immunoassays [18]. They primarily affect two-site immunoassays, where they lead to false-positive results in >80 % of cases, by bridging detection and capture antibody in the absence of analyte (Fig. 5.1). Occasionally, when HAB only bind to either capture or detection antibody, they can also cause a false low result. HAB fall into three major groups: (i) polyspecific antibodies, as are often seen after viral infections, which bind a variety of targets, including sometimes assays antibodies; these antibodies are common in the population (up to 30 % prevalence at any given time),

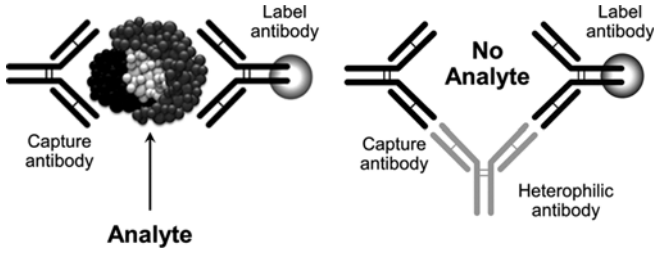

Fig. 5.1 Two-site immunoassay with bridging of two antibodies with antigen (*left*) and heterophile antibodies bridging the two antibodies (*right*) independent of the antigen, resulting in an increase in the bound-labeled antibody concentration

but rarely cause assay problems, because they have relatively low avidity to most assay antibodies; (ii) antibodies directed at species-conserved components of immunoglobulins; the prime example of this group is rheumatoid factor, which is an antibody against the Fc portion of immunoglobulins; these HAB are much less common than the polyspecific HAB, but because of their higher avidity to assay antibodies, they are more likely to cause problems; and (iii) high-affinity/avidity and high-titer antibodies that are specifically directed against mouse, rabbit, or goat IgG (common species for assay antibodies); these antibodies are rare; they require the patient to be specifically sensitized to mouse, rabbit, or goat (animal handling or diagnostic/therapeutic use of ABs) – but account for most HAB interferences. Cancer patients may be more likely than the general population to have interfering HAB [19].

In retrospect, it was clear that this patient did not have cancer-associated hypercalcemia, and perhaps, further testing was not needed in this regard. The positive PTHrP results should probably have been flagged as a "red herring" from the outset. The patient's history of hypercalcemia was too long and the hypercalcemia is too mild to be consistent with cancer-associated hypercalcemia, which is usually a preterminal event (median time to death of 30 days) that is associated with rapidly progressive, severe, and symptomatic hypercalcemia [20].

Outcome

The patient was reassured that she likely had mild, uncomplicated PHPT as what was observed. One year later, she continued to have stable, mild hypercalcemia and remained asymptomatic.

Clinical Pearls/Pitfalls

- The clinical and natural history of PHPT is very important when interpreting calcium biomarker laboratory results.
- PTH levels in PHPT are generally above the midpoint of the healthy population reference interval.
- Consider repeating PTH when the diagnosis of PHPT is unclear, especially since dynamic changes in calcium metabolism may occur in the presence of secondary contributing factors or if there is suspicion of assay interference.
- Consider additional non-PTH-mediated causes of hypercalcemia when the PTH is lower than expected for a diagnosis of PHPT.
- If repeat PTH measurement remains inappropriately in the lower half of the normal range despite continued hypercalcemia and other causes of hypercalcemia are not present, then serial sample dilution may be considered.
- When considering malignancy-related hypercalcemia, be mindful that a positive PTHrP result only has a high positive predictive value if the pretest probability for tumor-related hypercalcemia is high, i.e., when the patient has obvious progressive cancer and a short history of severe, progressive, and symptomatic hypercalcemia.
- Understand the laboratory assays you order and communicate with your laboratory if unexpected results are encountered.

Conflict of Interest All authors state that they have no conflicts of interest.

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