J Endocrinol Invest
DOI 10.1007/s40618-016-0553-2

REVIEW

How the reference values for serum parathyroid hormone concentration are (or should be) established?

J.-C. Souberbielle[1,2] · F. Brazier[1,2] · M.-L. Piketty[1,2] · C. Cormier[3] · S. Minisola[4] · E. Cavalier[5]

Received: 5 July 2016 / Accepted: 9 September 2016

Abstract Well-validated reference values are necessary for a correct interpretation of a serum PTH concentration. Establishing PTH reference values needs recruiting a large reference population. Exclusion criteria for this population can be defined as any situation possibly inducing an increase or a decrease in PTH concentration. As recommended in the recent guidelines on the diagnosis and management of asymptomatic primary hyperparathyroidism, PTH reference values should be established in vitamin D-replete subjects with a normal renal function with possible stratification according to various factors such as age, gender, menopausal status, body mass index, and race. A consensus about analytical/pre-analytical aspects of PTH measurement is also needed with special emphasis on the nature of the sample (plasma or serum), the time and the fasting/non-fasting status of the blood sample. Our opinion is that blood sample for PTH measurement should be obtained in the morning after an overnight fast. Furthermore, despite longer stability of the PTH molecule in EDTA plasma, we prefer serum as it allows to measure calcium, a prerequisite for a correct interpretation of a PTH concentration, on the same sample. Once a consensus is reached, we believe an important international multicentre work should be performed to recruit a very extensive reference population of apparently healthy vitamin D-replete subjects with a normal renal function in order to establish the PTH normative data. Due to the huge inter-method variability in PTH measurement, a sufficient quantity of blood sample should be obtained to allow measurement with as many PTH kits as possible.

Keywords Parathyroid hormone (PTH) · Vitamin D · Reference values · Primary hyperparathyroidism · Chronic kidney disease

Introduction

The measurement of parathyroid hormone (PTH) has become very frequent in routine clinical practice since the availability of numerous automated immunoassays with excellent analytical performances. This measurement is not, however, an easy task. Assessment of PTH concentration is of paramount importance in the exploration of disorders of calcium/phosphorus metabolism and in the monitoring of patients with chronic kidney disease (CKD). Correct interpretation of a PTH concentration necessitates at least to have a concomitant serum calcium concentration in order to evaluate whether PTH is adapted (high calcium/low PTH or low calcium/high PTH) or not (high calcium/high PTH or low calcium/low PTH) to the calcemia. It is now well known, however, that cases with normal calcium and high PTH [1], or high or low calcium with normal PTH, are frequently encountered in practice. In these cases, other biological parameters are necessary among which phosphatemia, calciuria, 25-hydroxyvitamin D (25OHD)

✉ J.-C. Souberbielle
Jean-claude.souberbielle@nck.aphp.fr

1 Service des Explorations Fonctionnelles, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75015 Paris, France

2 Université René Descartes, 75015 Paris, France

3 Rheumatology Department, Cochin University Hospital, 75014 Paris, France

4 Ordinario di Medicina Interna, Responsabile UOC Medicina Interna e Malattie Metaboliche dell'Osso (TMC 04), « Sapienza » Università di Roma, Rome, Italy

5 Clinical Chemistry Department, Sart Tilman University Hospital, Liège, Belgium

Published online: 01 October 2016

at least are mandatory. Like for any biological parameter, there is a need for well-validated PTH reference values. The aim of the present paper is to discuss the way the PTH reference values should be established as highlighted in the two most recent guidelines on asymptomatic primary hyperparathyroidism (PHPT) [2, 3].

The different PTH assays and what they measure

The human PTH molecule is an 84 amino acid peptide. Its metabolization occurs in the liver, releasing various amino-truncated fragments into the bloodstream that are called C-terminal PTH fragments because they have kept the carboxyl-terminal part of the native PTH molecule. These fragments are cleared from the circulation through the kidney and have a much longer half-life than the 1–84 PTH. They thus accumulate in the blood of patients with CKD.

The description of the first immunoassay for PTH was published in 1963. This assay, and those who used the same "competitive" design in the following years, used a single antibody directed towards the C-terminal part of the PTH peptide. While they have been important for a better comprehension of PTH physiology and helped for the diagnosis of PHPT and secondary hyperparathyroidism (SHPT), they lacked specificity and were flawed in all cases where C-terminal fragments accumulated such as in CKD. Furthermore, they lacked sensitivity in the low concentrations failing thus to demonstrate very low concentrations in non-parathyroid hypercalcemia or hypoparathyroidism. These assays, now called first-generation assays, are no longer used in clinical practice. Indeed, in 1987, Nichols Institute Diagnostics launched an immunoradiometric (IRMA) kit called Allegro [4]. This assay used a pair of antibodies, one coated to a bead and directed towards the 39–84 portion of the PTH molecule, and the other labelled with ^{125}I and directed towards the 13–24 portion of the PTH molecule, and was not influenced any more by the presence of C-terminal fragments. This "sandwich" assay and the other ones that became available after 1987 were globally called "intact" PTH assays as they were thought to only measure the full-length 1–84 PTH. In 1998, it was demonstrated that these so-called intact PTH assays recognized, with various cross-reactivities, a PTH molecule different from the 1–84 PTH that co-eluted in HPLC with a synthetic (7–84) PTH fragment [5]. In 1999, the first "third-generation" assay was developed by Scantibodies Laboratories [6]. This IRMA called "Whole" PTH assay, used an anti-C-terminal antibody similar to those of the "intact" assays, but an anti-N-terminal antibody directed towards the very first amino acids (1–4) of the peptide. During the recent years, several automated similar third-generation assays became available. While these assays do not measure the C-terminal fragments or the 7–84 PTH fragment, they, however, cross-react with a molecular form of PTH, called "amino-PTH" that is overproduced in parathyroid carcinoma [7]. Table 1 is a summary of what the different generations of PTH assays actually measure.

Analytical and pre-analytical problems with PTH assays

Lack of standardization of the PTH assays

Except in parathyroid carcinoma and some very rare cases of severe PHPT where amino-PTH is overexpressed, the second-generation PTH assays, which measure both the 1–84 and the 7–84 PTH, give theoretically higher values than third-generation assays that do not measure 7–84 PTH. In fact, this is only partly true as the various PTH assays are not standardized. In a previous study, we have shown that 15 different PTH assays gave dramatically different results in the same serum sample with a difference of almost 4 times between the kit producing the highest values and the kit producing the lowest values [8]. In this study, we also found that some second-generation assays gave results that were almost similar to third-generation assays. Thus, apparently different PTH concentrations measured in a single patient in different laboratories during a follow-up may be simply due to the use of different PTH kits by the clinical laboratories.

Sample time and sample stability

A circadian rhythm for PTH exists, showing a nocturnal acrophase, a mid-morning nadir and a smaller afternoon peak. In our opinion, a correct interpretation of a PTH result should mandatorily be performed in conjunction with total (or ionized) serum calcium concentration obtained on the same sample. As calcemia is influenced by food intake, especially if rich in calcium, samples for PTH should always be taken in the morning when the patient is in a fasting status (except in dialysis patients for whom the time of sampling will be dependent on the time of the dialysis session). Several studies, but not all, have suggested that PTH is more stable in EDTA plasma than in serum. To summarize, while EDTA PTH is said to be stable at room temperature for 24 h at least, serum PTH seems to be stable for 4–6 h only. This may appear as an advantage for using EDTA plasma. However, as measurement of calcium is impossible in EDTA plasma, using EDTA samples to measure PTH necessitates that two different tubes are obtained. We thus prefer serum in our practice. In fact, if we suppose that blood is drawn at 8:00 AM, it is likely that in all laboratories, the serum sample will be assayed at

Table 1 Main PTH circulating fragments, and whether they are measured (yes) or not (no) by the various PTH assay-generations

	First-generation assays	Second-generation assays	Third-generation assays
Most common identifications	C-PTH assays Mid-PTH assays	"Intact" PTH assays	Whole PTH assay, Ca-PTH assay BioIntact PTH assay
Methodology	Competition (mostly RIA)	Immunometry ("sandwich" assays)	Immunometry ("sandwich" assays)
1–84 PTH (also called intact PTH, whole PTH, CAP, full-length PTH, …)	Yes	Yes	Yes
7–84 PTH (also called "non-(1–84)" PTH, N-terminal truncated PTH, CIP)	Yes	Yes (with various cross-reactivity)	No
C-terminal fragments (various molecular forms which do not comprise the 1–34 amino acids)	Yes	No	No
"Amino" PTH (also called N-PTH or "atypical" PTH, possibly 1–84 PTH with phosphorylation of 17Ser)	Yes	Depends on the epitope of the anti-N-terminal Ab: *No* if the epitope is proximal (ex: 13–24); *Yes* if the epitope is distal (ex: 26–32)	Yes

It may be noted that the second-generation assays are named « intact » PTH assays, while, in addition to the intact 1–84 PTH molecule, they also measure 7–84 PTH

Ab antibody

12:00 or processed appropriately (whole blood centrifuged and serum stored at −20 °C until assayed). It must be also noted that serum and EDTA PTH measured with the same assay may be significantly different. This difference varies from one kit to another and may be huge. Some PTH assays give higher EDTA values than serum values, while it may be the opposite with other kits [9].

Establishing PTH reference values

The first step in establishing reference values for serum PTH is to recruit a healthy reference population. Exclusion criteria for this population can be defined as any situation possibly inducing an increase or a decrease in PTH concentration. Some of these conditions such as the use of a treatment and/or the existence of a symptomatic disease are easily identified at inclusion, but others are usually asymptomatic and may be ignored if not searched. Among these conditions, vitamin D insufficiency (low serum 25-hydroxyvitamin D [25OHD] concentration) is highly frequent in the general population [10] and should thus be prevalent in an otherwise apparently healthy group. If one admits that vitamin D insufficiency may induce an increase in PTH secretion, and that serum PTH concentration decreases (normalizes) when subjects are given vitamin D [11], it is then logical to exclude subjects with vitamin D insufficiency from a reference population recruited to establish normative data for PTH. This point has been strongly recommended in the two most recent guidelines on the diagnosis and management of asymptomatic primary hyperparathyroidism (PHPT) published in 2009 [2] and 2014 [3]. However, excluding vitamin D-insufficient subjects from the reference group requires measuring the 25OHD level beforehand in all subjects, a practice which greatly complicates the establishment of reference values and had not been taken into account in many previous studies which provided serum PTH reference values for different immunoassays [4, 12–15]. By doing this, however, it has been demonstrated in several studies that excluding subjects with a low serum 25OHD concentration from a reference population decreased the upper normal limit (ULN) for serum PTH compared to what is found in the whole population not taking vitamin D status into account [10, 16–27] (see Table 2). A point that deserves a consensus, however, is the 25OHD cut-off below which a 25OHD concentration may be considered "low". Indeed, at least two 25OHD cut-offs, 50 and 75 nmol/L, are debated. The 50 nmol/L cut-off is supported by the Institute of Medicine (IOM) report which is targeted towards the general (healthy) population in order to define optimal vitamin D intake (which vitamin D intake is necessary so that most individuals in the general population have a 25OHD concentration at or above 50 nmol/L?)

Table 2 Summary of studies that reported PTH reference values for different assays in "vitamin D-replete" subjects

References	Reference population that was recruited in the different studies	Conditions of blood sampling for PTH	25OHD concentration of the recruited reference population and 25OHD cut-off defining insufficient vitamin D status	PTH assay(s) used in these various studies	Manufacturer's PTH normative values and reference population as described in the package insert	PTH in the entire recruited reference population	PTH in the recruited reference population after exclusion of vitamin D-deficient subjects
Souberbielle [16]	280 Healthy elderly (140 women, 140 men) aged 60–79, living in the Paris area (northern France). Albeit complaint of various symptoms related to aging (asthenia, anxiety, loss of memory, pain), they were considered to be in good health and did not take drugs known to affect calcium metabolism	Fasting morning blood sample, performed in April–June. Dry tubes. Serums promptly frozen at −20 °C. Assayed in batches	The 25OHD assay was an in-house protein-binding assay that was recalibrated after this publication to match with the values obtained with the DiaSorin RIA 25OHD of the whole cohort was not specified. Insufficient vitamin D status defined as 25OHD ≤30 nmol/L (corresponding to 39 nmol/L in DiaSorin-equivalent). 40.4 % of the subjects had a 25OHD above this cut-off	Allegro Intact PTH, Nichols Institute Diagnostics, San Juan Capistrano, CA, second-generation	10–65 pg/mL 73 Healthy subjects from the Boston area	13–64 pg/mL	10–46 pg/mL In 113 subjects with 25OHD >39 nmol/L (DiaSorin RIA-equivalent)
				CAP, IRMA, Scantibodies Laboratory, Inc., Santee, CA, third-generation	5–39 pg/mL 128 EDTA plasma from apparently healthy blood donors	10–44 pg/mL	9–34 pg/mL In 113 subjects with 25OHD >39 nmol/L (DiaSorin RIA-equivalent)
Glendenning [17]	197 Healthy blood donors (98 women, 99 men) aged less than 60, living in Perth, Western Australia. Serum creatinine <120 µmol/L in all	Blood samples not obtained in a fasting state, season not reported	25OHD of the whole population was not specified	iPTH by chemiluminescent immunoassay (Immulite 2000; Diagnostics Products), second-generation	12–72 pg/mL Serum from 255 healthy "patients"	19–8.9–101 pg/mL	19–68 pg/mL In 147 subjects with 25OHD ≥50 nmol/L

Table 2 continued

References	Reference population that was recruited in the different studies	Conditions of blood sampling for PTH	25OHD concentration of the recruited reference population and 25OHD cut-off defining insufficient vitamin D status	PTH assay(s) used in these various studies	Manufacturer's PTH normative values and reference population as described in the package insert	PTH in the entire recruited reference population	PTH in the recruited reference population after exclusion of vitamin D-deficient subjects
Souberbielle [18]	927 Healthy adult individuals from different French geographic areas enrolled in a national survey for allergy risk. All had a normal protein-corrected serum calcium level	Blood samples obtained in a fasting state, in late June to late October	25OHD of the population was 13–151 nmol/L: 3–97 percentiles. Insufficient vitamin D status defined as 25OHD <50 nmol/L. 45.4 % of the subjects had a 25OHD above this cut-off	LIAISON intact PTH assay, second-generation	17.3–72.9 pg/mL 105 Healthy adults	3–80 pg/mL	3–51 pg/mL In 421 subjects with 25OHD ≥50 nmol/L
Aloia [19]	489 Women participants without comorbidities aged 47.8 ± 14.6, living in Mineola (New York area, USA)	Blood samples (probably) not obtained in a fasting state, season not reported	Mean 25OHD of the whole population was 51.5 nmol/L Various 25OHD cut-offs were tested	Allegro Intact PTH, Nichols Institute Diagnostics, San Juan Capistrano, CA, second-generation	10–65 pg/mL 73 healthy subjects from the Boston area	17.2–67.5 pg/mL	16.0–59.6 pg/mL In 124 subjects with 25OHD ≥70 nmol/L
Rejnmark [21]	2316 Women aged 17–87, living in Denmark. Blood samples from May 2003 to July 2007	Blood samples between 0800 and 1300 hours not in a fasting state in all subjects, season not reported EDTA plasma stored at −80 °C was used to measure PTH	25OHD of the population was 62 nmol/L (46–79): median (IQR). Deficient vitamin D status defined as 25OHD <30 nmol/L 90.4 % of the subjects had a 25OHD above this cut-off	Electrochemiluminescent immunoassay (ECLIA) on an automated instrument (Cobas e601; Roche Diagnostics), second-generation	15–65 pg/mL Reference population not specified	19–81 pg/mL	16–72.6 pg/mL In 525 women with 25OHD ≥80 nmol/L
La'ulu [20]	260 Healthy subjects, living in the Salt Lake City area (USA). 8 excluded. All included subjects had an eGFR (MDRD) >60 mL/mn/1.73 m^2 and a normal serum calcium level	Blood samples (probably) not obtained in a fasting state. 130 subjects had a blood sample in July–August, and 130 in February. Serum and EDTA plasma were tested	25OHD of the population was not specified. Various 25OHD cut-offs were tested. 243 had 25OHD ≥25 nmol/L, 198 had 25OHD ≥50 nmol/L, and 133 had 25OHD ≥75 nmol/L	Access 2 (Beckman Coulter, Fullerton, CA), second-generation	12–88 pg/mL 289 paired samples (serum and EDTA) from apparently healthy subjects. Exclusion of individuals with abnormal calcium, creatinine, and 25OHD	13.7–77.2 pg/mL	13.6–74.9 pg/mL In 133 subjects with 25OHD ≥75 nmol/L

Table 2 continued

References	Reference population that was recruited in the different studies	Conditions of blood sampling for PTH	25OHD concentration of the recruited reference population and 25OHD cut-off defining insufficient vitamin D status	PTH assay(s) used in these various studies	Manufacturer's PTH normative values and reference population as described in the package insert	PTH in the entire recruited reference population	PTH in the recruited reference population after exclusion of vitamin D-deficient subjects
				ARCHITECT i2000SR (Abbott Diagnostics, Abbott Park, IL), second-generation	8.7–77.1 pg/mL 143 plasma samples from apparently healthy adults	18.1–88.5 pg/mL	18.1–84.2 pg/mL In 133 subjects with 25OHD ≥75 nmol/
				ADVIA Centaur (Siemens Healthcare Diagnostics, Deerfield, IL), second-generation	14–72 pg/mL	11–70.6 pg/mL	10.9–69.1 pg/mL In 133 subjects with 25OHD ≥75 nmol/
				Modular E170 (Roche Diagnostics, Indianapolis, IN), second-generation	15–65 pg/mL Reference population not specified	17.3–73.5 pg/mL	17.1–72.9 pg/mL In 133 subjects with 25OHD ≥75 nmol/
				IMMULITE 2000 (Siemens Healthcare Diagnostics), second-generation	12–65 pg/mL Serum from 255 healthy "patients"	13.8–92.2 pg/mL	13.7–85.1 pg/mL In 133 subjects with 25OHD ≥75 nmol/
				LIAISON (DiaSorin, Stillwater, MN), second-generation	17.3–72.9 pg/mL 105 healthy adults	21.8–123.4 pg/mL	21.8–119.5 pg/mL In 133 subjects with 25OHD ≥75 nmol/
Cavalier [23]	120 Women 20–79 years, 120 men 19–80 years. All Caucasian. All had a 25OHD level ≥75 nmol/L, serum calcium between 2.15 and 2.60 mmol/L, serum phosphate between 0.74 and 1.51 mmol/L, and an eGFR (MDRD) >60 mL/mn/1.73 m^2. No drug potentially affecting calcium metabolism except native vitamin D3	Blood samples (dry tubes) obtained in the morning after an overnight fast. Blood centrifuged within 30 mn of sampling, serums aliquoted and store at −70 °C	25OHD mean concentration of the whole group not specified. All had a 25OHD concentration ≥75 nmol/L	Intact PTH Architect (Abbott) second-generation	15–68.3 pg/mL 143 plasma samples from apparently healthy adults	NA	16.3–64.7 pg/mL 240 subjects with 25OHD ≥75 nmol/L

Table 2 continued

References	Reference population that was recruited in the different studies	Conditions of blood sampling for PTH	25OHD concentration of the recruited reference population and 25OHD cut-off defining insufficient vitamin D status	PTH assay(s) used in these various studies	Manufacturer's PTH normative values and reference population as described in the package insert	PTH in the entire recruited reference population	PTH in the recruited reference population after exclusion of vitamin D-deficient subjects
				Access PTH intact (Beckman-Coulter). second-generation	12–88 pg/mL 289 paired samples (serum and EDTA) from apparently healthy subjects. Exclusion of individuals with abnormal calcium, creatinine, and 25OHD	NA	10.1–47.4 pg/mL 240 subjects with 25OHD ≥75 nmol/L
				N-Tact PTH SP IRMA (DiaSorin). second-generation	13–54 pg/mL 129 serum samples from apparently healthy young adults	NA	7.2–35.7 pg/mL 240 subjects with 25OHD ≥75 nmol/L
				LIAISON (DiaSorin,), second-generation (serum)	17.3–72.9 pg/mL 105 healthy adults	NA	21.3–68.2 pg/mL 240 subjects with 25OHD ≥75 nmol/L
				Intact PTH Vitros 5600 (Ortho Clinical Diagnostics)	7.5–53.5 pg/mL EDTA, heparin or serum samples from 240 subjects with normal calcium, TSH, creatinine and vitamin D levels	NA	108–47.5 pg/mL 240 subjects with 25OHD ≥75 nmol/L
				Elecsys 2010 (Roche Diagnostics); second-generation	15–65 pg/mL Reference population not specified	NA	13.7–50.2 pg/mL 240 subjects with 25OHD ≥75 nmol/L
				Total intact PTH IRMA (Scantibodies Laboratories); second-generation	14–66 pg/mL 165 EDTA plasma from apparently healthy blood donors	NA	7.8–49.7 pg/mL 240 subjects with 25OHD ≥75 nmol/L
				IMMULITE 2000 (Siemens Healthcare Diagnostics), second-generation	12–72 pg/mL Serum from 255 healthy "patients"	NA	5.4–57.1 pg/mL 240 subjects with 25OHD ≥75 nmol/L

Table 2 continued

References	Reference population that was recruited in the different studies	Conditions of blood sampling for PTH	25OHD concentration of the recruited reference population and 25OHD cut-off defining insufficient vitamin D status	PTH assay(s) used in these various studies	Manufacturer's PTH normative values and reference population as described in the package insert	PTH in the entire recruited reference population	PTH in the recruited reference population after exclusion of vitamin D-deficient subjects
				Liaison 1–84 PTH (DiaSorin); second-generation	5.5–38.4 pg/mL 74 individuals with 25OHD >75 nmol/L and serum calcium between 2.125 and 2.525 mmol/L	NA	4.6–25.8 pg/mL 240 subjects with 25OHD ≥75 nmol/L
				CAP, IRMA, Scantibodies Laboratory, Inc., Santee, CA, third-generation	5–39 pg/mL 128 EDTA plasma from apparently healthy blood donors	NA	6.8–30.8 pg/mL 240 subjects with 25OHD ≥75 nmol/L and eGFR >60
Fillée [22]	266 Healthy blood donors aged 18–65, living in the region of Brussels (Belgium)	Blood samples not obtained in a fasting state, performed in end of summer ($n = 157$) and winter ($n = 109$)	25OHD of the population was 51 nmol/L (34–70): median (IQR). Insufficient vitamin D status defined as 25OHD <50 nmol/L. 50.6 % of the subjects had a 25OHD above this cut-off	Access Intact PTH assay on a UniCel DxI 800 analyser (Beckman Coulter, Brea, CA, USA), second-generation	12–88 pg/mL 289 Paired samples (serum and EDTA) from apparently healthy subjects. Exclusion of individuals with abnormal calcium, creatinine, and 25OHD	17–78 pg/mL	15–70 pg/mL in 132 subjects with 25OHD ≥50 nmol/L
Deckers [24]	738 Healthy subjects (398 women, 340 men) aged 55–65, living in Netherland. LASA cohort	Blood samples obtained in the morning after light breakfast without dairy products, season not reported	25OHD of the population was 56.7 nmol/L (9–183): mean (range). Insufficient vitamin D status defined as 25OHD <50 nmol/L. 59 % of the subjects had a 25OHD above this cut-off	Intact PTH assay (Architect, Abbott Diagnostics, Abbott Park, IL), second-generation	15–68.3 pg/mL 143 plasma samples from apparently healthy adults	26–114 pg/mL	25–105 pg/mL in 438 subjects with 25OHD ≥50 nmol/L

Table 2 continued

References	Reference population that was recruited in the different studies	Conditions of blood sampling for PTH	25OHD concentration of the recruited reference population and 25OHD cut-off defining insufficient vitamin D status	PTH assay(s) used in these various studies	Manufacturer's PTH normative values and reference population as described in the package insert	PTH in the entire recruited reference population	PTH in the recruited reference population after exclusion of vitamin D-deficient subjects
Deckers [24]	633 Subjects from primary care (387 women, 244 men) aged 18–65, living in Netherland. NESDA cohort	Blood samples obtained in the morning after an overnight fast, season not reported	25OHD of the population was 67.8 nmol/L (8–179) nmol/L mean (range). Insufficient vitamin D status defined as 25OHD <50 nmol/L. 71 % of the subjects had a 25OHD above this cut-off	Intact PTH assay (Architect, Abbott Diagnostics, Abbott Park, IL), second-generation	15–68.3 pg/mL 143 plasma samples from apparently healthy adults	20–101 pg/mL	19–89 pg/mL in 450 subjects with 25OHD ≥50 nmol/L
Djennane [25]	435 Children aged 5–15 years living in Northern Algeria (Tizi-Ouzou) had a blood sample in March 2011. 408 of them had another blood sample in September 2011. They were apparently healthy and were recruited from school registry with the consent of their parents. All has normal serum calcium, phosphate and creatinine. Calcium intake was recorded in all	Blood samples obtained in the morning in a fasting state	25OHD was 71.4 nmol/L (48.5–79.5): median (IQR) in September, and 52.9 nmol/L (24.8–44.8) in March. Overall (March + September) 323 samples had a 25OHD >75 nmol/L	Elecsys 2010 (Roche Diagnostics); second-generation	15–65 pg/mL Reference population not specified	20.7–69.8 pg/mL	19.4–49.3 pg/mL In the 323 samples with 25OHD ≥75 nmol/L
Touvier [10]	1824 Healthy Caucasian adults (991 women, 833 men) aged 35–65, living in France. Participants in the SUVIMAX study	Fasting blood, obtained between October and April	25OHD of the population was 50 ± 25.75 nmol/L: mean ± SD. 771 Subjects had a 25OHD ≥50 nmol/L 293 Subjects had a 25OHD ≥575 nmol/L	Roche Cobas electrochemiluminescent immunometric assay (Roche Diagnostics), second-generation	15–65 pg/mL Reference population not specified	14.3–50.8 pg/mL	13.3–45.3 pg/mL In 293 subjects with 25OHD ≥75 nmol/L

Table 2 continued

References	Reference population that was recruited in the different studies	Conditions of blood sampling for PTH	25OHD concentration of the recruited reference population and 25OHD cut-off defining insufficient vitamin D status	PTH assay(s) used in these various studies	Manufacturer's PTH normative values and reference population as described in the package insert	PTH in the entire recruited reference population	PTH in the recruited reference population after exclusion of vitamin D-deficient subjects
Souberbielle [27]	898 Healthy adults (432 women, 466 men) aged 18–89 years, living in France. Participants of the VARIETE study (see extensive description of the inclusion/exclusion criteria in the paper)	Blood samples obtained in a fasting state, in all seasons	25OHD of the population was 59 nmol/L (47–70.8): median (IQR). Insufficient vitamin D status defined as 25OHD <75 nmol/L 20.4 % of the subjects had a 25OHD above this cut-off	Immunochemiluminometric assays on the LIAISON XL (DiaSorin, Stillwater, MN, USA), third-generation	<38.4 pg/mL 74 individuals with 25OHD >75 nmol/L and serum calcium between 2.125 and 2.525 mmol/L	10.1–37.9 pg/mL	9.4–28.9 pg/mL In 183 subjects In 293 subjects with 25OHD ≥75 nmol/L and eGFR >60
Li [26]	1436 healthy Chinese people aged 15–64, living in China. Extensive description of the population in the paper	Fasting morning venous blood, season not reported	25OHD of the population was 49.7 ± 20.4 nmol/L: mean ± SD. Insufficient vitamin D status defined as 25OHD <75 nmol/L 11.7 % of the subjects had a 25OHD above this cut-off	Chemiluminescence automated analyser (Roche Diagnostics), second-generation	15–65 pg/mL Reference population not specified	8.8–70 pg/mL	7.5–60.7 pg/mL 168 subjects with 25OHD ≥75 nmol/L

Note the definition of "vitamin D-replete" subjects differed in terms of serum 25OHD cut-off

NA not applicable

[28]. The 75 nmol/L cut-off is supported by the Endocrine Society and is intended for the care of the patients [29]. In our opinion, this 75 nmol/L cut-off value is the one that should be used when recruiting "vitamin D-replete" subjects to establish PTH normal values. This is not because we think that everybody needs a 25OHD concentration above 75 nmol/L, but rather because many reports and meta-analyses have concluded that serum PTH concentration may still be elevated in some subjects if their 25OHD concentration is below 70–80 nmol/L, and decreases when these subjects are given vitamin D [29]. Another point which should be taken into account in the inclusion criteria for establishing PTH reference values is renal function. It is generally accepted that PTH may rise in some patients when estimated glomerular filtration rate (eGFR), assessed with the MDRD or the CKDepi formula, is below 60 mL/mn/1.73 m^2 [30]. Such eGFR below 60 mL/mn/1.73 m^2 may be present but ignored in some apparently healthy subjects, especially in those aged more than 60 years.

Another issue concerning PTH reference values is whether the reference population should be stratified according to various factors such as age, gender, menopausal status, body mass index, and race. Indeed, it has been reported for example that serum PTH is higher in black than in white people [31], in overweight than in lean individuals [10], and in the elderly than in the young [32]. However, 25OHD is also known to be usually lower in black than white people [31], in overweight than in lean persons [10], and in the elderly than in the young [31], and this can explain part of the higher PTH concentration found in blacks, overweight, and elderly people. These differences in vitamin D status between young and old, whites and blacks, lean and overweight individuals should thus be re-evaluated in vitamin D-replete subjects. We found for example in [10] that the PTH concentration differed by weight status in the French general population (higher in overweight) but that this difference disappeared in subjects with higher vitamin D status (25OHD ≥75 nmol/L). However, age may be an independent determinant of PTH concentration with higher levels in older persons as suggested in several studies [33]. For example, our recent results in a cohort of French healthy subjects suggested that PTH reference values should be stratified for age, as subjects older than 60 years had higher PTH concentrations than younger subjects, independent of vitamin D status and renal function [27]. However, given the small number of "vitamin D-replete" subjects (i.e. with a 25OHD level ≥75 nmol/L) over 60 years old in this study, we were unable to provide separate reference values for younger and older subjects. Last but not least, dietary calcium intake of the subjects included in a reference population for PTH concentration should probably be considered. In an evaluation of the vitamin D status and its determinants of children/adolescents from Northern Algeria, we showed that for a given category of 25OHD concentration (all children whatever their 25OHD concentration, those with a 25OHD ≥50, and those with a 25OHD ≥75 nmol/L), low calcium intake (i.e. below the median intake of 623 mg/day found in our population) was significantly associated with higher mean PTH concentrations than in those with higher calcium intake [25]. Finally, it must be mentioned that questions remain concerning the PTH concentrations in children and in pregnant women. Several studies that measured PTH in healthy children reported concentrations that varied with age [34, 35]. However, these age-related variations were different across studies with higher values in teenagers in some studies [34] or in prepubertal children in others [35]. These discrepancies may be explained by the fact that these studies were usually not designed to evaluate specifically the paediatric PTH concentrations and thus did not take into account the various determinants of PTH secretion listed above. In our above-mentioned study performed in healthy children from Northern Algeria [25], the PTH values were perfectly similar to what we found in healthy adults with the same kit [23]. We thus believe that, assuming that the determinants of PTH concentration are taken into account when recruiting a paediatric reference population, PTH concentrations are similar in children and adults. The data concerning the PTH levels in pregnant women are also conflicting. While it is clear that, compared to non-pregnant women, calcium and phosphate levels are unchanged in pregnancy and that calciuria, 1,25-dihydroxyvitamin D, and PTHrP increase significantly [36], data on PTH measured with second-generation assays are conflicting as some studies showed a strong decrease [36–38], while others found no change [39–43].

Looking at Table 2, it may be noted that different authors who took vitamin D status into account when describing PTH reference values did not find similar ULN, even when using the same PTH kit. As an example, we can consider a widely used PTH assay, the Cobas/Elecsys/Modular kit from Roche Diagnostics, for which the manufacturer's ULN is 65 pg/mL. With this kit, we found a serum PTH ULN of 50 pg/mL in 240 healthy Belgian subjects with a serum 25OHD ≥75 nmol/L [23], 49.3 pg/mL in 323 healthy children from Northern Algeria with a 25OHD ≥75 nmol/L [25], and a plasma PTH ULN of 45.3 pg/mL in 293 healthy French adults with a 25OHD ≥75 nmol/L [10]. With the same kit, La'ulu found that the ULN of serum PTH values was 60.3 pg/mL when their analysis was restricted to 133 apparently healthy subjects with a 25OHD concentration ≥75 nmol/L [20], Li et al. [26] found a serum PTH ULN of 56.8 pg/mL in 168 healthy Chinese subjects with a 25OHD ≥75 nmol/L, while Rejnmark et al. [21] found that the plasma PTH ULN was 67 pg/mL in 525 Danish women with a 25OHD level ≥80 nmol/L. Explaining the

differences between these results, and specially those of the well-conducted study by Rejnmark, and our results is not obvious at first glance. Indeed, the fact that they explored only women should not explain their higher PTH limit than in some of our studies [10, 23, 25] as we did not find different PTH levels in men and in women. The larger age range of the Rejnmark's population compared to our studies [10, 23, 25] may explain some (but not much) of the difference because, as indicated above, age may be a significant determinant of PTH concentration with higher PTH levels in older compared to younger subjects. In our opinion, the most important reason for these different results resides in the way the blood samples were obtained (i.e. in the early morning after an overnight fast in our studies [10, 23, 25], and between 08:00 and 13:00 in a non-fasting state in the Rejnmark study [21]). Indeed, circadian variation of PTH concentration is well documented with higher concentration during the late morning–early afternoon period than during early morning hours [44–46]. As a possible consequence, the distribution of PTH values in the Rejnmark's study was not Gaussian (skewed to the right). As their mode of calculation of the reference range was based on the nonparametric percentile method, it is thus highly plausible that a subset of their population who had a blood sample at the latest hours influenced the distribution of the PTH values and was responsible for the higher ULN. In one of our recent studies on this topic [27], we used the Horn algorithm to determine the PTH reference range in a large population of healthy French subjects after elimination of the outliers [47]. In brief, this statistical method needs first that evaluation of the normality of the distribution of the PTH concentrations is performed (Kolmogorov test). If the distribution is non-Gaussian, the raw data are log-transformed, the Gaussian nature of the log-transformed data is tested, and the quartiles 1 and 3 (Q1 and Q3) and the interquartile range (IQR) of the log-transformed data are calculated (if the log-transformed data are not Gaussian, use the Box and Cox method to approximate a Gaussian distribution). Outliers are then defined as PTH concentrations below Q1–1.5 IQR and above Q3 + 1.5 IQR of the log-transformed values. Then, the 95 % confidence interval in the remaining subjects is calculated (after elimination of the outliers). Using this statistical method, the PTH ULN is lower than the one calculated when using the nonparametric method.

Clinical consequences of using PTH reference values established in vitamin D-replete subjects with a normal renal function

As stressed above, using PTH reference values which take vitamin D status and renal function into account will decrease the ULN when compared to what is generally obtained in an apparently healthy general population without considering vitamin D status and eGFR. The clinical value of decreasing the PTH ULN concentrations may, however, be questioned. The obvious consequence is that, in clinical practice, much more patients will be detected as having an increased PTH concentration. It is thus important to evaluate whether these patients have a potential reason for an increased PTH secretion, a question which, in many cases, will need additional explorations. In 2003 [48], we validated in 708 well-documented consecutive osteopenic patients the upper PTH limit of 46 ng/L that we found with the Nichols Allegro assay after exclusion of vitamin D-deficient subjects from a reference population (compared to the 65 ng/L ULN of the manufacturer) [16]. We showed that our proposed reference values increased the detection of high PTH levels in normocalcemic patients having a potential reason for an abnormal PTH secretion (better sensitivity), with no more than 3 % of patients with a PTH above 46 ng/L and no reason for an increased PTH secretion (no loss of specificity). The gain in sensitivity was important as among 348 patients with a potential cause of increased PTH, 46 (13.2 %) had a concentration above 65 ng/L, whereas 126 (36.2 %) had a concentration above 46 ng/L. Similarly, in a recent study on the effects of parathyroidectomy on bone mineral density, we found that more than half of our 39 patients with a surgically proven normocalcemic PHPT, and 40 % of those with a hypercalcemic but asymptomatic form of the disease, had a serum PTH concentration measured with the Roche Cobas automated assay below the upper normal value of 65 ng/L of the manufacturer [49].

Clinical consequences of using PTH reference values established according to our proposals do not resume to patients with endocrine/bone disorders and a normal renal function. Indeed, although not usually concerning the endocrinologists, we would like to address the case of chronic kidney disease (CKD) with emphasis on dialysis patients. It is well documented that PTH tends to increase as soon as the GFR decreases below 60 mL/mn/1.73 m^2. The reasons are multiple, including a decrease in calcitriol production due to phosphate retention and increased FGF23 secretion, a progressive shift to the right of the calcium set-point, and a resistance to PTH action for poorly understood reasons. In non-dialysis patients with CKD, the recommendation of the KDIGO guidelines [30] is to try to maintain the PTH concentration within the normal range, using primarily native vitamin D, calcium salts, and non-calcium phosphate binders, and keeping more active treatments such as calcitriol analogs or calcimimetics for patients in whom the primary treatment failed to decrease PTH. It must be noted that this recommendation is based on results mostly obtained with the Allegro intact PTH assay whose reference range was not established in vitamin D-sufficient subjects. It

should probably be revisited if excluding vitamin D-insufficient subjects and subjects with a (moderately) low GFR becomes the rule when establishing PTH reference values. Having said that, most nephrologists consider that a PTH concentration close to twice the ULN is not a problem in patients with advanced CKD (stages 4 and 5). The case is different in dialysis patients for whom it is a consensus to target higher PTH levels. According to the KDIGO guidelines [30], the PTH concentration of the patients treated by dialysis should be maintained within twice and nine times the ULN of the PTH assay. Indeed, both too low and too high PTH concentrations are better avoided in dialysis patients because low PTH levels are associated with low turnover bone disease and high PTH levels are associated with *osteitis fibrosa cystica* and increased mortality. The choice of the KDIGO expert panel to define the optimal PTH range for dialysis patients in terms of multiple of the ULN of a given PTH assay rather than in absolute concentration in pg/mL was an elegant way to overcome the huge inter-method variability of PTH results. However, as the PTH reference values for a given kit may greatly differ as a function of the reference population (see Table 2), classification of a dialysis patient according to the KDIGO target range (below, within, or above ×2–×9 ULN) may be influenced by the way the reference population for PTH concentration has been recruited. In a study published in 2012 [23], we measured serum PTH with 10 different kits in 149 haemodialysis patients and 240 healthy subjects with a 25OHD concentration ≥75 nmol/L and an eGFR >60 mL/mn/1.73 m^2. We showed that when using the manufacturer's ULN of these 10 PTH kits, many dialysis patients were classified differently by the 10 kits and that these discrepancies were significantly reduced when we used the ULN derived from our healthy population of 240 subjects to define the KDIGO target range.

Last, it should also be highlighted that several meta-analyses of prospective cohort studies performed in apparently healthy populations have reported that subjects in the highest quartile or quintile of PTH concentrations are at higher risk of cardiovascular events [50] and all-cause mortality [51]. After adjustment for different confounding factors, PTH was considered as an independent risk factor. This also argues for the necessity of obtaining appropriate threshold for normal PTH values.

Table 3 Summary of the various determinants of PTH concentration that should be considered when establishing PTH reference values, and how they could influence the reference range

Pre-analytical and post-analytical determinants	
Nature of the blood sample (Serum or EDTA plasma)	Some assays give higher values in EDTA plasma, while other assays give higher results in serum. PTH more stable in EDTA plasma, but serum allows measuring calcium (not possible in EDTA plasma). For this reason, serum is preferred
Time of sampling and fasting status	Due to circadian variations in calcium, phosphate, and PTH concentration, and to the decrease in PTH secretion after the consumption of calcium containing foods, it is recommended to use samples obtained in the morning in a fasting state
Mode of calculation of the reference range	The distribution of PTH concentration is usually not Gaussian, and several outliers are usually present when testing an apparently healthy population. It is recommended to use a statistical method that renders the distribution Gaussian and eliminate the outliers (i.e. the Horn method—see text)
Metabolic and endogenous determinants	
Vitamin D status (25OHD concentration)	PTH is usually increased in vitamin D-insufficient subjects. ULN is lower when vitamin D-insufficient subjects are excluded from the reference population
Renal function (eGFR)	PTH may be increased when GFR is <60 mL/mn/1.73 m^2. ULN is lower when subjects with impaired renal function are excluded from the reference population
Calcium intake (alimentary and/or supplemental)	PTH increased in case of chronic low calcium intake
Race	PTH higher in Blacks compared to white subjects. Independent determinant or due to the fact that Blacks have lower vitamin D status than whites? (more studies needed)
Weight status	PTH higher in overweight/obese compared to lean subjects. Independent determinant or due to the fact that obese have lower vitamin D status than lean subjects? (more studies needed)
Age	PTH higher in older (>60 years) compared to young subjects. Independent determinant or due to the fact that old subjects have lower vitamin D status and lower eGFR than young subjects? (more studies needed)
Pregnancy	Unclear: some studies report much lower PTH values in pregnant women compared to non-pregnant, while other studies report similar levels (more studies needed)
Paediatry	There are no firm data supporting that children have different PTH concentrations than young adults

Conclusion and perspectives

PTH measurement is now very frequently performed in routine clinical practice and is essential for a correct diagnosis of disorders of calcium/phosphorus metabolism and for the care of patients with CKD. The reference values for PTH concentration may significantly differ, especially in terms of ULN, in function of the inclusion/exclusion criteria that are used to recruit a reference population, with potential differences in the interpretation of a PTH concentration in a given patient. In our opinion, an important international multicentre work should be performed to recruit a very extensive reference population of apparently healthy vitamin D-replete subjects with a normal renal function in order to establish the PTH normative data for all the available PTH kits. Attention should be paid to several determinants of PTH concentration, especially calcium intake, and age with possible stratification according to age (Table 3). Due to the huge inter-method variability in PTH measurement, a sufficient quantity of blood sample should be obtained into allow measurement with as many assays as possible. Pre-analytical and analytical criteria must also be considered, especially the type of sample (serum or plasma—we prefer serum as stressed above), the time of sampling and the fasting/non-fasting status (we recommend early morning samples in a fasting state). Outliers should be detected by one of the recommended statistical methods and eliminated from the included subjects. These proposals correspond to our opinion, but we acknowledge that an international, multidisciplinary consensus is necessary. We must also remind the reader that a PTH concentration should always be interpreted in function of the concomitant calcemia (i.e. measured on the same sample than PTH [52]) and that a low calcium level associated with a low-normal PTH, or a high calcium level associated with a high-normal PTH, is indicative of a parathyroid disorder.

Compliance with ethical standards

Conflict of interest JCS reports lecture fees and/or travel/hotel expenses from DiaSorin, Roche Diagnostics, Abbott, Amgen, Shire, MSD, Lilly, Rottapharm, Meda. EC is consultant for IDS and DiaSorin and has received lecture fees from IDS, DiaSorin, Roche, Abbott, and Amgen. SM served as a speaker for Abiogen, Amgen, Diasorin, Eli Lilly, Italfarmaco, Fujii, Merck Sharp and Dohme, Takeda. He also served in advisory board of Amgen and Eli Lilly. FB, MLP, and CC have nothing to declare.

Ethical approval Not applicable to this review article.

Informed consent Not applicable to this review article.

References

1. Souberbielle JC, Cormier C, Cavalier E (2015) How to manage an isolated elevated PTH? Ann Endocrinol 76:134–141
2. Eastell R, Arnold A, Brandi ML, Brown EM, D'Amour P, Hanley D et al (2009) Diagnosis of asymptomatic primary hyperparathyroidism: proceedings of the third international workshop. J Clin Endocrinol Metab 94:340–350
3. Bilezikian J, Brandi ML, Eastell R, Silverberg S, Udelsman R, Marcocci C et al (2014) Guidelines for the management of asymptomatic primary hyperparathyroidism: summary statement from the fourth international workshop. J Clin Endocrinol Metab 99:3561–3569
4. Nussbaum SR, Zahradnik RJ, Lavigne JR, Brennan GL, Nozawa-Ung K, Kim LY et al (1987) Highly sensitive two-site immunoradiometric assay of parathyrin, and its clinical utility in evaluating patients with hypercalcemia. Clin Chem 33:1364–1367
5. Lepage R, Roy L, Brossard JH, Rousseau L, Dorais C, Lazure C et al (1998) A non (1–84) circulating parathyroid hormone fragment interferes significantly with intact PTH commercial assay in uremic samples. Clin Chem 44:4287–4290
6. John M, Goodman W, Gao P, Cantor T, Salusky I, Juppner H (1999) 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 84:4287–4290
7. Cavalier E, Daly AF, Betea D, Pruteanu-Apetrii P, Delanaye P, Stubbs P et al (2010) The ratio of parathyroid hormone as measured by third- and second-generation assays as a marker for parathyroid carcinoma. J Clin Endocrinol Metab 95:3745–3749
8. Souberbielle JC, Boutten A, Carlier MC, Chevenne D, Coumaros G, Lawson-Body E et al (2006) Inter-method variability in PTH measurement: implication for the care of CKD patients. Kidney Int 70:345–350
9. Joly D, Drueke T, Alberti C, Houillier P, Lawson-Body E, Martin K et al (2008) Variation in serum and plasma PTH levels in second-generation assays in hemodialysis patients: a cross-sectional study. Am J Kidney Dis 51:987–995
10. Touvier M, Deschasaux M, Montourcy M, Sutton A, Charnuax N, Kess-Guyot E et al (2014) 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 99:1196–1203
11. Björkman M, Sorva A, Tilvis R (2009) Responses of parathyroid hormone to vitamin D supplementation: a systematic review of clinical trials. Arch Gerontol Geriatr 48:160–166
12. Blind E, Schmidt-Gayk H, Scharla S, Flentje D, Fischer S, Göhring U et al (1988) Two-site assay of intact parathyroid hormone in the investigation of primary hyperparathyroidism and other disorders of calcium metabolism compared with a mid-region assay. J Clin Endocrinol Metab 67:353–360
13. Endres D, Villanueva R, Sharp C Jr, Singer F (1991) Immunochemiluminometric and immunoradiometric determinations of intact and total immunoreactive parathyrin: performance in the differential diagnosis of hypercalcemia and hypoparathyroidism. Clin Chem 37:162–168
14. Ratcliffe W, Heath D, Ryan M, Jones S (1989) Performance and diagnostic application of a two-site immunometric assay for parathyrin in serum. Clin Chem 35:1957–1961
15. Gao P, Scheibel S, D'Amour P, John M, Rao S, Schmidt-Gayk H et al (2001) Development of a novel immunoradiometric assay exclusively for biologically active whole parathyroid hormone 1–84: implication for improvement of accurate assessment of parathyroid function. J Bone Miner Res 16:605–614

16. Souberbielle JC, Cormier C, Kindermans C, Gao P, Cantor T, Forette F et al (2001) Vitamin D status and redefining serum parathyroid hormone reference range. J Clin Endocrinol Metab 86:3086–3090
17. Glendenning P, Vasikaran S (2002) Comment on: Vitamin D status and redefining serum PTH reference range in the elderly. J Clin Endocrinol Metab 97:946–947
18. Souberbielle JC, Fayol V, Sault C, Lawson-Body E, Kahan A, Cormier A (2005) Assay-specific decision limits for two new automated parathyroid hormone and 25-hydroxyvitamin D assays. Clin Chem 51:395–400
19. Aloia J, Feuerman M, Yeh J (2006) Reference range for serum parathyroid hormone. Endocr Pract 12:137–144
20. La'ulu S, Roberts W (2010) Performance characteristics of six intact parathyroid hormone assays. Am J Clin Pathol 134:930–938
21. Rejnmark L, Vestergaard P, Heickendorff L, Mosekilde L (2011) Determinants of plasma PTH and their implication for defining a reference interval. Clin Endocrinol 74:37–43
22. Fillée C, Keller T, Mourad M, Brikman T, Ketelslegers JM (2012) Impact of vitamin D-related serum PTH reference values on the diagnosis of mild primary hyperparathyroidism, using bivariate calcium/PTH reference regions. Clin Endocrinol 76:785–789
23. Cavalier E, Delanaye P, Vranken L, Bekaert AC, Carlisi A, Chapelle JP et al (2012) Interpretation of serum PTH concentrations with different kits in dialysis patients according to the KDIGO guidelines: importance of the reference (normal) values. Nephrol Dial Transpl 27:1950–1956
24. Deckers M, de Jongh R, Lips P, Penninx B, Milaschi Y, Smit J et al (2013) Prevalence of vitamin D deficiency and consequences for PTH reference values. Clin Chim Acta 426:41–45
25. Djennane M, Lebbah S, Roux C, Djoudi H, Cavalier E, Souberbielle JC (2014) Vitamin D status of schoolchildren in Northern Algeria, seasonal variations and determinants of vitamin D deficiency. Osteoporos Int 25:1493–1502
26. Li M, Lv F, Zhang Z, Deng W, Li Y, Deng Z et al (2016) Establishment of a normal reference value of parathyroid hormone in a large healthy Chinese population and evaluation of its relation to bone turnover and bone mineral density. Osteoporos Int 27:1907–1916
27. Souberbielle JC, Massart C, Brailly-Tabard S, Cormier C, Cavalier E, Delanaye P et al (2016) Serum PTH reference values established by an automated third-generation assay in vitamin D-replete subjects with normal renal function: consequences of diagnosing primary hyperparathyroidism and the classification of dialysis patients. Eu J Endocrinol 174:315–323
28. Ross C, Manson JE, Abrams S, Aloia J, Brannon P, Clinton S et al (2011) The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 96:53–58
29. Holick M, Binkley N, Bischoff-Ferrari H, Gordon C, Hanley D, Heaney R et al (2011) Evaluation, treatment and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 96:1911–1930
30. KDIGO C-M (2009) Work Group KDIGO Clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone disorder (CKD-MBD). Kidney Int 76(Suppl 113):S1–130
31. Bell NH, Greene A, Epstein S, Oexmann MJ, Shaw S, Shary JR (1985) Evidence for alteration of the vitamin D-endocrine system in blacks. J Clin Invest 76:470–473
32. Quesada JM, Coopmans W, Ruiz B, Aljam P, Jans I, Bouillon R (1992) Influence of vitamin D on parathyroid hormone in the elderly. J Clin Endocrinol Metab 75:494–501
33. Valcour A, Blocki F, Hawkins DM, Rao SD (2012) Effects of age and serum 25OHD-vitamin D levels on serum parathyroid hormone levels. J Clin Endocrinol Metab 97:3989–3995
34. Bailey D, Colantino D, Kuriakopoulou L, Cohen A, Chan MK, Armbruster D et al (2013) Marked biological variance in endocrine and biochemical markers in childhood: establishment of pediatric reference intervals using healthy community children from CALIPER cohort. Clin Chem 59:1393–1405
35. Soldin O, Dahlin J, Gresham E, King J, Soldin S (2008) Immulite 2000 age and sex-specific reference intervals for alpha fetoprotein, homocysteine, insulin, insulin-like growth factor-1, insulin-like growth factor binding protein-3, C-peptide, immunoglobulin E and intact parathyroid hormone. Clin Biochem 41:937–942
36. Kovacs C, Kronenberg H (1997) Maternal-fetal calcium and bone metabolism during pregnancy, puerperium, and lactation. Endocr Rev 18:832–872
37. Frolich A, Rudnicki M, Fischer-Rasmussen W, Olofsson K (1991) Serum concentration of intact parathyroid hormone during late human pregnancy: a longitudinal study. Eur J Obstet Gynecol Reprod Biol 42:85–87
38. Seely EW, Brown E, DeMaggio D, Weldon D, Graves S (1997) A prospective study of calciotropic hormones in pregnancy and post partum: reciprocal changes in serum intact parathyroid hormone and 1,25-dihydroxyvitamin D. Am J Obstet Gynecol 176:214–217
39. Saggese G, Baroncelli GI, Bertelloni S, Cipolloni C (1991) Intact parathyroid hormone levels during pregnancy, in healthy term neonates and in hypocalcemic preterm infants. Acta Paediatr Scand 80:36–41
40. Cross N, Hillman L, Allen S, Krause G, Vieira N (1995) Calcium homeostasis and bone metabolism during pregnancy, lactation, and postweaning: a longitudinal study. Am J Clin Nutr 61:514–523
41. More C, Bhattoa HP, Bettembuk P, Balogh A (2003) The effect of pregnancy and lactation on hormonal status and biochemical markers of bone turnover. Eur J Obstet Gynecol Reprod Biol 106:209–213
42. Uemura H, Yasui T, Kiyokawa M, Kuwahara A, Ikawa H, Matsuzaki T et al (2002) Serum osteoprotegerin/osteoclastogenesis-inhibitory factor during pregnancy and lactation and the relationship with calcium-regulating hormones and bone turnover markers. J Endocrinol 174:353–359
43. Kramer C, Ye C, Hanley A, Connelly P, Sermer M, Zinman B et al (2016) The relationship between parathyroid hormone and 25-hydroxyvitamin D during and after pregnancy. J Clin Endocrinol Metab 101:1729–1736
44. Fuleihan GEH, Klerman E, Brown E, Choe Y, Brown E, Czeisler C (1997) The parathyroid hormone circadian rhythm is truly endogenous—a general clinical research center study. J Clin Endocrinol Metab 82:281–286
45. Rejnmark L, Lauridsen A, Vestergaard P, Heickendorff L, Andreasen F, Mosekilde L (2002) Diurnal rhythm of plasma 1,25-dihydroxyvitamin D and vitamin D-binding protein in postmenopausal women: relationship to plasma parathyroid hormone and calcium and phosphate metabolism. Eur J Endocrinol 146:635–642
46. Smith E, Cai M, McMahon L, Holt S (2012) Biological variability of plasma intact and C-terminal FGF23 measurements. J Clin Endocrinol Metab 97:3501–3504
47. Horn PS, Feng L, Li Y, Pesce AJ (2001) Effect of outliers and nonhealthy individuals on reference interval estimation. Clin Chem 47:2137–2145
48. Souberbielle JC, Lawson-Body E, Hammadi B, Sarfati E, Kahan A, Cormier C (2003) The use in clinical practice of parathyroid

hormone normative values established in vitamin D-sufficient subjects. J Clin Endocrinol Metab 88:3501–3504
49. Koumakis E, Souberbielle JC, Sarfati E, Meunier M, Maury E, Gallimard E et al (2013) Bone mineral density evolution after successful parathyroidectomy in patients with normocalcemic primary hyperparathyroidism. J Clin Endocrinol Metab 98:3213–3220
50. van Ballegooijen A, Reinders I, Visser M, Brouwer I (2013) Parathyroid hormone and cardiovascular disease events: a systematic review and meta-analysis of prospective studies. Am Heart J 165:655–664
51. Yang B, Lu C, Wu Q, Zhang J, Zhao H, Cao Y (2016) Parathyroid hormone, cardiovascular and all-cause mortality: a meta-analysis. Clin Chim Acta 455:154–160
52. Minisola S, Pepe J, Piemonte S, Cipriani C (2015) The diagnosis and management of hypercalcemia. BMJ 2(350):h2723. doi:10.1136/bmj.h2723