Original Article

A NEW, VITAMIN D-BASED, MULTIDIMENSIONAL NOMOGRAM FOR THE DIAGNOSIS OF PRIMARY HYPERPARATHYROIDISM

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ABSTRACT

Objective: To refine the diagnostic criteria for primary hyperparathyroidism (1°HPT) to identify atypical patients, in whom serum calcium, parathyroid hormone (PTH), or both are within the "normal" range.

Methods: Total serum calcium, intact PTH, and 25-hydroxyvitamin D [25(OH)D] levels were measured in patients with 1°HPT and healthy patient groups. Multivariate analysis of healthy patient data first identified factors that significantly affected PTH levels and defined a new PTH reference range with a mathematical model. That nomogram was then validated for prediction of atypical 1°HPT in patients with surgically confirmed disease.

Results: On multivariate analysis, calcium ($P = .0002$), 25(OH)D ($P<.0001$), and age ($P = .015$) independently affected PTH. With these variables, we created a 4-dimensional nomogram that distinguished normal patients from those with hyperparathyroid states. Mathematically, this nomogram predicts 1°HPT when the measured serum PTH value is higher than PTH calculated by the following formula: PTH (pg/mL) = 120 – [6 × calcium (mg/dL)] – [0.52 × 25(OH)D (ng/mL)] + [0.26 × patient age (years)]. When applied to our surgical group of patients, this nomogram successfully identified 100% of patients (238 of 238) with classic 1°HPT, 84% (64 of 76) with normocalcemic 1°HPT, and 54% (20 of 37) with 1°HPT and normal PTH.

Conclusion: This study uniquely defines a patient-specific upper limit of normal for PTH based on the readily available variables of serum calcium, 25(OH)D, and patient age. Our nomogram may allow for more rapid definitive diagnosis and treatment of 1°HPT in patients with atypical presentations. **(Endocr Pract. 2012;18:124-131)**

Abbreviations:
1°HPT = primary hyperparathyroidism; **25(OH)D** = 25-hydroxyvitamin D; **PTH** = parathyroid hormone

INTRODUCTION

Primary hyperparathyroidism (1°HPT) is the most common cause of hypercalcemia in the outpatient population (1-3). Traditionally, the diagnosis of 1°HPT has depended on the demonstration of concomitantly elevated levels of serum total or ionized calcium (or both) and parathyroid hormone (PTH), in the setting of normal or high calcium excretion in the urine (1,4). A considerable number of patients, however, present with biochemical variables that do not fit this classic description but are nonetheless found to have 1°HPT (1,5-9). This includes at least the following 2 atypical presentations: normocalcemic 1°HPT (with a normal total serum calcium concentration but a high PTH level) and 1°HPT with high serum calcium values but PTH values that are "inappropriately" within the reference range. Normocalcemic 1°HPT has been relatively well characterized with respect to phenotype. Despite borderline laboratory values, these patients have kidney stones, develop osteoporosis, and have fractures (5,9). The other form of 1°HPT with high serum calcium and normal PTH levels has not been well characterized.

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With these shortcomings in the diagnosis of 1°HPT, several authors have sought to clarify the issue through the use of additional tests and by redefining the reference

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range for PTH. Oral and intravenous calcium suppression tests have been used to assist in these challenging diagnostic scenarios (10-12). Titon et al (12) used an infusion of 0.33 mmol/kg of calcium gluconate during a 3-hour period and measured serum PTH values at the beginning and end of the infusion and 3 hours after the infusion to determine a PTH suppression cutoff that distinguished patients with hyperparathyroidism from normal control subjects. Unfortunately, performance of such tests may necessitate an additional office visit and take several hours.

The role of vitamin D in the interpretation of diagnostic laboratory values for 1°HPT has also garnered some attention in the literature (13-19). Citing the high prevalence of vitamin D deficiency in "healthy" persons, Souberbielle et al (18,19) redefined the PTH reference range to <46 pg/mL (previously, 65 pg/mL) in a population of vitamin D-sufficient patients with osteopenia or osteoporosis. The authors proposed that the new reference range would facilitate the diagnosis of patients with mild 1°HPT. This reference standard, however, was defined in a group of patients with metabolic bone disease. In an attempt to replicate these findings in healthy patients, Aloia et al (20) measured relevant biochemical analytes in a cohort of 503 healthy women between the ages of 20 and 80 years. Although many factors were identified that were significantly predictive of PTH, the authors found that a substantial portion of healthy patients had PTH values above the reference standard proposed by Souberbielle et al (18,19). Thus, a general lowering of the reference standard could not be supported.

What is clear from the existing literature is that cases of "mild" 1°HPT with borderline laboratory results present a diagnostic challenge. Although numerous factors have been identified to affect PTH, as previously noted herein, such knowledge has not translated successfully into a modified reference standard with increased diagnostic accuracy and general applicability to all patients. Therefore, the purpose of the current study was to determine whether we could develop a new, multidimensional PTH nomogram that would yield clearer distinction between normal and disease phenotypes of parathyroid disorders, with the expectation that it should enhance the diagnostic accuracy in challenging 1°HPT scenarios.

METHODS

Patient Cohort Used in Nomogram Development

In an effort to construct a multidimensional nomogram for the diagnosis of 1°HPT, we based our initial analysis on a cohort of healthy normal subjects. We identified 1,000 consecutive patients with 25-hydroxyvitamin D [25(OH)D] measurements at the Cleveland Clinic in Cleveland, Ohio. Residual blood samples not required for clinical use from these patients are routinely stored for a brief period, in accordance with clinical pathology protocols, and then discarded. We maintained at −20°C the blood samples intended for disposal when they were no longer clinically needed. These samples would be the source of future measurement of total serum calcium and intact PTH concentrations. We considered the incorporation of ionized calcium values in our nomogram development, but this decision would have required recruitment of healthy persons for separate venipuncture and separate specimen processing and testing. Likewise, we considered the incorporation of 1,25-dihydroxyvitamin D levels, but this test is not performed at our institution. We reasoned that the most versatile and relevant nomogram would be one based on variables that are routinely assessed in patients with suspected 1°HPT and available at most institutions.

Using the *International Classification of Diseases, Ninth Revision* codes to query computerized medical records and confirmation by review of individual patient charts, we excluded from the study those patients with conditions that might affect calcium, 25(OH)D, and PTH metabolism. These exclusion criteria included renal failure, renal insufficiency, metabolic bone disease, any type of cancer, preexisting parathyroid disease, malnutrition, adrenal disorders, transplantation, sarcoidosis, diabetes insipidus, and hyperthyroidism. In addition, patients were excluded if they were taking any of the following medications: lithium, thiazide diuretics, cinacalcet, theophylline, high-dose vitamin D for repletion (ergocalciferol, 50,000 U), calcitriol, and bisphosphonates. We did not exclude or analyze any patients on the basis of race or sex. After this analysis was superimposed on the 1,000 patients whose blood samples were in storage, 222 healthy patients were available for further study. All these patients had normal serum creatinine measurements. Calcium and PTH measurements were performed on sequestered blood samples of these 222 patients. Institutional review board approval was obtained for this portion of the study, as well as for the study of the surgical cohort detailed in a subsequent section.

Biochemical Analysis

All 25(OH)D measurements were performed with a 2-step DiaSorin 25(OH)D assay (DiaSorin Inc., Stillwater, Minnesota). Serum intact PTH was quantified by using the ADVIA Centaur (Siemens USA Healthcare Diagnostics, Deerfield, Illinois) intact PTH assay, a 2-site sandwich immunoassay with antibodies against the N-terminal (1-34) and the 39-84 region of the molecule. Serum calcium measurements were performed with a Roche automated clinical chemistry analyzer (Roche Diagnostics, Mannheim, Germany) and use of a reaction with a cresolphthalein complexone in the presence of 8-hydroxyquinoline. This reaction forms a purple chromophobe, the intensity of

which, measured photometrically, is proportional to the calcium concentration.

Mathematical Modeling and Statistical Analysis

In combination with demographic data, the laboratory values from these healthy patients were used to construct a statistical model for the prediction of patient-specific "normal" PTH values. The data used to develop a predictive equation relating PTH to calcium and 25(OH)D were analyzed with use of SAS version 9.2 software (SAS Institute Inc., Cary, North Carolina). The variables to be used in the multivariate study were assessed for colinearity. In addition to the main variables, the data set allowed a test of the possible interaction between 25(OH)D levels and calcium levels as well as the interaction of 25(OH)D levels with the curvilinear relationship between calcium and PTH. None of these terms was significant. The regression model was developed by using stepwise regression methods. Both backward elimination and forward selection with replacement converged to the same model. The final model for defining patient-specific normal PTH values (referred to as the PTH nomogram) consisted of only those variables that were significant (P<.05).

Surgical Validation Cohort

From a prospectively maintained, institutional review board-approved database, we identified a cohort of 351 consecutive patients with 1°HPT who had complete preoperative total serum calcium, PTH, and 25(OH)D levels available. All patients referred to the endocrine surgery clinic have a serum albumin level measured for the purpose of correcting the total calcium level. None of these patients was found to have an abnormal serum albumin value. Measurements of serum albumin were not available for all the "healthy" control subjects, but no diagnoses were noted that would be expected to result in a below-normal level of serum albumin for the healthy cohort. All surgical cohort patients had undergone bilateral cervical exploration with intraoperative PTH guidance and parathyroid excision; pathologic confirmation of diseased glands showed that 226 had a single adenoma, 66 had double adenoma, and 59 had multigland hyperplasia. No patient in the surgical cohort had a "negative" result on neck exploration. The patients underwent assessment by both endocrinologists and surgeons in accordance with currently accepted treatment guidelines for parathyroid disease (summarized in reference 1, from the National Institutes of Health workshops). Moreover, no patient had a preoperative diagnosis of secondary hyperparathyroidism, which would have precluded a decision to proceed with surgical treatment. The 25(OH)D status was comparable among all disease categories found at operation: 25.5 ± 11.4 ng/mL for single adenomas, 26.4 ± 11.2 ng/mL for double adenomas, and 26.8 ± 13.4 ng/mL for multigland hyperplasia (P = no significant difference). The PTH nomogram was applied to this surgical patient population to determine its performance in the identification of 1°HPT in this setting, where disease was verified by ultimate surgical and histologic standards. In addition, subsets of surgical patients with borderline laboratory values were identified and examined separately to determine how the PTH nomogram performed in these difficult-to-diagnose populations.

RESULTS

Multivariate analysis was used to determine those factors that were significantly correlated with PTH in the 222 healthy patients. Significant independent predictors of PTH were total serum calcium (P = .0002), 25(OH)D (P<.0001), and age (P = .015) (Table 1). Sex was not a significant, independent predictor of PTH. As shown in Figure 1, upper limits of normal PTH levels were evident for each gradation of 25(OH)D levels: insufficient (10 ng/mL), low-normal (30 ng/mL), and well-repleted (50 ng/mL).

Mathematical modeling of the data set yielded the following predictive equation: PTH (pg/mL) = 90.73 – [6.07 × calcium (mg/dL)] – [0.52 × 25(OH)D (ng/mL)] + [0.26 × age (years)] (R^2 = 0.18; root mean square, 14.5). This equation calculates the expected PTH for a specific patient on the basis of his or her total serum calcium, 25(OH)D, and age measured on the same day. We then modified the equation to predict a patient-specific upper limit of normal PTH (with 95% confidence interval) and estimated this by adding 2 times the root mean square to the PTH value generated by the foregoing equation. Thus, the predictive equation for the upper limit of normal for PTH, or the PTH nomogram, becomes the following: PTH [upper limit of normal] (pg/mL) = 119.73 – [6.07 × calcium (mg/dL)] – [0.52 × 25(OH)D (ng/mL)] + [0.26 × age (years)]. As shown in Figure 2, this predictive equation is presented in a format that is easier to remember for clinical use, with numbers rounded to single digits (without affecting

Table 1
Independent Predictors of PTH: Multivariate Analysis

Variable	Coefficient	*P* value
Calcium	–6.075	.0002
25-Hydroxyvitamin D	–0.519	<.0001
Age	0.264	.0152
Sex	NA	NS

Abbreviations: NA = not applicable; NS = not significant; PTH = parathyroid hormone.

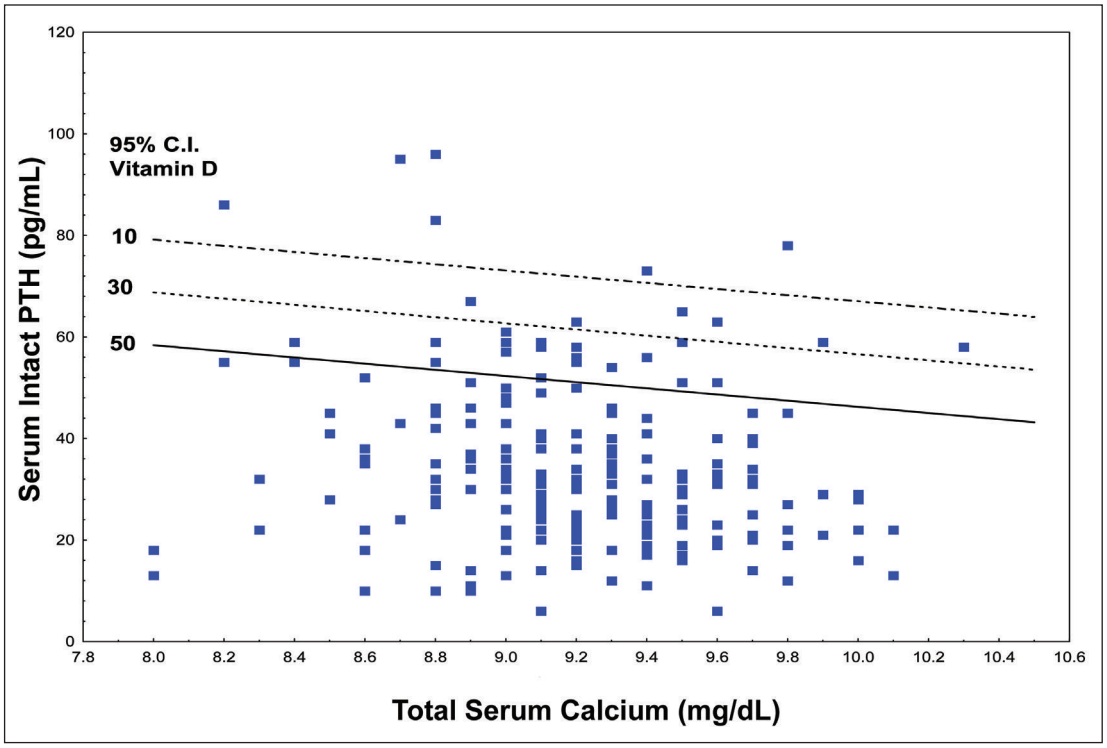

Fig. 1. Upper limits of normal levels for intact parathyroid hormone (*PTH*) at 25-hydroxyvitamin D levels of varying adequacy: 10 ng/mL (insufficient), 30 ng/mL (low-normal), and 50 ng/mL (well-repleted). *CI* = confidence interval.

accuracy of the calculation). For example, this equation would predict that a 54-year-old man with a total serum calcium level of 9.5 mg/dL and a 25(OH)D value of 20 ng/mL should have a maximal PTH concentration of 66 pg/mL; a higher measured value would predict parathyroid disease.

In applying this PTH nomogram to the entire series of patients with surgically confirmed 1°HPT, 331 of 351 surgical patients (94%) would have been correctly diagnosed on the basis of the initial preoperative laboratory results to indeed have 1°HPT ($P<.001$). All surgical patients with a classic laboratory presentation (total serum calcium >10.5 mg/dL and PTH >60 pg/mL) of 1°HPT were also clearly categorized as such by the nomogram (238 of 238 patients). In the original cohort of 222 patients deemed healthy by our screening criteria, 212 (95%) would also be classified as healthy by the nomogram. Ten individuals (5%) had an unexpectedly abnormal PTH measurement for which no cause was apparent in their clinical records. There are several potential explanations for this finding, including the following: (*1*) possible early, yet unrecognized 1°HPT; (*2*) an unrecognized or undocumented clinical condition affecting the calcium-PTH relationship that was not identified during screening for exclusion criteria; (*3*) false-positive findings within the nomogram; and (*4*) normal statistical variation, demonstrating the fact that "nomograms" such as the current one are designed to reflect the range of values that would encompass 95% of "normal" persons. Overall, the sensitivity, specificity, positive predictive value, and negative predictive value of the PTH nomogram to diagnose 1°HPT were 94%, 95%, 97%, and 92%, respectively.

Importantly, however, 76 of 351 patients presented with normocalcemic 1°HPT and 37 of 351 had normal PTH levels, demonstrating that 113 patients (32%) in our surgical cohort did not fulfill the classic criteria of 1°HPT and, by definition, had a more challenging diagnostic profile. Although these patients had atypical biochemical profiles before surgical intervention, they did have clinical symptoms and metabolic consequences consistent with 1°HPT that eventually led to surgical exploration. None of these patients underwent an operation in vain because they all had histologic confirmation of abnormal parathyroid glands, although the preoperative informed consent discussion with them quoted a higher risk for a potentially negative exploration. Thus, it was instructive to analyze how the PTH nomogram performed in these circumstances and whether it might have mitigated the surgical risks quoted to patients. In the subgroup with normocalcemic 1°HPT, the nomogram successfully identified 64 of 76 patients (84%). In addition, the model correctly classified 20 of 37 patients (54%) with "inappropriately" normal PTH values as having 1°HPT. Thus, the model reliably predicted normocalcemic 1°HPT and would have clarified a diagnosis

PTH [ULN (pg/mL)] =

120
- [6 x Total Serum Calcium (mg/dL)]
- [1/2 x 25-hydroxyvitamin D (ng/mL)]
+[1/4 x Age (years)]

Fig. 2. Predictive equation for expected normal parathyroid hormone (*PTH*) values. This equation provides a patient-specific upper limit of normal (*ULN*) PTH (R^2 = 0.18) based on clinical variables identified to affect PTH levels significantly in a cohort of healthy persons.

for at least half of the remaining atypical patients. The multidimensional nature of the PTH nomogram (Fig. 3) in graph format clearly depicts its ability to separate normal ranges from those of various disease states or phenotypes of hyperparathyroidism.

DISCUSSION

The results of this study support the notion that a diagnosis of 1°HPT can be strengthened by refining the interpretation of key biochemical variables relevant to this disease. We were able to identify clinical factors—total serum calcium, 25(OH)D, and age—that significantly affected PTH values. We developed a useful mathematical model for predicting normal PTH levels that is based on these multiple factors, not simply calcium values as is currently practiced. A unique aspect of our model is that the PTH nomogram generates a patient-specific upper limit of normal PTH, rather than providing generic and static PTH reference ranges.

The major motivation for developing such a model was the realization that the 2-dimensional, sigmoid relationship between total serum calcium and PTH levels, initially described in the 1970s, was insufficient to characterize fully all clinical scenarios of 1°HPT (21-23). This particularly applies to those patients with borderline or atypical laboratory values, inasmuch as the diagnosis of 1°HPT in patients with elevated serum calcium and intact PTH concentrations and urinary calcium excretion is straightforward. With use of existing 2-dimensional models, which usually define the normal PTH reference range as <60 pg/mL, patients with atypical 1°HPT are impossible to distinguish from those with secondary hyperparathyroidism and sometimes even those with hypercalcemia

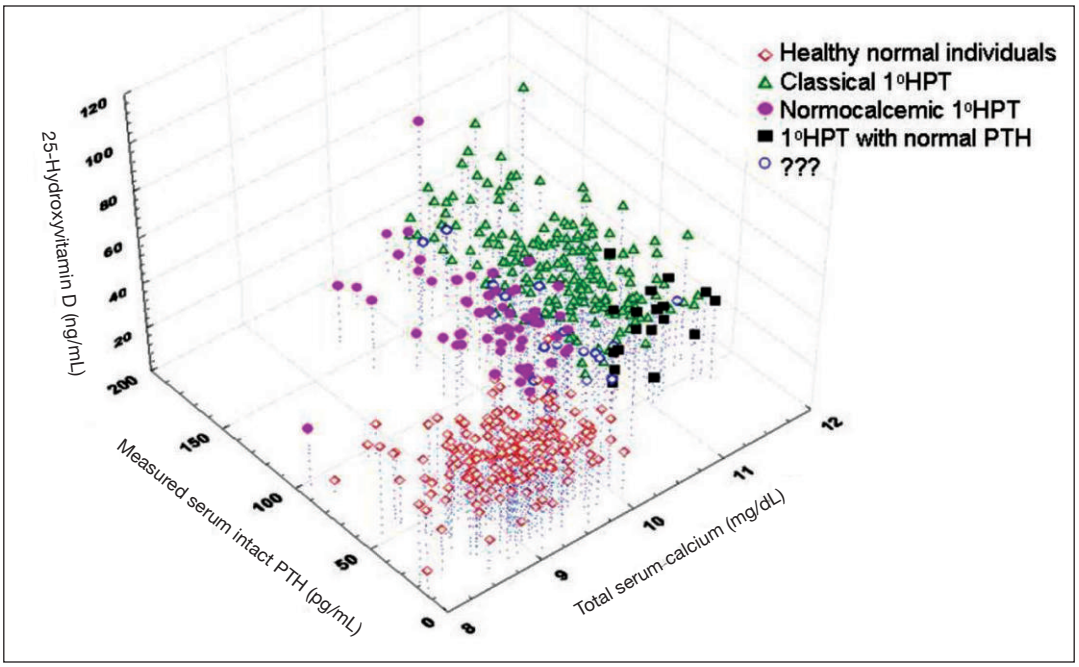

Fig. 3. Multidimensional representation of the normal range of parathyroid hormone (*PTH*) levels in healthy normal individuals and disease subtypes of primary hyperparathyroidism (*1°HPT*) in a surgical cohort with confirmed parathyroid disease.

of malignancy. Our expectation for this study was that multidimensional mathematical modeling of relevant biochemical factors may help support the 1°HPT diagnosis in patients with atypical or borderline laboratory values. In our patients with confirmed and treated 1°HPT, the PTH nomogram would, in fact, have correctly supported this diagnosis *before* surgical intervention in 84% of those with normocalcemic 1°HPT and 54% with hypercalcemia and inappropriately normal PTH values.

It is not surprising that about a third of our surgical cohort were in an atypical 1°HPT category. With the introduction of routine serum calcium measurements, the epidemiologic features of 1°HPT have shifted; the result is a greater proportion of cases of both asymptomatic hypercalcemia and mild serum calcium elevations being detected incidentally on routine blood studies (1,2,24). Another demographic trend has been observed: unlike the 90% prevalence of single adenomas seen in previous decades, modern series of patients undergoing parathyroid surgical treatment identify up to 20% to 30% with multigland hyperplasia (24-26).

Many patients with what is potentially "mild" or "early" 1°HPT have borderline laboratory results that pose diagnostic challenges. In a recent series of 60 consecutive patients with 1°HPT, Glendenning et al (27) found that 22% had normal serum calcium levels, with 8% having both serum calcium and PTH values in the reference range. Thus, there is a growing need and context for the application of our PTH nomogram or similar diagnostic aids. At our center, a substantial number of these patients were eventually offered surgical treatment and underwent neck exploration, with removal of pathologically confirmed abnormal parathyroid glands. Typically, these patients underwent follow-up for an extended period and had multiple laboratory measurements. Use of adjuncts such as ionized calcium and 25(OH)D or the presence of adverse consequences of the disease invariably contributed to the final decision. Thus, it is likely that our endocrinologists and endocrine surgeons used an informal, if not partially subconscious, version of this algorithm in deciding the course of management in these patients. As such, our study helps make explicit those factors that may enter into appropriate decision making in these challenging scenarios.

The traditional treatment regimen at our institution has been to correct vitamin D deficiency after a parathyroid surgical procedure. Because no patient underwent a negative surgical exploration, however, we do not believe that vitamin D treatment preoperatively would have influenced the ultimate treatment algorithm for these patients. Furthermore, it is important to emphasize again that the utility of the nomogram is to provide the expected PTH value for the given serum calcium and 25(OH)D measurements at a specific time. Treated secondary hyperparathyroidism should be reflected in improved calculated and measured PTH values.

One additional potential application of the PTH nomogram may be the assessment of eucalcemic elevation of PTH levels after parathyroid surgical treatment, a phenomenon noted in up to 40% of patients in some reported series (28-32). In some patients, this finding represents an adaptive response to underlying vitamin D deficiency or renal insufficiency. Indeed, Beyer et al (33) found the incidence of postoperative eucalcemic PTH elevations to be significantly lower in patients who received oral vitamin D supplementation in comparison with those who did not (14% versus 39%; $P<.04$). A proportion of these patients, however, will have persistent or recurrent 1°HPT. If one assumes that parathyroid function and calcium metabolism return to a baseline state after a curative surgical procedure, then the generation of a patient-specific upper limit of normal for PTH may help to sort out those patients who require further investigation.

Our study has some limitations that warrant discussion. Even with the use of multiple variables in the predictive model, the explained variance (R^2) is 0.18. This finding indicates that baseline "statistical noise" and additional factors beyond those we identified are likely influencing PTH levels. Despite this limitation, all factors used in our model were significantly correlated with PTH, and plotting the upper 95% confidence interval highlighted the difference between patients with 1°HPT and healthy control subjects. Other investigators such as Aloia et al (20) have identified body mass index ($R^2 = 0.09$), age ($R^2 = 0.01$), and 25(OH)D ($R^2 = 0.008$) as significant predictors of PTH in a model with overall R^2 of 0.11, and others have suggested that sex, race, and serum creatinine are potential factors (15,16,18-20,34,35). The potential exists to modify or improve our PTH nomogram through a larger scale study, with examination of a broader range of predictive variables. Although promising also in its retrospective evaluation of our surgical series of patients with 1°HPT, this predictive equation requires validation on a prospective basis. We are currently undertaking such a study. We are additionally examining whether the use of the PTH nomogram leads to fewer repeated laboratory tests for patients with atypical 1°HPT or shorter time frames between screening, diagnosis, and surgical intervention. These variables were not possible to examine in our current study.

A final factor that bears mentioning is measurement of ionized calcium. In this study, we used total serum calcium for the diagnosis of 1°HPT, with the rationale as stated in the "Methods" section. Although high ionized calcium levels have been reported in patients with suspected 1°HPT and normal total serum calcium (27,36), even then 18% of patients had laboratory values, either ionized calcium or PTH, within the normal range (27). Thus, in future modifications of our PTH nomogram, we may wish to examine

ionized calcium in an attempt to improve diagnostic accuracy further.

CONCLUSION

In summary, a significant proportion of patients with 1°HPT may have borderline laboratory results, which may present a diagnostic dilemma. The traditional, 2-dimensional nomogram with use of serum calcium and PTH levels may be inadequate for the assessment of such patients. As a result, patients may be misdiagnosed, need additional tests, or require follow-up with repeated blood studies in order to clarify the diagnosis. In the current study, we used mathematical modeling to develop a multidimensional nomogram in order to create patient-specific reference standards for PTH. This nomogram should not be confused as a model that predicts or calculates all 3 biochemical variables—calcium, PTH, and 25(OH)D levels. Rather, the nomogram provides a more biologically sensitive estimation of what PTH (and only PTH) ought to be, in light of the actual blood test measurements of serum calcium and 25(OH)D that physiologically regulate PTH release. Comparison of the estimated PTH from the nomogram with the actually measured serum PTH level can assist a clinician in thinking about patients with parathyroid diseases, especially when they do not conform to an easy diagnostic category. Application of our model to a retrospective series of patients with surgically proven parathyroid disease yielded promising results. This model deserves prospective validation and could potentially be modified through consideration of additional predictive variables and use of ionized calcium measurements.

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DISCLOSURE

The authors have no multiplicity of interest to disclose.

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