Published in : Clinical Chemistry and Laboratory Medicine (2023), pp. 1-8
doi.org/10.1515/cclm-2022-0942
Status : Postprint (Author's version)

DETERMINATION OF PARATHYROID HORMONE: FROM RADIOIMMUNOASSAY TO LCMS/MS

Etienne Cavalier

Department of Clinical Chemistry, CHU de Liège, CIRM, University of Liege, Domaine du Sart-Tilman, B-4000 Liège, Belgium, Phone: +324 3237692, Fax: +32 4 3238823, E-mail: Etienne.cavalier@chuliege.be. https://orcid.org/0000-0003-09472226

Abstract

Parathyroid hormone (PTH) determination is of paramount importance for the exploration of diseases related with calcium metabolism and for the follow-up of patients suffering from bone and mineral disorders associated with chronic kidney diseases (CKD-MBD). Unfortunately, the biologically active form of PTH, i.e. 1–84 PTH, circulates in the blood stream with many fragments and post-translationally modified forms, which decreases the specificity of immunoassays. The assays used to measure PTH, either from 2nd or 3rd generation, are not standardised, which may lead to interpretation errors and clinical consequences. Reference ranges for PTH have neither been always correctly established and the stability of the peptide is also a matter of concern. Fortunately, these last years, newer techniques using mass spectrometry (either high resolution or triple quadripole) coupled with liquid chromatography have been developed, which will help to standardise the different assays. Indeed, PTH assays standardisation is one of the task of the IFCC Committee for Bone Metabolism. Such standardisation will allow a better consistency in the interpretation of the results and will promote studies aiming at the establishment of correct reference ranges.

Keywords

mass spectrometry; parathormone; reference range; review; stability; standardisation; vitamin D.

Introduction

Role of parathyroid hormone

Serum calcium and phosphate concentrations are regulated by parathyroid hormone (parathormone, PTH), calcitriol (1,25(OH)2-vitamin D), and Fibroblast Growth Factor 23 (FGF23). PTH primarily affects the kidney and bone by interacting with the type 1 PTH/PTHrP receptor (PTH1R), a G-protein coupled receptor [1]. After binding PTH, the PTH/ PTHrP receptor transmits its signal by activating protein kinases A and C [2]. For this interaction, the very first N-terminal amino acids of the PTH peptide are essential [3]. In the kidney, PTH increases the synthesis of 1,25(OH)2 vitamin D and stimulates the reabsorption of calcium in the distal tubule. PTH also decreases renal reabsorption of phosphate in the proximal tubule via endocytosis of the sodium-

Published in : Clinical Chemistry and Laboratory Medicine (2023), pp. 1-8
doi.org/10.1515/cclm-2022-0942
Status : Postprint (Author's version)

dependent cotransporter Npt2a in the apical end of the tubule [4]. On bone, PTH acts via a receptor located on the surface of osteoblasts. Activation of this receptor stimulates the production of receptor activator for nuclear factor B ligand (RANK-L) in conjunction with Interleukin-1 and 1,25(OH)2 vitamin D, while inhibiting the expression of osteoprotegerin, an inhibitor of the RANK receptor found on the surface of pre-osteoclasts. The activation of the RANK receptor induces the transformation of pre-osteoclasts into active osteoclasts, leading to bone resorption and calcium (and phosphate) release by the bone. In addition, PTH promotes bone formation by aiding in the recruitment, proliferation, and differentiation of osteoblasts, as well as reducing their apoptosis. In order to treat osteoporosis, daily injections of PTH or its analogues (teriparatide, abaloparatide) are used in pharmacology to stimulate bone formation.

Synthesis and metabolism of PTH and its fragments

The human PTH gene is located on chromosome 11's short arm [5]. In the chief cells of the parathyroid glands, a large PTH polypeptide precursor hormone containing 115 amino acids (pre-pro PTH) is synthesised, which undergoes two successive proteolytic cleavages to yield PTH, the principal form of the hormone stored and secreted by the glands. Within 1 min of synthesis, the amino-terminal portion of preproPTH is cleaved and 25 amino acids are lost to yield the proparathyroid hormone (proPTH), an intermediate precursor with 90 amino acids. Within 15 min, Pro-PTH is transported to the Golgi apparatus, where a second cleavage of 6 AA in the terminal part occurs, resulting in the formation of the 84 amino acid, 9.5 kDa PTH peptide. The purpose and mechanism of the successive cleavages are unknown [6]; however, mutations that prevent cleavage in pre-proPTH can cause hypoparathyroidism [7], indicating the importance of the signal sequences for the peptide's proper activity.

Low extracellular calcium stimulates the release of (1–84) PTH in the blood. Once PTH is secreted, its half-life is 2–4 min. It is metabolised by the liver and cleared by the kidneys, which then releases fragments into circulation [8]. When the calcium-sensing receptor (CaSR) becomes saturated, PTH degradation also occurs within the parathyroid glands. However, when the CaSR is activated by elevated circulating Ca^{++} levels, intracellular Ca is released from the parathyroid cells, which inhibits the secretion of (1–84) PTH into the circulation. This inhibition is accompanied by an increased rate of NH2-terminal PTH peptide proteolysis. This degradation process generates large C-terminal fragments that are also secreted into the bloodstream [13]. These fragments have a longer half-life than (1–84) PTH [9], and they accumulate in the blood of CKD patients [10]. Using a high-resolution mass spectrometry approach, Kritmetapak et al. have recently characterised and quantified eight different PTH fragments (PTH28-84, 34–77, 34–84, 37–77, 37–84, 38–77, 38–84, and 45–84) present in the blood of CKD patients [11]. They observed a significant increase in circulating PTH fragment concentrations when glomerular filtration rate decreased to 17–23 mL/min/1.73 m^2. In addition, they confirmed their initial findings [12] that the 7–84 fragment, commonly regarded as the representative of large N-truncated fragments, was absent from the blood of CKD patients. We also confirmed this observation, as we did not detect 7–84 PTH in the plasma of CKD or haemodialysed patients using a recently developed LC/MS-MS method [13].

Earlier experimental data suggested that PTH fragments may have some biological effect, attempting to explain the "PTH resistance" frequently observed in CKD patients [14–20]. With Kritmetapak's LC-HRMS technique, none of the fragments used in these earlier studies have been identified (or their concentration was below the limit of quantification of the method). In a separate recent study, Kritmetapak et al. discovered that the LC-HRMS fragments could modulate the action of 1–84 PTH in mouse osteoblasts. Indeed, when

administered with 1–84 PTH, these fragments could either stimulate or inhibit the biological activity of 1–84 PTH. If these observations are of great interest, they warrant additional research.

Preanalytical considerations

PTH stability

This journal has published seminal articles highlighting the importance of the preanalytical phase in laboratory medicine [21–24]. As a matter of fact, the preanalytical phase is crucial for the correct interpretation of PTH results. PTH can be measured on EDTA plasma or serum. In a significant paper published in Clinical Chemistry and Laboratory Medicine [25], we examined the advantages of using one type of sample over another. Briefly, one of the primary benefits of using EDTA tubes for PTH is the reduction in time required for the pre-treatment of samples. This is essential for intraoperative PTH monitoring. Additionally, EDTA prevents coagulation-induced interferences and complexes divalent cations required for the activity of circulating proteases. However, the addition of anticoagulants or the presence of fibrinogen may interfere with certain analytical procedures. Also, simultaneous measurement of calcium (which is required for an accurate interpretation of PTH levels) or (bone) alkaline phosphatase is not possible with EDTA plasma. Hence, a second (serum gel) tube is required for a comprehensive biological investigation. PTH stability in EDTA and gel serum tubes has been the subject of numerous articles and a systematic review [26], but the results have been inconsistent. In fact, the conception of such studies varies, with various approaches regarding, for instance, the temperature of storage, sample type, patients or healthy subjects, definition of the "zero" point, length of storage, and whether or not samples are processed. The concept of "stability" is also debatable, as different thresholds can be used to determine whether or not PTH is stable in the sample. In fact, some studies use a purely statistical approach (Wilcoxon test or multifactor analysis of variance [27]), while others use an arbitrary cut-off of 10 or 20%, an approach that takes into account the analytical coefficient of determination of the method according to ISO Guide 5725-6 [28], or both the analytical and biological coefficients of variation according to WHO [29]. In a paper (which received the EFLM Walter Guder Preanalytical Award) published in this Journal, we demonstrated that PTH was more stable in EDTA than in serum gel tubes, but only when samples remained unprocessed for an extended period (18 h) at room temperature (25 °C), which can occur when samples are delivered from external care centres. PTH was not more stable in EDTA than in serum for other conditions [30]. Very few studies have accurately evaluated the long-term stability of PTH at −20 °C or −80 °C, and again, these studies used different protocols, making it difficult to interpret the results. PTH appears stable for 1 year in serum and plasma at −20 or −80 °C [31–33].

Additional preanalytic variables

Sampling site

For the follow-up of a haemodialysed patient, the question of the sampling site is particularly crucial. In fact, samples are typically obtained from such patients through the central venous catheter line or the arteriovenous fistula. Yet, results may vary significantly between peripheral and central sampling sites [34]. This has prompted the IFCC Working Group on PTH to recommend in a position paper published in this Journal

[26] that blood samples for PTH measurement should always be collected from the same sample site for intra- and inter-individual comparisons.

Sampling time

PTH has a bimodal rhythm, with a nocturnal acrophase, a morning nadir, and an afternoon peak, but the circadian rhythm varies considerably between individuals [26]. Due to contradictory findings in the scientific literature, it is unclear how dietary intake (and particularly calcium-rich foods) influences PTH levels. Nonetheless, the IFCC working group suggests that samples for PTH measurement should be collected between 10:00 and 15:00. As long as there is no definitive study demonstrating the absence of influence of food intakes on PTH concentrations, we believe it is reasonable to request a fasting status, particularly in the follow-up of osteoporotic patients when β-CTX is also requested [35].

In haemodialysed patients, samples should always be collected at the same time, but fasting is not required. In a recent study of more than 95,000 hemodialysed patients in the United States, the authors found that PTH (and phosphate) concentrations were lowest in the late morning and highest in the late afternoon, which clinicians may want to consider when initiating or modifying a therapy [36].

Biological variation

PTH is secreted tonically, with approximately 25% of the peptide secreted as small amplitude pulses approximately three times per hour [37]. This can contribute to the significant intra-individual variation of PTH and should be considered when interpreting PTH results (see below). Mean intra- and inter-individual variations of PTH are 15.7 and 23.5%, respectively, according to the Biological variation database (https://biologicalvariation.eu/). These results are very similar to those observed in haemodialysed patients (13.8%; [38]) and in the EuBIVAS study (15.2%; [39]).

Seasonal difference

PTH concentrations fluctuate with the seasons (lower in summer and higher in winter), likely reflecting the fluctuations of 25(OH)-vitamin D levels. In the summer, PTH levels were 25% lower in growing male adolescents [40], and 11% lower in patients with primary hyperparathyroidism [41] (both $p<0.0001$). Notably, patients receiving vitamin D supplements no longer exhibit this variation [42]. This finding is crucial for establishing PTH reference ranges (see below). Indeed, as recommended in the Guidelines for the management of asymptomatic primary hyperparathyroidism, PTH reference ranges should be established in subjects with adequate vitamin D levels [43].

Analytical considerations

Immunoassays for determining PTH

Berson and Yalow published the description of the first radio-immunoassay for parathyroid hormone in 1963 [44]. In the early 1970s, such immunoassays, now known as "first generation" assays, were utilised. They utilised a single antiPTH antibody directed against the C-terminal portion of the peptide and a ^{125}I-labeled peptide (generally of bovine origin) and experienced significant interferences, especially in haemodialysed patients, due to the presence of C-terminal fragments.

Published in : Clinical Chemistry and Laboratory Medicine (2023), pp. 1-8
doi.org/10.1515/cclm-2022-0942
Status : Postprint (Author's version)

In 1987, such assays were supplanted by the "Allegro" Immunoradiometric (IRMA) method developed by Nichols Diagnostics [45]. This IRMA employed a capture antibody directed against the (39–84) region of the PTH molecule coated on a plastic bead and a ^{125}I-labeled antibody recognising the (13–24) region of the peptide. Using a "sandwich" design decreased interference with the C-terminal or midfragments, and this "second-generation" assay kit and the ones that followed were considered to measure only the fulllength (1–84) PTH.

Several similar assays, either IRMA or fully automated chemiluminescent assays, appeared on the market in the years that followed. Some of them, such as the Allegro assay, use an anti-N-terminal antibody directed towards the proximal (13–24) portion of the hormone, whereas others, such as the Roche Elecsys intact PTH assay, recognise a more distal epitope in the (26–32) portion.

Unfortunately, it was quickly discovered that the second generation of PTH assays had limitations. In fact, a number of studies suggested that the degree of secondary hyperparathyroidism in CKD patients was overestimated, with some patients exhibiting elevated "intact" PTH concentrations despite displaying histological characteristics of low bone turnover [46, 47]. Lepage et al. demonstrated in 1998 that several "intact" PTH assays recognised a PTH moiety distinct from the (1–84) PTH, which co-eluted in HPLC with a synthetic (7–84) PTH fragment [48]. This fraction was subsequently referred to as the "non-(1–84) PTH" or, by extension, the "(7–84) PTH" (which has been further shown not to be present in human circulation, see above). Scantibodies Laboratories [49] designed the first "third-generation" PTH assay in 1999.

This IRMA, referred to as "Whole PTH assay" or "Bio-intact PTH," employed an anti-C-terminal antibody comparable to those of the "intact" PTH assays, but an anti-N-terminal antibody directed against the very first amino acids of the peptide (1–4). Therefore, this IRMA no longer measured the "non-1–84" PTH fragments. This kit made it possible to determine the amount of "non-(1–84)" PTH by subtracting the value of "whole" PTH from the "intact" PTH values.

Standardisation of higher order methods and PTH measurements

PTH assays are not yet standardised, which has a significant effect on the inter-analytical methods variability due to the fact that different assays yield different results. In 2010, an IFCC working group on PTH standardisation was established for this purpose. In 2017, the WG published its perspectives and priorities for enhancing PTH measurement [50]. According to the results of PTH (1–84) recovery, it was evident that inter-method agreement would improve if all PTH methods were calibrated against the same material. The Working Group had therefore proposed the use of a single internationally recognised standard, "such as" WHO PTH IS 95/646. This standard is composed of recombinant human Parathyroid hormone 1–84 and comes in ampoules that contain 100 µg of PTH 1–84. This standard cannot be enforced, however, because its complete traceability to a SI standard and its measurement uncertainty has not been assessed. In addition, there is currently no candidate reference measurement procedure for PTH determination. Two LCMS/MS have been published to date [51, 52], but they lack sensitivity and reproducibility must still be demonstrated. As stated previously, an HRMS-MS method for 1–84 PTH and PTH fragments was recently developed; however, this method lacks sensitivity for 1–84 PTH and has not been designed or validated as a potential reference method.

Very recently, Farré-Segura et al. have developed a LCMS-MS method for 1–84 PTH quantification without using any tryptic digestion or immunopurification. This method possesses most of the features of a candidate

Published in : Clinical Chemistry and Laboratory Medicine (2023), pp. 1-8
doi.org/10.1515/cclm-2022-0942
Status : Postprint (Author's version)

reference method and will be important to improve the standardisation of immunoassays, characterise quality controls and assess the value of PTH standards [13].

New PTH forms – modifications post-translational

Amino-PTH

In parathyroid carcinoma [53, 54] and in rare cases of severe primary hyperparathyroidism [55], a form of (1–84) PTH predicted to be phosphorylated on the serine (position 17) is overproduced, resulting in an inverted $\frac{3rd\ generation}{2nd\ generation}$ PTH ratio.

This form, known as amino-PTH, cross-reacts with the antibodies used in 3rd generation PTH kits but not with the antibodies used in 2nd generation kits (with the exception of the Roche intact PTH assay [56]). In normal individuals, approximately 10% of the circulating PTH is amino-PTH [57].

Thus, the $\frac{3rd\ generation}{2nd\ generation}$ PTH ratio should always be <1 because the large non-(1–84) fragments detected with 2nd generation kits represent a greater proportion of the circulating PTH than amino-PTH. The ratio is currently proposed as a new diagnostic tool for identifying patients with parathyroid carcinoma [58], and its inversion has been shown to be predictive of the recurrence of the cancer, even before the rise in calcemia [59].

Oxydised PTH: the end of a legend?

PTH contains two methionines, one at position 8 and the other at position 18. Numerous studies, primarily from the 1980s, demonstrated that oxidised PTH was inactive: it has a lower binding affinity to the PTH receptor and, when bound to the receptor, cannot generate cAMP; it loses its biological action on smooth muscle cells, cannot stimulate alkaline phosphatase activity in neonatal bone cells, and cannot regulate calcium and phosphate metabolisms in various animal models [60]. Since haemodialysed patients experience severe oxidative stress [61], Hocher et al. hypothesised that peptides such as PTH can be oxidised, thereby losing their ability to interact with their receptors [62]. Since immunoassays for PTH recognise both the oxidised (ox) and non-oxidised (n-ox) forms, the authors used antihuman oxPTH antibodies coated on a gel to separate the nonoxidised from the oxidised forms (Immunodiagnostik AG, Bensheim, Germany). After treatment, the non-oxidised form only was present in the samples and could be quantified with an immunoassay. Accordingly, they found, in haemodialysed patients, an increased survival in the highest n-oxPTH tertile compared with the lowest n-oxPTH tertile. Later, based on the correlation between intact PTH and oxPTH, Hocher et al. hypothesised that total intact PTH concentration was more of a reflection of oxidative stress than of the peptide's biological activity. Indeed, they found that only non-oxidised PTH but not oxidised PTH nor intact PTH was associated with graft loss in stable kidney transplant recipients and that only n-oxPTH should be quantified [63]. This prompted these authors to call for fourth generation PTH (immuno)assays, which would only recognise the n-oxPTH [64]. However, in a population of CKD patients, other authors found that 2nd generation PTH was more closely associated with cardiovascular events, CKD progression, and mortality than n-oxPTH [65]. As an indicator of bone turnover in patients with kidney failure, other authors have found that n-oxPTH provided no added value compared to total PTH [66]. Notably, Kritmetapak et al. did not detect oxidised PTH in a cohort of CKD patients using the HR-MS/MS method they developed [11]. With the extremely sensitive and specific LCMS/MS method we developed in our laboratory,

we were unable to detect any trace of oxidised PTH, even in samples obtained from haemodialised patients, calling into question the existence of any oxidised form and the results obtained by Hocher et al. [13].

Post-analytical considerations

Biological variability

To define the Analytical Performance Specifications (APS) and to calculate the Reference Change Values (RCV), that is, the minimal percentage of variation between two consecutive results which is considered biologically significant, biological variability is of utmost importance. The European Biological Variation Study Working Group found that the within-subject BV estimates differed significantly between men and women [13.0% (12.1–14.2%) and 15.2% (14.3–16.3%), respectively], whereas the between-subject estimates were comparable (men: 26.8% (21.4–35.1%), pre-menopausal women: 27.8% (22.7–36.1%)] [39]. Consequently, the Group has proposed APS values of 6.5 and 7.5% for the CV and bias, respectively, of PTH determination methods. Regarding the RCV, the authors proposed that a decrease of −26.7% would be considered biologically significant, while an increase of 36.5% would be considered biologically significant. These findings have significant clinical and analytical implications. From a clinical standpoint, an RCV of 36.5% indicates that there is no significant biological change in a patient's or subject's PTH result if the difference between the two results is less than 36.5%. If we consider a subject with a previous PTH value of 50 ng/L (the upper reference range of the 2nd generation Roche PTH assay), a change in his PTH concentration that is greater than 18 ng/L i.e. if the subject presents a PTH higher than 68 ng/L. Similarly, a PTH change in a haemodialysed patient with a baseline PTH of 300 ng/L will be deemed significant if it exceeds ≈110 ng/L (>410 ng/L). This is one of the reasons why the KDIGO guidelines insist on considering the trend of PTH variation rather than a single value [67].

Reference interval

The selection of subjects to determine accurate reference ranges is of the utmost importance. Patients suffering from primary or secondary hyperparathyroidism should logically be excluded from the reference population in order to establish accurate PTH reference values. This raises the question of which inclusion/exclusion criteria should be applied when recruiting a reference population to determine normal PTH levels. The exclusion criteria for this population include any condition that may induce an increase or decrease in PTH concentration. Low serum 25-hydroxyvitamin D (25OHD) concentration is highly prevalent in the general population [68] and should therefore be prevalent in a group recruited to establish normal PTH values that appears to be healthy. Excluding subjects with vitamin D deficiency from a reference population for serum PTH reference values seems logical and is strongly recommended in the two most recent guidelines for the diagnosis and management of asymptomatic PHPT [43]. Multiple studies have demonstrated that excluding subjects with a low serum 25OHD concentration from a reference population decreases the upper normal limit for serum PTH by 20– 35%, depending on the assay [69, 70]. In addition to 25(OH)D levels, renal function should be considered when determining PTH reference values. When estimated glomerular filtration rate (eGFR) is below 60 mL/min/1.73 m^2, PTH levels tend to rise. When establishing reference ranges, a creatinine measurement to determine the eGFR is required because a decreased renal function may be present but unrecognised in some apparently healthy subjects (especially those older than 60 years),

requiring a creatinine measurement to determine the eGFR. Other parameters such as age, BMI, dietary calcium intake, and ethnicity may also influence PTH reference ranges, and further research is required to determine whether PTH reference values should be stratified based on some of these parameters.

KDIGO guidelines recommend maintaining serum PTH levels in haemodialysed patients between two and nine times the upper normal limit (UNL) of the reference range [71]. Accordingly, it is of the utmost importance to establish correct upper limits for the PTH normal range. We established PTH reference ranges for 10 distinct PTH assays in a population of 120 healthy males and 120 healthy females with 25(OH)D levels >30 ng/mL, normal creatinine, calcium, and phosphate levels. When compared to the manufacturers' suggested reference ranges, we discovered that our UNL was consistently lower. The classification of 149 haemodialysed patients according to the KDIGO target range [70] was significantly improved by using the ULN we discovered rather than the one proposed by the manufacturers.

Conclusions

In clinical practise, PTH measurement is of paramount importance. It was one of the first immunoassays to be developed, and a growing interest in peptide research has supported its study ever since. Standardisation of assays, knowledge of post-translational modifications of the peptide, and the potential role played by circulating fragments will definitely be enhanced by the recent discoveries of the past few years and the enhancements in analytical determination by mass spectrometry. Clinical Chemistry and Laboratory Medicine has unquestionably played a pivotal role in the improvement of PTH determination, particularly by providing important and relevant recommendations for the preanalytical phase, which is the phase in Laboratory Medicine where the majority of problems arise.

Acknowledgments: We would like to thank Professor Pierre Delanaye for his careful review of the text.

References

1. Ramasamy I. Inherited disorders of calcium homeostasis. Clin Chim Acta 2008;394:22–41.
2. Jüppner H, Abou-Samra AB, Freeman M, Kong XF, Schipani E, Richards J, et al. A G protein-linked receptor for parathyroid hormone and parathyroid hormone-related peptide. Science 1991;254:1024–6.
3. Gardella TJ, Axelrod D, Rubin D, Keutmann HT, Potts JT,Kronenberg HM, et al. Mutational analysis of the receptor-activating region of human parathyroid hormone. J Biol Chem 1991;266:13141–6.
4. Pfister MF, Ruf I, Stange G, Ziegler U, Lederer E, Biber J, et al. Parathyroid hormone leads to the lysosomal degradation of the renal type II Na/Pi cotransporter. Proc Natl Acad Sci 1998;95:1909–14.
5. Naylor SL, Sakaguchi AY, Szoka P, Hendy GN, Kronenberg HM, Rich A, et al. Human parathyroid hormone gene (PTH) is on short arm of chromosome 11. Somat Cell Genet 1983;9:609–16.
6. Habener JF, Rosenblatt M, Kemper B, Kronenberg HM, Rich A, Potts JT. Pre-proparathyroid hormone; amino acid sequence, chemical synthesis, and some biological studies of the precursor region. Proc Natl Acad Sci USA 1978;75:2616–20.

7. Arnold A, Horst SA, Gardella TJ, Baba H, Levine MA, Kronenberg HM. Mutation of the signal peptide-encoding region of the preproparathyroid hormone gene in familial isolated hypoparathyroidism. J Clin Invest 1990;86:1084–7.

8. Segre GV, Perkins AS, Witters L, Potts JT. Metabolism of parathyroid hormone by isolated rat kupffer cells and hepatocytes. J Clin Invest 1981;67:449–57.

9. Yamashita H, Gao P, Cantor T, Futata T, Murakami T, Uchino S, et al. Large carboxy-terminal parathyroid hormone (PTH) fragment with a relatively longer half-life than 1-84 PTH is secreted directly from the parathyroid gland in humans. Eur J Endocrinol 2003;149:301–6.

10. D'amour P, Lazure C, Labelle F. Metabolism of radioiodinated carboxyterminal fragments of bovine parathyroid hormone in normal and anephric rats. Endocrinology 1985;117:127–34.

11. Kritmetapak K, Losbanos LA, Hines JM, O'Grady KL, Ulmer CZ, Vesper HW, et al. Chemical characterization and quantification of circulating intact PTH and PTH fragments by high-resolution mass spectrometry in chronic renal failure. Clin Chem 2021;67:843–53.

12. Singh RJ, Hines JM, Lopez MF, Krastins B, Hoofnagle a. N. Mass spectrometric immunoassay raises doubt for the existence of parathyroid hormone fragment 7–84. Clin Chem 2015;61:558–60.

13. Farré-Segura J, Le Goff C, Lukas P, Cobraiville G, Fillet M, Servais AC, et al. Validation of an LC-MS/MS method using solid-phase extraction for the quantification of 1-84 parathyroid hormone : toward a candidate reference measurement procedure. Clin Chem 2022;68: 1399–409.

14. Sneddon WB, Magyar CE, Willick GE, Syme CA, Galbiati F, Bisello A, et al. Ligand-selective dissociation of activation and internalization of the parathyroid hormone (PTH) receptor: conditional efficacy of PTH peptide fragments. Endocrinology 2004;145:2815–23.

15. Slatopolsky E, Finch J, Clay P, Martin D, Sicard G, Singer G, et al. A novel mechanism for skeletal resistance in uremia. Kidney Int 2000;58: 753–61.

16. Divieti P, Inomata N, Chapin K, Singh R, Jüppner H, Bringhurst FR. Receptors for the carboxyl-terminal region of PTH(1-84) are highly expressed in osteocytic cells. Endocrinology 2001;142:916–25.

17. Divieti P, John MR, Jüppner H, Bringhurst FR. Human PTH- (7–84) inhibits bone resorption in vitro via actions independent of the type 1 PTH/PTHrP receptor. Endocrinology 2002;143:171–6.

18. Kukreja SC, D'Anza JJ, Wimbiscus SA, Fisher JE, McKee RL, Caulfield MP, et al. Inactivation by plasma may be responsible for lack of efficacy of parathyroid hormone antagonists in hypercalcemia of malignancy. Endocrinology 1994;134:2184–8.

19. Murray TM, Rao LG, Muzaffar SA, Ly H. Human parathyroid hormone carboxyterminal peptide (53–84) stimulates alkaline phosphatase activity in dexamethasone-treated rat osteosarcoma cells in vitro. Endocrinology 1989;124:1097–9.

20. Sutherland MK, Rao LG, Wylie JN, Gupta A, Ly H, Sodek J, et al. Carboxyl-terminal parathyroid hormone peptide (53–84) elevates alkaline phosphatase and osteocalcin mRNA levels in SaOS-2 cells. J Bone Miner Res 2009;9:453–8.

21. Lippi G, Guidi GC, Mattiuzzi C, Plebani M. Preanalytical variability: the dark side of the moon in laboratory testing. Clin Chem Lab Med 2006; 44:358–65.

22. Lippi G, Blanckaert N, Bonini P, Green S, Kitchen S, Palicka V, et al. Causes, consequences, detection, and prevention of identification errors in laboratory diagnostics. Clin Chem Lab Med 2009;47:143–53.

23. Lippi G, Becan-McBride K, Behúlová D, Bowen RA, Church S, Delanghe J, et al. Preanalytical quality improvement: in quality we trust. Clin Chem Lab Med 2013;51:229–41.

24. Lippi G, Banfi G, Church S, Cornes M, De Carli G, Grankvist K, et al. Preanalytical quality improvement. In pursuit of harmony, on behalf of European federation for clinical Chemistry and laboratory medicine (EFLM) working group for preanalytical phase (WG-PRE). Clin Chem Lab Med 2015;53:357–70.

25. Cavalier E, Plebani M, Delanaye P, Souberbielle J-C. Considerations in parathyroid hormone testing. Clin Chem Lab Med 2015;53:1913–9.

26. Hanon EA, Sturgeon CM, Lamb EJ. Sampling and storage conditions influencing the measurement of parathyroid hormone in blood samples: a systematic review. Clin Chem Lab Med 2013;51:1925–41.

27. Valcour A, Zierold C, Blocki FA, Hawkins DM, Martin KJ, Rao SD, et al. Trueness, precision and stability of the LIAISON 1–84 parathyroid hormone (PTH) third-generation assay: comparison to existing intact PTH assays. Clin Chem Lab Med 2018;56:1476–82.

28. ISO Guide 5725-6:1994. Accuracy (trueness and precision) of measurement methods and results — part 6: use in practice of accuracy values; n.d.

29. World Health Organization. Use of anticoagulants in diagnostic laboratory investigations; 2002.

30. Schleck ML, Souberbielle JC, Delanaye P, Plebani M, Cavalier E. Parathormone stability in hemodialyzed patients and healthy subjects: comparison on non-centrifuged EDTA and serum samples with second and third-generation assays. Clin Chem Lab Med 2017;55:1152–9.

31. Cavalier E, Carlisi A, Bekaert AC, Rousselle O, Chapelle JP, Delanaye P. New insights on the stability of the parathyroid hormone as assayed by an automated 3rd generation PTH assay. Clin Chim Acta 2012;413:353–4.

32. Cavalier E, Delanaye P, Hubert P, Krzesinski JM, Chapelle JP, Rozet E. Estimation of the stability of parathyroid hormone when stored at −80°C for a long period. Clin J Am Soc Nephrol 2009;4:1988–92.

33. Cavalier E, Delanaye P, Carlisi A, Krzesinski JM, Chapelle JP. Stability of intact parathyroid hormone in samples from hemodialysis patients. Kidney Int 2007;72:370–2.

34. Vulpio C, Bossola M, Speranza D, Zuppi C, Luciani G, Di Stasio E. Influence of blood sampling site on intact parathyroid hormone concentrations in hemodialysis patients. Clin Chem 2010;56:489–90.

35. Morris HA, Eastell R, Jorgensen NR, Cavalier E, Vasikaran S, Chubb SAP, et al. IFCC-IOF working group for standardisation of bone marker assays (WG-BMA), clinical usefulness of bone turnover marker concentrations in osteoporosis. Clin Chim Acta 2017;467:34–41.

36. Ginsberg C, Miller LM, Ofsthun N, Dalrymple LS, Ix JH. Differences in phosphate and parathyroid hormone concentrations over the day among patients on hemodialysis. J Am Soc Nephrol 2022;33:2087–93.

37. Samuels MH, Veldhuis JD, Kramer P, Urban RJ, Bauer R, Mundy GR. Episodic secretion of parathyroid hormone in postmenopausal women: assessment by deconvolution analysis and approximate entropy. J Bone Miner Res 1997;12:616–23.

38. Cavalier E, Delanaye P, Moranne O. Variability of new bone mineral metabolism markers in patients treated with maintenance hemodialysis: implications for clinical decision making. Am J Kidney Dis 2013;61:847–8.

39. Bottani M, Banfi G, Guerra E, Locatelli M, Aarsand AK, Coşkun A, et al. European Biological Variation Study (EuBIVAS): within- and between- subject biological variation estimates for serum biointact parathyroid hormone based on weekly samplings from 91 healthy participants. Ann Transl Med 2020;8:855.

40. Guillemant J, Cabrol S, Allemandou A, Peres G, Guillemant S. Vitamin D-dependent seasonal variation in growing male adolescents. Bone 1995;17:513–6.

41. Nevo-Shor A, Kogan S, Joshua BZ, Bahat-Dinur A, Novack V, Fraenkel M. Seasonal changes in serum calcium, PTH and vitamin D levels in patients with primary hyperparathyroidism. Bone 2016;89:59–63.

42. Cong E, Walker MD, Kepley A, Zhang C, McMahon DJ, Silverberg SJ. Seasonal variability in vitamin D levels No longer detectable in primary hyperparathyroidism. J Clin Endocrinol Metab 2015;100:3452–9.

43. Bilezikian JP, Brandi ML, Eastell R, Silverberg SJ, Udelsman R, Marcocci C, et al. Guidelines for the management of asymptomatic primary hyperparathyroidism: summary statement from the fourth international workshop. J Clin Endocrinol Metab 2014;99:3561–9.

44. Berson SA, Yalow RS, Aurbach GD, Potts JT. Immunoassay of bovine and human parathyroid hormone. Proc Natl Acad Sci 1963;49:613–7.

45. Nussbaum SR, Zahradnik RJ, Lavigne JR, Brennan GL, Nozawa-Ung K, Kim LY, et al. Highly sensitive two-site immunoradiometric assay of parathyrin, and its clinical utility in evaluating patients with hypercalcemia. Clin Chem 1987;33:1364–7.

46. Quarles LD, Lobaugh B, Murphy G. Intact parathyroid hormone overestimates the presence and severity of parathyroid-mediated osseous abnormalities in uremia. J Clin Endocrinol Metab 1992;75:145–50.

47. Wang M, Hercz G, Sherrard DJ, Maloney NA, Segre GV, Pei Y. Relationship between intact 1–84 parathyroid hormone and bone histomorphometric parameters in dialysis patients without aluminum toxicity. Am J Kidney Dis 1995;26:836–44.

48. Lepage R, Roy L, Brossard JH, Rousseau L, Dorais C, Lazure C, et al. Anon-(1–84) circulating parathyroid hormone (PTH) fragment interferes significantly with intact PTH commercial assay measurements in uremic samples. Clin Chem 1998;44:805–9.

49. John MR, Goodman WG, Ping G, Cantor T, Salusky IB, Juppner H. A Novel Immunoradiometric assay detects full-length human PTH but not amino-terminally truncated fragments: implications for PTH measurements in renal failure. J Clin Endocrinol Metab 1999;84: 4287–90.

50. Sturgeon CM, Sprague S, Almond A, Cavalier E, Fraser WD, AlgecirasSchimnich A, et al. Perspective and priorities for improvement of parathyroid hormone (PTH) measurement – a view from the IFCC Working Group for PTH. Clin Chim Acta 2017;467:42–7.

51. Kumar V, Barnidge DR, Chen LS, Twentyman JM, Cradic KW, Grebe SK, et al. Quantification of serum 1–84 parathyroid hormone in patients with hyperparathyroidism by immunocapture in situ digestion liquid chromatography-tandem mass spectrometry. Clin Chem 2010;56: 306–13.

52. Lopez MF, Rezai T, Sarracino DA, Prakash A, Krastins B, Athanas M, et al. Selected reaction monitoring-mass spectrometric immunoassay responsive to parathyroid hormone and related variants. Clin Chem 2010;56:281–90.

53. Rubin MR, Silverberg SJ, D'Amour P, Brossard JH, Rousseau L, Sliney J, et al. An N-terminal molecular form of parathyroid hormone (PTH) distinct from hPTH(1–84) is overproduced in parathyroid carcinoma. Clin Chem 2007;53:1470–6.

54. Cavalier E, Daly AF, Betea D, Pruteanu-Apetrii PN, Delanaye P, Stubbs P, et al. The ratio of parathyroid hormone as measured by third- and second-generation assays as a marker for parathyroid carcinoma. J Clin Endocrinol Metab 2010;95:3745–9.

55. Räkel A, Brossard JH, Patenaude JV, Albert C, Nassif E, Cantor T, et al. Overproduction of an amino-terminal form of PTH distinct from human PTH(1–84) in a case of severe primary hyperparathyroidism: influence of medical treatment and surgery. Clin Endocrinol 2005;62:721–7.

56. Souberbielle JC, Friedlander G, Cormier C. Practical considerations in PTH testing. Clin Chim Acta 2006;366:81–9.

57. D'Amour P, Brossard JH, Rousseau L, Roy L, Gao P, Cantor T. Amino-terminal form of parathyroid hormone (PTH) with immunologic similarities to hPTH(1–84) is overproduced in primary and secondary hyperparathyroidism. Clin Chem 2003;49:2037–44.

58. Schulte KM, Talat N. Diagnosis and management of parathyroid cancer. Nat Rev Endocrinol 2012;8:612–22.

59. Caron P, Maiza JC, Renaud C, Cormier C, Barres BH, Souberbielle JC. High3rd generation/2nd generation PTH ratio in a patient with parathyroid carcinoma: clinical utility of 3rg generation/2nd generation PTH ratio in patients with primary hyperparathyroidism. Clin Endocrinol 2008;70: 533–8.

60. Hocher B, Yin L. Why current PTH assays mislead clinical decision making in patients with secondary hyperparathyroidism. Nephron 2017;136:137–42.

61. Himmelfarb J, Stenvinkel P, Ikizler TA, Hakim RM. The elephant inuremia: oxidant stress as a unifying concept of cardiovascular disease in uremia. Kidney Int 2002;62:1524–38.

62. Hocher B, Armbruster FP, Stoeva S, Reichetzeder C, Grön HJ, Lieker I, et al. Measuring parathyroid hormone (PTH) in patients with oxidative stress – do we need a fourth generation parathyroid hormone assay? PLoS One 2012;7:e40242.

63. Lu YP, Zeng S, Chu C, Hasan AA, Slowinski T, Yin LH, et al. Non-oxidized PTH (n-oxPTH) is associated with graft loss in kidney transplant recipients. Clin Chim Acta 2020;508:92–7.

64. Hocher B, Zeng S. Need for better PTH assays for clinical research and patient treatment. Clin Chem Lab Med 2017;56:13–5.

65. Seiler-Mussler S, Limbach AS, Emrich IE, Pickering JW, Roth HJ, Fliser D, et al. Association of nonoxidized parathyroid hormone with cardiovascular and kidney disease outcomes in chronic kidney disease. Clin J Am Soc Nephrol 2018;13:569–76.

66. Ursem SR, Heijboer AC, D'Haese PC, Behets GJ, Cavalier E, Vervloet MG, et al. Non-oxidized parathyroid hormone (PTH) measured by current method is not superior to total PTH in assessing bone turnover in chronic kidney disease. Kidney Int 2021;99:1173–8.

67. KDIGO 2017 Clinical Practice Guideline Update for the Diagnosis. Evaluation, prevention, and treatment of chronic kidney disease– mineral and bone disorder (CKD-MBD). Kidney Int Suppl 2017;7:1–59.

68. Touvier M, Deschasaux M, Montourcy M, Sutton A, Charnaux N, Kesse-Guyot E, et al. Interpretation of plasma PTH concentrations according to 25OHD status, gender, age, weight status, and calcium intake: importance of the reference values. J Clin Endocrinol Metab 2014;99: 1196–203.

69. Souberbielle JC, Fayol V, Sault C, Lawson-Body E, Kahan A, Cormier C. Assay-specific decision limits for two new automated parathyroid hormone and 25-hydroxyvitamin D assays. Clin Chem 2005;51: 395–400.

70. Cavalier E, Delanaye P, Vranken L, Bekaert AC, Carlisi A, Chapelle JP, et al. Interpretation of serum PTH concentrations with different kits in dialysis patients according to the KDIGO guidelines: importance of the reference (normal) values. Nephrol Dial Transplant 2011;27:1950–6.

71. Moe SM, Drueke TB. For the K.W. Group, KDIGO clinical practice guideline for the diagnosis, evaluation, prevention and treatment of chronic kidney disease mineral and bone disorder (CKD-MBD). Kidney Int 2009;76:S1–28.