Clinical Guidelines and PTH Measurement: Does Assay Generation Matter?

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ABSTRACT PTH is an important regulator of calcium and phosphate homeostasis and bone remodeling. It is metabolized into PTH fragments, which are measured to a different extent by PTH assays of different generations because of differences in fragments recognized and lack of assay standardization. PTH is measured in the workup of several conditions, and clinical guidelines provide recommendations concerning these measurements. This review provides an overview of the impact of differences between PTH assays, applying distinct clinical guidelines for primary and secondary hyperparathyroidism and perioperative use of PTH measurements. Guidelines deal with PTH measurement in different ways, recommending either trend monitoring, the use of a fold increase of the upper reference limit, or an absolute PTH cutoff value. For classic primary hyperparathyroidism (PHPT), the type of PTH assay used will not affect diagnosis or management because the precise concentration of PTH is less relevant. In chronic kidney disease, the guideline recommends treating secondary hyperparathyroidism above a twofold to ninefold PTH increase, which will result in different clinical decisions depending on the assay used. For patients after bariatric surgery, guidelines state absolute cutoff values for PTH, but the impact of different generation assays is unknown because direct comparison of PTH assays has never been performed. During parathyroid surgery, PTH measurements with a third-generation assay reflect treatment success more rapidly than second-generation assays. Increased awareness among clinicians regarding the complexity of PTH measurements is warranted because it can affect clinical decisions. ***(Endocrine Reviews 40: 1468 – 1480, 2019)***

PTH is an important regulator of calcium and phosphate homeostasis and bone remodeling. Its measurement is important for three different purposes. The first is the diagnostic workup of hypocalcemia and hypercalcemia. In patients with hypercalcemia, PTH differentiates between hyperparathyroidism, in which PTH is abnormally high compared with what would be expected with regard to the calcium concentration, and other causes of hypercalcemia, in which PTH is low. The second is the analysis of secondary hyperparathyroidism (SHPT), in which PTH concentrations are elevated because of hypocalcemia, hyperphosphatemia, or vitamin D deficiency. SHPT may develop in patients with chronic kidney disease (CKD) and in patients who have undergone bariatric surgery. These patients are likely to develop various degrees of metabolic bone disease (MBD). To date, the best available hormonal parameter to detect metabolic bone disease is PTH. Therefore all guidelines related to those conditions recommend measurement of PTH to monitor development and degree of MBD. Third, PTH measurements are performed during and after parathyroid and thyroid surgery.

PTH is currently measured by automated sandwich-type immunoassay methods of different generations. These assays use different antibodies against PTH and recognize not only biologically active full-length PTH but also PTH fragments. Therefore, differences between laboratory results can arise. Moreover, the process of standardization of PTH assays has not yet been finished. This entails calibration of the assay against an internationally recognized reference material or reference measurement procedure. The lack of standardization contributes to differences in PTH concentrations between laboratories even when the same assay generation is used.

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ESSENTIAL POINTS

- PTH is measured by automated immunoassay methods of different generations
- PTH assays differ in the antibodies used and consequently the extent to which PTH fragments and PTH isoforms are measured, in addition to biologically active PTH
- Standardization of the PTH assay is ongoing, and lack of standardization leads to remarkable differences in PTH concentrations measured in different laboratories
- Clinical guidelines concerning primary and secondary hyperparathyroidism and perioperative use of PTH were screened for recommendations on PTH measurement
- Guidelines deal with PTH measurement in different ways, recommending either trend monitoring, the use of a fold increase of the upper reference limit, or an absolute PTH cutoff value
- Both laboratory experts and clinicians should be aware of the differences between the PTH assays because they can affect clinical decisions, and guidelines should be applied with caution

The aim of this review is to discuss different methods of PTH measurement, and associated pitfalls, in the context of clinical guidelines, and to increase awareness among clinicians and laboratory specialists of the complexity of PTH measurements in clinical care. International guidelines for primary hyperparathyroidism, for patients at risk for SHPT (CKD and post–bariatric surgery), and for perioperative use of PTH, were screened for recommendations on the use of PTH assays and implications of their use in clinical care.

A literature search was performed in PubMed with combinations of the following terms: *PTH, parathyroid hormone, bariatric surgery, chronic renal failure, mineral bone metabolism, perioperative PTH,* and *primary/secondary hyperparathyroidism*. Guidelines were retrieved from the Web sites of the relevant medical associations. Additionally, publications of the working group on PTH from the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) were included.

The Role of PTH in Calcium Regulation

Calcium and phosphate regulation

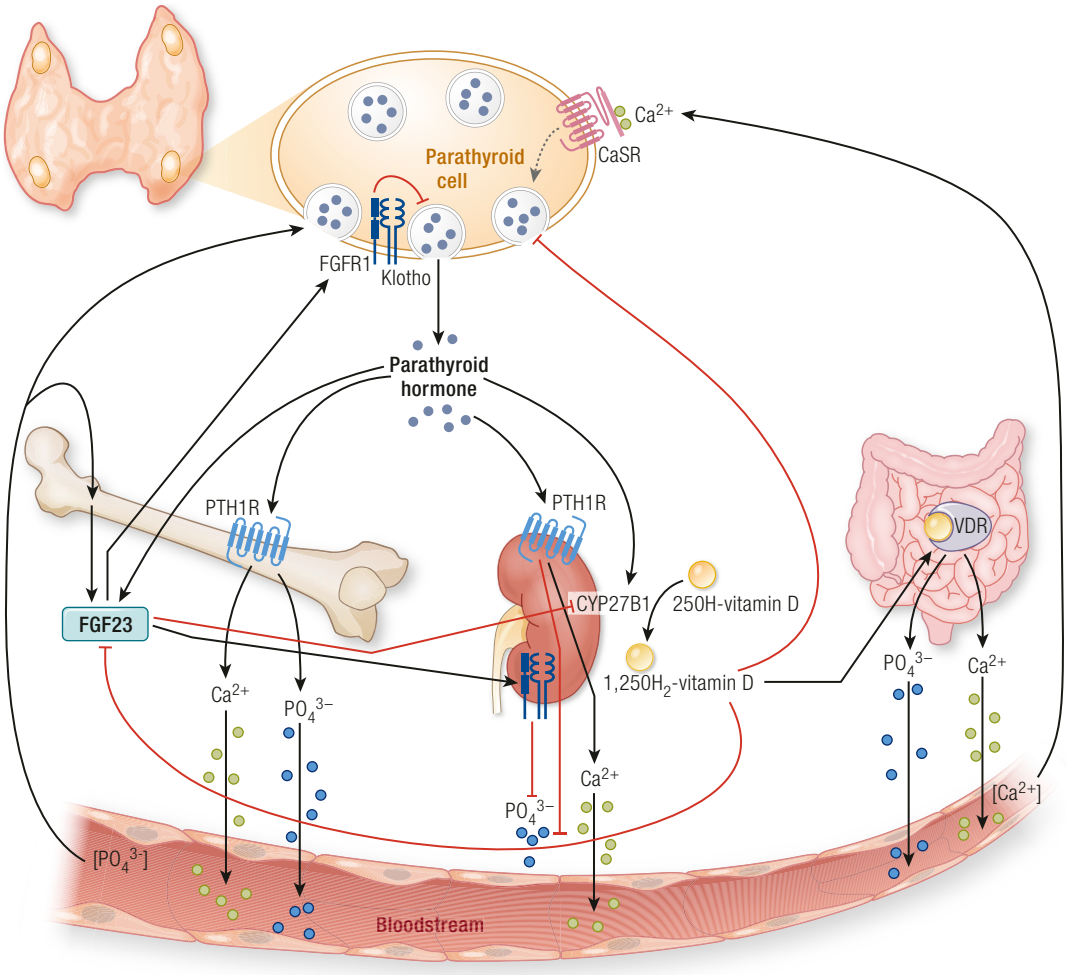
Normal serum concentrations of phosphate and calcium are maintained through interactions of three central hormones: PTH, 1,25-dihydroxyvitamin D, and fibroblast growth factor 23 (FGF23). These hormones act on four primary target organs: bone, kidney, intestine, and parathyroid glands (Fig. 1). Hypocalcemia is sensed via the calcium-sensing receptor on the parathyroid glands, resulting in release of PTH. Subsequently, PTH increases calcium concentration in three ways. First, PTH stimulates the enzyme 1-α-hydroxylase (CYP27B1) to hydroxylate 25-hydroxyvitamin D into 1,25-dihydroxyvitamin D, which in turn increases intestinal absorption of calcium. Second, in the distal and proximal renal tubule, PTH binding to the PTH receptor (PTH1R) increases calcium absorption and decreases phosphate reabsorption. Third, PTH binding to PTH1R in the bones stimulates the release of phosphate and calcium from the bones into the circulation. Overall, these actions result in an increased calcium concentration without affecting the phosphate concentration. In addition, the FGF23 hormone is secreted by osteocytes and osteoblasts and is involved in calcium and phosphate homeostasis. FGF23 is released in response to high concentrations of phosphate, PTH, and 1,25-dihydroxyvitamin D. In the kidneys, FGF23 binding to the FGF23 receptor with its cofactor Klotho increases phosphate excretion into the urine and inhibits CYP27B1, thereby decreasing the concentration of 1,25-dihydroxyvitamin D. Furthermore, FGF23 inhibits PTH release from the parathyroid glands by binding to the FGF23 receptor/Klotho complex (1).

PTH fragments and posttranslationally modified forms

PTH is synthesized in the chief cells of the parathyroid glands as a 115–amino acid peptide precursor, preproPTH. Cleavage of preproPTH results in proPTH, which is cleaved into the 84–amino acid peptide PTH, the biologically active form that is secreted into the circulation. After secretion from the granules of the chief cells, PTH exerts its effects through interaction with the PTH1R. The first 34 amino acids and the formation of an alpha helix are crucial for its biological activity (2, 3).

PTH circulates not only in the form of the full-length 84–amino acid peptide but also as multiple fragments, consisting mostly of the C-terminal part and therefore called C-terminal fragments. These C-terminal fragments arise mainly from metabolism by the liver (4, 5) but also from direct secretion from the parathyroid gland (3). C-terminal fragments are cleared primarily by the kidney and accumulate in patients with kidney disease. The plasma half-life of full-length PTH is very short (2 to 4 minutes), but the half-life of C-terminal fragments is five to ten times longer with normal kidney function and

Figure 1. Regulation of calcium and phosphate homeostasis. Binding of PTH to PTH1R in the bones results in the release of calcium and phosphate from the bones into the circulation. Binding to PTH1R in the kidneys increases calcium absorption, stimulates phosphate excretion, and stimulates hydroxylation of 25-hydroxyvitamin D into 1,25-dihydroxyvitamin D. 1,25-Dihydroxyvitamin D increases intestinal absorption of calcium through binding to the vitamin D receptor. In turn, hypocalcemia, low 1,25-dihydroxyvitamin D, and hyperphosphatemia increase PTH secretion. FGF23 binds to the FGF23 receptor/Klotho complex, which results in increased phosphate excretion into the urine and decreased 1,25-dihydroxyvitamin D by inhibition of CYP27B1. Furthermore, FGF23 suppresses PTH secretion by the parathyroid gland. This figure is a simplified version of the regulation of calcium and phosphate homeostasis. Many processes occur simultaneously. Only relevant pathways and receptors are depicted. PO_4^{3-} represents all variants of phosphate and phosphorus in the bloodstream. CaSR, calcium-sensing receptor; FGFR, fibroblast growth factor receptor; VDR, vitamin D receptor.



even longer in the presence of kidney disease (6). The relative amount of C-terminal fragments in relation to full-length PTH differs between people, but in patients with kidney disease this relative amount is higher than in healthy people (7, 8). In addition, calcium concentration influences the ratio of C-terminal fragments to full-length PTH (3). This ratio decreases in response to hypocalcemia, whereas in hypercalcemia adaptation of the parathyroid gland leads to a larger decline in the secretion of full-length PTH compared with PTH fragments (9).

The exact lengths of the different truncated fragments of PTH have been identified by mass spectrometry analysis, most in patients with kidney disease (Fig. 2) (12, 13). Other C-terminal fragments were identified by HPLC analysis and subsequent radioactive protein sequencing (11). The most abundant fragment observed was the 7–84 PTH fragment (10). In HPLC experiments, this fragment was found to comigrate with a synthetic 7–84 PTH fragment and is also called non–(1–84) PTH (10). Of note, this specific fragment was not detected by mass spectrometry analysis, which can be explained by the design of the method. PTH fragments do not activate the PTH1R receptor; however, there is some evidence that these fragments have some biological activity through binding a distinct class of receptors (3). In contrast to the PTH1R receptor, which has been well characterized, receptors for PTH fragments have not yet been cloned (18).

Figure 2. The different forms of PTH. Numbers indicate affected amino acids. *Detected after subcutaneous injection or intravenous infusion of recombinant human PTH. MS, mass spectrometry; N, healthy volunteers; Ref, reference number.

Every protein can undergo posttranslational modifications. For PTH it has been shown that it can be phosphorylated and oxidized (19, 20). Phosphorylation of several serine residues (Fig. 2) results in phosphorylated PTH that is unable to activate the PTH receptor *in vitro* (16). Oxidation of PTH occurs in methionine at positions 8 and 18 and is particularly found in patients with kidney disease (15, 21). Because of the altered structure, this oxidized PTH cannot activate the PTH receptor. This form of PTH is thus considered to be biologically inactive (21, 22). In parathyroid carcinoma a posttranslational modified form of PTH is overproduced (23, 24). This form is in some articles referred to as amino-PTH and hypothesized to be modified in the amino acid region 12–18 (17). However, this modified form is not specific for parathyroid carcinoma because it has been identified in healthy people as well as in patients with primary hyperparathyroidism (PHPT) and in patients with kidney disease (17).

Measurement of PTH: Current Assays and Pitfalls

Immunoassays

First-generation PTH assays use a single polyclonal antibody directed against the C-terminal part or midterminal part of PTH. Because these assays measure many biologically inactive C-terminal fragments, currently more specific PTH assays are used.

Second-generation assays are called intact PTH assays and differ from first-generation assays by the use of an extra antibody. In this sandwich immunoassay, one antibody is directed against the C-terminal part and a second antibody is directed against the *N*-terminal part of PTH (Fig. 3, top). This way, the assay was supposed to measure only full-length PTH. However, because the *N*-terminal antibody is not directed at the first four amino acids, this assay was discovered to measure also C-terminal fragments,

mainly the PTH fragment 7–84 (10). Within second-generation assays there are still two different antibody designs for recognition of the *N*-terminal part of PTH. The antibody can be directed to the amino acids 12–24 or the amino acids 26–32 (25). Depending on the assay design, different fragments are measured.

Third-generation PTH assays are called whole or bio-intact PTH assays. The sandwich-type immunoassay uses an *N*-terminal antibody directed against the first four amino acids of PTH and a C-terminal antibody similar to second-generation assays. Therefore, only full-length PTH is measured, and fragments such as 7–84 PTH are not detected. Although third-generation assays seem more specific than second-generation assays, these assays can detect PTH forms that are posttranslationally modified, like the form found overproduced in parathyroid carcinoma. PTH measurements by second- and third-generation assays can vary up to 47% (26, 27). These differences are important to consider when interpreting PTH concentrations. Characteristics of second- and third-generation assays are summarized in Table 1.

One important aspect in diagnostic measurements is the comparability between different assays. Comparability can be increased by standardization of assays. Standardization is defined as the achievement of equivalent results by different clinical laboratory tests conducted by different laboratories using calibration that can be traced to a reference measurement procedure (28). It is a complex and time-consuming procedure that involves international collaboration and cooperation of all partners, including *in vitro* diagnostics companies. The IFCC has formed a working group that aims to improve comparability of PTH measurements. One of the objectives has been to achieve standardization of PTH assays by calibrating assays against the same international standard (29, 30), which resulted in better agreement between different assays (31).

Mass spectrometry assays

Another objective of the IFCC working group is to facilitate the development of a candidate reference measurement procedure to standardize methods against this reference method (30). Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is a promising candidate as a reference measurement method, because the combination of chromatography with detection based on mass/charge ratio is far more specific than immunoassays (12, 13,

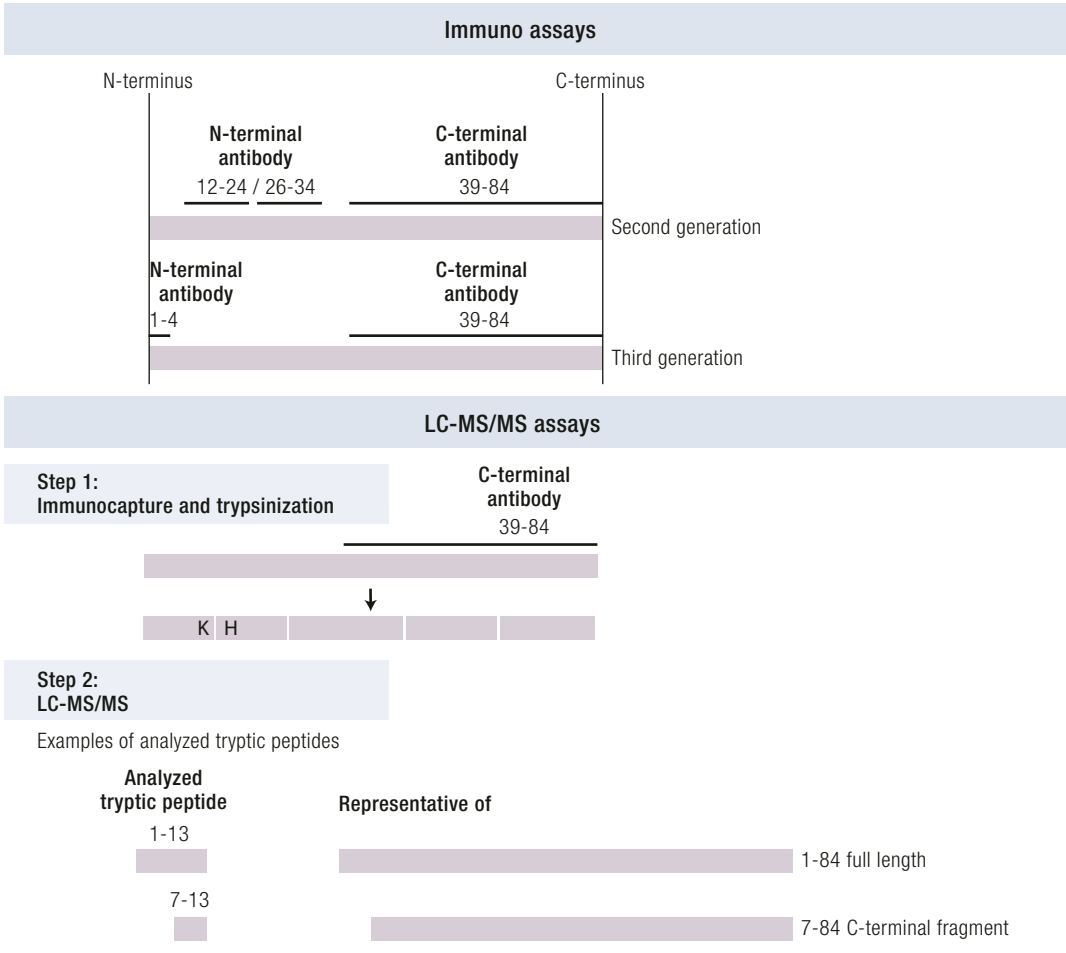


Figure 3. Technical aspects of different PTH assays. (Top) Antibody recognition sites in second- and third-generation assays. Numbers indicate amino acid numbers of the epitope at which the antibody is directed. (Bottom) Principle of LC-MS/MS–based PTH assays. The first step involves immunocapture by a C-terminal antibody against PTH, followed by digestion by trypsin. The second step involves LC-MS/MS analysis, where separation is based on the mass/charge ratio. The tryptic peptide 1–13 is representative of 1–84 full-length PTH, and the tryptic peptide 7–13 is representative of the 7–84 C-terminal fragment. Of note, this 7–84 fragment is not identified by LC-MS/MS, possibly because of a modification in the 7–13 part, which results in a different mass/charge ratio. H, histidine; K, lysine.

Table 1. Characteristics of Second- and Third-Generation PTH Assays

	Second-Generation Assays	Third-Generation Assays
Assay name	Intact PTH	Whole PTH
		Bio-intact PTH
Technique	Sandwich-type immunoassay	Sandwich-type immunoassay
Antibodies	*N*-terminus (amino acids 12–24 or 26–32)	*N*-terminus (amino acids 1–4)
	C-terminus (amino acids 34–84)	C-terminus (amino acids 34–84)
Cross-reactivity	C-terminal fragments	Amino PTH
	Oxidized PTH	Oxidized PTH
Standardization	Companies use different international standards	Companies use different international standards

25, 32). Mass spectrometry–based assays for PTH involve sample preparation before LC-MS/MS analysis. The serum is enriched for PTH because PTH is a low-abundance protein. Current enrichment methods use immune affinity extraction with a C-terminal PTH antibody (32). The enrichment step is followed by protein digestion via trypsin, because mass spectrometry analysis is currently possible only with small proteins. This cleavage step will result in tryptic peptides with different lengths that can subsequently be quantified by mass spectrometry, based on mass/charge ratio. Depending on the selected tryptic peptide that is analyzed, one is able to discriminate different PTH fragments (Fig. 3). Although mass spectrometry–based assays are specific, analytical sensitivity has still to be optimized, because mass spectrometry has a lower analytical sensitivity (limit of quantitation) compared with immunoassays (30). In addition, because for most enrichment methods an antibody recognizing PTH is used, the outcome depends on the choice of the antibody.

Selection of the tryptic peptide used for identification determines which form of PTH will be measured. Trypsin cleaves peptides after an arginine or lysine amino acid (33) generating, among other peptides, the 1–13 tryptic peptide. Most methods use this 1–13 fragment as representative of full-length PTH. This implies that any (posttranslational) modification after amino acid 1–13 is measured as full-length unmodified PTH. Any modification that interferes with biological activity is not taken into account. Of note, the 7–84 PTH fragment has not been detected yet by mass spectrometry analysis. Detection of this form of PTH is based on analysis of the 7–13 tryptic peptide (25). Either a modification in the 7–13 region that alters the mass/charge ratio of this tryptic peptide or a modification that influences the ability of trypsin to cut after position 13 could possibly lead to a lack of detection of this 7–84 PTH fragment. Therefore, both knowledge of the exact structure of the protein to be analyzed and correct design of the assay are crucial for correct measurement. Therefore, a better understanding of the clinical relevance of the PTH isoforms, including phosphorylated and oxidized PTH, is necessary to determine the best reference method (30). Optimization of the mass spectrometry method is needed as well, before it can serve as a reliable reference method.

Analytical aspects of PTH measurements and reference values

Preanalytical conditions are important and may influence PTH measurement (Table 2) (34). Measurement of PTH in serum vs plasma results in different PTH concentrations, so laboratories should measure PTH in either serum or plasma and not use both interchangeably (30, 35). The IFCC working group recommends measurement of PTH in EDTA plasma and not in serum, because PTH is most stable in EDTA plasma (30). Also, PTH assays can be influenced by biotin supplements (vitamin H, also known as vitamin B7 or B8) (36). This interference could lead to underestimation as well as overestimation depending on the design of the PTH assay.

The biological variation of PTH is substantial. The within-subject variation of PTH is ~20% in healthy people and up to 30% in patients on hemodialysis (37, 38). This implies that the difference in PTH concentration must exceed 54% in healthy people and 72% in patients on hemodialysis to state that the concentration of PTH has decreased or increased, and this difference is not attributable to biological variation (37).

The determination of PTH reference values is challenging because many factors influence PTH concentrations, as summarized in Table 2. PTH concentrations are race dependent: PTH is higher in black compared with white people (39). PTH is also correlated with body mass index (BMI) (40). Additionally, PTH increases with age, possibly because of the steady decline in glomerular filtration rate (GFR), leading to a higher PTH concentration in people >60 years old (41). Because PTH is increased in vitamin

D–deficient people due to secondary hyperparathyroidism, reference values of PTH are 20% lower if established in healthy people with replete vitamin D status compared with healthy people with unknown vitamin D status (42). Several studies have shown that excluding vitamin D–deficient subjects leads to a lower upper reference value of PTH (42–45). In practice, it is hard to use vitamin D status in PTH reference value experiments because there is no generally accepted cutoff value for vitamin D deficiency.

Patient Groups and Applicable Guidelines

Primary hyperparathyroidism

PHPT is defined as hypercalcemia and an elevated or inappropriately normal concentration of PTH and is commonly caused by hyperplasia or an adenoma in one or more parathyroid glands. Because of elevated PTH, calcium is removed from the bones, which can result in the development of osteoporosis. In addition, these patients have an increased risk of kidney stone formation due to hypercalciuria. The only curative treatment of PHPT is parathyroidectomy, which is indicated for symptomatic patients and patients with osteoporosis, impaired kidney function (GFR $<$60 mL/min/1.73 m^2), kidney stones, hypercalciuria, and patients $<$50 years of age or when calcium has increased to more than 0.25 mmol/L above the upper limit of normal (46). Because PTH concentration is not included in the decision criteria for performing surgery, differences in PTH measurements are not an issue in this respect (46, 47). However, PTH measurement is important to set the diagnosis PHPT because it discriminates between PHPT and other causes of hypercalcemia, such as bone metastases or sarcoidosis.

The American Association of Endocrine Surgeons guideline for management of PHPT does not mention differences in PTH assays and does not give recommendations on this issue (48). In contrast, the international workshop on diagnosing asymptomatic PHPT does consider differences in PTH assay measurements and recommends using assay-specific reference values (49, 50). The workshop states that PTH measurement can be performed with second- and third-generation PTH assays because both assay generations have similar diagnostic sensitivity for PHPT. This conclusion is based on four clinical studies (27, 51–53). One study by Gao *et al.* (27) recommended measuring PTH with third-generation assays. This recommendation was based on their HPLC experiments, which showed a higher percentage of PTH fragments in patients with PHPT compared with normal subjects. The percentage of PTH fragments in this patient group is not consistent and can vary from 20% to 90%.

Overestimation of PTH by second-generation assays would be of minor consequence in patients with hypercalcemic PHPT because in these patients the distinction between hypercalcemia due to PTH overproduction and PTH-independent causes of

Table 2. Aspects Influencing PTH Concentration

	Aspect	Effect on PTH Concentration
Biological aspects	Race	PTH concentration is higher in black people compared with white people.
	Age	PTH concentration increases with increasing age (because of a concomitant decline in GFR).
	BMI	PTH concentration is higher in obese patients.
	Vitamin D deficiency	Vitamin D deficiency increases the PTH concentration.
	Biotin supplements used by patients	Depending on the assay design used, PTH concentrations could be overestimated or underestimated, or no interference is observed.
Preanalytical aspects	Serum or EDTA plasma	PTH is more stable in EDTA plasma than in serum.
	Storage conditions	PTH is more stable at 4°C than at room temperature.
	Sampling site	PTH concentration depends on the sampling site. PTH concentrations are higher in central blood than in peripheral blood.
	Sampling time	PTH has a circadian rhythm. The optimal sampling time is unknown.
Analytical aspects	Assay design	Assay design, in particular the antibodies used, determine the extent to which PTH fragments and posttranslational modified PTH is measured in addition to PTH that is biologically active.
	Standardization of the PTH assay	Depending on the standardization procedure performed, PTH concentrations could be overestimated or underestimated.
Postanalytical aspects	Reference values	Reference values are influenced by vitamin D status, BMI, race, age, and the assay used.

hypercalcemia is clear cut. However, accurate measurement of PTH is important in identifying patients with normocalcemic primary hyperparathyroidism. This variant of PHPT is characterized by an elevated PTH concentration in combination with normal calcium values. Even though the calcium concentration is within the reference range, these patients are still at risk for developing complications associated with classic PHPT (54). Because PTH elevation is crucial for establishing this diagnosis, accurate measurement is essential.

Another proposed use of PTH measurement is the distinction between parathyroid carcinoma and benign disease. Third-generation assays measure a posttranslationally modified form (in the 12–18 amino acid region) overproduced in parathyroid carcinoma (23). Because third-generation assays usually give lower PTH values than second-generation assays, a ratio of third- to second-generation assay PTH measurements >1 could indicate presence of this modified form and may point toward parathyroid carcinoma (55). However, the use of this ratio has several limitations, most prominently the fact that PTH concentrations already differ between different assays of the same generation, influencing the ratio. In addition, its use is limited by the practical consideration that most laboratories do not offer both generation assays. Furthermore, the 12–18 posttranslationally modified form of PTH is also present in healthy people and patients with PHPT or CKD.

Chronic kidney disease

Patients with CKD are at risk for developing SHPT. Declining kidney function results in disturbance of calcium and phosphate homeostasis, because the kidneys play a critical role in maintaining calcium and phosphate within normal ranges. If the kidneys fail to do so, a compensatory mechanism is initiated that eventually results in SHPT. The pathophysiological mechanism is complex and involves different pathways. Ultimately, PTH increases in response to hyperphosphatemia, hypocalcemia, lowered 1,25-dihydroxyvitamin D concentration, and elevated FGF23. The calcium and phosphate imbalance comes at the cost of other organs and contributes to the development of CKD-associated MBD. SHPT requires treatment because abnormal bone turnover can result in skeletal and mineral disorders, such as renal osteodystrophy. The gold standard to diagnose renal osteodystrophy is bone biopsy. Because this is an invasive and costly procedure, PTH is used as a first screening test to detect skeletal and mineral disorders in these patients (56, 57).

C-terminal PTH fragments accumulate in the circulation with increasing stages of kidney disease. Recognition of the different fragments is antibody specific and therefore assay specific, resulting in differences in PTH concentrations even when the same generation assay is used. This complicates the follow-up of patients in different hospitals.

In patients with CKD, a third-generation assay demonstrates ~50% (43% to 47%) lower PTH concentrations compared with a second-generation assay (26, 27). Several studies comparing 15 different commercially available PTH assays (both second and third generation) showed more than twofold differences between the lowest and the highest value of PTH within the same patient (58–60). Even a 4.2-fold difference within a single patient has been described (59). The Kidney Disease: Improving Global Outcomes guideline takes these differences in PTH measurements across assays into account. For patients on dialysis, it recommends maintaining PTH concentration in the range of 2 to 9 times the upper normal limit for the specific assay (61, 62). Even with this wide range, it is difficult to interpret PTH concentrations. Using assay-specific reference values, one study with 149 patients on dialysis found that 7.4% of these patients have more than nine times the upper limit of PTH values with one assay and 26.2% with another assay (58). The guideline states that in patients with moderate to end-stage CKD who are not on dialysis, the optimal PTH concentration is unknown (61, 62). To provide clinicians with guidance, the Kidney Disease: Improving Global Outcomes guideline recommends following trends of PTH concentrations to determine the optimal treatment. Finally, it recommends using second-generation assays because of the widespread availability of these assays.

One could argue that third-generation assays, which do not recognize C-terminal fragments, are an improvement for patients with CKD. Several clinical studies have addressed the relationship between measurements with different-generation PTH assays and metabolic bone disease. Of note, some studies refer to differences between second- and first-generation assays, even though the assays compared are of the third and second generation, causing further confusion (56, 63). These clinical studies have not convincingly shown an advantage of third-generation assays compared with second-generation assays in the diagnosis of low, normal, and high bone turnover (56, 57, 63). Although PTH measurement may improve when assays are standardized based on preanalytical conditions and measure bio-intact PTH (64), it is still remarkable that most clinical studies did not show an improvement of third-generation assays in predictive value of bone turnover or other clinical outcomes. One possible explanation is comeasurement of an oxidized form of PTH that is not biologically active (21). Patients with renal failure have increased oxidative stress and high levels of oxidized PTH (21). Indeed, a clinical study in patients with renal failure showed that measuring nonoxidized PTH correlated better with clinical outcomes, including mortality due to cardiovascular complications, than measuring both oxidized and nonoxidized PTH (65).

The standard treatment of SHPT includes dietary modification to reduce phosphate intake, use of phosphate binders, vitamin D supplementation, and adjustment of the dialysis regimen. In patients with SHPT who do not respond to standard therapy and in whom surgery is contraindicated, cinacalcet can be prescribed. Cinacalcet is a calcimimetic agent, which increases the sensitivity of the calcium-sensing receptors, thereby decreasing PTH production (66). The National Institute for Health and Care Excellence guideline recommends starting cinacalcet treatment when PTH reaches 85 pmol/L (corresponding to 802 pg/mL, ~10 × the upper reference limit) and when SHPT is refractory to other treatments and surgery is contraindicated (67). This cutoff value is based on several clinical trials, but the assays used in these studies are not described in the guideline. Given the aforementioned challenges in PTH measurement, stating absolute cutoff values in guidelines should be avoided.

Bariatric patients

As the prevalence of obesity continues to rise, bariatric surgery is increasingly performed. After partial bypass of the small intestine (*e.g.*, gastric bypass surgery), absorption of calcium and vitamin D is decreased, which may result in secondary hyperparathyroidism. The prevalence of SHPT 5 years after bariatric surgery is reported to be as high as 63%, depending on the type of surgery performed (68). Guidelines recommend monitoring PTH after bariatric surgery on a regular basis to evaluate for calcium and vitamin D deficiency and starting supplementation to prevent metabolic bone disease (69–71). Serum calcium concentration often remains normal after bariatric surgery (72). Even if calcium intake or absorption is inadequate, normal calcium concentration can be maintained as a result of compensatory mechanisms such as bone resorption and decreased calcium excretion. Therefore, PTH may be more sensitive in detecting clinically relevant

Table 3. Overview of Guideline Recommendations on PTH Measurements and PTH Assays

Patient Category	General Recommendations on Measurement of PTH	Specific Recommendation on PTH Assay Generation	Guideline
PHPT	PTH measurements for PHPT diagnosis, follow-up after parathyroidectomy, follow-up of parathyroid carcinoma	Not mentioned	American Association of Endocrine Surgeons, 2016 (48)
Asymptomatic PHPT	PTH measurements for PHPT diagnosis	Second- and third-generation, use assay-specific reference values	International workshop on PHPT management, 2014 (49, 50)
Secondary hyperparathyroidism in CKD	Therapeutic decisions should be based on trends in PTH concentrations. In patients on dialysis, maintain PTH levels 2–9 times upper reference limit	Second-generation assay	Kidney Disease, Improving Global Outcomes, 2017 (61, 62)
	Start cinacalcet treatment if intact PTH reaches 85 pmol/L (802 pg/mL) when SHPT is refractory to other treatments and surgery is contraindicated	Assay generation is not mentioned. The advice to measure intact PTH suggests the use of a second-generation assay	National Institute for Health and Care Excellence, 2007 (67)
Secondary hyperparathyroidism after bariatric surgery	Intact PTH measurements every 6 mo if surgery influences gastrointestinal absorption	Assay generation is not mentioned. The advice to measure intact PTH suggests the use of a second-generation assay	Endocrine and Nutritional Management of Post-Bariatric Surgery Patient, 2010 (74)
	PTH measurements if surgery influences gastrointestinal absorption	Not mentioned	Interdisciplinary European Guidelines on Metabolic and Bariatric Surgery, 2014 (76), and American Association of Clinical Endocrinologists/The Obesity Society/ American Association of Metabolic and Bariatric Surgery, 2013 (75)
	Routine presurgery and postsurgery screening for calcium and vitamin D deficiency: PTH > 6.9 pmol/L (65 pg/mL) indicates calcium or vitamin D deficiency	Assay generation is not mentioned. The advice to measure intact PTH suggests the use of a second-generation assay	American Association of Metabolic and Bariatric Surgery (73)
Perioperative use of PTH measurement	PTH measurements 4 h after thyroid surgery	Not mentioned	Australian Endocrine Surgeons, 2007 (83)
	Intensive calcium monitoring after thyroid surgery not needed if PTH > 1.6 pmol/L (15 pg/mL)	Not mentioned	American Thyroid Association, 2018 (84)

calcium deficiency. The same holds true for the detection of vitamin D deficiency (72). According to the guideline of the American Society for Metabolic and Bariatric Surgery, PTH concentration >6.9 pmol/L (65 pg/mL) indicates calcium or vitamin D deficiency (73). The rationale for this cutoff value is not provided in the guideline, nor is the PTH assay mentioned that should be used.

As in SHPT in CKD, PTH is used as a surrogate marker for changes in bone mineral density (69–71). Although several studies have shown progressive deterioration in bone quality and elevated fracture risk after bariatric surgery, the pathologic mechanism is not clear. PTH does not seem to be a reliable marker of bone disease in bariatric patients because changes in bone mineral density occur independently of changes in PTH (74–78). This finding suggests that additional mechanisms are responsible for this phenomenon. Some studies have suggested the use of other bone turnover markers to evaluate for bone disease after bariatric surgery (69, 73).

In general, for patients after bariatric surgery it is unknown what the appropriate cutoff values of PTH are. Because no comparison of second- and third-generation assays has been performed in bariatric patients, the clinical implication of the differences is unknown. Because of the differences between assays, absolute cutoff values should be avoided in guidelines. Because PTH is measured regularly, clinical decisions are based on PTH changes over time. For an adequate evaluation of the trend of PTH, subsequent measurements should be performed with the same PTH assay.

Perioperative use of PTH measurements

During parathyroidectomy, measuring PTH concentrations provides surgeons with information about whether the parathyroid tissue causing the elevated PTH concentration has been removed. After successful resection, PTH concentration drops instantly because PTH has a half-life of only several minutes (79). Because second-generation assays recognize C-terminal fragments that have a longer half-life, third-generation assays seem more useful for this purpose (80). Indeed, perioperative measuring of PTH with a third-generation assay has shown a more rapid decrease in PTH concentrations compared with measuring PTH with a second-generation assay (81). The difference is more pronounced in patients with SHPT (due to kidney disease) compared with patients with PHPT. Generally a 50% decline in PTH compared with the preoperative level is used as a cutoff to define treatment success. In patients with SHPT in whom parathyroidectomy is being performed, it takes time for PTH concentrations to drop below the 50% cutoff after removal of the last hyperplastic gland. With third-generation assays, this drop can be observed after 10 minutes, whereas it takes an additional 5 minutes if a second-generation assay is used (81).

After thyroid surgery, PTH measurement is used as a predictor of postoperative hypoparathyroidism resulting in hypocalcemia (82). During thyroid surgery parathyroid glands can be damaged or inadvertently removed, resulting in transient or even permanent postoperative hypoparathyroidism. The Australian Endocrine Surgeons guidelines advise measurement of PTH 4 hours after surgery (83). This recommendation is based on studies in which only second-generation assays were used. According to the American Thyroid Association Surgical Affairs Committee, there is no need for intensive calcium monitoring when the PTH concentration is >1.6 pmol/L (15 pg/mL) measured ≥20 minutes after surgery. This cutoff value is based on several clinical studies that do not always state the type of assay used (84). Of note, in this guideline, PTH measurements are referred to as intact PTH. Although this suggests the use of second-generation assays, the exact assay design is not specified. Because antibodies can be directed at the 12–24 or 26–32 epitopes (Fig. 3), significant differences in PTH concentrations may still occur. Considering the properties of a third-generation assay, one could argue that PTH measured with this assay generation best reflects parathyroid function in the first postoperative hours and is more reliable in predicting postoperative hypocalcemia. However, studies comparing different-generation PTH assays in patients undergoing thyroid surgery have not been performed.

To use follow-up PTH measurements during and after surgery, it is most important to use the same assay to allow proper comparison.

An overview of recommendations on measurement of PTH in international guidelines is presented in Table 3.

Conclusions

The clinical interpretation of PTH measurements is challenging, because it is complicated by comeasurement of PTH fragments and posttranslationally modified PTH variants, depending on the assay used. The biological function of these fragments and modified forms has not yet been completely elucidated.

Over the years, the PTH assay has evolved from an assay measuring various fragments to an assay measuring primarily, but not exclusively, biologically active PTH. Both second- and third-generation PTH assays are used in clinical care. In most patients, second-generation assays will measure higher PTH concentrations compared with third-generation assays. These differences exist because second-generation assays measure C-terminal fragments and third-generation assays do not. Furthermore, the different assay generations recognize other

(posttranslational) modified forms. Even assays of the same generation differ in PTH concentrations measured, because the various methods have not been standardized. Standardization of PTH assays by the IFCC working group is in progress but has not yet been finished. An accurate reference method could be used to reduce assay variability. LC-MS/MS seems a promising candidate to serve as a reference method. However, mass spectrometry–based methods have yet to be optimized.

The observed difference between second- and third-generation assays is most pronounced in patients with CKD because of the impaired renal clearance of PTH fragments. Depending on the assay used, applying guideline recommendations will result in different clinical decisions in a number of patients. This will especially be the case if absolute cutoff values are used that are provided in these guidelines. During parathyroid surgery, third-generation PTH assays have the advantage of reflecting treatment success more rapidly than second-generation assays. For the diagnosis and management of PHPT the precise concentration of PTH is less relevant, and therefore the assay used is also less relevant. For patients who have undergone bariatric surgery, PTH might be used as a marker for clinically relevant hypocalcemia and vitamin D deficiency. PTH does not seem to be a reliable marker for bone disease in these patients. Because a comparison of second- and third-generation assays has never been performed in bariatric patients, the clinical impact of differences between assays remains unknown.

In general, because of the differences between PTH assays, guidelines should avoid stating absolute cutoff values. In addition, for all patients in whom multiple measurements of PTH are performed, the interpretation does require insight into technical differences between PTH assays. For a correct interpretation of the PTH trend over time, the measurements should be performed with the same assay in the same laboratory with assay-specific reference values.

Regardless of which assay is the most accurate to use, clinicians and laboratory specialists should be aware of the differences between second- and third-generation PTH assays stated in this review, to correctly interpret PTH measurement results, apply the various guidelines, and improve patient care.

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Abbreviations

BMI, body mass index; CKD, chronic kidney disease; CYP27B1, 1-α-hydroxylase; FGF23, fibroblast growth factor 23; GFR, glomerular filtration rate; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry; MBD, metabolic bone disease; PHPT, primary hyperparathyroidism; PTH1R, PTH receptor; SHPT, secondary hyperparathyroidism.