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Serum PTH reference values established with an automated 3rd-generation assay in vitamin D-replete subjects with normal renal function. Consequence for the diagnosis of primary hyperparathyroidism and the classification of dialysis patients.

Short-title : PTH reference values in vit D-replete subjects

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Abbreviations

PTH : parathyroid hormone ; PHPT : primary hyperparathyroidism ; SHPT : secondary hyperparathyroidism ; N-PHPT : normocalcemic primary hyperparathyroidism; CKD-MBD : chronic kidney disease (CKD)-related mineral and bone disorders (MBD) ; ULN : upper limit of normal ; 25OHD : 25-hydroxyvitamin D ; eGFR : estimated glomerular filtration rate ; BMI : body mass index ; BMD : bone mineral density ; LOQ : limit of quantification ; IQR : interquartile range

Abstract

Objective: To determine PTH reference values in French healthy adults, taking into account serum 25OHD, renal function, age, gender, and BMI.

Participants and main biological measurements: We studied 898 healthy subjects (432 women) aged 18-89 years with a normal BMI and eGFR, 81 patients with surgically proven primary hyperparathyroidism (PHPT), and 264 dialysis patients. 25OHD and 3rd-generation PTH assays were implemented on the LIAISON XL platform.

Results: Median PTH and 25OHD values in the 898 healthy subjects were 18.8 ng/L and 23.6 ng/mL respectively. PTH was lower in subjects with 25OHD $\geq$30 ng/mL than in those with lower values. Among the 183 subjects with 25OHD $\geq$30 ng/mL, those aged $\geq$60 years (n=31) had higher PTH values than younger subjects, independently of 25OHD, BMI and eGFR ($p<0.001$). Given the small number of subjects aged $\geq$60 years, we adopted the 95% CI of PTH values for the entire group of 183 vitamin D-replete subjects (9.4-28.9 ng/L) as our reference values. With 28.9 ng/L as the upper limit of normal (ULN) rather than the manufacturer's ULN of 38.4 ng/L, the percentage of PHPT patients with "high" PTH values rose to 90.1% from 66.6% ($p<0.001$), and 18.6% of the dialysis patients were classified differently in view of the KDIGO target range (2 to 9 times the ULN).

Conclusion: When only subjects with 25OHD $\geq$30 ng/mL were included in the reference population, the PTH ULN fell by 22.4%, diagnostic sensitivity for PHPT improved, and the classification of dialysis patients was modified.

Key words: parathyroid hormone; vitamin D; reference values; primary hyperparathyroidism; dialysis

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Introduction

With the advent of automated assays, serum parathyroid hormone (PTH) is frequently measured in clinical practice. Second-generation assays cross-react with N-terminal truncated PTH fragments (7-84 PTH), while third-generation assays do not detect 7-84 PTH but measure, in addition to 1-84 PTH, a post-translational form called amino-PTH, that is overproduced in many patients with parathyroid carcinomas [1, 2]. Guidelines for the diagnosis of asymptomatic primary hyperparathyroidism (PHPT) [3], and also the KDIGO guidelines [4], emphasize that 2nd- and 3rd-generation PTH assays have similar clinical value for the diagnosis of PHPT, and for the follow-up of chronic kidney disease (CKD)-related mineral and bone disorders (MBD). As a result, more and more clinical laboratories worldwide are using 3rd-generation PTH assays routinely.

A serum PTH concentration above the upper limit of normal [ULN] reflects either secondary hyperparathyroidism (SHPT) when associated with hypocalcemia, or PHPT when associated with hypercalcemia. In patients with a normal total calcemia, an elevated PTH level may correspond either to SHPT or to normocalcemic PHPT (N-PHPT). PHPT is all the more probable in case of high normal serum calcium levels [5]. A definite proportion of patients who fall in this subgroup have elevated ionized calcium. In dialysis patients, KDIGO guidelines recommend maintaining serum PTH within 2 to 9 times the ULN [4]. The definition of the PTH ULN is therefore of prime importance for the care of these numerous patients, and this raises questions as to the inclusion/exclusion criteria that should be applied when recruiting a reference population to establish PTH normal values. The exclusion criteria should include any situation potentially inducing an increase or decrease in the PTH concentration. This includes a low serum 25-hydroxyvitamin D (25OHD) concentration, which is highly frequent in the general population [6] and is thus likely to be prevalent in an apparently healthy group recruited to establish normal PTH values. Excluding subjects with low 25OHD from a reference population for serum PTH reference values is strongly recommended in the two most recent guidelines on the diagnosis and management of asymptomatic PHPT [7, 8]. We have demonstrated in several studies that this lowers the serum PTH ULN by 20-35% depending on the assay [6, 9-12].

Another point which should be taken into account is renal function. Indeed, PTH levels can rise when the eGFR is below 60 mL/min/1.73 m² [4], and some apparently healthy subjects, especially those older than 60 years, may have a low eGFR.

Another issue is whether the PTH reference population should be stratified according to factors such as age, gender, menopausal status, body mass index (BMI), and race.

The aim of this study was to determine PTH reference values for an automated 3rd–generation assay in French healthy adults, stratifying the results according to vitamin D status, renal function, gender, age and BMI. We also determined the frequency of high PTH levels in a series of patients with surgically proven PHPT, and the classification of dialysis patients according to KDIGO guidelines.

Subjects and methods

Subjects

We enrolled healthy volunteers who participated in the VARIETE study, a population-based cross-sectional study designed to recruit a reference population normal serum IGF-I values in adults (ClinicalTrials.gov identifier: NCT01831648). They were recruited between January 2011 and February 2012 by the clinical research units of 10 university hospitals distributed throughout France. Inclusion criteria were a normal physical work-up (weight, height, blood pressure, nutritional status and gonadal/sexual status), normal laboratory values determined after an overnight fast (plasma sodium, potassium, calcium, phosphate, creatinine, glycemia, total cholesterol, liver enzymes, TSH, blood cell counts, albuminemia, prothrombin time, and HIV and HCV serology), age 18-89 years and BMI between 19 and 28 kg/m², and a written informed consent to participate in the study. The exclusion criteria were a medical history of thyroid, renal, hepatic, cardiovascular, pulmonary, intestinal or psychiatric disorders, cancer, epilepsy, intercurrent illness occurring during the week preceding inclusion, current consumption of tobacco or other toxics, and treatment potentially modifying IGF-I or calcium/phosphorus metabolism (antiandrogens or antiestrogens, loop diuretics, hydrochlorothiazide, CYP-inducing drugs). In addition to the blood samples necessary for the screening biological evaluation, 50 mL of whole blood and 30 mL of EDTA blood was obtained from each subject. Blood was promptly centrifuged (3000 rpm at 4°C), and serum or plasma was aliquoted in polypropylene tubes that were immediately stored at -80°C. This study was funded by Programme Hospitalier de Recherche Clinique, French Ministry of Health, N° P081216 / IDRCB 2009-A00892-55, and was approved by the Paris-Sud Ethics Committee in November 2009.

We also obtained serum samples from 81 consecutive patients with PHPT before parathyroid surgery. These patients were osteoporotic (low bone mineral density [BMD] and/or low-trauma fracture) and were initially referred to our tertiary care centre for etiological diagnosis of abnormal calcium/phosphorus or related hormone levels detected during a screening biological evaluation aimed at ruling out secondary causes of osteoporosis. We requested that the physician who referred the patient prescribe vitamin D if the 25OHD concentration was low, before investigations in our unit. Based on the pharmaceutical forms available in France, the recommended supplementation scheme comprised four 100,000 IU vitamin D3 vials (one vial every other week) in case of serum 25OHD concentration <20 ng/mL, and two vials in case of 25OHD concentration between 20 and 29.9 ng/mL. According to the procedure in our unit, an oral calcium load test was performed in those with total calcium serum levels <3 mmol/L at their initial work up (n=78). In those with normal serum calcium level, the oral test was followed by an *IV* calcium load test as described in [13]. In all patients, an insufficient fall in serum PTH when serum ionized calcium level rose well above the upper normal limit during the test was observed, confirming the diagnosis of PHPT. All had preoperative parathyroid imaging (echography and sestaMIBI scintigraphy) which was positive in 45 cases (44.4%) and negative or discrepant (negative at one of the test and positive at the other one) in 36 Cases (55.6%). As they all had osteoporosis, these 81 consecutive patients were addressed to the surgeon and underwent successful parathyroidectomy, as confirmed by pathological examination of parathyroid tissue removed during surgery.

Finally, we collected sera from 264 hemodialysis patients managed in the same dialysis department, just before a dialysis session.

Laboratory methods

The biological parameters of the healthy volunteer screening evaluation were determined locally by the laboratories attached to the clinical research units, using standard chemistry. The CKDepi formula was used to evaluate eGFR [14]. PTH and 25OHD measurements were centralized and done in batches by means of immunochemiluminometric assays on the LIAISON XL (DiaSorin, Stillwater, Mn, USA), using serum samples that had never been thawed. According to data obtained by one of us (CM), intra-assay coefficient of variation (CV) were 3.2% at 25.7 ng/L and 3.2% at 284 ng/L for the 3rd-generation PTH assay, and 2.2% at 17.9 ng/mL and 2.7% at 51.9 ng/mL for the 25OHD assay. Inter-assay CV were 12.2% at 16.9 ng/L and 9.9% at 160 ng/L for the 3rd-generation PTH assay, and 9% at 17.9 ng/mL and 7.9% at 34 ng/mL for the 25OHD assay. Limit of quantification was 4 ng/L and 4 ng/mL for the 3rd-generation PTH assay, and for the 25OHD assay respectively.

Statistical analysis

Quantitative variables are reported as median, quartile (Q) 1 (25th percentile), Q3 (75th percentile), and interquartile range (IQR). Associations between the serum PTH concentration and other quantitative variables were assessed by simple regression. The LOWESS representation was used to smooth the relationship between PTH and 25OHD. Variables significantly associated with PTH were included in a model for multiple regression analysis. Variables significantly associated with PTH in multiple regression analysis were then stratified into arbitrarily defined subgroups, and the mean PTH values in these subgroups were compared by ANOVA. To determine the PTH reference range we first detected outliers, defined as PTH concentrations below Q1-1.5 IQR and above Q3+1.5 IQR after log transformation of the raw values [15]. We then calculated the 95% confidence interval in the remaining subjects after eliminating the outliers. Percentages were compared by means of the chi² test. A p value <0.05 was considered significant.

Results

Healthy subjects

Nine hundred seventy-two Caucasian subjects were initially recruited. Two were excluded because their informed consent was not available, and another 60 were excluded because of abnormal values in the screening evaluation. Among the remaining 910 subjects, no serum sample was available for PTH testing in 12 cases. The study population thus consisted of 898 subjects, whose main characteristics are summarized in Table 1. The median PTH value in these 898 subjects was 18.8 ng/L (Q1: 15.2 ng/L; Q3: 24.0 ng/L; IQR: 8.8 ng/L). After excluding nine outliers (eight high values, one low value), the range of PTH values (2.5^{th}-97.5^{th} percentile) was 10.1-37.9 ng/L, with no significant difference between men and women (18.8 ng/L [15.2-24.0] and 19.1 ng/L [15.4-24.0] respectively; p=0.13). In simple regression analysis, serum PTH correlated negatively with serum 25OHD (r=-0.29; p<0.001), phosphate (r=-0.19; p<0.001), calcium (r=-0.16; p<0.001) and eGFR (r=-0.25; p<0.001), and positively with age (r=0.37; p<0.001), and BMI (r=0.20; p<0.001). In multiple regression analysis, only the 25OHD level and age remained significantly correlated with PTH. PTH concentrations in the 898 subjects are shown in Table 2 according to age and 25OHD concentrations. Figure 1 shows the relationship between 25OHD and PTH concentrations, represented by the LOWESS curve. No obvious inflection point (i.e. a 25OHD concentration above which PTH no longer decreases) is visible in this curve. It should be noted that few subjects had “high” 25OHD levels as only 5 (0.5%) had a concentration above 50 ng/mL.

As PTH concentrations were significantly lower in subjects with serum 25OHD $\geq$ 30 ng/mL (n=183) than in the other three 25OHD groups (Table 2), we used only these 183 subjects to establish our PTH reference range. The median PTH concentration in these 183 subjects was 17.0 ng/L (Q1: 13.5 ng/L; Q3: 21.5 ng/L; IQR: 8.0 ng/L). After excluding six outliers with high values, the range (2.5^{th}-97.5^{th} percentile) of PTH concentrations was 9.4-28.9 ng/L. In simple regression analysis, serum PTH levels in this group of 183 subjects correlated positively with age (r=0.30; p<0.001) and BMI (r=0.23; p=0.002), and negatively with eGFR (r=-0.22; p=0.002), but not with serum 25OHD (p=0.32). In multiple regression analysis, only age remained significantly associated with serum PTH. The median PTH concentration in subjects less than 60 years old (n=152) was 17.8 ng/L (2.5^{th}-97.5^{th} percentile: 9.1-28.5 pg/mL), a value significantly lower than in subjects aged 60 years or more (n=31) who had a median PTH concentration of 21.5 ng/L (p<0.001). No significant difference was found between subjects aged 18-29 years and those aged 30-59 years (p=0.09). In the 31 older subjects, the estimated range of PTH values was11-33 ng/mL. However, as this subgroup was small, we considered that it should not be used as reference values. Thus, for the following analyses in PHPT and dialysis patients, we used the range of PTH values obtained in the whole group of 183 subjects with serum 25OHD $\geq$ 30 ng/mL as our reference, that is 9.4-28.9 ng/L. Interestingly, in the entire initial population of 898 apparently healthy subjects, 26 (2.9%) had PTH concentrations >38.4 ng/L, corresponding to the ULN given by the kit manufacturer, while 114 (12.7%) had PTH concentrations >28.9 ng/L. Thus, 88 of our 898 apparently healthy subjects (9.8%) would be considered as having elevated PTH concentrations when using our upper normal limit of 28.9 ng/L, but normal PTH concentrations using the manufacturer’s ULN.

PHPT patients

The main characteristics of these 81 patients are presented in Table 3. All had a 25OHD serum concentration $\geq$20 ng/mL, and 52 had a 25OHD concentration $\geq$30 ng/mL. Using the manufacturer's ULN of 38.4 ng/L, 27 (33.3%) of our 81 PHPT patients had a “normal” PTH concentration. The percentage of “normal” PTH values (9.9%, 8/81) was significantly lower (chi^2=13.1; $p<0.001$) when using our ULN of 28.9 ng/L. Figure 2 shows the relationship between serum total and ionized calcium levels in the 81 PHPT patients. Although well correlated (r=0.86; $p<0.001$), some discrepancies in the classification of the patients between both calcemia were noted. Twenty seven (33.3%) of our 81 PHPT patients had normal total calcemia ($\leq$2.60 mmol/L) and 12 (14.1%) had normal ionized calcemia ($\leq$1.30 mmol/L). One had normal ionized calcemia and high total calcemia. Thus, 11 (13.6%) of these 81 patients were considered as having true N-PHPT. None of these11 N-PHPT patients had a PTH concentration below 28.9 ng/L, while four (36.4%) had values below 38.4 ng/L. Among the 27 patients with normal total calcemia, 12 had a PTH concentration below 38.4 ng/mL (5 of them with a ionized calcemia $\leq$1.30 mmol/L) and three had values below 28.9 ng/L.

Dialysis patients

The KDIGO guidelines recommend maintaining dialysis patients' serum PTH concentrations between two and nine times the ULN of the kit used in the laboratory. We thus determined the percentage of our 264 dialysis patients who had PTH levels below, within and above this target range, based on the manufacturer’s ULN of 38.4 ng/mL (76.8-345.6 ng/L) and on our ULN of 28.9 ng/L (57.8-251.1 ng/L). Their median PTH concentration was 179.5 ng/L [81.5-272.0] (range 12.4-1750 ng/L). Forty-eight patients (18.2%) were classified differently with the two PTH ULN values used to calculate the KDIGO target range (Table 4).

Discussion

We established PTH reference values for an automated 3rd-generation assay in a large group of French Caucasian healthy volunteers. When we included only subjects with 25OHD ≥ 30 ng/mL and eGFR ≥ 60 mL/min/1.73 m², as recommended [3], the PTH ULN was 22.4% lower than the ULN usually applied by clinical laboratories using this kit (28.9 ng/mL instead of 38.4 ng/L).

One question addressed in recent guidelines for the diagnosis of asymptomatic PHPT is whether a 25OHD of 20 or 30 ng/mL should be considered as the concentration below which a subject should be excluded from a reference population for serum PTH values [3]. Indeed, the higher the 25OHD cut-off defining low 25OHD levels, the lower the PTH ULN (as an example, the ULN that we calculated in our healthy subjects with a 25OHD concentration ≥20 ng/mL from the present study was 32.1 ng/L). It must be underlined that the 20 ng/mL cut-off, supported by the Institute of Medicine, is intended to establish optimal vitamin D intake in the general (healthy) population [16], while the 30 ng/mL cut-off is supported by the Endocrine Society and is intended for use in patient management [17]. Since these 2011 recommendations, the debate about the 25OHD threshold has continued [18, 19] with experts defending the 20 ng/mL value [20] and other the 30 ng/mL value [21]. The choice is important, as it has a huge influence on the number of subjects that may be included in a “vitamin D-replete” reference population for normal PTH values. Indeed, at least in France, approximately half the general healthy population and almost 80% have a 25OHD below 20 ng/mL and 30 ng/mL, respectively [6]. In our opinion, the 30 ng/mL cut-off should be used when recruiting “vitamin D-replete” subjects to establish PTH normal values, not because 25OHD concentrations should always be above 30 ng/mL but rather because this cut-off would have more diagnostic value for detecting HPT (either primary or secondary) when interpreting a PTH concentration. Indeed, in the highly frequent case of a normocalcemic patient with an elevated PTH, the clinical question is whether the elevated PTH is due to vitamin D insufficiency or to N-PHPT (after all other causes of SHPT have been excluded). Many reports have concluded that PTH concentrations are sometimes elevated in subjects with 25OHD concentrations below 28-32 ng/mL [22]. Having said that, we recognize that the debate on the optimal cut-off defining vitamin D sufficiency is “hot”, and that some scientists who do not accept the 30 ng/mL value will not accept our PTH ULN of 28.4 ng/L with this 3rd generation assay. For them, a PTH ULN of 32.1 ng/L (calculated in the population with 25OHD$\geq$20 ng/mL) would be more appropriate.

Another important question is whether the PTH reference values should be stratified for factors known to be associated with PTH levels, such as race, BMI and age. Indeed, serum PTH levels are higher in black than white people [23], in overweight individuals than lean [24], and in the elderly than the young [25]. This may simply be due to differences in vitamin D status, as 25OHD levels are usually lower in blacks, in overweight persons, and in the elderly. The present study included only Caucasian subjects, and we were thus unable to determine whether race is independently associated with PTH levels. We found that BMI was not an independent determinant of PTH levels, in keeping with our findings in another cohort of healthy French subjects [6]. Our results suggest that PTH reference values should be stratified for age, as subjects older than 60 years had higher PTH concentrations than younger subjects, independently of vitamin D status and renal function. However, given the small number of “vitamin D-replete” subjects over 60 years old, we were unable to provide separate reference values for younger and older subjects.

As stressed above, taking vitamin D status into account when establishing PTH reference values leads to a lower ULN than generally obtained in apparently healthy general populations. The obvious consequence is that above-normal concentrations will be found more often in clinical practice. On the one hand, this will improve the diagnostic sensitivity of PTH assay, as witnessed by the higher frequency of elevated PTH concentrations among our patients with surgically proven PHPT. Even if high calcium and PTH in the upper normal range is usually accepted as a diagnostic criterion of PHPT (the PTH is abnormally high-normal in face of hypercalcemia), many doctors feel more “comfortable” with the diagnosis of PHPT when both parameters are elevated. However, even with our lower ULN, almost 10% of our PHPT patients still had “normal” PTH values, emphasizing the need to interpret serum PTH concentrations with respect to calcemia. Importantly, our PHPT diagnoses were based on total and ionized calcemia, a calcium load test, and a lower PTH ULN. It may be noted that the calcium load test is not a standard diagnostic procedure for PHPT in most units. Indeed, high PTH and (even moderately) high total or ionized calcemia is usually considered sufficient. It is however systematic in our unit and has proved to be extremely helpful in normocalcemic patients. The superiority of ionized calcium over total calcium for the diagnosis of PHPT has been reported by others [26] and is confirmed by our data showing that a proportion of our surgically-proven PHPT patients had an elevated pre-surgery ionized serum calcium and a normal serum total serum calcium level (see Figure 2). Assuming that ionized calcium is seldom measured routinely in clinical practice, and that most laboratories use a higher PTH ULN than ours, the diagnosis of PHPT would probably have been missed in 12 (12.8%) of our 81 patients, as they had both normal total calcemia and PTH <38.4 ng/L. On the other hand, use of our PTH reference values might lead to lower specificity. In a previous study, we verified that PTH reference values for the Nichols Allegro PTH assay established in vitamin D-replete subjects did not affect diagnostic specificity: as expected, PTH concentrations were "above-normal" in only 3% of 360 consecutive osteoporotic patients with no reason for having high PTH levels, based on their medical charts and extensive biological investigation [27]. We must underline that our PHPT population does not reflect PHPT patients in general. First, all had osteoporosis, and, second, the proportion of normocalcemic patients may seem very high. This is probably due to a selection bias related to the fact that our unit is specialized in the exploration of calcium/phosphorus metabolism in patients with bone diseases, and that many of these patients were referred because of very mild calcium/phosphorus and/or PTH abnormalities detected during the work-up of osteoporosis (to exclude a secondary cause of bone fragility). Furthermore, although it is clearly stated in the recent guidelines for the management of asymptomatic PHPT that "N-PHPT is now a well-recognized variant of PHPT" [7], and that "N-PHPT is part of the diagnostic spectrum of PHPT, and we need to ensure a correct diagnosis…" [3], there is a lack of recommendations concerning the treatment of this entity (Surgery or not ?). In these patients, our practice is to propose parathyroidectomy if they meet one or several of the indications for parathyroid surgery that are proposed in the guidelines[7]. As all our PHPT patients had osteoporosis, they were all addressed for parathyroid surgery to the same experienced surgeon, even in case of negative preoperative imaging. We have previously shown that hypercalcemic PHPT and N-PHPT patients had a similar BMD gain at the spine and at the hip one year after parathyroidectomy[13].

The KDIGO recommendation to maintain PTH levels between two and nine times the ULN in dialysis patients deserves some discussion. SHPT is frequently associated with CKD and may be considered an appropriate adaptive response to decreasing GFR aimed at maintaining calcium/phosphorus homeostasis. However, SHPT may have deleterious consequences for bone turnover and mineralization and, in its severe forms, may lead to osteitis fibrosa cystica. SHPT may also become autonomous, leading to tertiary (hypercalcemic) hyperparathyroidism. However, many patients with CKD do not exhibit a sufficient increase in PTH levels with often a low bone turnover. This so-called adynamic bone disease is associated with a tendency to hypercalcemia and an increased risk of vascular calcification.

Thus, PTH levels should be neither too high nor too low in CKD patients, especially in case of dialysis, leading experts to propose an optimal range for PTH serum levels. However, marked inter-method variability in serum PTH values precludes the use of a PTH target range (expressed in ng/L) applicable to all PTH assay methods [28-30]. This is why KDIGO proposes a PTH target range based on multiples of the ULN rather than on absolute concentrations. The range of 2 to 9 times the ULN was chosen because several studies showed that values below and above these limits were frequently associated with severely impaired bone turnover on biopsy [31], and with increased cardiovascular morbidity and mortality [32, 33]. Although a PTH target range based on ULN multiples is a pragmatic way of overcoming the inter-method variability of PTH measurement, the way in which normal PTH values are established is of paramount importance. Indeed, with a given PTH assay the ULN may vary significantly depending on the reference population. Here, 18.6% of our dialysis patients were classified differently with our ULN of 28.9 ng/L compared to the manufacturer's ULN of 38.4 ng/L. This is consistent with what we have previously found on comparing the manufacturers' reference ranges for 10 different PTH kits with the reference ranges that we established in the same population of vitamin D-replete Belgian healthy subjects for the 10 kits [9]. This variability may influence the therapeutic choices of nephrologists, who are used to adapting the dosages of PTH treatments, such as active vitamin D and calcimimetics, according to KDIGO recommendations for PTH values.

It must be underlined that, as in our previous studies on the same topic [6, 9-12], the blood samples used here were obtained in the morning (7:30-9:30 AM) after an overnight fast. Indeed, the ULN for a 2nd-generation PTH assay obtained in healthy persons sampled in a non fasting state over a larger time span was higher than in our studies [34] (see discussion in [6]).

The main strengths of our study pertain to the large number of healthy subjects; the population-based recruitment with strict inclusion criteria; the ability to stratify PTH concentrations by 25OHD status, gender, renal function, and BMI; centralization of PTH assay in a single laboratory; and the use of a 3rd-generation assay, which is increasingly employed worldwide. Its limitations must also be acknowledged. First, although large, our population of healthy subjects was insufficient to propose separate reference values for younger and older adults. Second, we restricted the study to Caucasian adults and were therefore unable to determine whether PTH reference values should be stratified according to ethnicity. Third, we did not rule out certain causes of PTH elevation in apparently healthy adults that might influence PTH ULN, such as very low calcium intake or renal calcium leakage. However, we believe that the use of the Horn algorithm, which allowed us to identify and eliminate outliers, minimized this problem. Fourth, as the control of PTH secretion is very complex, other variables not considered in the present study such as daily calcium intake or plasma FGF23 may influence PTH normative data. Fifth, as indicated above, our PTH ULN only applies for this 3rd-generation PTH assay, and if the target 25OHD serum level of 30 ng/mL is accepted.

In conclusion, we confirm that serum PTH reference values are highly dependent on the characteristics of the reference population, especially vitamin D status, renal function, and age. Inclusion of only vitamin D-replete subjects with an eGFR $\geq$ 60 mL/min/1.73 m² reduced the upper normal limit of the reference range by 22.4% compared to the usual reference values of the LIAISON 3rd-generation PTH assay. This had two consequences: 1) it significantly increased the prevalence of elevated PTH values in patients with surgically proven PHPT and, thus, the diagnostic sensitivity of PTH assay for PHPT, and 2) it modified the classification of dialysis patients based on the PTH target range recommended by KDIGO guidelines. As massive inter-method variability in PTH assay results has been demonstrated, more studies are needed to establish PTH reference values for all available assays, using the same large population of vitamin D-replete healthy subjects with normal eGFR and stratifying the data according to age and, possibly, ethnicity.

Declaration of interest

JCS reports lecture fees and/or travel/hotel expenses from DiaSorin, Roche Diagnostics, Abbott, Amgen, Shire, MSD, Lilly, Rottapharm.

CM reports lecture fees and travel/hotel expenses (DiaSorin)

EC is consultant for IDS and DiaSorin and has received lecture fees from IDS, DiaSorin, Roche, Abbott and Amgen

PD is consultant for IDS and has received lecture fees and/or travel expenses from DiaSorin, Amgen, Shire, Fresenius, Menarini, Sanofi.

SB-T, CC, and PC declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Legends

Figure 1: Relationship between serum PTH and 25OHD concentrations in 898 healthy French subjects. The solid curve is the Lowess representation of the relationship.

Figure 2 : Pre-surgery serum total calcium levels of the 81 surgically-proven PHPT patients plotted against their pre-surgery ionized serum calcium level. The vertical line marks the ULN of total serum calcium level in our laboratory (2.60 mmol/L). The horizontal line marks ULN of ionized serum calcium level in our laboratory (1.30 mmol/L). Dots in the upper left quadrant correspond to patients with an elevated ionized calcemia and a normal total calcemia. One patient had a slightly elevated total calcemia (2.62 mmol/L) and a high normal ionized calcemia (1.30 mmol/L).

Table 1. Characteristics of the healthy subjects participating in the VARIETE study.

	Median [Q1-Q3] (min-max)
Gender: men/women	466/432
Age (years)	32 [24-54] (18- 89)
BMI (kg/m²)	22.9 [21.1-24.8] (18.5-28)
Serum 25OHD (ng/mL)	18.8 [15.2-24.0] (7.4-79.0)
Serum PTH (ng/L)	23.6 [18.8-28.3] (5.2-59.4)
Serum calcium (mmol/L)	2.30 [2.21-2.39] (2.10-2.60)
Serum phosphate (mmol/L)	1.10 [0.97-1.22] (0.75-1.51)
Serum albumin (g/L)	43.0 [40.0-46.0] (32.6-50)
eGFR (CKDepi) (mL/min/1.73 m²)	101 [88-114] (60-144)

Table 2: PTH concentrations in the normal subjects of the VARIETE study, according to age and serum 25OHD levels.

		n	PTH (ng/L): median [Q1-Q3]	P value
Age:	a) 18-29 years	411	16.9 [14.1-21.2]	versus b) and c): p<0.001
	b) 30-59 years	307	18.9 [15.3-24.4]	versus a) and c): p<0.001
	c) ≥ 60 years	180	23.5 [18.9-31.7]	versus a) and b): p<0.001
25OHD	a) <12 ng/mL	66	26.5 [18.6-33.7]	versus b), c), and d): p<0.001
	b) 12-19.9 ng/mL	208	20.5 [16.4-26.5]	versus a), c), and d): p<0.001
	c) 20-29.9 ng/mL	441	18.0 [15.1-22.7]	versus a) and b): p<0.001; versus d): p=0.04
	d) ≥30 ng/mL	183	17.0 [13.5-21.5]	versus a) and b): p<0.001; versus c): p=0.04

Table 3 : Main characteristics of the 81 patients with PHPT

Gender : men/women	4/77
Age (years)	67 [57-76] (min : 28-max: 91)
Serum total calcium (mmol/L)	2.64 [2.55-2.75]
Serum ionized calcium (mmol/L)	1.38 [1.32-1.44]
Serum 25OHD (ng/mL)	32 [26-39]
Serum 3rd-generation PTH (ng/L)	46.0 [36.6-65.5]

Table 4: Classification of dialysis patients (n=264) according to the KDIGO target range (two to 9 times the PTH ULN) based on our ULN of 28.9 ng/L and on the manufacturer's ULN of 38.4 ng/L

	Patients (n [%]) with a PTH concentration below, within or above the KDIGO target range based on the manufacturer's ULN (76.8-345.6 ng/L)	Patients (n [%]) having a PTH concentration below, within or above the KDIGO target range based on our ULN (57.8-251.1 ng/L)
Below the target range	64 (24.2%)	50 (18.9%)
Within the target range	154 (58.3%)	134 (50.8%)
Above the target range	46 (21.6%)	80 (30.3%)

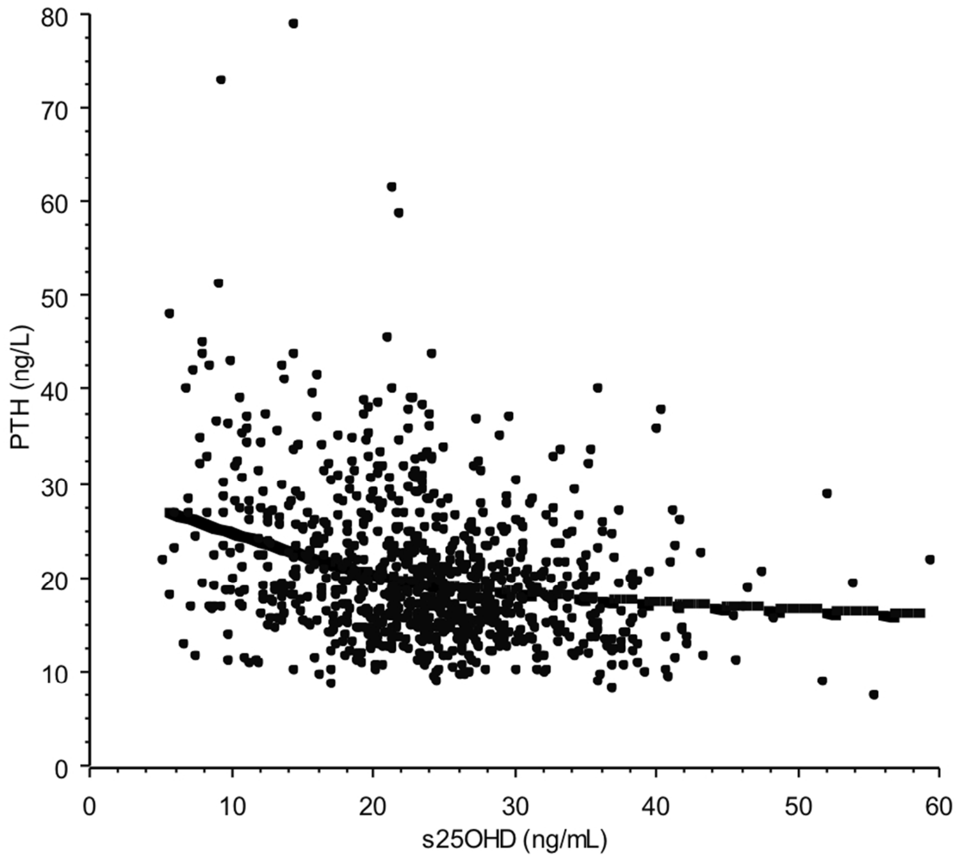

Figure 1: Relationship between serum PTH and 25OHD concentrations in 898 healthy French subjects. The solid curve is the Lowess representation of the relationship.
172x155mm (150 x 150 DPI)

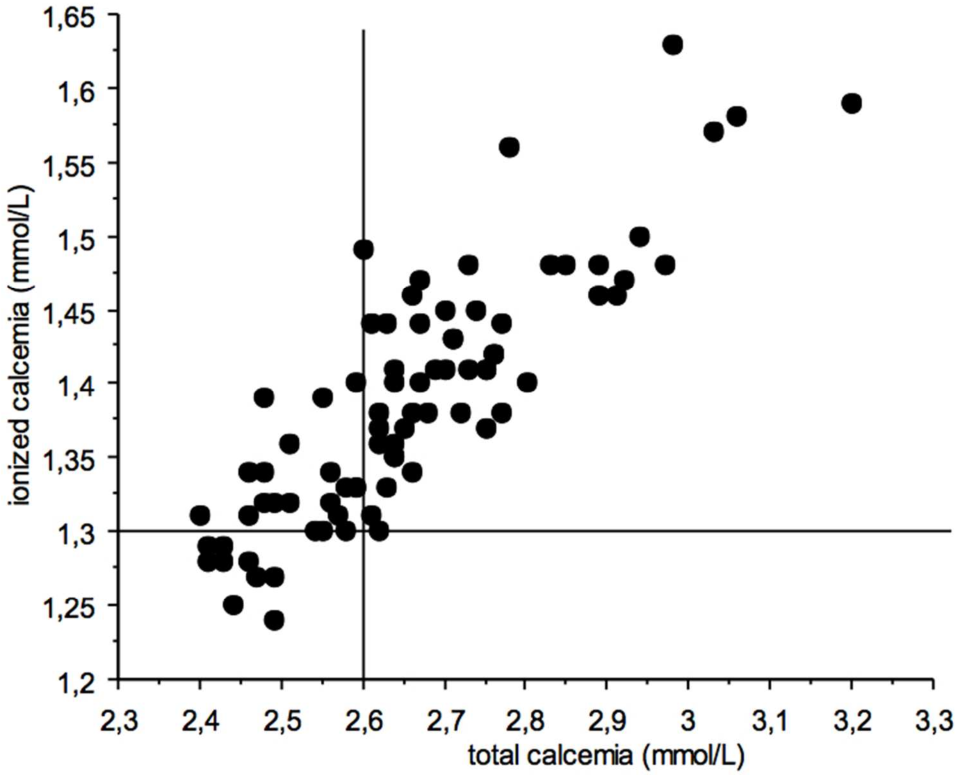

Figure 2 : Pre-surgery serum total calcium levels of the 81 surgically-proven PHPT patients plotted against their pre-surgery ionized serum calcium level. The vertical line marks the ULN of total serum calcium level in our laboratory (2.60 mmol/L). The horizontal line marks ULN of ionized serum calcium level in our laboratory (1.30 mmol/L). Dots in the upper left quadrant correspond to patients with an elevated ionized calcemia and a normal total calcemia. One patient had a slightly elevated total calcemia (2.62 mmol/L) and a high normal ionized calcemia (1.30 mmol/L).
130x108mm (150 x 150 DPI)