Variability of Parathyroid Hormone and Other Markers of Bone Mineral Metabolism in Patients Receiving Hemodialysis

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Background and objectives: Clinical management of mineral bone disorder in patients with kidney failure is guided by biochemical targets, in particular parathyroid hormone (PTH) concentration. The biologic variation of PTH and other bone mineral markers was measured in hemodialysis patients to better define their role in management.

Design, setting, participants, & measurements: Intact PTH, biointact (whole-molecule) PTH, calcium, albumin-adjusted calcium, phosphate, and alkaline phosphatase (ALP) were measured in nonfasting samples obtained twice a week (both short-dialysis interval) over a 6-week period in 22 stable hemodialysis patients. Concurrently, samples were obtained from 12 healthy volunteers. Intraindividual coefficients of variance (CV_I) were calculated and used to derive the reference change value (RCV) required to be 95% certain that a change has occurred.

Results: CV_I of all markers was significantly ($P < 0.05$) greater in patients than in healthy volunteers. For phosphate, ALP, and PTH this implies that an increased number of samples is required to estimate an individual's homeostatic set point. CV_I of intact PTH was 25.6% in hemodialysis patients and 19.2% in healthy volunteers. A greater RCV should be used for patients (72%) compared with healthy volunteers (54%). Ideally 26 specimens should be measured to estimate a patient's intact PTH homeostatic set point (within ±10%) with 95% probability. The CV_I of biointact PTH was at least as high as that for intact PTH.

Conclusions: The uncertainty of PTH estimation in an individual significantly undermines its value as a tool in the management of chronic kidney disease-mineral bone disorder using current management approaches.

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Almost universally, patients with ESRD have disturbances in bone and mineral metabolism (1). This group of disorders, termed chronic kidney disease-mineral and bone disorder (CKD-MBD) encompasses pathogenically linked biochemical abnormalities, bone diseases, and vascular and soft tissue calcification (2) and contributes to morbidity (3,4) and mortality (2,5,6). Common laboratory manifestations include disturbances in calcium, phosphate, and parathyroid hormone (PTH) concentrations (7), and these markers are the subjects of routine monitoring with accompanying target ranges (8–10). In particular, management is guided by PTH concentration, which has effectively replaced alkaline phosphatase (ALP) as a marker of bone turnover. U.K. and U.S. guidelines recommend maintenance of PTH within a range of 150 to 300 ng/L (8,9), although this has proved difficult to achieve (11). Recent guidelines from Kidney Disease Improving Global Outcomes (KDIGO) suggest that a broader range of PTH concentrations (approximately 150 to 675 ng/L) may be tolerated with management changes being initiated in response to trends in PTH concentration (10).

Hitherto, management guidelines for CKD-MBD have given little consideration to biologic variability. Laboratory analytes are subject to three main sources of variation: preanalytical, analytical, and biologic. If sources of preanalytical variation are minimized (*e.g.*, by standardizing sampling procedures), then intraindividual biologic variation (CV_I) can be estimated by subtracting analytical variation (CV_A) from total variation (CV_T) (12). CV_I is the random fluctuation around an individual's homeostatic set point. Numerical data on biologic variation can be used to evaluate the significance of changes in serial results and the number of samples required to produce a precise estimate of the homeostatic set point (12). CV_I can be used to determine how much an analyte's concentration must vary by between two results before the change is considered significant: This is termed the critical difference or reference change value (RCV).

There is an extensive literature concerning biologic variation of markers (see http://www.westgard.com/biodatabase1.htm, accessed December 1, 2009), but there are few data on PTH variability and this has only been reported in healthy subjects (13,14). The biologic variation of some analytes in disease differs from that of healthy subjects (15,16), and RCVs calculated

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from healthy individuals may be lower than appropriate for specific clinical situations, resulting in "false-positive" signals for patients being monitored. This could lead to inappropriate medical decisions (*e.g.*, an unnecessary change in treatment dose).

In the area of CKD-MBD, a further confounder may be the nature of the analyte being detected. Targets for PTH concentration are based on the use of so-called "intact" PTH assays, which were initially thought to detect full-length PTH (1-84) (17). However, it is now appreciated that they also detect large N-terminally truncated fragments, predominantly PTH (7-84), which does not have the same biologic efficacy (18,19). Recently assays have been developed with the ability to detect whole-molecule PTH (1-84) without crossreactivity with PTH (7-84) (20). For the purposes of this paper, we have described these assays as "intact" and "biointact," respectively.

A recent international guideline emphasized the need for increased understanding of intraindividual variation in the laboratory markers of CKD-MBD (10). The aims of the study presented here were to establish

1. The biologic variation of PTH and other markers of bone mineral metabolism in hemodialysis patients and whether this differs from that in health.
2. How many measurements are required to establish a true homeostatic set point in any individual?
3. How much does the concentration need to vary by to truly represent any underlying change (*i.e.*, what is the RCV)?
4. The influence of PTH (7-84) on observed biologic variation.

Materials and Methods

Twenty-two patients receiving thrice-weekly hemodialysis for renal failure at the Kent Kidney Care Centre were recruited and gave consent. The hemodialysis prescription was unchanged throughout the study and all patients had been on hemodialysis for at least 3 months. The number of subjects selected was typical of biologic variation studies, which are relatively robust to the effects of sample size (12). To standardize preanalytical variables, only patients that normally dialyze on Monday, Wednesday, and Friday were selected. To minimize the influence of diurnal variation (21), time of blood collection was standardized for individual subjects. The exclusion criteria were patients receiving cinacalcet, aged less than 18 years, known poor compliance with therapy, terminal illness, metastatic malignancy, bone cancer, myeloma, and significant cognitive impairment. Patients were issued with their usual pharmacotherapy prescription specifically to cover the study period: Most were receiving 1α-hydroxycholecalciferol (Alfacalcidol) and Calcichew. Dietary intake was assessed at baseline through self-reporting (a 3-day food diary) and at study end using a 24-hour recall diary. No changes to dietary or pharmacologic prescription were made during the study period. The study had ethical approval from the East Kent Research Ethics Committee (08/H1103/45).

Twelve members of staff who had a negative screening test for proteinuria (Multistix 10 SG, Siemens Healthcare, Surrey, United Kingdom) and estimated GFR (22) $\geq$60 ml/min per 1.73 m^2 also volunteered to participate in this study.

Nonfasting blood samples were collected predialysis from the vascular access (fistula) of the patients before their Wednesday and Friday dialysis sessions for 6 weeks (*i.e.*, 12 samples per individual). Blood was collected from patients that dialyzed in the morning ($n = 13$) between 8:00 and 10:00 a.m. and from patients that dialyzed in the afternoon ($n = 9$) between 1:00 and 3:00 p.m. Concurrently, samples were collected from the 12 healthy volunteers on Wednesday and Fridays (between 9:30 and 11:30 a.m.). Samples were collected using standard venepuncture and phlebotomy procedures including the use of a tourniquet. Blood was collected in EDTA vacutainers and serum separator tubes (both Becton-Dickinson Ltd. Vacutainer Systems) following the manufacturer's recommended order of draw. Plasma/serum was separated within 4 hours of venepuncture and stored at −80°C until assayed. Intact and biointact PTH are stable under these conditions (23,24).

Before analysis, samples were thawed at room temperature and mixed by inversion. For measurements of all analytes, each sample from each individual was measured in duplicate in random order in a single batch. A single operator performed all analyses using a single instrument and a single batch of reagents, controls, and calibrators.

Serum calcium, albumin, phosphate, and total ALP were measured using colorimetric assays on an Architect c8000 system (Abbott Laboratories). The between-batch coefficients of variation (CV) were <5% in all cases. Plasma intact PTH was measured using a chemiluminescent immunoassay on an Abbott Architect ci8000 system (reference range 9 to 73 ng/L). The between-batch CVs ($n = 19$) were 5.0%, 4.6%, and 2.6% for PTH concentrations of 8, 54, and 208 ng/L, respectively. Plasma biointact PTH was measured using a human PTH 1-84 ELISA (Immutopics, Inc., San Clemente, CA) using two antibodies targeted to the 1-4 and 39-84 regions of PTH. The between-batch CVs ($n = 12$) were 1.9% and 9.6% for PTH concentrations of 12 and 114 ng/L, respectively. The biointact PTH assay was standardized against the Architect ci8000 PTH assay.

Data Analyses

Comparisons between dietary intakes, weight, and Kt/V at the start and end of the study were done using paired *t* test or Wilcoxon match pairs signed ranks test as appropriate. Variation analyses were performed on natural logarithmic transformed data after exclusion of outliers using Cochran and Reed tests (25). The Cochran test did not highlight any outliers among duplicate measurements; however, it did identify results for four samples from different subjects (three hemodialysis, all analytes; and one healthy volunteer, ALP) as outliers among within-subject variances. The Reed test identified calcium concentrations for one healthy volunteer as an outlier. Statistical analyses were performed excluding these outliers. Linear regression analysis was used to examine the possibility of persistent trends (increasing or decreasing concentration) over time in analyte concentrations during the study ("Hawthorne effect") (26). With the exception of phosphate in the healthy controls, exclusion of subjects demonstrating such trends had no effect on the estimated variance components (data not shown).

CV_A, CV_I, and RCV ($P < 0.05$) were calculated as described previously (12) using general linear model ANOVA (Minitab). The number of specimens required to estimate the homeostatic set point of an individual within ±10% with a confidence of 95% was calculated and this analysis was adjusted to also consider ±20% and ±30% limits. Subdivision of the cohort of 22 patients into approximate tertiles on the basis of the individual mean PTH concentration of the native data was undertaken to facilitate an exploratory analysis as to whether biologic variation differs at differing concentrations of PTH. The cohort of patients was also subdivided into two groups with mean PTH concentrations of <300 ng/L ($n = 13$) and ≥300 ng/L ($n = 9$). A cutoff of 300 ng/L was used based on recommended target ranges (8,9). Variation among patients who dialyzed in the morning was compared with that among those who dialyzed in the afternoon. The *F*-test was used to compare variances of two independent samples and variables; that is,

the variance components obtained for the healthy volunteers with those obtained for the hemodialysis patients and the variance components obtained for the subgroups of the hemodialysis patients (*e.g.*, PTH <300 ng/L and ≥300 ng/L) for intact PTH.

Results

The hemodialysis patients were significantly older ($P < 0.05$) than the healthy volunteers (Table 1). Among the hemodialysis patients, Kt/V did not vary during the study and there were no differences in terms of energy, protein, calcium, or phosphate intakes or weight ($P > 0.05$). Ten patients had vascular disease (peripheral vascular disease, cardiovascular disease, ischemic heart disease) and four had diabetes mellitus.

The CV_I for calcium and albumin-adjusted calcium was significantly higher for patients than for the healthy volunteers ($P < 0.05$; Table 2). However, the RCVs were small for both groups and only one specimen is required to determine the homeostatic set point of an individual. For phosphate and ALP, the higher CV_I observed for the hemodialysis patients contributed to a larger number of samples being required to determine the homeostatic set point (N.B. see footnote to Table 2). Although CV_A values were significantly different ($P < 0.05$) between the two groups for all three analytes, they were effectively of similar magnitude.

For both intact and biointact PTH, CV_I was higher ($P < 0.05$) in hemodialysis patients compared with healthy volunteers, resulting in a higher RCV. For example, in hemodialysis patients a baseline intact PTH concentration of 300 ng/L must increase to >516 ng/L before it can be considered a significant change. Among healthy volunteers and patients, biologic variability of biointact PTH appeared higher than that of intact PTH (Figures 1 and 2, Table 2).

CV_I for PTH was lower ($P < 0.05$) in patients with higher (tertile 3 or ≥300 ng/L) compared with lower (tertiles 1 and 2 or PTH <300 ng/L) intact PTH concentrations (Table 3). Con-

Table 1. Characteristics of study subjects and serum/plasma analyte concentrations

	Healthy Volunteers	Hemodialysis Patients
n	12	22
Age, years	38.5 (13.3), 24 to 61	67.7 (12.3), 42 to 90[a]
Women, *n*	8	11
Weight, kg (study beginning)	NA	78.2 (16.5), 53.5 to 113.0
Weight, kg (study end)	NA	78.5 (16.3), 54.5 to 113.0
Dialysis adequacy (Kt/V, study beginning)	NA	1.42 (0.31)
Dialysis adequacy (Kt/V, study end)	NA	1.44 (0.34)
Cause of renal failure, *n*	NA	Small kidneys/unknown etiology (7), GN (4), membranous nephropathy (3), pyelonephritis (2), diabetes mellitus (2), renal vascular disease (2), IgA nephropathy (1) and Wegener's granulomatosis (1)
Duration of ESRD, months	NA	11, 3 to 120[b]
Calcium concentration, mmol/L	2.39 (0.10)	2.29 (0.14)
Albumin-adjusted calcium concentration, mmol/L	2.36 (0.10)	2.42 (0.14)
Phosphate concentration, mmol/L	1.11 (0.14)	1.54 (0.42)
ALP activity, U/L	62 (21)	99 (55)
Intact PTH concentration, ng/L	51.9 (23.0)	303 (274)
Biointact PTH concentration, ng/L	27.5 (11.4)	131 (109)
Daily dietary intake		
Energy, kcal/kg (study beginning)	NA	21.4 (7.9), 10.0 to 38.0
Energy, kcal/kg (study end)	NA	20.2 (7.0), 9.0 to 32.0
Protein, g/kg (study beginning)	NA	0.80, 0.30 to 1.30[b]
Protein, g/kg (study end)	NA	0.80, 0.30 to 1.10[b]
Phosphate, mg (study beginning)	NA	982 (259), 524 to 1482
Phosphate, mg (study end)	NA	978 (282), 524 to 1430
Calcium, mg (study beginning)	NA	723 (201), 337 to 1186
Calcium, mg (study end)	NA	707 (298), 203 to 1359

The mean values were obtained from all of the data for all of the analytes. The mean age was calculated from baseline data. NA, not applicable to the healthy volunteers.

[a]The age of the hemodialysis patients was significantly different ($P < 0.05$) than that of the healthy volunteers.

[b]Values for continuous variables are expressed as mean (SD), range (where given), or median, range.

Table 2. Estimation of variance components for healthy volunteers and hemodialysis patients including the RCV to be considered as significant ($P < 0.05$) and the number of specimens required to estimate the homeostatic set point of an individual (within ±10%, 20%, and 30% with a confidence of 95%)

	Healthy Volunteers						Hemodialysis Patients					
				Number of Specimens						Number of Specimens		
	CV_A (%)	CV_I (%)	RCV (%)	±10%	±20%	±30%	CV_A (%)	CV_I (%)	RCV (%)	±10%	±20%	±30%
Calcium	0.52	1.81	5.3	1	1	1	0.62[a]	2.90[a]	8.2	1	1	1
Albumin-adjusted calcium	0.54	1.36	4.1	1	1	1	0.68[a]	2.53[a]	7.3	1	1	1
Phosphate	1.01	10.2[b]	28	4	1	1	0.71[a]	14.6[a]	41	8	2	1
ALP	1.22	5.83	16	1	1	1	0.89[a]	9.87[a]	27	4	1	1
Intact PTH	3.52	19.2	54	15	4	2	3.61	25.6[a]	72	26	6	3
Biointact PTH	4.24	23.8	67	22	6	2	6.32[a]	30.2[a]	86	37	9	4

[a]Significantly different than the equivalent variance component in healthy volunteers ($P < 0.05$).
[b]Note that for phosphate, five of the healthy controls demonstrated a persistent change (decrease or increase) over time during the study using regression analysis (data not shown). When these subjects were excluded, the difference between healthy controls and HD patients was eliminated (CV_I and RCV for healthy controls 15.5% and 43%, respectively).

Figure 1. Box plots showing median (horizontal line), upper and lower quartile (large rectangle), and range (represented by the whiskers) plasma intact PTH concentrations for individual hemodialysis patients (upper panel, subjects 1 to 22) and healthy volunteers (lower panel, subjects 23 to 34).

sequently, fewer specimens are required to estimate an individual's homeostatic set point at higher PTH concentration. The CV_I for intact PTH was significantly ($P < 0.05$) lower for patients that dialyzed in the afternoon compared with the morning, suggesting that biologic variability of PTH among patients may be lower in the afternoon (Table 3).

Discussion

To our knowledge this is the first study to describe the biologic variation of markers of CKD-MBD in patients receiving maintenance hemodialysis. These data have implications for clinical decision-making and guideline targets.

The estimates of biologic variation we observed for calcium, phosphate, and ALP among healthy volunteers closely resemble previously published data (see http://www.westgard.com/biodatabase1.htm) (15,27). Although a reasonable estimate of the homeostatic set point of an individual can be obtained from a single sample for calcium and ALP, even among healthy volunteers, four samples are required to estimate the set point of phosphate. For all three of these markers, biologic variation was higher among hemodialysis patients. However, a single sample would still suffice to gain an estimate of the true calcium concentration, but multiple samples are

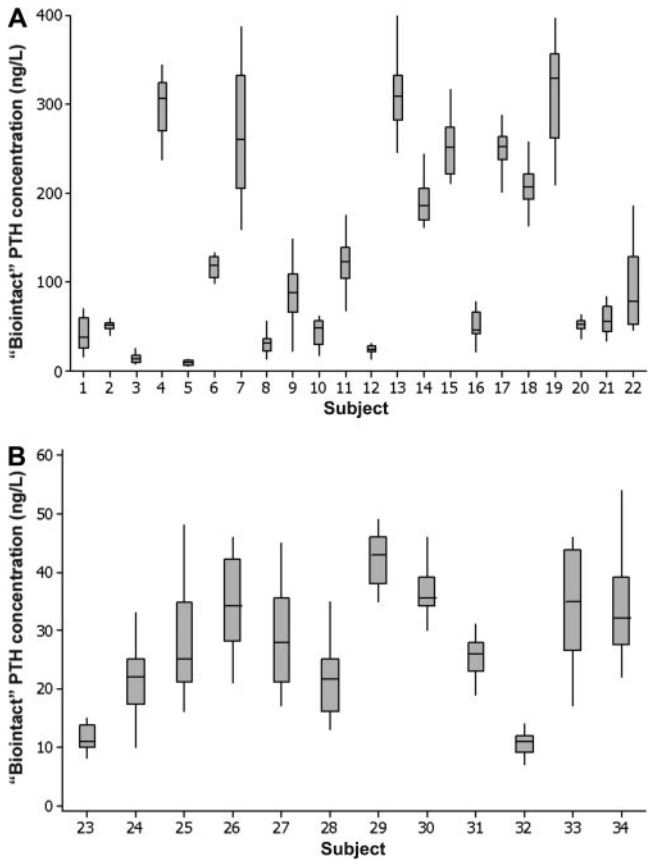

Figure 2. Box plots showing median (horizontal line), upper and lower quartile (large rectangle), and range (represented by the whiskers) plasma biointact PTH concentrations for individual hemodialysis patients (upper panel, subjects 1 to 22) and healthy volunteers (lower panel, subjects 23 to 34).

required to estimate the set point for phosphate and ALP. Given the underlying pathology and the extensive efforts undertaken to maintain phosphate control and bone homeostasis in hemodialysis patients, it is unsurprising that these markers should show higher intraindividual variation than in healthy individuals. Serum ALP was historically used to predict bone turnover in CKD-MBD, but its use was limited by its nonspecificity for bone disease. Although still routinely measured, it is now seen as an adjunct to PTH measurement with no clearly defined management targets (10). Its lower biologic variability vis-à-vis PTH (see below) should possibly lead to a reappraisal of its value in this area. In the study presented here, total ALP activity was measured: Evaluation of the biologic variation of bone-specific ALP would be interesting.

There have been two recent studies of the biologic variation of intact PTH in healthy subjects. Among ten healthy subjects (four men, median age 21 years, range 19 to 27 years, mean PTH 52 ng/L) sampled once a week for 6 weeks between 12:30 and 2:30 p.m., using a Nichols Advantage assay, a CV_I of 25.9% was obtained (13). In the second study of 20 healthy subjects (10 men, median age 37 years, range 19 to 60 years, median PTH 29 ng/L) sampled weekly for 5 weeks between 8:45 and 9:30 a.m. and using a Beckman Access 2 immunoassay system (Beckman-Coulter, High Wycombe, United Kingdom), a CV_I of 25.3% was obtained (14). These estimates using two different assays at different times of day are not dissimilar to the variability we observed in our healthy volunteers, with the implication being that, even in the healthy state, at least 15 samples are required to obtain an estimate of the homeostatic set point of PTH for an individual and that concentrations must change by more than 50% before they can be assumed to be real.

Among hemodialysis patients, biologic variation of intact and biointact PTH was higher than among the healthy volunteers, suggesting that for intact PTH, at least 26 samples are required to obtain an estimate of the homeostatic set point and that concentrations must change by more than 70% before that change can be considered significant. As for ALP (discussed above), it is unsurprising that the biologic variability of PTH is higher in patients with CKD-MBD, although to some extent one might have expected the known extended half-life of PTH in uremia to have mitigated against this (28,29). The frequency of pulsatile PTH secretion is increased in uremic individuals and

Table 3. Estimation of variance components for intact PTH in hemodialysis patients

Intact PTH	Number of Patients	Intact PTH Concentration (mean (SD)), ng/L	CV_A (%)	CV_I (%)	RCV (%)	Number of Samples		
						±10%	±20%	±30%
Tertile 1	7	60.8 (34)	3.69	31.7	88	39	10	4
Tertile 2	8	206 (111)	3.36	27.2	76	29	7	3
Tertile 3	7	660 (161)	3.87	4.5	16	1	1	1
<300 ng/L	13	104 (73)	3.46	31.2	87	38	9	4
≥300 ng/L	9	592 (110)	3.89	13.9[a]	40	8	2	1
AM patients	9	187 (227)	3.46	32.1	89	40	10	4
PM patients	13	383 (276)	3.74	19.8[b]	56	16	4	2

Variance components were used to calculate the critical difference for changes in serial results (RCV) to be considered as significant ($P < 0.05$) and the number of samples required to estimate the homeostatic set point of an individual (within ±10%, 20%, and 30% with a confidence of 95%).

[a]Significantly different than the CV_I in patients with a mean PTH <300 ng/L ($P < 0.05$).

[b]Significantly different than the CV_I in patients who dialyzed in the morning ($P < 0.05$).

this may be one factor contributing to increased biologic variation (28). Clearly, in clinical practice such multiple sampling is impractical. However, there is little doubt that at the individual patient level, therapeutic changes are instituted on a routine basis as a result of observed changes in PTH concentrations that are far below the 70% RCV. The limitation of using single estimates of markers in the management of CKD-MBD has recently been acknowledged by KDIGO (10). The workgroup concluded that "Owing to assay and biologic variation issues, …trends in laboratory values should be preferentially used over single values for determining when to initiate and/or adjust treatments." The group suggests that in dialysis patients PTH concentrations should be maintained in the range of approximately 2 to 9 times the upper limit of normal for the assay and that marked changes within this range should prompt an initiation or change in therapy to avoid progression to concentrations outside of this range (10). Our data support this recommendation and provide the first published data in hemodialysis patients to provide a basis for understanding exactly what a "marked" change represents.

There are no previously published data concerning the biologic variability of biointact PTH. We have observed that the variation of biointact (whole-molecule) PTH is slightly greater than that of the mixture of intact PTH and other peptide fragments detected by intact PTH assays. The difference between the two assays was relatively small and should not be overinterpreted. However, one could hypothesize that the difference is related to greater variation of biologically active PTH (1-84) subject to feedback regulation in the biointact assay, whereas in conventional intact PTH assays PTH (1-84) is measured against a background of PTH (7-84), representing a relatively unregulated retained fragment (29).

Our results provide preliminary evidence that biologic variation of PTH may be lower at higher concentrations of PTH and that variability may be lower in the afternoon than in the morning. However, there are limitations to this aspect of our study. First, numbers of patients in the subgroups were relatively small. Furthermore, the mean PTH concentration of the afternoon dialysis patients was higher than that of the morning patients. Hence the afternoon *versus* morning difference may simply reflect the effect of PTH concentration on variability, or *vice versa*. These questions could be answered by future studies comparing afternoon and morning dialysis patients who have been matched for mean PTH concentration or by studying more patients standardized to morning or afternoon dialysis.

There was a significant difference in age between our healthy volunteer cohort and our group of hemodialysis patients. Differences may therefore represent the effect of age rather than disease and its treatment. Nevertheless, our patients represent a fairly typical cohort in terms of age (11), energy (30), protein (30), phosphate (31) and calcium (32) intake, and percentage achievement of biochemical targets (11). Samples were collected in the nonfasting state, which may have influenced the variations we observed. However, the biologic variation estimates we obtained are valid and relevant to clinical practice.

It is important for nephrologists to be aware of the high biologic variation of these markers and to use that knowledge when considering treatment-changing decisions. It should also be remembered that these estimates of variation represent minimum estimates. Although in all cases the assays we have studied met desirable quality specifications defined by biologic variation (*i.e.*, the CV_A was $<50\%$ of the CV_I and in most cases was $<25\%$ of the CV_I), (33), in clinical practice interassay analytical variation will also be brought to bear on the situation, resulting in greater observed variation of bone markers.

In conclusion, the biologic variation of PTH is high and many samples are required to estimate an individual's homeostatic set point. This variation is not due to the use of nonspecific intact PTH assays. Changes in treatment should not be based on a single estimation of PTH but, instead, PTH concentrations should be interpreted using serial measurements; population-based reference ranges (and clinical target ranges derived from them) are not appropriate. Further studies are required to confirm whether the biologic variation of PTH differs at different intact PTH concentrations, at different times of the day, and using different dialysis modalities.

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Disclosures

None.

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