

Assessment of Urinary Bone Markers for Monitoring Treatment of Osteoporosis

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Background: The usefulness of urinary markers of bone turnover in monitoring therapy depends on their within-person variability compared with their responses to therapy. The aim of this study was to ????

Methods: We measured variation during a whole year of cross-linked N-terminal telopeptide of collagen I (NTx) and urinary deoxypyridinoline (DPD) as ratios to creatinine concentration and after log-transformation of the ratios, in untreated women stratified into three bone density classes, of which the lowest was osteoporotic. We also measured changes in bone mineral density at the lumbar spine (LSBMD) and hip (FNBMD) in untreated women with normal bones and in those with moderate osteopenia and calculated the reference change value (RCV; or least significant change) at $P < 0.05$ for all of these measures. We made the same measurements on women treated with bisphosphonates, estrogen replacement (HRT), or calcium and examined their individual responses to treatment compared with RCV.

Results: After 12 months on bisphosphonates, LSBMD changed more than RCV (2.55%) in 47% of women compared with 44% of those on HRT and 13% of those on calcium. Response of FNBMD was less. Log NTx (RCV = -28%) responded to bisphosphonates in 78%, regardless of BMD, but less often to HRT (67%). Log DPD (RCV = -30%) responded to bisphosphonates less frequently (53% at 12 months).

Conclusions: NTx has advantages over DPD in monitoring therapy for osteoporosis when mailed urine samples are used.

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Extended treatment of an individual patient with a bone-active agent for osteoporosis calls for some evidence that

the treatment is working. In the osteoporotic patient, a change of spinal bone density from vertebrae L1 or L2 to L4 has been used to demonstrate a response, but its use has been restricted by the occurrence of deformities in the lumbar spine, particularly in the elderly; by the limited response of bone density to some treatments (1); and because the time to show a significant change may be as long as 2 years or more (2). Changes in measurements of markers of bone resorption and formation, which are often increased in postmenopausal women with osteoporosis, have been proposed as a means of detecting early responses to treatment (3). Groups of patients treated with estrogens or bisphosphonates show significant decreases of these markers (4).

Even if the response of a treatment group is statistically significant, this is not useful clinically unless the change in an individual patient is greater than would be expected on the basis of known variability. This degree of change is known in laboratory medicine as the reference change value (RCV)¹ and in the bone marker field and elsewhere as the least (or minimum) significant change or, sometimes, critical difference. It is the minimum change in the quantity that would imply a true biological response. Hereafter in this report it is referred to as RCV. Variation of factors affecting bone turnover, including seasonal variation of vitamin D, may affect bone marker activity (5); they therefore should be followed for as long as possible in patients receiving no bone-active agents. Within-person variability may also vary among different populations or groups. Intraassay, interassay, and within-person variances, and hence RCV, have been reported for several markers.

Hannon et al. (6) measured several markers on several occasions for up to 24 weeks in a few apparently healthy individuals. Urinary C- and N-terminal telopeptides of

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¹ Nonstandard abbreviations: RCV, reference change value; CTx and NTx, C- and N-terminal telopeptides of collagen 1, respectively; HRT, estrogen replacement therapy; DPD, deoxypyridinoline; BMD, bone mineral density; DXA, dual-energy x-ray absorptiometry; LSBMD, lumbar spine bone mineral density; FNBMD, femoral neck bone mineral density; and Cr, creatinine.

collagen 1 (CTx and NTx) have been measured in larger numbers of individuals at 1-year intervals for 3 years (7). Any effect of seasonal changes would not have been reflected in the estimates of variability from such a study. Garnero et al. (3) measured a range of bone markers over 15 months in postmenopausal osteoporotic women, but these women were not without treatment as they were the control arm of an alendronate trial and were receiving calcium supplements. The calcium supplements transiently depressed urinary NTx in that study. Ravn and coworkers (8, 9) have also shown a significant decrease in urinary NTx in nonosteoporotic postmenopausal women taking calcium supplements. Moreover, calcium and vitamin D were found by Chapuy et al. (10) to reduce the incidence of hip fractures in institutionalized individuals. Hence, calcium supplements are likely to modify NTx responses, and it is important to investigate variation of marker response through the year in patients taking no treatment.

The time course and magnitude of any response are likely to vary according to the treatment and the marker used. Comparison of the reported responses of bone markers to estrogen replacement therapy (HRT) and alendronate is difficult because of differences in timing of sample collection and methods of calculation, but serum intact procollagen I C- and N-terminal propeptides and urinary free deoxypyridinoline (DPD) may respond more rapidly to HRT, and urinary NTx and CTx may respond better after alendronate treatment (6, 11). Methods of data manipulation and calculation of the RCV are, however, important. For example, changes calculated from baseline will give fewer responders than changes expressed as a percentage of the mean of baseline and final measurements (7).

We examined the response of urinary NTx and DPD and bone density in the spine in patients taking no treatment and in those taking HRT, bisphosphonates, or calcium supplements only, following an osteoporosis screening and treatment initiative. Treatment was determined by the general practitioner, and patients were followed for 12 months. This allowed us to calculate the RCV for each of the markers and determine how they responded.

Materials and Methods

PATIENTS

Women older than 50 years were referred from medical practices, having been identified as suitable for bone density measurement by their doctor. All patients underwent a distal forearm scan of the nondominant arm at the doctor's surgery by use of a mobile densitometer (DTX100; Osteometer), and 60% of them were found to have a forearm bone mineral density (BMD) <0.419 g/cm². These women were asked to take part in the study. A total of 355 women took part, but 51 of these were excluded from the analysis because they had previously been treated with medication known to affect bone me-

tabolism. An additional 53 were excluded because they stopped taking medication or changed it during the study period.

A total of 251 women were included in the analysis. These were divided into classes according to their BMD, initially at the forearm. This was done for the purpose of clinical triage (12). Women whose distal forearm BMD was <0.34 g/cm² formed class A (osteoporotic). Those whose forearm BMD was >0.34 g/cm² but <0.419 g/cm² were invited to have a dual-energy x-ray absorptiometry (DXA) scan (QDR 4500; Hologic Inc.). These women were allocated either to class B (osteoporotic), if the BMD at either the lumbar spine (LSBMD) or neck of the femur (FNBMD) was more than 2.5 SD below normal peak bone density, or otherwise to class C (normal). LSBMD and FNBMD were measured again at month 12 in classes B and C. Normal peak bone density was the mean LSBMD (vertebrae 2, 3, and 4) or FNBMD of healthy Caucasian females 23–42 years of age without a history of fracture (13). A BMD less than 2.5 SD below the value for peak bone mass was taken as indicating osteoporosis as defined by the WHO (14) and was 0.78 g/cm² in the spine and 0.60 g/cm² at the femoral neck.

Treatment was advised for all women in the osteoporotic classes. Local Ethics Committee approval was obtained for this study.

In a separate small study to assess shorter-term variation, NTx was measured in similar mailed urine samples from 45 patients referred to the metabolic bone disease clinic for assessment. They were asked to mail two samples, at least 2 weeks apart, before any treatment was begun. None of those included here were known to have recently undergone a change in therapy or medical history that might affect their bone turnover. Many had osteoporosis or other mild metabolic bone diseases, but at that time their diagnosis was not complete. Evident cases of Paget disease of bone were excluded.

BIOCHEMICAL MARKERS

Second morning void urines were collected at baseline and at 3, 6, and 12 months posttreatment. Samples were mailed by the patient to the laboratory on the day of collection. On receipt, the pH was measured to check that conditions for creatinine (Cr) loss (pH between 4.5 and 5.5) had not arisen (15), and the samples were stored at -20 °C until analyzed.

NTx was measured by ELISA (Osteomark; Ostex International Inc.) in a microtiter plate format. The supplied protocol, which precludes control of the across-plate variation that we have sometimes observed with this assay, was modified by inclusion of a third set of calibrators in a different part of the plate. This assay yields an estimate in terms of bone collagen equivalents in nmol/L. Urinary DPD was measured by coated-tube RIA (γ -BCT DPD RIA; Immuno Diagnostic Systems Limited). This assay measures free DPD and probably some that is bound to small peptides. To compensate for variable

urine dilution all concentrations are expressed as ratios to Cr, which was measured by the Jaffe reaction on a microtiter plate.

The total analytical imprecision (CV_a) for NTx was 14% at 360 nmol/L and 7.8% at 1330 nmol/L. For DPD, it was 12% at 38 nmol/L and 6.5% at 98 nmol/L, and for Cr, it was 5.2% at 3.0 nmol/L and 5.7% at 8 nmol/L. The corresponding values for NTx/Cr were therefore 15% and 5.6% and for DPD/Cr were 13% and 8.7%, using a pooled CV for Cr. These data were derived from clinical urine collections stored frozen in small samples used as quality controls, usually three in each run. At least eight runs were used to derive the CVs for each analyte. None of these women had high-turnover bone disease such as Paget disease; therefore, estimates of imprecision for high NTx or DPD results in this study are neither available nor appropriate.

STATISTICS

Comparisons between classes or groups were analyzed by use of *t*-tests for continuous variables (log-transformed as appropriate) or χ^2 tests for discontinuous data. Changes of all the measured quantities are expressed as a percentage of the baseline value of the individual patient. Evaluation of changes with time within a treatment group were analyzed by paired *t*-tests, and correlations between markers were determined by linear regression analysis. Distributions were tested for conformance with a gaussian distribution by use of the Lilliefors version (which standardizes the data) of the Kolmogorov–Smirnov test. The numbers of patients treated with HRT were too small to allow meaningful inferences when divided among all

BMD classes; therefore, classes A and B (osteopenic) were sometimes pooled for comparison with class C (normal).

The RCV (95% confidence) was estimated using the relationship $RCV = 2.33 \times CV_{gmed}$, where CV_{gmed} is the CV derived from the median of all within-person variances in the appropriate reference group (16). We used a one-tailed estimate because only a decrease in the bone markers or an increase in BMD is clinically relevant (17). The *F*-test (variance ratios) was used to estimate the significance of differences between the variances or RCVs of groups.

Results

PERFORMANCE OF MAILED URINE SAMPLES

The pH of all mailed urines fell within the range 5.5–7.5; it is therefore unlikely that significant Cr loss by conversion to creatine had occurred during transit (15). We found by use of divided samples and mailing portions from different areas at different times of the year that mailed specimens give the same Cr, DPD, and NTx results as those frozen immediately after collection (data not shown).

BASELINE VALUES

Baseline values for the three BMD classes are shown in Table 1. Distal forearm BMD decreased with age, from a median of 0.393 (range, 0.359–0.416) g/cm² at 50–59 years to 0.318 (0.202–0.411) g/cm² in women older than 80 years. The differences between each decade were statistically significant. None of the other measures showed significant associations with age. The women in class A, as well as having lower BMD, which was the basis for the

Table 1. Baseline values for all women by forearm BMD group.

	Class A (osteoporotic)	Class B	Class C (normal)
Age, years			
Median (n)	71.9 (85)	68.9 ^a (59)	71.4 ^b (107)
Range	60.5–90.0	57.0–85.5	49.7–92.5
Distal forearm BMD, g/cm ²			
Median (n)	0.301 (85)	0.373 ^b (59)	0.382 ^{b,d} (107)
Range	0.20–0.337	0.342–0.418	0.341–0.419
LSBMD (DXA), g/cm ²			
Median (n)		0.787 (58)	0.910 ^e (106)
Range		0.535–1.267	0.781–1.265
FNBMD (DXA), g/cm ²			
Median (n)		0.577 (59)	0.690 ^e (107)
Range		0.485–0.902	0.602–0.942
NTx, nmol/mmol Cr			
Median (n)	82.6 (85)	58.7 ^c (59)	55.2 ^b (107)
Range	23.1–296	15.9–186	15.2–210
DPD, nmol/mmol Cr			
Median (n)	5.94 (85)	5.81 (59)	5.99 (107)
Range	1.00–16.9	1.76–18.7	3.56–11.8

^{a–c} Compared with class A: ^a*P* < 0.01; ^b*P* < 0.0001; ^c*P* < 0.001.

^{d,e} Compared with class B: ^d*P* < 0.05; ^e*P* < 0.0001.

Table 2. Variability of BMD and bone markers in untreated patients (one-tailed).

	LSBMD	FNBMD	NTx	DPD
CV _{gmed} , %	1.10	1.43	25.4	11.6
RCV, %	2.55	3.34	59.2	27.0
RCV log/antilog (downward), %			-27.8	-30.2
Relevant limit, ^a %	102.55	103.34	72.2	69.8

^a Upper limit for bone density; lower limit for bone resorption markers.

grouping, tended to be slightly older and had distinctly higher NTx/Cr ratios but similar DPD/Cr ratios.

UNTREATED PATIENTS

A total of 131 of the women received no bone-active agents before or during the study period. Of these, 24% were in class A (osteoporotic), 13% were in class B, and 63% were in class C. The variations of age, BMD, NTx/Cr, and DPD/Cr among the different BMD classes were very similar to those seen in the total study population. They form a reference set from which within-person variation was estimated for four collections of urine and two DXA measurements of BMD. From these data, we estimate that the intraindividual CVs in our untreated patients were 4.8% for DPD/Cr and 15% for NTx/Cr, after removal of the component for analytical variation. The median variances were used to calculate a value for the RCV for each analyte, expressed as a percentage of initial value. This was determined for those women in the reference set for whom all four urine collections or two BMD measurements were completed. However, analysis of the distributions of the urine markers by use of the Kolmogorov-Smirnov test showed that the distributions of NTx/Cr (mean $P = 0.0002$ for the four time periods) and, to a

lesser extent, DPD/Cr (mean $P = 0.0052$) were positively skewed. Logarithmic transformation of these data for the reference set yielded distributions that were more gaussian ($P = 0.72$ and 0.15 , respectively). The RCV for the urine markers was therefore calculated using the log-transformed values. The RCV for each of the markers is shown in Table 2. Where they are derived from logarithms, the RCV is asymmetric with respect to direction of change, and only the values for the relevant downward direction are stated. There were no significant differences among the BMD classes for any of the measured quantities (F -test), although the RCV for DPD/Cr was somewhat larger in class A (-44.0%) compared with classes B and C (-35.7% and -39.7% , respectively; $P > 0.25$ and $0.1 < P < 0.25$, respectively). There were no significant differences among the variances of the intraindividual CVs of the BMD classes, however (F -test). Furthermore, we could find no evidence of heterogeneity in the distributions of the individual variances within the classes, based on the criterion of Harris and Brown (18).

The within-person variation of log NTx/Cr in the small short-term study was less than in the main study ($P < 0.025$ by F -test of mean variances). With a mean interval of 26 days, the one-tailed RCV derived from the 45 pairs of (log)NTx/Cr was -22.6% .

RESPONSES TO TREATMENT

At baseline, patients who were subsequently prescribed bisphosphonates had significantly lower BMD at all sites than the untreated women ($P < 0.0001$; Table 3), and greater DPD/Cr ratios ($P < 0.05$). The HRT-treated women also had a lower BMD. This was significant for LSBMD and FNBMD ($P < 0.005$) but not at the forearm. Class A patients who were treated with bisphosphonates

Table 3. Baseline values for treated and untreated patients.

	No treatment	Bisphosphonates	Calcium	HRT
Age, years				
Median (n)	69 (131)	70 (87)	67 (18)	69 (15)
Range	50-90	57-85	56-93	59-78
Distal forearm BMD, g/cm ²				
Median (n)	0.375 (131)	0.342 (87) ^a	0.363 (18)	0.363 (15)
Range	0.208-0.419	0.202-0.414	0.299-0.414	0.291-0.408
LSBMD, g/cm ²				
Median (n)	0.913 (100)	0.826 (45)	0.889 (12)	0.746 (9)
Range	0.535-1.265	0.617-1.267	0.730-1.105	0.565-0.912
FNBMD, g/cm ²				
Median (n)	0.697 (100)	0.591 ^a (45)	0.683 (12)	0.584 ^b (9)
Range	0.485-0.942	0.485-0.751	0.491-0.845	0.522-0.705
NTx, nmol/mmol Cr				
Median (n)	60.6 (131)	64.7 (87)	73.3 (18)	77.0 (15)
Range	15.2-29.6	19.3-289	32.3-190	15.9-184
DPD, nmol/mmol Cr				
Median (n)	5.71 (131)	6.22 ^c (87)	6.05 (18)	5.82 (15)
Range	1.00-13.1	3.34-16.9	3.84-11.8	4.38-18.7

^{a-c} Compared with no treatment: ^a $P < 0.0001$; ^b $P < 0.0005$; ^c $P < 0.05$.

had significantly higher NTx/Cr ratios [median (range), 73.6 (25.1–288) nmol/mmol Cr] at baseline than those in class B [54.8 (19.3–181) nmol/mmol Cr; $P = 0.023$]. The median NTx/Cr ratio of class C patients treated with bisphosphonates [43.8 (28.6–189) nmol/mmol Cr] was smaller than for those in class B but not significantly different ($P = 0.92$). There were no other significant differences among the excretion of bone markers under any of the treatment regimes at baseline and none among the ages. When the patients treated with bisphosphonate were combined with those treated with HRT and compared with the combined untreated and calcium-treated patients, there were still no differences in NTx or age at baseline. Because the numbers of women taking HRT or calcium were small, they have not been classified according to bone density for analysis, whereas there were enough patients taking bisphosphonates for this to be done.

POPULATION RESPONSES OF BONE RESORPTION MARKERS TO TREATMENT

BMD. LSBMD but not FNBMD increased after 12 months of bisphosphonate treatment ($P \leq 0.0001$). BMD also increased at both sites after HRT, but the change was not statistically significant. Treatment with calcium had no effect on BMD at either site.

NTx. The NTx/Cr ratio decreased at all times with bisphosphonate treatment in BMD classes A and B ($P < 0.0001$), but in class C the increase was not significant at 3 months ($P = 0.029$) but was at 6 and 12 months ($P = 0.036$ and 0.020 , respectively; Table 4). In women treated with HRT, NTx/Cr decreased from baseline, but although statistically significant from 3 months ($P = 0.0036$), the decrease was not marked until 12 months ($P = 0.0003$). In women treated with calcium, NTx/Cr decreased slightly

compared with baseline at month 6 ($P = 0.011$) but was not significantly different from baseline at month 12. The other classes showed no effect of calcium.

DPD. Among the BMD classes treated with bisphosphonates, there were no significant differences in baseline DPD/Cr ratio. The response of DPD/Cr to bisphosphonates at 12 months was significant only in class A (osteoporotic) patients ($P < 0.005$). At this time, no decrease was seen in patients in classes B and C, although the smaller numbers in class C would make it harder to detect. On HRT, DPD/Cr in the combined classes decreased continuously from baseline ($P = 0.0013$ at 12 months; Table 4). Treatment with calcium had no effect on DPD/Cr.

INDIVIDUAL RESPONSES IN RELATION TO THE RCV CRITERION

BMD. Forty patients took bisphosphonates and had repeated DXA scans (classes B and C). In 47% of these, the LSBMD changed by more than the RCV. FNBMD changed by this much in 29%. LSBMD changed significantly in 44% of patients treated with HRT, which invoked a response of FNBMD in 33%. Of those who took only calcium, LSBMD changed significantly in 13% and FNBMD in 9%.

NTx. Bisphosphonate treatment produced a significant change in NTx/Cr more often than in DPD/Cr, and the response was not sensibly dependent on the BMD class. Of the class A patients, 68% showed a significant NTx/Cr response at 3 months, increasing to 80% at 12 months ($n = 81$). The equivalent responses in class B were 57% and 75% ($n = 31$), and in class C were 78% and 80% ($n = 10$). HRT was not quite as effective as bisphosphonates, with 53% responding at 3 months, increasing to 67% at 12 months compared with 64% and 78% for the whole of the

Table 4. Effects of bisphosphonates and HRT on bone resorption markers.

	Month	Bisphosphonates			HRT (all classes)
		Class A	Class B	Class C	
NTx, % of baseline					
Median (n)	3	58.2 ^a (40)	57.7 ^a (35)	66.8 (10)	70.6 ^b (15)
Range		8.52–186	15.3–131	31.8–198	27.7–141
Median (n)	6	47.6 ^a (40)	52.6 ^a (33)	45.6 ^b (9)	80.1 ^c (15)
Range		9.00–219	13.0–134	32.2–115	13.0–128
Median (n)	12	40.1 ^a (40)	43.5 ^a (33)	45.3 ^b (10)	55.3 ^d (15)
Range		8.62–154	20.4–189	16.9–128	16.3–102
DPD, % of baseline					
Median (n)	3	83.6 (40)	81.4 ^b (35)	87.6 ^c (10)	86.6 ^e (15)
Range		51.7–289	58.2–162	71.1–116	48.9–120
Median (n)	6	79.6 ^c (40)	79.6 ^b (33)	88.0 (9)	79.0 ^b (15)
Range		12.4–164	56.3–160	54.1–134	50.9–117
Median (n)	12	77.5 ^b (40)	77.4 (33)	91.6 (9)	68.6 ^b (15)
Range		36.7–171	53.7–234	51.6–226	34.6–109

^{a–e} Compared with baseline: ^a $P < 0.0001$; ^b $P < 0.005$; ^c $P < 0.05$; ^d $P < 0.0005$; ^e $P < 0.01$.

bisphosphonate-treated group ($P = 0.45$ and 0.35 , respectively). With calcium, only NTx/Cr changed by more than RCV, and only for a few women, but the proportion of women showing a change in NTx/Cr larger than the RCV was greatest at month 3 (29% of 17) decreasing to 18% at month 12.

DPD. The proportion of patients whose DPD/Cr responded to bisphosphonate was much smaller than for NTx/Cr in all BMD classes and did not increase markedly after 3 months. From 23% of 40 in class A, 37% of 35 in class B, and 0 of 10 in class C at 3 months, it progressed only to 33% in class A, 31% in class B, and 20% in class C at 12 months. This difference was significant for class A ($P \leq 0.0001$ for each period.) and also for classes B and C (24%) at all time points ($P = 0.0005$ and 0.007 at 12 months). The proportion of women in whom DPD/Cr responded to HRT was not significantly different from the proportion in whom NTx/Cr responded, increasing from 40% ($n = 15$) at 3 months ($P = 0.46$) to 53% at 12 months ($P = 0.45$).

Eighty percent of the HRT-treated patients whose NTx/Cr decreased more than the RCV at month 12 also showed a response of DPD/Cr at 12 months, and these women accounted for all the DPD/Cr responders in the HRT-treated group. Only 50% of the NTx/Cr responders showed a response of BMD, and these patients accounted for 50% of all responders at the lumbar spine and 66% of responders at the femoral neck. After bisphosphonate treatment, the women whose NTx/Cr responded at month 12 included 96% of the DPD/Cr responders and 88% of the LSBMD responders. Of our class B patients, 77% of LSBMD responders also responded with NTx/Cr at 6 months but only 31% with DPD/Cr.

Discussion

This was not a blind, controlled trial, which means that only limited conclusions may be drawn about the diagnostic use of bone markers or the effectiveness of therapy, but these were not the primary aims of the study. We wished to determine whether such markers could be used to assess the response of patients on a time scale shorter than is possible with sequential bone density measurements and also whether this was related to the type of therapy or the status of the patients' bones. One would expect that the treatment prescribed by the general practitioner would have some relation to the baseline condition of the patient. This was the case. In particular, bisphosphonate tended to be prescribed more often for the most osteopenic women. Nonetheless, there was a range of therapy for all the BMD classes, although the numbers of women receiving HRT were disappointingly small in that they allowed only very limited statistical inferences to be made. Another weakness is that, for ethical reasons, we did not do DXA scans on the women with the lowest bone densities, whose bones might have been expected to show the greatest response to therapy.

This means that our data relating changes of BMD with urine markers are limited to moderately osteoporotic women or those whose BMD is normal. Because we wished to find out whether biochemical tests could be useful for following individual patients, we related our results to estimates of the smallest change that would be outside the range predicted from the known variability of each test. The estimation of the RCV is not unequivocal. On the question of whether a one-tailed or a two-tailed test should be used, recent reports (17) recognized that only a decrease in bone resorption marker excretion is clinically significant (an increase would be ignored), and therefore this is the hypothesis being tested and a one-tailed test is appropriate. Our clinical practice would agree with this. However, we found that the results with our data were not very sensitive to the limits set. Much more influential was our correction, by taking logarithms, for the skewed distribution of the measured changes, which is also evident in data from other groups. Making this correction increased the rate of recognition of significant changes in patients' NTx/Cr ratios in particular. The method of calculating RCV will give different values according to whether the difference is related to changes from baseline or changes from the mean $[(\text{baseline} + \text{final})/2]$ (7). We have avoided relating the changes to any function of the values of the marker beyond baseline to avoid the possible inference of negative values after treatment (6). Many other variants of the calculation have been used, including choices between confidence limits of 90% and 95%, between one-tailed and two-tailed tests, and use of mean within-person CV (CV_w) or, more correctly, CV_w derived from mean or median variance (16). For the purposes of clinical decision-making and patient management, different confidence limits might be appropriate.

If the RCV test is to be of use then we need to be sure that the within-person variations from which it is calculated are similar to the variability of the patient to which it is applied. Fraser and Harris (16) have recommended testing for the homogeneity of the distributions of within-person variances, but the means of doing this are obscure for most practitioners and even then could not assure that an individual patient would not have an unusual variability. Our study has shown that the within-person variation of NTx/Cr in our osteoporotic patients, measured over a whole year, is no greater than in those with normal bone density. On the other hand, the variation of DPD/Cr among the most osteoporotic women (class A) was slightly more than among those with normal or less diminished BMD, although this was not statistically significant. The distributions of these variances are homogeneous by the test suggested by Harris and Brown (18), but the best test of all would be the estimation of the variation in an individual patient before treatment is commenced. This will rarely be practical.

A slow cyclical variation of bone density and biochemical markers of bone turnover in postmenopausal women

has recently been recognized, with a period of ~7 years (19). This cycle time is much longer than the observation periods that we and others have used to estimate long-term within-person variation. It does, nonetheless, mean that the variation in bone density, as well as markers, will differ between individuals depending on their position in the cycle and also that the variation may change over a period of years for any one patient. This effect, which imposes a nonrandom element on the previously assumed random biological variability, would tend to degrade the performance of all of these measures of response to treatment. It might be estimated by considering the phase of the cycle from knowledge of the time since menopause, and if this could be allowed for, the apparent random biological variation might be smaller than it presently seems. It would be useful to know whether the variability itself (as opposed to the cyclical change) of bone markers is affected by this cycle.

If variation were measured over an interval much shorter than 6 months, then a smaller CV might be expected because the influence of seasonal variation would not be seen. Our results with a mean interval of 26 days confirm this. The same would be true if measurements were made at intervals of only whole years. It is clearly important to estimate variability under the same circumstances as will be used clinically for repeated tests. Among the reports cited in this report, the RCV (as least significant change) has been given as between 31% and 68% for NTx/Cr and between 26% and 43% for DPD/Cr, calculated according to a range of formulae as described above.

We have recognized a vulnerability of the commercial assay for NTx/Cr and its given protocol to undetected across-plate variation, and we have taken measures to control this. This kind of analytical error can give the appearance of biological variation. Although the effect on the RCV, which is derived from total variation, is the same regardless of the source, there is a danger that if the source of variation is misidentified in this way, then the false conclusions will be drawn that the variation cannot be reduced and that it may vary among different individuals.

We have used urine samples collected by each patient in her home, thus minimizing any possible influence of stress or disturbance of daily rhythms. We do not know whether the home environment and patient involvement are any more conducive to reliable collection than a hospital setting, but it is not impossible. Our main reason for using this system is the much greater convenience for our patients, who come from a wide area. We have found that preanalytical variation is no greater problem with mailed urine samples than with those collected in, or brought to, the hospital. It would be statistically advantageous to collect and analyze replicate samples over a shorter time period as a means of reducing variation and hence RCV. However, even using mailed samples, this might strain patient compliance in the context of long-

term treatment for a nonacute condition. Moreover, the assays for all the recently developed bone resorption markers are expensive, although automated analysis should reduce costs in the future.

Fink et al. (11) found that first and second morning urinary DPD/Cr ratios correlated well with each other, but this was not so for NTx/Cr. This might mean that the collection protocol is more important for NTx/Cr than for DPD/Cr. The same study also found that 88% of osteoporotic women whose LSBMD changed significantly after 12 months on alendronate also showed a significant decrease in NTx/Cr at 4 months but only 44% for free DPD/Cr. Our most osteoporotic women (class A) did not have repeated BMD measurements; therefore, we cannot compare them directly. In class B (mildly osteoporotic), however, of those who showed a BMD response almost three times as many responded with NTx/Cr as with DPD/Cr.

In conclusion, urinary NTx/Cr can be used to monitor therapy for osteoporosis using mailed urine samples. By 12 months, more than three fourths of patients show a response in excess of the RCV.

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