Assay-Specific Decision Limits for Two New Automated Parathyroid Hormone and 25-Hydroxyvitamin D Assays

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Background: The recent development of nonradioactive automated assays for serum parathyroid hormone (PTH) and 25-hydroxyvitamin D (25OHD) has made measurement of these two hormones possible in many laboratories. In this study, we compared two new assays for PTH and 25OHD adapted on an automated analyzer, the LIAISON®, with two manual immunoassays used worldwide.

Methods: We studied 228 osteoporotic patients, 927 healthy individuals, 38 patients with primary hyperparathyroidism, and 167 hemodialyzed patients. Serum PTH was measured with the Allegro® and the LIAISON assays, and 25OHD was measured with DiaSorin RIA and the LIAISON assay. Regression analysis was used to calculate decision thresholds for the LIAISON assays that were equivalent to those of the Allegro PTH and DiaSorin 25OHD assays.

Results: The 25OHD concentrations obtained with the LIAISON assay and the RIA in osteoporotic patients were well correlated (r = 0.83; P < 0.001). Regression and Bland–Altman analyses suggested that the LIAISON 25OHD assay reads lower than the DiaSorin RIA at low concentrations but higher at high concentrations. However, the cutoff (50 nmol/L) used in our laboratories to define vitamin D insufficiency with the DiaSorin RIA is applicable to the LIAISON 25OHD assay. In 927 healthy individuals, the 3rd–97th percentile intervals were 3–80 ng/L and 13–151 nmol/L for the LIAISON PTH and 25OHD concentrations, respectively. However, 506 individuals (54.6%) were vitamin D-insufficient; we therefore considered only the 421 individuals with a LIAISON 25OHD >50 nmol/L as eligible for the reference population for the LIAISON PTH assay. In this group, the 3rd–97th percentile interval for LIAISON PTH was 3–51 ng/L. Considering upper reference limits of 46 and 51 ng/L for the Allegro and LIAISON assays, respectively, the frequency of above-normal PTH concentrations in patients with primary hyperparathyroidism was similar in both assays. Regression analysis between serum PTH measured by the Allegro and LIAISON assays in 167 hemodialyzed patients and the corresponding Bland–Altman analysis of these data suggest that the LIAISON PTH assay tends to read higher than the Allegro assay at low concentrations but lower at high concentrations (>300 ng/L).

Conclusions: Because clinical decision limits for both PTH and 25OHD should be assay specific, we propose equivalences between these assays and two manual assays used worldwide. These assay-specific decision limits should help potential users of the LIAISON PTH and 25OHD assays.

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Parathyroid hormone (PTH)4 and 25-hydroxyvitamin D (25OHD) are frequently measured in routine practice. Until recently, these analytes were measured by RIA/radiocompetition assays and/or HPLC in specialized laboratories. New automated assays made their measurement possible in many more laboratories. It should be stressed, however, that although these new assays can ease daily laboratory practice, they need careful analytical

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4 Nonstandard abbreviations: PTH, parathyroid hormone; 25OHD, 25-hydroxyvitamin D; and PHPT, primary hyperparathyroidism.
and clinical evaluation and a definition of specific reference values before being used broadly.

Population-based reference values for 25OHD vary with season (1), latitude (2), age (2), and skin pigmentation (3). Health-based reference values for serum 25OHD have been proposed to replace population-based reference values, with a cutoff at 50 nmol/L defining vitamin D insufficiency (4, 5). Although higher than most lower limits of the commonly used reference values, this cutoff is still considered too low by many specialists (6–9). Until a consensus is reached, we currently use this cutoff of 50 nmol/L in our laboratories. Because PTH may be increased in patients with vitamin D insufficiency and decreases when vitamin D-insufficient individuals are given vitamin D, it seems logical to exclude those with vitamin D insufficiency from a reference population for serum PTH. However, because vitamin D insufficiency is usually clinically silent, 25OHD must be measured beforehand, and individuals with a concentration below the threshold defining vitamin D insufficiency should be excluded from the reference population. Applying these criteria, we showed that the upper reference limit for serum PTH obtained with the Allegro® PTH assay (Nichols Institute) or with the Whole PTH assay (Scantibodies Laboratories) becomes ~30% lower than the upper limit usually considered (46 ng/L instead of 65 ng/L with the Allegro assay and 34 ng/L instead of 44 ng/L with the Whole PTH assay) (10). The differences in the concentrations obtained with these two assays highlight that epitope recognition may influence the PTH reference interval.

Reference values and decision thresholds for serum PTH and 25OHD may vary among assays and should thus be assay specific (11, 12). In the present work, we focused on reference values and clinical decision limits for two new automated assays for 25OHD and PTH (LIAISON®, DiaSorin). We first compared 25OHD concentrations measured in osteoporotic patients with the LIAISON assay and with the DiaSorin RIA and developed assay-specific decision limits for the LIAISON 25OHD assay. We then measured 25OHD and PTH with the LIAISON assays in a large population of healthy individuals and considered only those with a 25OHD concentration >50 nmol/L as the reference population for the LIAISON PTH assay. Finally, we studied the LIAISON PTH assay in patients with surgically confirmed primary hyperparathyroidism (PHPT) and established equivalences between the LIAISON PTH assay and the Nichols Allegro PTH assay in hemodialyzed patients.

Materials and Methods

PATIENTS AND HEALTHY INDIVIDUALS

The two 25OHD assays, the RIA and the LIAISON, were compared on sera from 228 consecutive Caucasian osteoporotic patients seen in our osteoporosis clinic to rule out a secondary cause of low bone mass. In these patients, osteoporosis was initially diagnosed on the basis of a bone mineral density T-score below −2.5 SD or on the basis of a low trauma fracture associated with a bone mineral density T-score below −1 SD. None of these patients had clinical signs of osteomalacia. They were residents of the Paris region (latitude 49 degrees North), and most of them had been exposed to sunshine during the previous months because blood samples were obtained in summer (from late June to late October 2003).

Serum PTH and 25OHD were measured with the LIAISON assays in 927 healthy individuals from different French geographic areas enrolled in a national survey of allergy risk. Blood samples were obtained throughout the year, and sera were thawed once for the centralized measurement (in Laboratoire Marcel Mérieux, Lyon, France) of total IgE. The remaining quantity of serum (at least 1 mL) was kept frozen at −20 °C until the combined measurement of PTH and 25OHD with the LIAISON analyzer and serum total calcium and proteins by standard methods. All of the individuals had a normal protein-corrected serum calcium. They gave oral informed consent for the anonymous measurement of biochemical markers other than total IgE in the remaining serum.

PTH was also measured with both the LIAISON PTH assay and the Nichols Allegro PTH assay in 38 consecutive patients with surgically confirmed PHPT and in 167 hemodialyzed patients. The PHPT patients were initially referred to our bone clinic because they were osteoporotic, to rule out a secondary cause of osteoporosis. Because PHPT was then diagnosed in these patients, they were treated surgically under guidelines from a recent consensus development conference on asymptomatic PHPT designating densitometric osteoporosis as a criterion for surgery in PHPT (13).

LABORATORY METHODS

The LIAISON is an automated analyzer with which a result for both PTH and 25OHD can be obtained in 35 min (25 min if only PTH is desired) from a single serum sample (minimum quantity of serum needed to measure both analytes is 450 µL).

The LIAISON intact PTH assay is a direct immunochemiluminescent sandwich assay that uses two affinity-purified polyclonal antibodies, one directed against the N-terminal portion (residues 1–34) of the PTH molecule (the labeled antibody) and the other (the capture antibody) directed against the C-terminal end (residues 39–84). This assay is said by the manufacturer to measure both the intact 1–84 PTH molecule and the 7–84 PTH fragment with 100% and ~80% cross-reactivity, respectively.

The LIAISON 25OHD assay is a direct competitive immunochemiluminescent assay. Reagents include an antibody specific to vitamin D coated on magnetic particles and 25OHD conjugated to an isoluminol derivative and diluted in phosphate buffer. The anti-25OHD antibody is directed against the N-terminal portion (residues 1–34) of the 1–84 PTH molecule with 100% and 30% cross-reactivity. Reagents include an antibody specific to vitamin D coated on magnetic particles and 25OHD conjugated to an isoluminol derivative and diluted in phosphate buffer. The anti-25OHD antibody is directed against the N-terminal portion (residues 1–34) of the 1–84 PTH molecule with 100% and 30% cross-reactivity.
During incubation in an acidic assay buffer containing 100 mL/L acetonitrile, the 25OHD present in serum is dissociated from its binding protein and competes with the labeled vitamin D for binding sites on the antibody.

The analytical characteristics of the two LIAISON assays as provided by the manufacturer in the product inserts are presented in Table 1.

As outlined above, PTH and 25OHD were also measured in our patients with the Allegro intact PTH assay (Nichols Institute) and with the 125I 25OHD RIA (DiaSorin) for which analytical and clinical performance have been described elsewhere (14, 15).

**RESULTS**

Data are presented as the mean (SD). Assay methods were compared by simple regression analyses and Bland–Altman plots. Regression analyses were used to calculate equivalences between assays for decision limits, whereas Bland–Altman analyses were used to demonstrate possible intermethod bias. The difference in means between groups was assessed by ANOVA. P ≤0.05 was considered significant.

The 25OHD concentrations obtained with the LIAISON assay and the RIA in our osteoporotic patients were well correlated (Fig. 1). Of these 228 patients from whom blood samples were obtained in summer, 78 (34.2%) were considered vitamin D-insufficient (DiaSorin RIA 25OHD value ≤50 nmol/L). Equivalences between the DiaSorin 25OHD RIA and the LIAISON 25OHD assay were calculated from the equation presented in Fig. 1. For example, 25OHD RIA concentrations of 12.5, 25, 50, 100, and 200 nmol/L corresponded to 6, 21, 50, 108, and 225 nmol/L in the LIAISON assay. These data and Bland–Altman plots (not shown) suggest that the LIAISON 25OHD assay reads lower than the DiaSorin RIA at low concentrations but higher at high concentrations. It is of note, however, that the cutoff of 50 nmol/L used at present in our laboratories to define vitamin D insufficiency with the DiaSorin RIA is applicable to the LIAISON 25OHD assay.

In the 927 healthy individuals, we found the expected significant negative correlation between the LIAISON PTH values and the LIAISON 25OHD values (r = −0.21; P <0.0001), whereas serum calcium (protein corrected) was correlated with neither the LIAISON PTH (r = 0.02; P = 0.54) nor the LIAISON 25OHD (r = 0.012; P = 0.71). In the whole population of 927 individuals, the 3rd to 97th percentile intervals were 3–80 ng/L and 13–151 nmol/L for the LIAISON PTH and the LIAISON 25OHD assay, respectively. However, 506 of these 927 individuals (54.6%) had a LIAISON 25OHD ≤50 nmol/L; we therefore considered only the 421 remaining individuals with a LIAISON 25OHD >50 nmol/L eligible as the reference population for the LIAISON PTH assay. In this group, the mean (SD) LIAISON PTH [23.1 (15.4) ng/L] was significantly lower (P <0.001) than in the group with 25OHD ≤50 nmol/L [32.6 (24.2) ng/L], with a 3rd to 97th percentile interval of 3–51 ng/L, and the PTH concentrations were no longer correlated with 25OHD (r = 0.053; not significant). Furthermore, the mean LIAISON PTH concentration for those individuals with a LIAISON 25OHD between 50 and 75 nmol/L [23.3 (16.2) ng/L] was not different from the mean LIAISON PTH for those with a LIAISON 25OHD >75 nmol/L [23.0 (14.8) ng/L].

As expected, the LIAISON PTH concentrations were higher in the PHPT patients [81.6 (45.2) ng/L] and in the

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<th>Table 1. Analytical characteristics of the LIAISON assays as provided by DiaSorin in the product inserts.</th>
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<td><strong>LIAISON 25OHD</strong></td>
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* The analytical sensitivity was calculated as 2 SD from 10 replicate measurements of the zero calibrator.

* The intraassay CV is the SD/mean of four replicate measurements of five serum pools, whereas the interassay CV is the SD/mean of the same five serum pools assayed in duplicate, twice per assay, in one assay per day, for 5 operator days. The mean LIAISON PTH values of these five serum pools were 67, 159, 303, 568, and 1290 ng/L, and the mean LIAISON 25OHD concentrations were 12.0, 37.5, 91.5, 34.0, and 67.0 nmol/L. The high CVs obtained with the LIAISON 25OHD assay were obtained at the lowest concentration (mean value, 12 nmol/L).

* Linearity was assessed by testing serial dilutions of three serum pools added to the zero calibrator.
hemodialyzed patients [246.9 (228.8) ng/L] than in the reference population \((P < 0.001\) for both groups). Considering upper reference cutoffs of 46 and 51 ng/L for the Allegro Nichols and the LIAISON PTH assays, respectively, 4 of our 38 patients with surgically confirmed PHPT had a normal PTH concentration in both assays, 3 had a normal concentration in the Allegro assay but a high concentration in the LIAISON assay, whereas 4 had a high concentration in the Allegro assay but a normal concentration in the LIAISON assay (Fig. 2). In any PHPT patients with a normal PTH concentration, PTH was in fact inappropriately in the high-normal range when compared with hypercalcemia.

The results of the regression analysis between Allegro PTH and LIAISON PTH values in the 167 hemodialyzed patients are shown in Fig. 3. The equation presented in Fig. 3 allowed calculation of equivalent values between assays in these patients. For example, equivalent LIAISON values for Allegro concentrations of 50, 150, 300, 500, and 1000 ng/L are 90, 169, 288, 446, and 842 ng/L, respectively. These equivalences and Bland–Altman plots (not shown) suggest that the LIAISON PTH assay tends to read higher than the Allegro assay at low concentrations but lower at high concentrations (>300 ng/L).

**Discussion**

We have developed in this study corresponding clinical decision thresholds between the DiaSorin 25OHD RIA and the LIAISON 25OHD assays and between the Nichols Allegro PTH and the LIAISON PTH assays.

Serum 25OHD is the best indicator of vitamin D status (4), but there is currently no consensus on a threshold below which vitamin D insufficiency can be defined. We currently use a threshold of 50 nmol/L with the DiaSorin RIA because several authors have reported that a serum 25OHD >50 nmol/L is sufficient to prevent secondary hyperparathyroidism (4, 5). Our results were consistent with this threshold because PTH concentrations were not correlated with 25OHD in the healthy individuals with 25OHD >50 nmol/L and were not higher in those with a serum 25OHD between 50 and 75 nmol/L than in those with 25OHD >75 nmol/L. We find this cutoff at 50 nmol/L exactly applicable to the LIAISON 25OHD assay, but this is probably fortuitous, and we keep in mind that many authorities regard this concentration as indicating vitamin D insufficiency. Indeed, it has been proposed (7, 8) that 25OHD should be >75 nmol/L to take advantage of effects of vitamin D not related to calcium metabolism (16–22). Furthermore, other authors have suggested that 25OHD should be ≥75 nmol/L to prevent secondary hyperparathyroidism (6, 23). Because most studies from which these thresholds are derived have used the DiaSorin RIA for measuring 25OHD, we propose assay-specific decision thresholds for the LIAISON 25OHD assay in comparison with the DiaSorin RIA (for example, 75 nmol/L with the RIA corresponds to 79 nmol/L with the LIAISON 25OHD assay). These equivalences need confirmation because the high CVs reported by the manufacturer may induce poor reproducibility of our findings. Assuming that a LIAISON 25OHD of 50 nmol/L is used to define vitamin D insufficiency, 34.2% of our 228 osteoporotic patients (blood obtained in summer) and 54.6% of the 927 healthy patients (blood obtained throughout the year) were vitamin D-insufficient. These proportions increase to 66.2% and 76.8% in the osteoporotic patients and healthy individuals, respec-
tively, if a threshold of 79 nmol/L is used. This confirms that vitamin D insufficiency is an endemic problem in France.

When establishing reference values for serum PTH, it seems logical to exclude from the reference population any person with a condition potentially leading to an increased PTH concentration. Vitamin D insufficiency is one condition that may increase PTH, but to know whether an apparently healthy individual is vitamin D-insufficient, serum 25OHD must be measured. However, vitamin D status has not been taken into account in most published studies on PTH reference values (14, 24–28). We found that in healthy individuals with 25OHD >50 nmol/L, the upper limit of the LIAISON PTH reference interval was 36% lower than when vitamin D status was not taken into account in the same population (51 ng/L instead of 80 ng/L), a result highly comparable to our previous findings with the Allegro PTH assay and the Whole PTH assay (10). We also found that the Allegro assay and the LIAISON PTH assay yielded similar clinical information for the diagnosis of PHPT. However, despite using a narrower reference interval than is typically used, we found, like others (28–30), that although PTH is disproportionately high for a given calcium concentration, it is not clearly above normal in every patient with PHPT. This emphasizes the need for simultaneous measurement of serum calcium and PTH.

Finally, we propose equivalences between the Allegro PTH assay and the LIAISON PTH assay in hemodialyzed patients. PTH measurements are part of the routine evaluation of such patients to identify renal osteodystrophy subtypes and to adjust treatment with calcium/vitamin D. In these patients, the decision limits are based on studies that compared results of bone biopsies with PTH concentrations measured with the Allegro assay (31) and suggested that intact PTH concentrations should be maintained between 150 and 300 ng/L. Although confirmed in the guidelines for the management of renal osteodystrophy (32), it is not indicated that this target range is specific for the Allegro assay only. We found that the equivalent range with the LIAISON PTH assay is 169–288 ng/L. It should be stressed that choosing a PTH assay today for the follow-up of hemodialyzed patients is a challenge. Indeed, “intact” PTH assays such as the Allegro or the LIAISON PTH assay recognize a non-(1–84) PTH fragment (33) that accumulates in renal failure (34). The recent development of assays that measure the 1–84 PTH form only (35) has been identified as a real improvement in the noninvasive diagnosis of bone disease in hemodialyzed patients by some (36) but not all authors (37, 38). Deciding whether these new assays will replace the intact assays in routine practice deserves further studies and a published consensus (32, 39).

In summary, we have evaluated two new automated PTH and 25OHD assays and propose equivalences between these assays and two manual assays used worldwide. These assay-specific decision limits could help potential users of the LIAISON PTH and 25OHD assays.

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