Effects of Age and Serum 25-OH-Vitamin D on Serum Parathyroid Hormone Levels

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Context: Several studies define optimal serum 25-hydroxyvitamin D (25-OHD) levels based on serum PTH level reaching an asymptote. However, results differ widely, ranging from 25-OHD levels of 12–44 ng/ml; many studies are constrained by small sample size.

Objective: The objective of the study was to determine the relationship between serum PTH and 25-OHD levels and age in a very large reference laboratory database.

Design: This was a detailed cross-sectional analysis of 312,962 paired serum PTH and 25-OHD levels measured from July 2010 to June 2011.

Results: Median PTH levels and the proportion of patients (PTH ≥ 65 pg/ml), from 63 successive 25-OHD frequency classes of 5000 patients, provide smooth, exceptionally well-fitted curves ($R^2 = 0.994$ and $R^2 = 0.995$, respectively) without discernible inflection points or asymptotes but with striking age dependencies. Serum 25-OHD was below the recent Institute of Medicine sufficiency guidance of 20 ng/ml in 27% (85,000) of the subjects. More importantly, 40 and 51% of subjects (serum 25-OHD <20 and 10 ng/ml, respectively) had biochemical hyperparathyroidism (PTH ≥ 65 pg/ml).

Conclusions: This analysis, despite inevitable inherent limitations, introduces several clinical implications. First, median 25-OHD-dependent PTH levels revealed no threshold above which increasing 25-OHD fails to further suppress PTH. Second, the large number of subjects with 25-OHD deficiency and hyperparathyroidism reinforces the Third International Workshop on Asymptomatic Primary hyperparathyroidism’s recommendations to test for, and replete, vitamin D depletion before considering parathyroidectomy. Third, strong age dependency of the PTH-25-OHD relationship likely reflects the composite effects of age-related decline in calcium absorption and renal function. Finally, this unselected large population database study could guide clinical management of patients based on an age-dependent, PTH-25-OHD continuum. (J Clin Endocrinol Metab 97: 3989–3995, 2012)

Osteoporosis and primary hyperparathyroidism (PHPT) are the two most common bone and mineral disorders (1–3) whose clinical expression is often influenced by the prevailing vitamin D and calcium nutritional status. In addition, many more patients suffer bone loss due to secondary hyperparathyroidism associated with chronic vitamin D depletion and age-related decline in kidney function.

Integral to the appropriate clinical management of individuals with these conditions is an accurate assessment of the mediators governing normal calcium homeostasis, 25-hydroxyvitamin D (25-OHD) and PTH. Although prolonged, severe vitamin D depletion (serum 25-OHD level <15 ng/ml) results in rickets and osteomalacia, less severe vitamin D deficiency, commonly referred to as vitamin D insufficiency, is associated with increased PTH.
levels, accelerated cortical bone loss and an increased risk of fractures.

It is well established that individuals with serum levels of 25-OHD less than 15 ng/ml can be considered as suffering from definitive vitamin D deficiency (4, 5). There is emerging general consensus that levels much higher than this minimal threshold are required for optimal skeletal health (4, 5). Several surrogate clinical indicators such as intestinal calcium absorption, bone mineral density and rates of bone loss, falls, and fractures have been proposed as the physiological criteria to define vitamin D sufficiency (6). In addition, a number of other studies have suggested that the serum 25-OHD concentration for optimal skeletal health could be defined as the level at which PTH declines to a minimum. However, to date, this latter approach has failed to provide a definitive serum 25-OHD threshold for optimal health but instead unveiled a range of values that vary from 12 ng/ml (7) to 15 ng/ml (8) to 20 ng/ml (9–13) to 30 ng/ml (10, 14–16) all the way up to 44 ng/ml (17, 18).

Common to most published studies on the PTH-25-OHD relationship is the finding of a rapid initial drop in PTH as the serum 25-OHD levels exceed the minimal level of 10 ng/ml, followed by a slower decline in serum PTH with increasing 25-OHD levels. However, a significant limitation of these studies was their smaller sample size (<100 to ~30,000), such that frequently the data did not provide adequate resolution to determine whether the declining PTH level with increasing 25-OHD reached a true asymptote. Also, various mathematical models have been used to assess the PTH-25-OHD relationship. One common approach has been to fit the data to a segmented line model in which one steep line is fitted to the lower, 25-OHD portion of the curve with a second, more horizontal line to the higher, 25-OHD portion. Several other publications fitted the data assuming that the relationship plateaus beyond some higher level of 25-OHD. Here, the inclusion of at least 10-fold more PTH-25-OHD paired values (~313,000) than previous studies provides a higher resolution of this relationship, which discloses an unbroken continuity of decreasing PTH with increasing 25-OHD. In addition, we examine the extent to which the PTH-25-OHD relationship is influenced by aging.

Materials and Methods

Study population and data

Laboratory Corp. of America Holdings is an independent clinical laboratory testing company with majority operations in the United States. Data on laboratory tests and demographic variables are available from a computerized database. The study population includes 312,962 individuals for whom, upon clinical referral, had 25-OHD and PTH tests performed between July 1, 2010, and June 30, 2011. Where available, calcium and creatinine levels were also obtained.

Laboratory analysis

Laboratory tests for serum PTH and 25-OHD were performed in multiple Laboratory Corp. laboratories throughout the United States using identical methods and instruments. Laboratory Corp. participates in both Vitamin D External Quality Assessment Scheme and College of American Pathologists proficiency surveys for 25-OHD testing, and the College of American Pathologists proficiency surveys for creatinine, calcium, and PTH testing. Serum 25-OHD was measured using the Liaison 25-OH Vitamin D TOTAL Assay (DiaSorin USA, Stillwater, MN), a competitive one-step backfill chemiluminescence assay with a measurement range of 4–150 ng/ml and functional sensitivity of 4.0 ng/ml or less. The intra- and interassay precision of this assay are 8.9 and 12.8%, respectively, with reported cross-reactivity values of 100% for both 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3. Serum PTH was measured using the Elecsys E-170 PTH, Intact assay (Roche Diagnostics, Indianapolis, IN), a two-site electrochemiluminescence immunoassay with a measurement range of 1.2 to 5000 pg/ml and functional sensitivity of 6.0 pg/ml. The intra- and interassay precision is 1.1 and 3.3%, respectively. Serum calcium was measured using the Modular D analyzer assay (Roche Diagnostics), a 0-cresolphthalein complexome colorimetric assay with a measurement range of 0.2–20.0 mg/dl. The intra- and interassay precision is 0.6 and 1.4%, respectively. Serum creatinine was measured using the Modular D analyzer assay (Roche Diagnostics), a kinetic colorimetric assay with a measurement range of 0.2–25.0 mg/dl. The intra- and interassay precision is 1.2 and 2.6%, respectively.

Optimal split-point analysis

A secondary analysis of the data was rendered following the methodology of Hawkins (19). The method was applied to find optimal 25-OHD segment boundaries between patient groupings within which the PTH is relatively constant but between which it varies. The segmentation reported, based on the percentage of patients with PTH above 65 pg/ml, provides the maximum likelihood fit to a model of 18 segments of binomial data with the probability changing from one class to the next.

Results

The 312,962 patients were split by their 25-OHD values into 63 groups, each containing 5,000 patients, and the median PTH of each was calculated. The median was chosen as the measure of location because the distribution of PTH is heavily right skewed, making the median more representative than the mean. The graph of the median PTH vs. the 25-OHD is shown in Fig. 1A. Also shown on the graph is a smooth curve given by the equation $PTH = 11.9 + 140.6 \times (25-OHD)^{-0.46}$, which captures the shape of the points well ($R^2 = 0.994$). In light of the large size of each of these groups, there is very little random variability in the plotted points. As the points themselves and the
fitted curve show, the relationship between PTH and 25-OHD decreases smoothly with no evidence of either inflection points or of a horizontal asymptote.

Figure 1B shows for the same 63 groups of size 5000 the percentage of patients exceeding the widely used PTH clinical threshold of 65 pg/ml, along with the fitted curve, percentage high PTH = 57.2 + 166.7 \times (25-\text{OHD})^{-0.21} \ (R^2 = 0.995). The points in this graph also show little random variability, and the graph shows a smoothly decreasing relationship with no evidence of either inflection points or horizontal asymptotes.

Next, the data were broken down into four age classes (0 to 20, 20–40, 40–60, and older than 60 yr old) and the same graphs constructed for each of these separate age groups. Smooth curves fitted to these graphs are superimposed in Fig. 2A, which shows the median PTH, and Fig. 2B, which depicts the percentage of the patients with PTH values exceeding 65 pg/ml.

Discussion

Evaluation of patients with osteoporosis and primary hyperparathyroidism, the two most common disorders of bone and mineral metabolism, dictates assessment of vitamin D and calcium nutrition. Clinicians routinely measure serum PTH and 25-OHD levels, but the interpretation of the results requires a clear understanding of the relationship between these two hormones as well as the potential influence of age and renal function, among others. Furthermore, the distinction between secondary hyperparathyroidism due to vitamin D depletion and normocalcemic primary hyperparathyroidism due to parathyroid adenoma requires detailed assessment of calcium and vitamin D nutrition (20). In addition, parathyroid adenoma weight and disease expression are significantly influenced by the vitamin D nutritional status (21, 22). Finally, vitamin D depletion is more common among patients with low bone mass, osteoporosis, and PHPT than in the general population.

However, clinical perspectives about the optimal level of 25-OHD that best supports skeletal and extraskeletal health as well as related concerns, vary widely. Recently, Sai et al. (23) published a comprehensive review of 70 studies investigating the level of serum 25-OHD at which the serum PTH level reached a plateau or was maximally suppressed. They found one, seven, eighteen, six, ten, four, and three studies supporting inflection points at serum 25-OHD levels of less than 10, 10–15, 15–20, 20–25, 25–30, 30–35, 35–40, and 40–50 ng/ml, respectively. Eight studies, including their own, failed to find a threshold level of serum...
25-OHD at which serum PTH levels plateau, and three studies failed to find a relationship between serum PTH and 25-OHD levels at all.

Even using the conservative estimate of 20 ng/ml of 25-OHD as the level assuring sufficiency, greater than a quarter (27% or 85,000) of the individuals in this study fell below this threshold. Of more direct clinical relevance, a full 40% (27,950 of the 70,000) and 51% (7,650 of the 15,000) of the subjects with serum 25-OHD levels below 20 and 10 ng/ml, respectively, had biochemical hyperparathyroidism with serum PTH levels of greater than 65 pg/ml, the upper threshold of normality. The 27,950 subjects with serum 25-OHD less than 20 ng/ml and elevated PTH levels represent 9% of the entire study group. To the extent this cross-sectional analysis of samples from clinically referred subjects represents the U.S. population at large, these are not insignificant frequencies.

In contrast, we found significant continuing declines in serum PTH levels beyond 20 and 30 ng/ml of 25-OHD: neither curve depicted in Fig. 1, A and B, reaches a horizontal asymptote, even when extended to a serum 25-OHD level of 70 ng/ml. Furthermore, both figures intuitively convey the appearance of smooth continuous curves that reflect the high resolution power of the same 313,000 paired PTH and 25-OHD values; indeed, all but 0.5% of the random scatter (noise) is unaccounted for by the regressions. No apparent inflection points are evident that could be construed as a putative level marking a mechanistically plausible departure point (cutoff) where PTH values relative to 25-OHD levels rise with a significantly greater slope from any evident point of coinciding discontinuity. We found highly significant differences in the proportion of subjects with PTH greater than 65 pg/ml in each of 18 25-OHD segments, based on a statistical split point analysis (19), ranging from 5.5 up to as high as 81 ng/ml (Table 1).

The availability of highly empowered continuous curves depicting the interrelationship between PTH and 25-OHD levels affords the opportunity to explore beyond the conventional inflection points that have been based on discontinuous data. More likely, the continuous nature of the regressions

![Graph showing PTH Median vs 25-OHD Frequency Class Mean](https://example.com/graph1.png)

![Graph showing Percent PTH > 65 pg/mL vs 25-OHD Frequency Class Mean](https://example.com/graph2.png)
caused by 25-OHD deficiency (24); whereas the former are recalcitrant, the PTH values in SHPT drop back to within normal range after replenishment of vitamin D stores (25). In view of the frequencies of elevated PTH depicted in Table 2, assessment of 25-OHD levels and replenishment should be accomplished for optimal management of patients with primary hyperparathyroidism before curative parathyroidectomy is recommended (26).

Perhaps a more surprising finding from our study is the large number of individuals with normal PTH levels (<65 pg/ml) despite frank vitamin D deficiency: 49% or 7,350 of 15,000 subjects with 25-OHD less than 10 ng/ml, which represents 2.4% of the entire 313,000 subject study population (Fig. 1A). Consistent with the perception that normal PTH, vitamin D-deficient subjects are rare, clinical practice has generally afforded these subjects with minimal, if any, further care. In this context, the role of magnesium deficiency in the blunted PTH response is of potential clinical significance (27). Aside from general health benefits, magnesium replenishment could serve to unmask nascent hyperparathyroidism in normal PTH, vitamin D-deficient subjects.

Recently, when applied to a surgically confirmed group of patients with primary hyperparathyroidism, a nomogram was reported, which successfully identified 100% (238 of 238) with classical, 96% (64 of 67) with normocalcemic primary hyperparathyroidism, and 53% (21 of 40) with normal serum PTH levels (28). Although the velocity of clinical inferences leveraged from these data and the broader applicability of the nomogram await validation, the ability to develop a patient-specific normal limit for PTH levels based on serum calcium and 25-OHD levels, and age is of great clinical significance. In that regard, both the reported nomogram and the current study results could facilitate movement of the clinical community away from the fixed values of 20 ng/ml vs. 30 ng/ml vs. 40 ng/ml to define vitamin D sufficiency (23) toward the management of patients based on an age-dependent PTH-25-OHD continuum.

Our conclusions are inevitably limited by the lack of clinical information about the subjects tested. The fact that less than 5% of test requests (14,809 of 312,962) included serum calcium measurements could be interpreted to suggest that most were ordered as part of osteoporosis/low bone density evaluation and not as a follow-up of previously discovered hypercalcaemia. This assumption is supported by the fact that only 17% of individuals in whom serum calcium was measured (2,510 of 14,809) had hypercalcaemia. The even smaller subset of 2,178 samples that included serum creatinine measurements (0.70% of total) revealed that only 19% had evidence of renal insufficiency (422 of 2,178). Offsetting

### Table 1. Optimal split point analysis of PTH vs. 25-OHD

<table>
<thead>
<tr>
<th>25-OHD range</th>
<th>Segment</th>
<th>From</th>
<th>To</th>
<th>Count</th>
<th>&gt;65 ng/ml (%)</th>
<th>Z-score</th>
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<tr>
<td></td>
<td>0</td>
<td>5.5</td>
<td>2360</td>
<td>62.8%</td>
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<tr>
<td></td>
<td>2</td>
<td>5.6</td>
<td>70</td>
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<td>55.1%</td>
<td>5.61</td>
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<tr>
<td></td>
<td>3</td>
<td>7.1</td>
<td>9961</td>
<td>48.2%</td>
<td>6.23</td>
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<td>6</td>
<td>14.7</td>
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<td>20.1</td>
<td>17239</td>
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<td></td>
<td>14</td>
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<td>41.8</td>
<td>29308</td>
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<td>48.1</td>
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<td>80.7</td>
<td>9832</td>
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<tr>
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<td>18</td>
<td>80.8</td>
<td>100</td>
<td>2097</td>
<td>9.9%</td>
<td>3.56</td>
</tr>
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</table>

na, Not available.

in Fig. 1, A and B, reflect the underlying adaptive mechanics of calcium homeostasis.

Our data further suggest that hyperparathyroidism also increases continuously both with age and with decreasing 25-OHD levels (Figs. 2, A and B). Here the parent frequency classes from Fig. 1, A and B, were maintained but partitioned by age. With advancing age, elevated serum PTH levels become increasingly more pronounced with decreasing 25-OHD levels (Table 2). This may reflect the likelihood of younger individuals (<20 and 20–40 yr) having relatively fewer confounding variables (declining renal function and primary hyperparathyroidism) affecting this relationship. Given that the PTH suppressive effect of vitamin D is less strong in the younger individuals, the ability to develop a patient-specific normal limit for PTH levels based on serum calcium and 25-OHD levels, and age is of modest concern, considering its infrequent recognition clinically.

The measurement of serum 25-OHD levels can serve to differentiate PHPT and normocalcemic hyperparathyroidism from secondary hyperparathyroidism (SHPT).

### Table 2. Proportion of individuals with elevated PTH levels (>65 pg/ml) stratified by 25-OHD and age

<table>
<thead>
<tr>
<th>Age</th>
<th>&gt;60 yr (%)</th>
<th>40–60 yr (%)</th>
<th>20–40 yr (%)</th>
<th>&lt;20 yr (%)</th>
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<tr>
<td>25-OHD (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>37</td>
<td>25</td>
<td>16</td>
<td>12</td>
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<tr>
<td>30</td>
<td>29</td>
<td>18</td>
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<td>60</td>
<td>17</td>
<td>10</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>
these limitations is the sheer magnitude of the laboratory database and the statistical power.

Regardless of the clinical reason for testing, this large reference laboratory’s experience reveals the kinds of results clinicians are using in clinical practice and points to the areas in which further studies to better characterize patient subsets could prove both instructive and informative. Several potentially important and clinically relevant observations can be made. First, increasing serum 25-OHD levels are associated with decreasing PTH levels. Although this finding is not new, plotting median PTH levels of a very large sample set throughout the continuum of serum 25-OHD levels clearly reveals no discernible threshold whatsoever above which increasing 25-OHD levels fail to further suppress PTH.

Second, the sheer magnitude of the proportion of individuals older than 60 yr of age with deficient or insufficient 25-OHD levels who were found have PTH levels above the upper limit of the reference range. Our limited clinical data about the study patient population does not allow us to discern whether these individuals have PHPT, normocalcemic PHPT, or SHPT. However, the large number of abnormal results clearly reinforces the need for 25-OHD levels to be measured and taken into consideration in the differential diagnosis of PHPT as recommended by the Third International Workshop on Asymptomatic Primary Hyperparathyroidism (29).

Third, the strong impact of the PTH-25-OHD relationship corroborates the results from earlier studies (30, 31). The clinical implications of this association have been, to some extent, built into current practice guidelines in recommending parathyroidec- tomy for individuals younger than 50 yr of age than older individuals with PHPT (29, 32). The increased impact of vitamin D deficiency on serum PTH levels could reflect the depletion of calcium stores with declining calcium absorption and/or renal function with aging. An interesting alternative hypothesis posits that prolonged 25-OHD deficiency could induce secondary hyperparathyroidism, which could ultimately become autonomous. It has been hypothesized that long-term vitamin D deficiency could cause parathyroid gland tissue hyperplasia or induce somatic mutations leading to the development of parathyroid adenomas (21, 33). Extensive clinical studies will be required to understand the physiology that causes the association of PTH level with 25-OHD level to be stronger with advancing age. Elevated serum PTH levels in the presence (or absence) of frank vitamin D depletion have been linked with various skeletal (1, 2) and nonskeletal consequences (34–37). Nevertheless, a stronger understanding of the PTH-25-OHD dynamic relationship should serve to facilitate our approach to prevention and treatment of disease.

Acknowledgments

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References