Establishment of reference values in a healthy population and interpretation of serum PTH concentrations in hemodialyzed patients according to the KDIGO Guidelines using the Lumipulse® G whole PTH (3rd generation) assay

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Establishment of reference values in a healthy population and interpretation of serum PTH concentrations in hemodialyzed patients according to the KDIGO Guidelines using the Lumipulse® G whole PTH (3rd generation) assay

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A B S T R A C T

Background: 3rd generation PTH assays only detect the bioactive 1–84 fragment. Since standardization is still lacking, each new PTH assay requires to establish reference values and to assess the impact in the medical care of the mineral and bone disorders in hemodialyzed patients.
Methods: Using Fujirebio Lumipulse G wPTH assay, serum PTH levels were measured in a population of 439 healthy subjects from France and Belgium PTH levels were also determined in 119 hemodialyzed patients. These patients were classified according to the KDIGO recommendation.
Results: Reference range was found to be 6.5 (90%CI: 6.0–7.0) – 41.8 (90% CI: 38.1–43.7). In hemodialysis patients, Passing-Bablock regression between 3rd generation PTH from Fujirebio and DiaSorin was DiaSorin = 1.01 xFujirebio-2.4 with a slope not different from 1.0(95%CI: 0.96–1.04) and a non-significant intercept, ranging from −6.0 to 0.1. Hemodialysis patients with a PTH concentration below 2-fold the Upper Limit of Normality (ULN), within the KDIGO range and upper 9-fold upper limit were respectively 33.6%, 54.6%, 11.8% (Fujirebio Lumipulse) and 36.1%, 51.3% and 12.6% (Diasorin Liaison).
Conclusion: We determined a reference range with the 3rd generation PTH assay from Fujirebio. In a hemodialysis population, 3rd generation assays from Fujirebio and DiaSorin provide similar results. To the best of our knowledge, this is the first time that we can show similar PTH results obtained by 2 different 3rd generation PTH assays in healthy subjects and hemodialyzed patients without mathematically processing them.

1. Introduction

Serum parathyroid hormone (PTH) is frequently measured in clinical practice. Indeed, assessment of PTH concentration is of paramount importance in the exploration of disorders of calcium/phosphorus metabolism and in the monitoring of patients suffering from chronic kidney disease (CKD). Unfortunately, PTH measurement and clinical interpretation of the results is not an easy task. From an analytical point of view, different generations of PTH assays are present on the market. The older ones, called “intact” PTH or 2nd generation PTH assays are known to cross-react with N-terminal truncated PTH fragments, generally called (7–84) PTH or non-(1–84) PTH that are suspected to increase in CKD [1]. The more recent ones are called 3rd generation assays and do not detect the non-(1–84) PTH but measure however a post-translational form called amino-PTH, that is overproduced in many patients suffering from parathyroid carcinomas [2]. Standardization of these assays is still lacking and we are still waiting for a reference method for PTH determination, as well as commutable calibrators, even if an International Standard, namely the WHO 95/646, is available [3]. In practice, results obtained with the 2nd or 3rd generation assays are not transposable, especially for CKD patients, which is most confusing for clinicians [4]. We have however recently shown that standardization of 3rd generation PTH assays from DiaSorin and Roche with the WHO 95/646 Standard could reduce significantly inter-

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method variability [5].

From a clinical perspective, the Guidelines for the diagnosis and management of asymptomatic primary hyperparathyroidism (GDMAPHP) [6] as well as the KDIGO Guidelines [7], emphasize that 2nd and 3rd generation PTH assays have similar clinical values for the diagnosis of primary hyperparathyroidism and for the follow-up of CKD-related mineral and bone disorders (CKD-MBD). Hence, a correct establishment of PTH reference ranges is of particular interest to rapidly detect a secondary hyperparathyroidism and to properly classify hemodialyzed patients according to multiples of the Upper Limit of Normal (ULN), as recommended by the KDIGO [7–9].

In this context, Fujirebio (Tokyo, Japan) has recently launched a new 3rd generation PTH assay on the Lumipulse platform. The aim of this study was to determine PTH reference values for this new automated method in a French (Montpellier) and Belgian (Liege) vitamin D replete populations. We also classified dialysis patients according to KDIGO Guidelines (which recommends maintaining the hemodialysis (HD) patients at 2–9 times the ULN) with the Fujirebio and DiaSorin 3rd generation PTH assays.

2. Material and methods

2.1. Patients

We selected a reference population of 269 healthy subjects from Montpellier (France) and 184 healthy subjects from Liège (Belgium) that presented normal calcium and phosphorus levels, eGFR > 60 mL/min/1.73 m^2 and 25(OH)-vitamin D (25(OH)D) levels > 30 ng/mL as recommended [6] to establish PTH reference range. We also selected a population of 119 hemodialyzed patients from CHU Lapeyronie (Montpellier) undergoing dialysis three times a week. Blood was obtained just before a dialysis session and was centrifuged within 30 min of blood sampling. Serum was aliquoted and stored at −80 °C until assayed.

2.2. PTH assays

The Lumipulse G whole PTH (3rd generation) assay is a chemiluminescent sandwich method using a pair of polyclonal goat anti-PTH antibodies, one targeted against the C-terminal and the other against the first amino-acids of the peptide. The coefficient of variation (CV) of the method ranges from 1.4 to 6.9%, the limit of detection (LOD) is 0.6 pg/mL and the limit of quantification (LOQ) is 4.0 pg/mL. Expected range on serum (p2.5–p97.5) established by Fujirebio on 133 apparently healthy white Europeans presenting 25(OHD) values > 30 ng/mL, eGFR > 60 mL/min/1.73m^2 and normal calcium and phosphorus levels is 5.5–31.9 pg/mL. The method is claimed to be traceable to the NIBSC WHO 95/646 recombinant, human, 1–84 PTH Standard (1st International Standard).

The Liaison (1–84) PTH immunoassay (3rd generation) (DiaSorin, Stillwater, MN,USA) is a two-step automated sandwich chemiluminescent immunoassay that also uses two polyclonal antibodies targeted against the C-terminal and the first amino acids of the peptide. The CV of the method is 5.5%, 4.1%, 4.0% and 4.7% at 10.6, 33.5, 378 and 1662 pg/mL, respectively. The LOD is 1.7 pg/mL and the LOQ is 4.0 pg/mL. The expected range established on serum by DiaSorin using 74 apparently healthy adults from the Midwest of the USA presenting 25(OH)D values > 30 ng/mL and normal calcium and phosphorus levels is 5.5–38.4 pg/mL. The ULN, however, has been challenged in white European populations and found to be (p97.5) at 28.8 pg/mL [9] and 28.9 pg/mL [8]. The method has been calibrated against an internal, synthetic human 1.84 PTH without any other specification.

2.3. Statistics

Non-parametric statistics were used when distribution was not Gaussian according to the D'Agostino-Pearson test and results were expressed as median (95% confidence interval). We used Medcalc (Oostende, Belgium) to calculate the Mann-Whitney tests and the the lower and upper limits of normality were calculated as the p2.5 and p97.5 of the distribution by the non-parametric percentile method, according to the CLSI C28-A3 Guideline. Agreement between classification of the hemodialyzed patients was assessed with the kappa test. A p value < 0.05 was considered as significant.

3. Results

3.1. Healthy population

The reference population in Montpellier consisted in 136 healthy men and 133 healthy women, with a median age of 43 years (95% CI: 39–46.0) ranging from 18 to 89 years old. The median 25(OH)D value was 41 (95%CI: 40.0–43.0; range: 30–100) ng/mL. No gender related difference was observed for age or 25(OH)D values.

The median Fujirebio Lumipulse 3rd generation PTH value in these 269 subjects was 16.3 pg/mL (Q1: 12.0 pg/mL; Q3: 23.5 pg/mL; and IQR: 11.5 pg/mL). The upper and lower limits of normality of PTH values (2.5th–97.5th percentile) were 6.1 (90%CI: 5.6; 6.8) – 41.6 (36.9; 44.7) pg/mL according to the CLSI C28-A3 Guideline. No gender related difference was observed.

In Liege, the reference population consisted in 58 healthy men and 126 healthy women, with a median age of 58.5 (95% CI: 56.0–61) years ranging from 18 to 94 years. The median 25(OH)D value was 35.9 (90%CI: 34.1–37.2; range: 30–59.8) ng/mL. Like in Montpellier, there was no significant difference between age and 25(OH)D values in males vs. females.

The median PTH value in these 184 subjects was 19.1 pg/mL (Q1: 14.7 pg/mL; Q3: 23.6 pg/mL; and IQR: 8.9 pg/mL). The upper and lower limits of normality of PTH values (2.5th–97.5th percentile) were 8.8 (90%CI: 4.4; 9.5) – 42.7 (35.2; 47.5) pg/mL according to the CLSI C28-A3 Guideline. No gender related difference was observed.

Altogether, the Fujirebio Lumipulse 3rd generation PTH reference range, established in 453 Individuals according to the non-parametric percentile method, was found to be 6.5 (90%CI: 6.0–7.0) – 41.8 (90% CI: 38.1–43.7) pg/mL.

The subjects from Liege were older ($p < 0.0001$) and presented lower 25(OH)D ($p < 0.0001$) and higher PTH ($p = 0.0045$) values compared to those from Montpellier. In simple regression analysis, serum PTH levels in the whole group of 439 subjects correlated positively with age ($r = 0.28$; $p < 0.001$) but not with serum 25(OH)D. The median PTH concentration in subjects < 60 years old (n = 306) was 16.5 pg/mL (2.5th–97.5th percentile: 6.2–32.4 pg/mL), a value significantly lower than in subjects aged 60 years or more (n = 133) who had a median PTH concentration of 19.0 pg/mL ($p < .001$). This had however little impact on the reference range since it was calculated at 6.2–33.0 in older vs. 6.3–34.7 pg/mL in younger individuals.

3.2. Hemodialyzed patients

The main characteristics of the 129 patients are summarized in Table 1.

PTH (either Fujirebio or DiaSorin) was significantly correlated with Calcium ($r = -0.19$; $p < 0.05$), age ($r = 0.188$; $p < 0.05$) and bone alkaline phosphatase ($r = 0.478$; $p < 0.0001$) but not with 25(OH)D or phosphorus. In multiple regression, PTH only remained associated with bone alkaline phosphatase.

According to the KDIGO, the interval encompassing 2 to 9 times the ULN determined in the present study will be 84–378 pg/mL for the Fujirebio whole PTH assay. For the DiaSorin assay, it will be 76–342 pg/mL according to DiaSorin reference range or 58–260 pg/mL according to Souberbielle et al. [8] and Cavalier et al. [9]. The classification of patients is presented in Table 2. Compared to Fujirebio, the

Table 1
Main characteristics of the 129 hemodialyzed patients. Continuous data are represented as median (25th -75th percentile).

Gender (men/women)	85/44
Age (years)	73 (63.2–82.0)
Serum total calcium (mmol/L)	2.24 (2.13–2.30)
Serum phosphorus (mmol/L)	1.24 (0.93–1.65)
Serum 25(OH)D (ng/mL)	31.0 (17.3–42.0)
Bone alkaline phosphatase (µg/L)	22.0 (16.5–34.3)
Serum 3rd generation Fujirebio Lumipulse PTH (pg/mL)	114.7 (63.0–252.6)
Serum 3rd generation DiaSorin Liaison XL PTH (pg/mL)	107.0 (58.4–232.5)

The Passing-Bablock regression between 3rd generation PTH assays from Fujirebio and DiaSorin was DiaSorin = 1.01 × Fujirebio −2.4 with a slope not different from 1.0 (95%CI: 0.96–1.04) and an intercept not different from 0.0, ranging from −6.0 to 0.1 (Fig. 1).

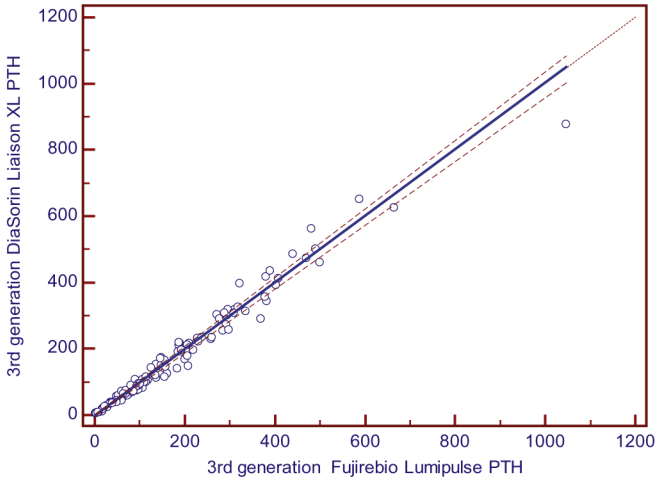

Fig. 1. Comparison of 3rd generation PTH assays from Fujirebio and DiaSorin obtained in 129 hemodialyzed patients (results are in pg/mL).

Table 2
Classification (in %) of 119 hemodialyzed patients according to the KDIGO 2–9 times the ULN with the 3rd generation Fujirebio PTH reference range and the 3rd generation DiaSorin PTH reference range according to DiaSorin and to Souberbielle et al. [8] or Cavalier et al. [9].

	< 2 ULN	2–9 ULN	> 9 ULN
Fujirebo	33.6	54.6	11.8
DiaSorin	36,1	51,3	12,6
[8,9]	26,1	52,1	21,8

discordance rate was 5.0% when we used the DiaSorin reference range and 9.2% when we used the one published in [8,9]. There was no significant difference between those percentages. The kappa test was used to evaluate agreement between the classifications of the patients according to Fujirebio's 84–378 pg/mL and DiaSorin IFU values (76–342 pg/mL) or those published in [8,9] (58–261 pg/mL) and was found at 0.914 and 0.833, respectively.

4. Discussion

In this paper, we evaluated for the first time the reference range of the Fujirebio Lumipulse 3rd generation PTH assay. These reference ranges were established on a large (n > 400) population of healthy vitamin D sufficient individuals from two centers located in Belgium and in the south of France and found it to be 6.5 (90%CI: 6.0–7.0) – 41.8 (90% CI: 38.1–43.7)pg/mL. We also measured the PTH of 119 hemodialyzed patients with the 3rd generation PTH assay from Fujirebio and the 3rd generation PTH assay from DiaSorin.

Establishment of reference range and defining a reference population is not an easy task [10]. In this study, we used the recommendations of the GDMAPHP [6] that request using a population presenting 25(OH)D levels > 30 ng/mL to calculate the PTH reference range. Souberbielle et al. have performed the same study with DiaSorin PTH on the VARIETE population in 183 subjects with a 25(OH)D concentration > 30 ng/mL and an eGFR > 60 mL/min and found a range of 9.4–28.9 pg/mL (after excluding 6 outliers). We also published in 2011 a reference range obtained in 240 healthy individuals for DiaSorin 3rd generation PTH that was 6.6–28.8 pg/mL according to the CLSI C28-A3 [9]. What is important, and unfortunately frequently overlooked, in studies dealing with reference ranges, is the 90% confidence interval observed on the limits. In [9], this 90% CI for the ULN was 26.6–33.5 pg/mL. In other words, it means that if we select another reference population according to the same criteria, there is 90% chance that the ULN is comprised between 26.6 and 33.5 pg/mL. In the present study using the Fujirebio 3rd generation PTH assay, we found that the ULN was 41.8 pg/mL, with a 90% CI ranging from 38.1 to 43.7 pg/mL. This value is close to the ULN value proposed by DiaSorin. Even if the ULN is not comprised in the 90% CI of [9], we have to admit that there is some overlap between the 90%CI of the two methods. This finding, and the fact that the Passing-Bablock regression between the two methods has a slope not different from of 1.0 and an intercept not different from 0.0 may lead us to conclude that both methods are standardized on the same calibrator – or at least that DiaSorin calibrator is not far away from WHO IS 95/646 International Standard. Surprisingly this is not at all the case with Roche Cobas 3rd generation PTH assay, which is also claimed to be calibrated against WHO IS 95/646, but which gives a ULN of 49 pg/mL. However, we recently demonstrate that restandardization of automated third generation PTH assays with the WHO 1–84 PTH Standard significantly reduces inter-method variability [5]. In conclusion, there remains a problem of commutability of the standards used to calibrate PTH and this problem should be solved in order to harmonize reference ranges.

The great advantage of 3rd generation PTH assays over “intact” PTH methods is that they do not recognize the non-(1–84) PTH that is supposed to accumulate in CKD patients. Hence, standardization of these assays in the full spectrum of patients is possible whereas it remains questionable with “intact” (2nd generation) PTH assays that recognize other fragments than 1–84 PTH with various cross-reactivities.

PTH determination is routinely performed in hemodialyzed patients for the diagnosis and management of mineral bone diseases. Indeed, PTH concentrations that are either too high or too low are better avoided in these patients leading the experts to propose an optimal range for PTH serum concentrations. Hence, KDIGO Guidelines propose a classification of HD patients according to multiples of the ULN of PTH. This however raises some questions regarding the ULN value to be used since some manufacturers have not taken 25(OH)D into consideration when recruiting the reference population [9,11–15]. We have shown that, using a well-defined, vitamin D replete population with a eGFR > 60 mL/min/1.73m^2 to establish the reference range could definitely harmonize the classification of HD patients according to the KDIGO [9]. In this study, we found a percentage of misclassification ranging from 5.0 to of 9.2% between DiaSorin and Fujirebio, a value similar to what we observed when we compared different PTH assays in [9].

In conclusion, we have established a reference range in a healthy population of French and Belgian Caucasians with the 3rd generation PTH assay from Fujirebio and found it to be quite similar to the one we previously established for the DiaSorin 3rd generation PTH assay. To the best of our knowledge, this is the first time that we can show similar PTH results obtained by 2 different 3rd generation PTH assays in healthy subjects and hemodialyzed patients without mathematically processing them by applying a coefficient of correction like in Souberbielle et al. [16], for instance. This, for us, is one of the great advantages of working with 3rd generation PTH assays.

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