Introduction
Parathyroid hormone (PTH) determination has now become a “routine” test in clinical chemistry, and the majority of manufacturers propose this parameter in their panel menu, either on fully automated instruments or for manual enzyme-linked immunosorbent assays (ELISAs). Indeed, PTH is requested for the diagnosis of primary and secondary hyperparathyroidism (together with calcium serum levels), and for the follow-up of hemodialyzed patients that suffer from chronic kidney diseases-mineral and bone disorders (CKD-MBD), a pathology that encompasses low or high bone turnover along with vascular calcifications. More generally, PTH determination is recommended in any bone pathology and disease linked with calcium levels. However, PTH determination remains challenging. The difficulty starts with the pre-analytical phase: PTH stability, for instance, is a matter of intense debate (1). It is particularly tricky when it comes to PTH results interpretation. Indeed, if a clinician discusses a patient’s thyroid-stimulating hormone (TSH) levels at 6 mUI/L with a colleague, they will both be sure that the value is elevated. The situation will certainly be the same for a 25(OH)D value at 25 ng/mL, a PSA at 5 µg/L, or an ultrasensitive troponin T at 30 ng/L. However, if a nephrologist working in my hospital relates the clinical case of a hemodialyzed patient presenting PTH levels at 100 ng/L in a nephrology congress, this will indicate “normal” value since it is approximately 3 times higher than the upper limit of normality whereas most of his or her colleagues will think that the patient is presenting a low bone turnover. This discrepancy is due to a simple reason: our laboratory has made the choice to work with a 3rd generation PTH assay while most other laboratories (still) work with 2nd generation (or “intact”) PTH assays, that either present cross-reactivity to PTH fragments or simply provide higher results because of a lack of standardization. Despite this, none of the nephrologists and clinical chemists present in the audience will know if this PTH at 100 ng/L is biologically active (because it is non-oxidized) or not. In the following review, some pitfalls of PTH interpretation will be explored.

Keywords: Parathyroid hormone (PTH); standardization; reference range; biological variation

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PARATHYROID HORMONE RESULTS INTERPRETATION IN THE BACKGROUND OF VARIABLE ANALYTICAL PERFORMANCE

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Abstract: Parathyroid hormone (PTH) determination is a difficult task. In this paper, we highlight the importance of PTH standardization and the impact of establishing robust reference ranges for the accurate interpretation of PTH results. We also discuss the biological variation of PTH and its impact on the calculation of least significant change (LSC) and on targets for analytical validation of the methods. Finally, we look at the different fragments and forms of PTH that are important in clinical practice.

Keywords: Parathyroid hormone (PTH); standardization; reference range; biological variation
Gaussian curve of the distribution of values observed in a “healthy” population. The values encompassed between these two limits correspond to 95% of the “healthy” population. From a strict statistical perspective, this is only true if the number of subjects included in the study is infinite. Since it is not possible to perform such studies on an infinite number of healthy subjects, the CLSI EP28-A3c guideline for defining, establishing and verifying reference intervals requires the inclusion of 120 healthy individuals of the “local” population (per age or sex class if necessary) to establish reference ranges. However, if a maximum of 2.5% of the subjects is outside each limit in an infinite population, this percentage raises to 4.6% outside each limit if calculations are performed on 120 individuals. This leads to the concept of the “90% confidence range” that should encompass each limit, but the 90% confidence interval is not frequently provided by laboratories on the result protocols. As such, the “90% confidence range” would also be completely abstruse for most clinicians. Selecting a population of 120 healthy individuals is very complicated—or impossible—for most laboratories, and so the CLSI guidelines then allow the “validation” (or “verification”) of the reference ranges by third parties (manufacturers, textbooks, publications etc.). The ISO 15189 Guideline recommends that this verification should be performed on a periodic basis. In practice, this verification only requests the participation of 20 healthy individuals from the “local” population. The test is performed, and if 90% of the observations fall into the third party’s 95% reference range, the range is considered as valid. The verification is much more affordable than the establishment of the reference range, but raises a few questions, particularly as it relates to the PTH.

First, what is considered a “local” population? Cities are now almost completely cosmopolitan, and ethnicity and cultural habits can definitely change the reference ranges from one population to the next, even if they live in the same city. For example, African American (AA) subjects present higher PTH levels than their Caucasian counterparts (2-5). It is not clear whether this is due to a high prevalence of vitamin D deficiency in AA subjects (6) since 25(OH)D has not always been measured in these studies, to a physiological “PTH resistance”, or if it is even linked to a diverse recognition of fragments by the 2nd generation (“intact”) PTH assays. Indeed, we have recently shown that African subjects living in Abidjan, Côte d’Ivoire, presented similar reference ranges to French and Belgian subjects when a 3rd generation PTH assay was used; however, when a 2nd generation PTH assay was used (7), the African subjects’ upper reference ranges were then comparatively higher (7).

Food consumption patterns (particularly calcium and protein) and other habits also have an impact on PTH reference ranges, which leads to the second question: what is a “normal” population in terms of PTH? We have already shown that selecting subjects presenting 25(OH)-vitamin D serum levels above 30 ng/mL in the reference population decreased the upper limit of PTH normality compared to studies where individuals from the general population were included without taking vitamin D sufficiency into consideration (8,9). The direct clinical impact of using PTH reference ranges established in a vitamin-D-replete population was a better classification of hemodialyzed patients according to the KDIGO guidelines and an earlier detection of secondary hyperparathyroidism due to vitamin D deficiency (8,10). From a practical point of view, most laboratories worldwide use the reference ranges provided by manufacturers. These reference ranges are not necessarily established properly, as detailed above. Yet, by “verifying” them in a vitamin-D-replete population of 20 individuals free from secondary hyperparathyroidism, the values obtained will always be encompassed in the manufacturer’s range, and will be wrongly used by the laboratory. I strongly encourage each laboratory to verify that the reference ranges they use have correctly been established in a vitamin-D-replete population, as recommended in The Guidelines for the Management of Asymptomatic Primary Hyperparathyroidism (11).

The main characteristics of the major PTH assays available on the market are presented in Table 1.

**Biological variability and least significant change (LSC)**

Biological variability is of paramount importance in defining optimal, desirable and minimal bias and coefficients of variation of analytical methods, which then allows calculation of allowable total error. It is also largely used to calculate the LSC, which is the minimal percentage of variation between two successive results that is considered as biologically significant. Establishment of intra- and inter-individual variability (CVw and CVg) is very complicated and the majority of laboratorians refer to the Westgard website that presents a desirable biological variation database (https://www.westgard.com/biodatabase1.htm). From this website, one can see that CVw and CVg for PTH...
Table 1 Characteristics of different commercial PTH assays

<table>
<thead>
<tr>
<th>Name of the assay</th>
<th>Manufacturer</th>
<th>Intra-assay CV (%)</th>
<th>Inter-assay CV (%)</th>
<th>Reference range (Manufacturer) (pg/mL)</th>
<th>Reference population</th>
<th>Automated 2nd or 3rd generation</th>
<th>Tracer</th>
<th>Epitope, type and origin of coated Ab</th>
<th>Epitope, type and origin of labelled Ab</th>
<th>Detection limit (pg/mL)</th>
<th>Highest measurable value (pg/mL)</th>
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<tr>
<td>2nd generation assays</td>
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<tr>
<td>Intact PTH Architect</td>
<td>Abbott (Abbott Park, IL)</td>
<td>&lt;6.1</td>
<td>&lt;6.4</td>
<td>15.0–68.3</td>
<td>143 plasma samples from apparently healthy adults</td>
<td>Yes</td>
<td>2nd Acridinium ester</td>
<td>Polyclonal goat</td>
<td>Polyclonal goat</td>
<td>3.0</td>
<td>3,000</td>
</tr>
<tr>
<td>Access PTH intact</td>
<td>Beckman-Coulter (Brea, CA)</td>
<td>&lt;2.6</td>
<td>&lt;5.8</td>
<td>12–88</td>
<td>289 paired samples (serum and plasma EDTA) from apparently healthy men and women aged 19–67 years old. Exclusion of individuals with abnormal calcium, creatinine and 25-OH vitamin D levels</td>
<td>Yes</td>
<td>2nd Alkaline phosphatase</td>
<td>Polyclonal goat</td>
<td>Monoclonal</td>
<td>1.0</td>
<td>3,500</td>
</tr>
<tr>
<td>N-tact PTH SP IRMA</td>
<td>DiaSorin (Stillwater, MN)</td>
<td>&lt;3.6</td>
<td>&lt;4.9</td>
<td>13–54</td>
<td>129 serum samples from apparently healthy fasting young adults</td>
<td>No</td>
<td>2nd</td>
<td>¹²⁵I</td>
<td>Polyclonal goat 39–84</td>
<td>0.7</td>
<td>2,000</td>
</tr>
<tr>
<td>Liaison N-tact</td>
<td>DiaSorin (Stillwater, MN)</td>
<td>&lt;5.0</td>
<td>&lt;6.2</td>
<td>17.3–72.9</td>
<td>105 healthy adults</td>
<td>Yes</td>
<td>2nd Isoluminol</td>
<td>39–84</td>
<td>1–4</td>
<td>1.0</td>
<td>2,000</td>
</tr>
<tr>
<td>Intact PTH Vitros 5600</td>
<td>Ortho Clinical Diagnostics (Rochester, NY)</td>
<td>&lt;2.0</td>
<td>&lt;7.5</td>
<td>7.5–53.5</td>
<td>EDTA, heparin plasma or serum from 240 patients presenting normal calcium, TSH, creatinine and vitamin D levels</td>
<td>Yes</td>
<td>2nd Horseradish peroxidase</td>
<td>Polyclonal goat 39–84</td>
<td>Polyclonal 1–34 goat</td>
<td>2.8</td>
<td>5,000</td>
</tr>
<tr>
<td>Elecsys 2010</td>
<td>Roche (Mannheim, Germany)</td>
<td>&lt;2.7</td>
<td>&lt;6.5</td>
<td>15–65</td>
<td>Not specified</td>
<td>Yes</td>
<td>2nd Ruthenium</td>
<td>Monoclonal</td>
<td>Monoclonal 26–32</td>
<td>1.2</td>
<td>5,000</td>
</tr>
<tr>
<td>Immulite 2000Xpi Intact PTH</td>
<td>Siemens Healthcare Diagnostics (Deerfield, IL)</td>
<td>&lt;5.7</td>
<td>&lt;8.8</td>
<td>12–65</td>
<td>Serum from 255 apparently healthy patients</td>
<td>Yes</td>
<td>2nd Alkaline phosphatase</td>
<td>Monoclonal</td>
<td>44–84</td>
<td>3.0</td>
<td>2,500</td>
</tr>
</tbody>
</table>

Table 1 (continued)
<table>
<thead>
<tr>
<th>Name of the assay</th>
<th>Manufacturer</th>
<th>Intra-assay CV (%)</th>
<th>Inter-assay CV (%)</th>
<th>Reference range (Manufacturer) (pg/mL)</th>
<th>Reference population</th>
<th>Automated 2nd or 3rd generation</th>
<th>Tracer</th>
<th>Epitope, type and origin of coated Ab</th>
<th>Epitope, type and origin of labelled Ab</th>
<th>Detection limit (pg/mL)</th>
<th>Highest measurable value (pg/mL)</th>
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</thead>
<tbody>
<tr>
<td><strong>3rd generation assays</strong></td>
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</tr>
<tr>
<td>Liaison 1-84</td>
<td>DiaSorin (Stillwater, MN)</td>
<td>&lt;5.9</td>
<td>&lt;9.0</td>
<td>5.5–38.4</td>
<td>74 individuals with 25-OH vitamin D levels &gt;75 nmol/L and serum calcium levels comprised between 2,125 and 2,525 mmol/L</td>
<td>Yes</td>
<td>3rd</td>
<td>Isoluminol</td>
<td>Polyclonal C-terminal</td>
<td>Polyclonal N-terminal</td>
<td>1.7</td>
</tr>
<tr>
<td>Roche Cobas 1-84 PTH</td>
<td>Roche Diagnostics GmbH (Mannheim)</td>
<td>&lt;7.4 on &lt;9.4 on cobas e411, e411, &lt;3.5 on &lt;6.2 on cobas e601 and e601, and e602</td>
<td>14.9–56.9</td>
<td>596 apparently healthy patients, chemistry and hematology results normal, no VTD intake and normal calcium values</td>
<td>yes</td>
<td>3rd</td>
<td>Ruthenium</td>
<td>Biotinylated Monoclonal monoclonal PTH specific Ab</td>
<td></td>
<td>5.5</td>
<td>2,300</td>
</tr>
<tr>
<td>Fujirebio Lumipulse Whole PTH</td>
<td>Fujirebio INC (Tokyo, Japan)</td>
<td>&lt;4.1</td>
<td>&lt;4.1</td>
<td>5.5–31.9 on serum, 4.8–36.3 on EDTA</td>
<td>133 apparently healthy patients, VTD &gt;75 nM, Ca, P and DFG normal</td>
<td>Yes</td>
<td>3rd</td>
<td>ALP</td>
<td>Ab polyclonal anti PTH Goat</td>
<td></td>
<td>0.6</td>
</tr>
<tr>
<td>Vidas PTH 1-84</td>
<td>bioMerieux SA (Marcy-l’Etoile, France)</td>
<td>&lt;6.5%</td>
<td>&lt;12.5%</td>
<td>9.2–44.6</td>
<td>N=491</td>
<td>Yes</td>
<td>3rd</td>
<td>Enzyme linked fluorescent assay</td>
<td>NA</td>
<td>NA</td>
<td>2.2</td>
</tr>
</tbody>
</table>

PTH, parathyroid hormone.
in plasma is 25.3% and 43.4%, respectively, whereas it is 25.9% and 23.8% in serum, respectively. These data are based on two papers published in 2008, one from Viljoen et al. (12) and the second by Ankrah-Tetteh et al. (13). The details of these studies can be found in Table 2. Since 2008, other papers have evaluated the biological variation of PTH in healthy subjects and in hemodialyzed patients. The results of these studies can be found in Table 2. The medians for the CVw are very similar between healthy subjects and hemodialyzed patients, whereas the CVg is much higher in hemodialyzed patients, which is logically expected. From these data, we can calculate that the LSC is around 60% for both patients and subjects. From an analytical perspective, the desirable analytical coefficient of variation (CV) will be around 11% while the desirable bias will be 8.8% for healthy subjects and 15.9% in hemodialyzed patients.

These results have important clinical and analytical implications. From a clinical perspective, an LSC at 60% means that there is no significant biological change in a patient or a subject’s PTH result if the increase (or decrease) is not higher than 60%. If we consider a subject with a previous PTH value at 50 ng/L (the upper reference range of the 2nd generation Roche PTH assay), a significant change in this subject’s PTH concentration will be considered as biologically significant if it is higher than 30 ng/L (i.e., if the subject presents a PTH higher than 80 ng/L or lower than 20 ng/L). In the same vein, a PTH change in a hemodialyzed patient that presented a PTH at 300 ng/L will be considered as significant if it is higher or lower than 180 ng/L (>480 or <120 ng/L). This is one of the reasons why the KDIGO guidelines insist on the trend of PTH variation instead of taking a single value into consideration.

From an analytical perspective, a desirable CV at 11% means that a value at 50 ng/L can comprise a measure between 44.5 and 55.5 ng/L. Hopefully, according to the UK NEQAS external control for PTH, most of the PTH methods present mean CVs of about 6%, which corresponds to the optimal CV (0.25× CVw). However, the differences obtained between methods giving the lowest values and those giving the highest values—even if we consider 2nd generation PTH assays only—far exceed the desirable bias. Also, mean recovery of synthetic 1–84 PTH by different assays participating at the UK-NEQAS control varies from 100% (DiaSorin Liaison 3rd generation PTH) to more than 250% (Siemens Immulite or Future Diagnostics STAT) (16). These results show the urgent need for PTH standardization.

**PTH stability**

The pre-analytical phase is of paramount importance for PTH determination. Many papers and systematic reviews have been published on PTH stability in EDTA plasma and serum (17). Unfortunately, the results of these studies are discrepant, and the best sample to use for PTH determination, as well as sample handling and storage conditions, remains controversial (1). For instance, Morales García et al. (18), and Jane Ellis et al. (19) showed that using EDTA tubes could maintain PTH stability during a longer period without the necessity of immediately freezing the samples. On the contrary, Joly et al. recommend serum over EDTA plasma (20), while Parent et al. showed that both media could be used if samples were quickly processed (21). The main advantages of using EDTA tubes is that the stability of the peptide may be increased due to the inactivation of metalloproteases by the chelation of divalent ions (22). Another explanation for the apparent higher stability in EDTA is that the clotting process releases thrombin in the serum which, in turn, can cleave the peptide between the Arg in position 44 and the Asp in position 45, making it invisible for the antibodies used in immunoassays (23). The major advantage of using serum over EDTA plasma relates to the fact that the clinical interpretation of PTH concentration must be performed together with calcium concentrations. Since calcium cannot be determined in EDTA plasma, another (serum) sample would be needed if EDTA was used. In fact, the concept of PTH stability mainly relies on the way that this stability is evaluated, either by a purely statistical approach, or using an “acceptable change limit” (ACL) (according to the ISO Guide 5725-6) that takes analytical variation into consideration (24), or even using the total change limit concept (according to the WHO guideline on the “use of anticoagulants in diagnostic laboratory investigations”) that takes both biological and analytical variation to decipher whether a decrease of PTH is biologically significant or not (25). Accordingly, Schleck et al. showed that PTH seemed to be more stable in EDTA than in serum gel tubes but only when samples had to stay unprocessed for a long period of time (18 hours) at room temperature (25 °C), which can happen when samples are delivered from external care centres. For all the other conditions, using serum gel tubes is recommended since the calcium measurement necessary for a good PTH results interpretation can be achieved on the same sample (26). These findings were later confirmed by Valcour et al. (27).
<table>
<thead>
<tr>
<th>First author and reference</th>
<th>Population</th>
<th>Assay</th>
<th>Scheme</th>
<th>CVw (%)</th>
<th>CVg (%)</th>
<th>LSC (%)</th>
<th>Desirable CV (%)</th>
<th>Desirable bias (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td></td>
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<tr>
<td>Viljoen 2008 (12)</td>
<td>20 healthy individuals (10 M and 10 F); median age =37 years</td>
<td>Beckman Access (2nd generation)</td>
<td>Between 8:45 and 9:30 on the same day of the week, weekly for 5 weeks. Plasma</td>
<td>25.3</td>
<td>43.4</td>
<td>70.1</td>
<td>12.7</td>
<td>12.6</td>
</tr>
<tr>
<td>Ankrah-Tetteh 2008 (13)</td>
<td>10 healthy individuals (4 M and 6W); median age 21 years old</td>
<td>Nichols Allegro</td>
<td>Between 12:30 and 14:30 for 6 weeks</td>
<td>25.9</td>
<td>23.8</td>
<td>71.8</td>
<td>13.0</td>
<td>8.8</td>
</tr>
<tr>
<td>Gardham 2010 (14)</td>
<td>12 healthy volunteers</td>
<td>Abbott ci800 (2nd generation), plasma</td>
<td>Between 9:30 and 11:30 on Wednesdays and Fridays for 6 weeks</td>
<td>19.2</td>
<td>NP</td>
<td>53.2</td>
<td>9.6</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>12 healthy volunteers</td>
<td>Immutopics 1-84 PTH (3rd generation), plasma</td>
<td>Between 9:30 and 11:30 on Wednesdays and Fridays for 6 weeks</td>
<td>23.3</td>
<td>NP</td>
<td>64.6</td>
<td>11.7</td>
<td>NA</td>
</tr>
<tr>
<td>Cavalier (unpublished)</td>
<td>22 healthy, fasting volunteers</td>
<td>Roche Cobas (2nd generation)</td>
<td>Between 8 and 9:30, on Tuesday and Thursday for 6 weeks</td>
<td>16.9</td>
<td>27.7</td>
<td>46.8</td>
<td>8.5</td>
<td>8.1</td>
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<td>Median</td>
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<tr>
<td>Hemodialyzed patients</td>
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<tr>
<td>Gardham 2010 (14)</td>
<td>22 patients</td>
<td>Abbott ci800 (2nd generation), plasma</td>
<td>Before dialysis session on Wednesdays and Fridays for 6 weeks</td>
<td>25.6</td>
<td>NP</td>
<td>71</td>
<td>12.8</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>22 patients</td>
<td>Immutopics 1-84 PTH (3rd generation), plasma</td>
<td>Before dialysis session on Wednesdays and Fridays for 6 weeks</td>
<td>30.2</td>
<td>NP</td>
<td>83.7</td>
<td>15.1</td>
<td>NA</td>
</tr>
<tr>
<td>Adapted from Cavalier 2013 (15)</td>
<td>17 patients</td>
<td>DiaSorin Liaison (2nd generation), serum</td>
<td>Twice a week for over 6 weeks</td>
<td>19.8</td>
<td>55.5</td>
<td>54.9</td>
<td>9.9</td>
<td>14.7</td>
</tr>
<tr>
<td></td>
<td>17 patients</td>
<td>DiaSorin Liaison (3rd generation), serum</td>
<td>Twice a week for over 6 weeks</td>
<td>19.0</td>
<td>65.8</td>
<td>52.7</td>
<td>9.5</td>
<td>17.1</td>
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<tr>
<td>Median</td>
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*Calculated as $2^{1/2} \times 1.96 \times CV_w$. PTH, parathyroid hormone.
**PTH standardization**

As can be seen from a review of the relevant literature above, PTH standardization is mandatory. For this purpose, an International Federation of Clinical Chemistry (IFCC) working group on PTH standardization was created in 2010. In 2017, the working group published the perspectives and priorities for the improvement of PTH measurement (16). From the results of PTH 1-84 recovery, it is clear that if all PTH methods were calibrated against the same material, between-method agreement would improve. Hence, the working group has proposed the use of a single international recognized standard “such as” WHO PTH IS 95/646. This standard is composed of recombinant human PTH 1-84 and comes in ampoules that contain 100 µg of PTH 1-84. However, this standard cannot be imposed yet because its commutability must be formally assessed beforehand. This step will be done according to the protocol proposed by the IFCC working group on commutability. Also, a candidate reference measurement procedure for PTH determination does not yet exist. To date, two LCMS/MS have been published so far (28,29), but they lack sensitivity, and reproducibility still needs to be demonstrated. The first method, published by Kumar et al. (28) shows a linear range of the assay at 39.1–4,560 ng/L, along with a limit of detection and a limit of quantification of 14.5 and 39.1 ng/L, respectively. The intra-assay CVs ranged from 6% to 11%, and the interassay CVs ranged from 7% to 17%. The second method, published by Lopez et al. (29) shows a limit of detection of 8 ng/L and a limit of quantification ranging from 16–31 ng/L. The CVs ranged from 5% to 9%.

Unfortunately, it remains impossible today to assess with precision the “true” PTH value of a given sample. On top of that, the peptide (or fragment) that needs to be measured has yet to be clarified (see below).

**PTH and PTH fragments: which one(s) should we measure?**

Once secreted, the half-life of PTH is 2–4 minutes. Its metabolization is performed through uptake by the liver, kidneys and the parathyroid gland itself (30,31). This degradation process leads to the production of large C-terminal fragments, generally called “non-(1-84) PTH” or “(7-84) PTH”, which have a higher half-life than 1-84 PTH (31). They thus accumulate in the blood of patients suffering from CKD (32). In normal individuals, they account for 15% to 30% of the total PTH, but in CKD patients, this percentage can be as high as 70% to 80%. Experimental data have demonstrated that these fragments can have an opposed biological action: non-(1-84) PTH has been shown to down-regulate the biological activity of 1-84 PTH and cause internalization of the PTH1R receptors, without activating them (33). This is of importance in uremic patients in whom high levels of these fragments can be found, and could probably explain part of the apparent tissue resistance to PTH that characterizes chronic renal failure. Indeed, if PTH resistance is not completely understood, two theories have emerged to explain it. The first one, developed by Slatopolsky et al. (34), is based on the fact that PTH 7-84 antagonizes the calcemic actions when given in a 1:1 molar ratio with PTH 1-84 to parathyroïdectomized rats, hence acting as a competitive inhibitor of PTH 1-84 for PTH1R. The second theory is based on the studies published by Divieti et al. (35,36) which introduce evidence for a new PTH receptor that could interact with the carboxyl-terminal part of the peptide (C-PTHR). Indeed, 7-34 PTH analogues do not lower calcium in hypercalcemic mice (37), suggesting that the end of the peptide is important for inhibitory action. On the other hand, amino-truncated fragments stimulate alkaline phosphatase activity in vitro and induce the expression of mRNAs for alkaline phosphatase and calcitonin (38,39). This hypothesis is elegant, but the C-PTHR receptor has still to be discovered and phenotyped. We have indirect proof of its existence from experiments demonstrating how PTH 19-84 can bind osteoblastic and osteolytic cell lines from mice KO for the PTH1R (35). Unfortunately, all these experimental findings have never definitively been confirmed in vivo and no new information has been published this last decade on the topic.

**New PTH forms**

**Amino-PTH**

A form of 1-84 PTH phosphorylated on serine (position 17) has been found to be overproduced in parathyroid carcinoma (40,41) and in rare cases of severe primary hyperparathyroidism (42), leading to an inversion of the 3rd generation PTH ratio.

This form, called amino-PTH, cross reacts with the antibodies used in the 3rd generation PTH but not with the antibodies used in the 2nd generation kits [except the Roche intact PTH assay, to some extent (43)]. In normal
individuals, amino-PTH represents approximately 10% of the circulating PTH (44). Thus, the 3rd generation PTH ratio should always be <1 because the large non-(1-84) fragments detected with the 2nd generation kits represent a larger proportion of the circulating PTH than amino-PTH does. The ratio is now proposed as a new diagnostic tool to detect patients suffering from parathyroid carcinoma (45), and its inversion has been shown to be predictive of the recurrence of the cancer, even before the rise of calcemia (46).

Oxidized PTH

PTH peptide possesses two methionines, one on position 8 and the other on position 18. These methionines are prone to be oxidized, especially in hemodialysis (HD) patients who suffer from very intense oxidative stress (47). Many studies, mainly from the eighties, have shown that oxidized PTH is inactive. It has a lower binding affinity to the PTH receptor and, when bound to the receptor, is unable to generate cAMP; it loses its biological action on smooth muscle cells, cannot stimulate alkaline phosphatase activity in neonatal bone cells and, finally, cannot regulate calcium and phosphate metabolisms in different animal models (48). Immunoassays for PTH recognize both the oxidized and non-oxidized forms, and the only way to measure the non-oxidized form is to perform a chromatography on an affinity column with fixed antibodies that selectively capture oxidized PTH, thus allowing the measurement of the non-oxidized PTH with any PTH assay. It seems however that the antibodies used in the different available PTH assays recognize the oxidized PTH to varying extents due to difference in cross-reactivities (or matrix effect) (49). Also, whether PTH oxidation occurs in vivo or in vitro remains another important puzzle to be solved. A recent study has indirectly answered the question by proving that non-oxidized PTH was stable in EDTA plasma and that oxidation did not occur after 180 minutes until centrifugation (49). In the future, a fully standardized, fourth generation PTH assay that would easily measure the non-oxidized 1-84 PTH might thus be of interest. In fact, one study has already demonstrated the case by proving that non-oxidized PTH was stable in EDTA plasma and that oxidation did not occur after 180 minutes until centrifugation (49). In the future, a fully standardized, fourth generation PTH assay that would easily measure the non-oxidized 1-84 PTH might thus be of interest. In fact, one study has already demonstrated that patients in the highest versus the lowest non-oxidized PTH tertile presented increased survival, and that higher non-oxidized PTH reduced the odds for death (50). These data were confirmed in the results from patients in the EVOLVE trial: non-oxidized PTH, but not oxidized or intact PTH, had a predictive value for cardiovascular events and all-cause mortality (data not yet published, but presented as a poster at the 2014 ASN by Hocher et al.). These results, however, were opposed by another study in which a cohort of CKD patients were followed over 5.1 years for the occurrence of acute heart failure, atherosclerotic events, CKD progression, or all-cause death. The results showed that there was no association of nonoxidized PTH with any of the clinical outcomes examined (51). However, these patents presented an e-GFR range between 89 and 15 mL/min/1.73 m² and were not hemodialyzed patients. Indeed, HD patients present a much more intensive oxidative stress compared to non-HD CKD patients.

Conclusions

PTH determination is a very difficult task and many obstacles stand in the way of standardizing and defining the peptides of interest to measure; also, appropriate reference ranges are necessary for an informed interpretation of clinical results and a multicenter, multiethnic study will be necessary once the measurements have been standardized. When working with PTH, this is unfortunately the price to be paid for avoiding confusion and providing better care to patients.

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Footnote

Conflicts of Interest: E Cavalier is a consultant for IDS, DiaSorin and Fujirebio.

References


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