

Evaluation of a Fully Automated Serum Assay for C-Terminal Cross-Linking Telopeptide of Type I Collagen in Osteoporosis

PATRICK GARNERO,^{1,2*} OLIVIER BOREL,¹ and PIERRE D. DELMAS¹

Background: Biochemical markers of bone turnover can provide prognostic information about the risk of osteoporotic fracture and are useful tools for monitoring efficacy of antiresorptive therapy. A serum-based automated assay may be of better clinical value than urinary markers because of lower imprecision and day-to-day within-person variability. Our aim was to evaluate the technical and clinical performances of a new, fully automated assay for serum C-terminal cross-linking telopeptide of type I collagen (CTX), a marker of bone resorption.

Methods: Serum CTX was measured on the Elecsys 2010 automated analyzer (Roche). Results were compared with those of the manual ELISA. We measured serum CTX concentrations in 728 healthy women, ages 31–89 years. We investigated the ability of this assay to predict the rate of postmenopausal forearm bone loss evaluated by four repeated bone mineral density measurements using dual-x-ray absorptiometry in 305 women followed prospectively for 4 years. Finally, in a cohort of healthy, untreated, postmenopausal women, we compared baseline serum CTX in 55 women who subsequently had a fracture (20 vertebral and 35 peripheral fractures) with values in the 380 women who did not fracture during a mean 5 years of follow-up.

Results: The within- (n = 21) and between-run (n = 21) CVs were <4.1% and 5.7%, respectively. In 728 healthy women, serum CTX concentrations (automated) correlated with those of the manual ELISA ($r = 0.82$; $P < 0.0001$). The median long-term within-person variability assessed by four repeated measurements over 3 months in 18 postmenopausal women was 9.4%. Compared with 254 premenopausal women, serum CTX was

39% ($P < 0.0001$) higher in 45 perimenopausal women and 86% ($P < 0.0001$) higher in 429 postmenopausal women (mean age, 64 years). Baseline serum CTX correlated negatively with changes of bone mass measured at the mid ($r = -0.23$; $P < 0.0001$) and distal ($r = -0.27$; $P < 0.0001$) radius. Postmenopausal women with serum CTX greater than the mean + 2 SD values in premenopausal women accounted for 42% of the population, lost bone at the mid radius on average eightfold more rapidly than the other women ($-0.27\% \pm 2.92\%$ vs $-2.25\% \pm 3.95\%$; $P < 0.0001$), and had increased risk of fracture with a relative risk (95% confidence interval) of 1.8 (1.01–3.1) after adjustment for physical activity.

Conclusions: The automated assay for serum CTX is precise and predicts rate of bone loss and fracture risk in postmenopausal women. Because it is convenient to use and has high throughput, this serum bone resorption marker may be useful for the investigation of patients with osteoporosis.

© 2001 American Association for Clinical Chemistry

Biochemical markers of bone turnover have improved in sensitivity and specificity in recent years [for a review, see Ref. (1)]. In osteoporosis, markers of bone turnover are increasingly used to monitor the efficacy of osteotropic treatments and to predict fracture risk [for a review, see Ref. (2)]. Although all sensitive markers of bone formation are serum-based tests and include osteocalcin, bone-specific alkaline phosphatase, and the N-terminal propeptide of type I collagen (1, 2), most of available indices of bone resorption are urine markers. Among the different bone resorption markers, degradation products of type I collagen have demonstrated their superiority (1). These markers include the nonreducible pyridinoline and deoxypyridinoline cross-links (3, 4), which can be measured in hydrolyzed urine sample by HPLC or ELISA (5, 6), and the related type I collagen telopeptides (7, 8).

An important issue for the clinical use of biochemical markers relates to their intraindividual reproducibility. This variation has been reported to be relatively high for

¹ INSERM Research Unit 403, Hôpital E Herriot, Pavillon F, 69437 Lyon Cedex 03, France.

² Synarc, 69003 Lyon, France.

*Author for correspondence. Fax 33-4-72-68-65-09; e-mail patrick.garnero@synarc.com.

Received September 20, 2000; accepted January 12, 2001.

urinary resorption markers (9, 10). One way to reduce the precision error of bone markers and hence improve their clinical utility is to use a serum-based assay. Indeed, measuring a marker in serum rather than in urine provides better reproducibility because the variable ionic strength of urine samples and the need to correct for creatinine excretion may introduce some variability in the results. This partly explains the recent development of a serum-based immunoassay for bone resorption markers including the C-terminal (11) and N-terminal (12) cross-linking telopeptides of type I collagen (CTX³ and NTX, respectively) and more recently the osteoclast-specific 5b isoenzyme of tartrate resistant acid phosphate (13). Recent data indicated an approximately twofold lower inpatient variability of serum CTX and NTX compared with urinary measurements (14, 15). Another way to reduce variability is to use fully automated analyzers that are suitable for routine use in clinical chemistry. The aim of this study was to evaluate the technical and clinical performances of a new automated analyzer for serum CTX using a cohort of 728 healthy women followed prospectively.

Materials and Methods

SUBJECTS

We studied 728 healthy female volunteers, ages 30–89 years, who had been enrolled in an epidemiological study of the determinants of bone loss (Ofely study). None of these women had a disease or were receiving treatment that could interfere with bone metabolism, including estrogen replacement therapy. Past and present physical activity was recorded by questionnaire. The monthly hours of physical exercise, the daily walking distance, and the number of stairs climbed; the weekly hours of house work; and the physical demands of professional work (sitting, light, medium, and heavy) were recorded to calculate an individual activity score. Details and the inclusion and exclusion criteria of these women have been described previously (16–19). For each woman, fasting blood samples were collected at baseline between 0730 and 0930 to measure serum CTX with a manual ELISA and an automated analyzer. Analyses were performed on frozen samples that were stored at -80°C for a period of 6–7 years. This study was approved by the local ethics committee, and informed consent was obtained from all participants. According to the objectives, we analyzed subgroups of this population.

AGE- AND MENOPAUSE-RELATED CHANGES IN SERUM CTX

Women were separated in three groups according to their menopausal status.

Premenopausal women. This group included 254 women, ages 31–58 years (mean age, 39 years), with regular menses and human follicle-stimulating hormone (FSH) concentrations <16.7 IU/L. Serum FSH was measured with a two-site immunochemiluminometric assay (Magic Lite FSHTM; Ciba Corning[®]).

Perimenopausal women. This group included 45 women, ages 39–56 years (mean, 50.4 years), with irregular menses and serum FSH >16.7 IU/L, which corresponds to the mean + 1 SD of the premenopausal population.

Postmenopausal women. This group included 429 women, ages 45–89 years (mean, 64 years), with no menses for at least 12 months. The longest time period since last menses was 40 years (mean time since menopause, 14 years).

SERUM CTX AND RATE OF POSTMENOPAUSAL BONE LOSS

To analyze the relationship between baseline serum CTX concentrations and the rate of forearm bone loss, we investigated 305 postmenopausal women (mean age, 64 years; range, 50–88 years) who had a completed 4-year follow-up and who did not receive any treatments, including hormone replacement therapy, that could interfere with bone metabolism during the 4 years of follow-up as described previously (17).

SERUM CTX AND FRACTURE RISK

The relationship between baseline serum CTX concentrations and the risk of incident fracture was analyzed in 429 healthy untreated postmenopausal women as described previously (18). At baseline, women responded to a detailed questionnaire that included questions on history of fragility fractures, physical activity, calcium intake, and smoking habits (18). Subsequently, each woman was seen every year for a maximum of 6 years. At each visit the occurrence of fractures within the previous year was registered. For women who did not continue, mail was sent every year to identify the occurrence of fracture. All peripheral fractures were confirmed by radiographs. Lateral x-ray films of the thoracic and lumbar spine were obtained at baseline for all women and at follow-up for 79% of the women after an average of 3.8 years. All vertebral prevalent and incident fractures were identified by semiquantitative morphometry by two individuals who were unaware of the baseline results. A vertebra was classified as having a prevalent fracture on the baseline radiograph if any of the vertical height (anterior, middle, and/or posterior) was reduced by $>20\%$. A new fracture was defined by a decrease $\geq 20\%$ and at least 4 mm in any vertebral height of one or more thoracic or lumbar vertebrae between follow-up and baseline x-ray films (20).

³ Nonstandard abbreviations: CTX and NTX, C- and N-terminal cross-linking telopeptides of type I collagen, respectively; FSH, follicle-stimulating hormone; BMD, bone mineral density; LSC, least significant change; RR, relative risk; and 95% CI, 95% confidence interval.

During a mean 5.0 ± 1.3 years of follow-up, 21 vertebral fractures and 37 peripheral osteoporotic fractures (12 wrist, 5 hip, 5 rib, 5 ankle, 3 patella, 3 humerus, 1 sacrum, 1 pelvis, and 2 metatarsi) were recorded in 55 women (18).

INTRAINDIVIDUAL VARIABILITY OF SERUM CTX

To study intraindividual variations of serum CTX, we investigated 18 healthy untreated postmenopausal women, ages 56–72 years. Fasting serum samples collected between 0730 and 0930 were obtained at baseline, day 2, day 3, month 2, and month 3 and stored at -80°C until measurement.

SERUM CTX ASSAYS

ELISA for serum CTX (serum ELISA-CTX). Serum CTX was measured by a two-site ELISA (Serum Crosslaps one step; Osteometer Biotech), which uses two monoclonal antibodies raised against an amino acid sequence specific to part of the β -isomerized C-telopeptide of type I collagen (Glu-Lys-Ala-His- β Asp-Gly-Gly-Arg; Crosslaps antigen) (11). Briefly, calibrators, controls, or unknown serum samples are added to microtiter wells coated with streptavidin, followed by a mixture of a biotinylated antibody and a peroxidase-conjugated antibody. A complex of CTX antigens, biotinylated antibody, and peroxidase-conjugated antibody is generated, and the complex binds to the streptavidin surface via the biotinylated antibody. The amount of antigen is quantified by the use of a chromogenic peroxidase substrate. The intra- and interassay CVs are $<8\%$.

Automated assay for serum CTX (serum automated-CTX). Serum CTX was measured on the Elecsys 2010 automated analyzer (Roche Diagnostics GmbH, Mannheim, Germany) using the β -Crosslaps/serum reagents. This assay is specific for cross-linked β -isomerized type I collagen C-telopeptide fragments and uses two monoclonal antibodies, each recognizing the Glu-Lys-Ala-His- β Asp-Gly-Gly-Arg peptide (Crosslaps antigen). Briefly, 50 μL of

serum and a biotinylated monoclonal antibody against the Crosslaps antigen are incubated together. A second antibody labeled with a ruthenium complex is then added together with streptavidin-coated microparticles. A sandwich complex is formed that binds to the solid phase (the microparticles) via biotin-streptavidin interactions. These microparticles are then magnetically captured onto the surface of an electrode. Application of the voltage on this electrode then induces a chemiluminescent emission, which is measured by a photomultiplier and compared to a calibration curve, which is instrument-specific and is generated by two-point calibration, and a master curve provided via the bar code of the reagent. In total, the duration of this automatic process is 18 min.

BONE DENSITOMETRY

Bone mineral density (BMD) of the mid and distal radius was measured by dual x-ray absorptiometry on a QDR 2000 device (Hologic, Inc). The mid area of the radius is composed mainly of cortical ($>95\%$) bone, and the distal area comprises both cortical ($\sim 75\%$) and trabecular ($\sim 25\%$) bone. The short-term in vivo imprecision (CV) of dual x-ray absorptiometry was 1.2% and 0.6% for the mid and distal radius, respectively.

STATISTICAL ANALYSES

As shown on Fig. 1 and as expected in postmenopausal women, CTX concentrations measured either by ELISA or automated analyzer were not normally distributed with a frequency distribution skewed to the left. Consequently, CTX data were log-transformed before statistical analysis. Correlations between the different variables were assessed by linear regression analysis. The least significant change (LSC) at a significance of $P < 0.05$ was calculated by a two-tailed test according the formula: $\text{LSC} = 1.96 \times \sqrt{2} \times \text{CV}_i$ (9), where CV_i is the intraindividual variability assessed over 3 months. The effect of menopausal status on serum CTX concentrations was determined by ANOVA. Comparisons between pre- and postmenopausal women were also assessed using the t -score, which

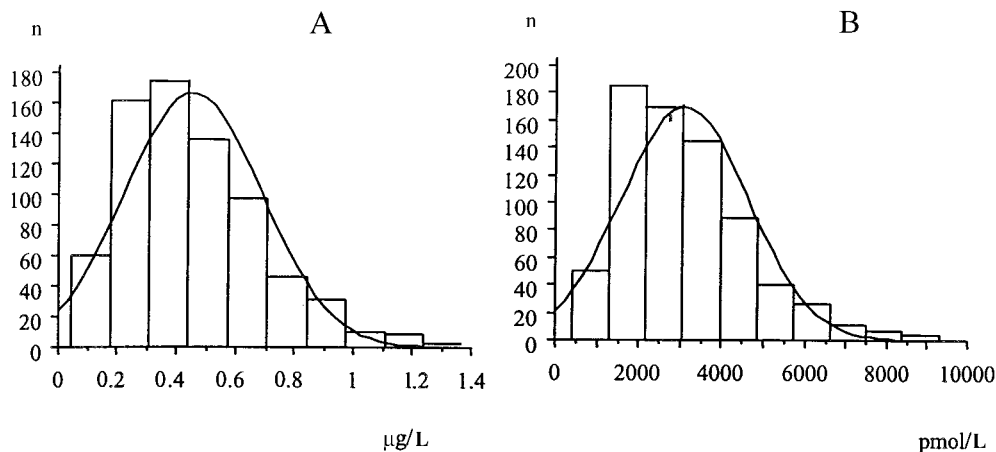


Fig. 1. Frequency distribution of serum CTX values obtained by automated (A) and manual (B) assays in 728 healthy women.

is the number of SD from the premenopausal mean. The formula for the *t*-score is: $(x_i - M)/SD$, where x_i is the CTX concentration of an individual postmenopausal woman, M is the mean of the premenopausal reference population, and SD is the standard deviation of the reference group.

To analyze the relationship between baseline concentrations of serum CTX and the rate of postmenopausal bone loss, individual rates of change in BMD were calculated. It was assumed that the expected change in BMD was linear over the 4-year follow-up period. BMD values at baseline, year 2, year 3, and year 4 were regressed on time to yield a rate of change in BMD for each subject. The relationship between baseline concentrations of serum CTX and the rate of change was assessed by linear regression analyses. The mean + 2 SD of serum CTX concentrations of the premenopausal women was defined as the upper limit of the premenopausal range and was used as a cutoff limit between low and high bone turnover in postmenopausal women, as suggested previously (17,18). Differences in the rate of BMD loss between women with low and high bone turnover at baseline were assessed by the Student *t*-test.

The relationships between baseline serum CTX concentrations and the risk of incident fracture were analyzed by logistic-regression analysis before and after adjustment for potential confounding factors.

All analyses were performed using the Statistical Analysis Software (SAS).

Results

ANALYTICAL PERFORMANCE OF THE AUTOMATED ASSAY FOR SERUM CTX

The intraassay CV assessed by 21 measurements of two serum samples (mean serum CTX values, 0.210 and 1.600 $\mu\text{g/L}$), with aliquots being randomly distributed, was 4.1% and 1.2%, respectively. The interassay CV evaluated by repeated measurements ($n = 21$) of four serum samples (mean serum CTX values, 0.201, 0.834, 1.551 and 3.221 $\mu\text{g/L}$) was <5.7%. The lower detection limit, calculated as the concentration 2 SD above that of the lowest calibrator, was 0.01 $\mu\text{g/L}$.

Dilution test. Three serum samples were diluted 1:2, 1:4, 1:8, and 1:16 with assay buffer. As shown on Table 1, the dilution recovery was 86–119% (mean, 104%).

Recovery test. Known amounts of β -Crosslaps antigen calibrator were added to three serum samples. The recovery was 91–101% (Table 2).

CORRELATION OF THE AUTOMATED SERUM CTX ASSAY WITH THE MANUAL ELISA

Serum CTX concentrations were measured in the 728 healthy women using both the manual ELISA and the automated analyzer, and a highly significant correlation was obtained ($r = 0.82$; $P < 0.0001$) between the two assays

Table 1. Results of dilution test for the automated serum CTX assay.

Sample	Dilution	Serum CTX, $\mu\text{g/L}$		Recovery, %
		Expected	Measured	
I	Undiluted	0.940	0.940	100
	1:2	0.470	0.558	119
	1:4	0.240	0.277	115
	1:8	0.120	0.124	103
	1:16	0.060	0.058	97
II	Undiluted	1.920	1.920	100
	1:2	0.960	1.036	108
	1:4	0.480	0.494	103
	1:8	0.240	0.228	95
	1:16	0.120	0.103	86
III	Undiluted	2.883	2.883	100
	1:2	1.442	1.586	110
	1:4	0.721	0.836	116
	1:8	0.360	0.410	114
	1:16	0.180	0.176	98

(Fig. 2). The intercept of the regression line was significantly different from 0 ($P < 0.001$).

LONG-TERM INTRAINDIVIDUAL VARIATION OF AUTOMATED SERUM-CTX

Intraindividual variability of serum automated-CTX was assessed in 18 untreated postmenopausal women over 3 months. At baseline, the women had an average serum CTX concentration of $0.516 \pm 0.217 \mu\text{g/L}$. The median intraindividual CV (CV_i) for serum CTX values was 9.4% (range, 4.1–27%). On the basis of this CV_i, the LSC was determined to be 27%, meaning that an individual should display a $\geq 27\%$ decrease of serum CTX concentrations when receiving antiresorptive therapy to have a <5% chance of the decrease being the result of random variation in marker concentration.

CHANGES IN SERUM CTX CONCENTRATIONS WITH AGE AND MENOPAUSE

In premenopausal women, there was no significant correlation between serum automated-CTX and age (Fig. 3 and Table 3). Serum automated-CTX was 39% ($P < 0.0001$)

Table 2. Recovery for the automated serum CTX assay.^a

Sample	CTX, $\mu\text{g/L}$			Recovery, %
	Endogenous	Added	Measured	
I	0.192	0.020	0.193	91
	0.192	0.730	0.931	101
II	0.383	0.020	0.379	94
	0.383	0.730	1.090	98
III	0.570	0.020	0.584	99
	0.570	0.730	1.313	101

^a Known amounts of β -Crosslaps calibrator were added to two serum samples. Recovery is expressed as a percentage of the theoretical value (endogenous + added).

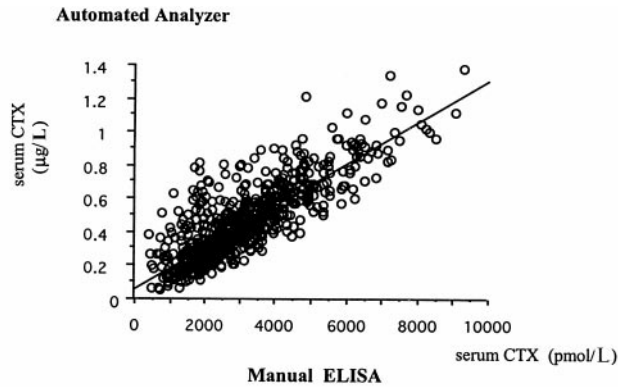


Fig. 2. Correlation between serum CTX concentrations measured by the manual ELISA (*x*-axis) and the automated assay (*y*-axis) in 728 healthy women.

Equation for the line: $y = 0.073 + 0.000122x$; $r = 0.82$; $n = 728$.

higher in perimenopausal women and 86% ($P < 0.0001$) higher in postmenopausal women than in premenopausal women (Fig. 3 and Table 3). When expressed as a *t*-score, serum automated-CTX discriminated postmenopausal from premenopausal controls with a mean *t*-score of 1.88. No significant change of serum automated-CTX with age was observed in postmenopausal women (Fig. 3 and Table 3).

SERUM CTX CONCENTRATIONS TO PREDICT RATE OF POSTMENOPAUSAL BONE LOSS

At baseline, serum automated-CTX correlated negatively with both mid and distal radius BMD: $r = -0.24$ ($P < 0.0001$) and $r = -0.18$ ($P = 0.0017$) for mid and distal radius, respectively.

Table 3. Serum automated-CTX results per age group in pre- and postmenopausal women.

Group	Age, years	n	Serum CTX, ^a µg/L
Premenopausal women	30–34	52	0.328 ± 0.145
	35–39	98	0.278 ± 0.119
	40–44	53	0.291 ± 0.132
	45–49	34	0.340 ± 0.165
	≥50	17	0.271 ± 0.144
All		254	0.299 ± 0.137
Postmenopausal women	45–54	60	0.528 ± 0.224
	55–59	113	0.582 ± 0.220
	60–64	71	0.573 ± 0.214
	65–69	51	0.539 ± 0.203
	70–74	71	0.525 ± 0.228
	75–79	35	0.562 ± 0.273
	≥80	28	0.589 ± 0.249
All		429	0.556 ± 0.226 ^b

^a Mean ± SD.

^b $P < 0.0001$ vs mean value in the 254 premenopausal women.

In the whole population, baseline serum automated-CTX correlated significantly with changes of bone mass assessed prospectively over 4 years at both the mid ($r = -0.23$; $P < 0.0001$) and the distal ($r = -0.27$; $P < 0.0001$) radius. Results obtained for serum CTX measured by ELISA were similar: $r = -0.20$ and -0.24 ($P < 0.0001$) for the mid and distal radius, respectively. In 51 women within 5 years of menopause, the rate of BMD loss was faster than in the whole population: -0.62% vs -0.22% per year for mid radius and -0.78% vs -0.59% per year for distal radius in the early and whole postmenopausal

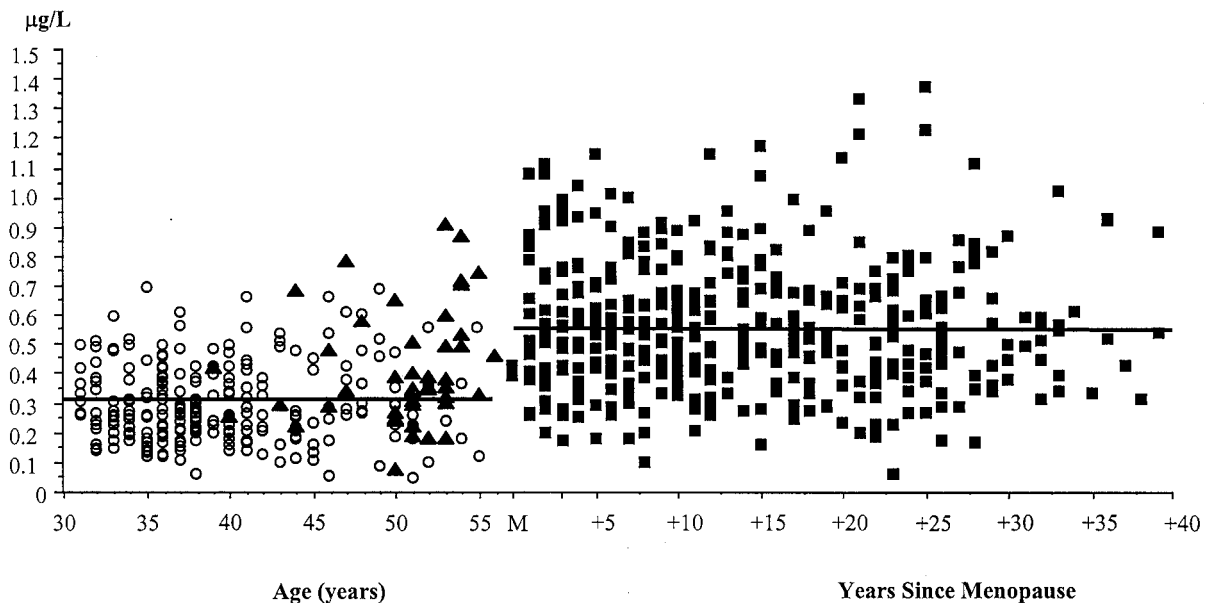


Fig. 3. Age- and menopause-related changes in serum CTX measured by the automated assay.

○, premenopausal women ($r = 0.04$; $P = 0.94$ vs age); ▲, perimenopausal women; ■, postmenopausal women ($r = -0.026$; $P = 0.94$ vs years since menopause). Horizontal lines indicate the means. M, menopause.

populations, respectively. When the analysis was restricted to these early postmenopausal women, the negative correlation between baseline serum automated-CTX concentrations and rate of bone loss was higher: $r = -0.47$ and -0.49 ($P < 0.001$) for mid and distal radius, respectively.

Postmenopausal women with high turnover at baseline, i.e., with serum automated-CTX greater than the mean + 2 SD values in premenopausal women, accounted for 42% of the population and over 4 years lost bone at the distal ($-3.87\% \pm 4.36\%$) and mid radius ($-2.25\% \pm 3.94\%$) more rapidly ($P < 0.001$) than women with low bone turnover ($-1.64\% \pm 3.2\%$ and $-0.27\% \pm 2.90\%$ for distal and mid radius, respectively; Fig. 4). When we used serum CTX measured by ELISA, the difference in bone loss over 4 years between high and low turnover was of a magnitude similar to the results obtained by serum automated-CTX [$-4.49\% \pm 4.65\%$ vs $-2.04\% \pm 3.47\%$ and $-2.78\% \pm 4.22\%$ vs $-0.63\% \pm 3.15\%$ ($P < 0.0001$) for distal and mid radius, respectively].

SERUM CTX CONCENTRATIONS TO PREDICT OSTEOPOROTIC FRACTURES IN POSTMENOPAUSAL WOMEN

At baseline, women who had osteoporotic fractures during the study were older (67 vs 64 years; $P = 0.004$), had slightly lower physical activity (physical activity score, 12.1 vs 13.8; $P = 0.004$), and had a lower BMD at the femoral neck, spine, and radius ($P < 0.0001$ for all sites) than controls but did not differ in body weight, height, and body mass index. An increased physical activity score was associated with decreased fracture risk with a relative risk (RR) of 0.61 [95% confidence interval (95% CI), 0.45–0.84] for each SD increase in the score. As expected, the proportion of women with prevalent osteoporotic

fractures was also higher among women who had fractures during the study (31% vs 13%; $P = 0.002$). Serum automated-CTX did not correlate with age and was not different between women with and without prevalent fractures (data not shown), but was moderately correlated with physical activity score ($r = 0.13$; $P = 0.006$). Women with serum automated-CTX greater than the mean + 2 SD values in premenopausal women had an increased, although not statistically significant, risk of fracture compared with the other groups, with a RR of 1.6 (95% CI, 0.95–2.7). After adjustment for physical activity, the RR of fracture associated with high serum automated-CTX increased slightly and was significant [RR (95% CI), 1.8 (1.01–3.1)]. The corresponding RR (95% CI) values obtained with serum CTX (ELISA) were 1.6 (0.92–2.9) and 2.0 (1.1–3.6) before and after adjustment for physical activity, respectively.

Discussion

In this study, we performed an extensive evaluation of the clinical performance of a new automated assay for serum CTX in osteoporosis. We found that this assay exhibits good analytical performance and predicts rate of bone loss and fracture risk in postmenopausal women with performance similar to that of the manual ELISA.

The automated assay for serum CTX is fast, and the intra- and interassay CVs are $<4\%$ and 6% , respectively, which compare favorably with the CVs for the manual ELISA (11). We observed a high correlation between serum CTX concentrations measured with this automated assay and those obtained with the manual ELISA ($r = 0.82$). The manual ELISA uses SI units, whereas serum CTX values in the automated system are expressed in $\mu\text{g/L}$. It was not possible, however, to correlate the units used in the two assay systems because each assay may

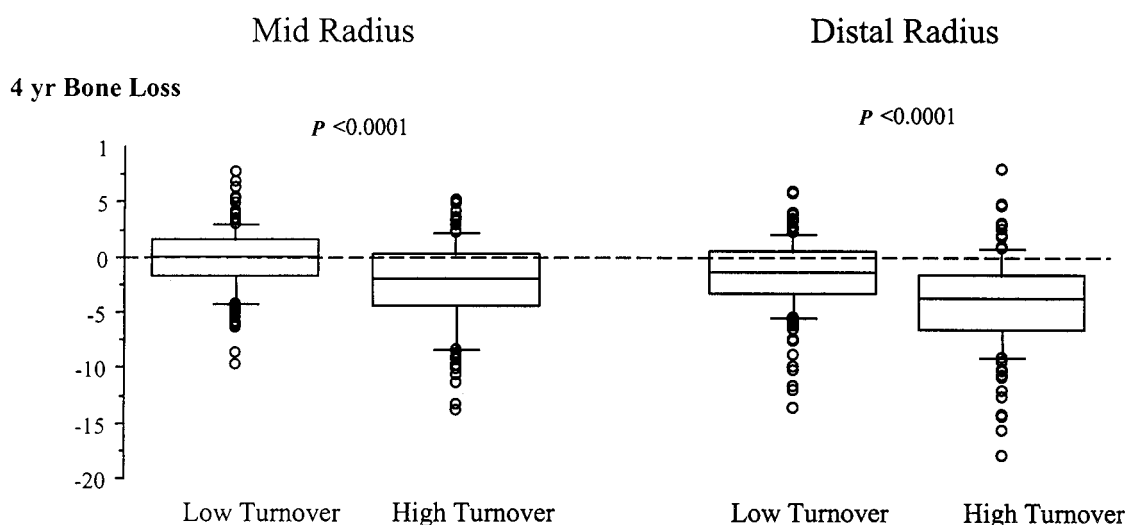


Fig. 4. Mid and distal radius BMD loss in 305 healthy untreated postmenopausal women with low and high bone turnover at baseline.

High turnover was defined as serum automated-CTX greater than the mean + 2 SD values for premenopausal controls. The top and bottom of each box represent 75th and 25th percentiles, respectively. The upper and lower bars represent 90% and 10% limits, respectively, and the outliers are shown as \circ . *, $P < 0.0001$ vs rate of bone loss in the low turnover group.

detect several differing molecular entities, precluding the determination of a specific molecular weight. Indeed, the enzymatic degradation of type I collagen by the osteoclasts generates several fragments of different molecular sizes, which would be measured by the immunoassays provided that they contain the 8-amino acid sequence used to raise the antibodies.

The long-term intraindividual variation of serum automated-CTX values in postmenopausal women was 9.4%, which is comparable to that of serum CTX by ELISA (11.5%) (14) and serum NTX (7.5%) (15). These data indicate that serum CTX measurements obtained in fasting subjects demonstrate lower intraindividual variability and consequently a lower LSC than the corresponding urinary measurements (9, 10).

As reported previously in other studies and in the same population with other markers of bone formation and bone resorption (16), we found no significant changes with age in serum automated-CTX concentrations in premenopausal women, ages 30–58 years. We believe that values obtained in this large population of well-characterized estrogen-replete women taken from the general population could represent an adequate reference range for this new marker. In women characterized by irregular bleeding and increased FSH concentrations, a pattern that is frequent in perimenopausal women (21), we found a significant increase of serum automated-CTX. This suggests that bone resorption increases before cessation of menses in women with estrogen deficiency and is consistent with data obtained for other biochemical markers in the same cohort (16). This increase in bone turnover that can be detected by serum CTX is likely to be responsible for the loss of trabecular and cortical bone observed in some of these women (22). The 39% increase of serum automated-CTX observed in perimenopausal women was larger than that reported previously in the same population for serum bone alkaline phosphatase (17%) and in the same order of magnitude as that of urinary CTX and NTX (30–40%). Thus, serum automated-CTX could be a sensitive index to identify perimenopausal women with high bone resorption who would be at risk for bone loss.

At the time of menopause, serum automated-CTX increased by 86%, a pattern consistent with the accelerated bone loss occurring within the first years following menopause (23). After this marked increase, we found no age-related decline. Such a sustained bone turnover has been found previously in this study with other bone formation and urinary resorption markers (16) and also in other populations (24, 25). In most previous studies, however, the bone resorption rate was assessed by urinary markers corrected for urinary creatinine. Because decreased muscle mass, and therefore low urinary creatinine value, is common in elderly women, one could speculate that increased bone resorption markers in advanced age may result from lower urinary creatinine. Our study, performed in a large cohort of healthy postmenopausal women using a serum assay, suggests that the rate of bone

resorption remains high up to 40 years after menopause. However, we cannot exclude that increased serum CTX in elderly women could result in part from decreased clearance of this marker because of alterations in renal function.

In osteoporosis, the main cause for concern is increased risk of fractures. Several prospective studies have found that a decrease by 1 SD in BMD values measured by dual x-ray absorptiometry was associated with a two- to four-fold increase in the risk of fractures of the hip, wrist, or spine. In that context, an important issue is whether combined use of bone remodeling markers and BMD measurements could improve the accuracy of fracture risk evaluation. Increased bone turnover can be related to decreased bone strength as a result of two mechanisms. Several studies have shown that increased concentrations of bone markers are associated with increased rate of bone loss at the spine, forearm, and calcaneus in the subsequent years and thus a lower bone mass later in life (17, 26–28). In addition, recent data obtained in large prospective studies have shown that increased concentrations of bone resorption markers are associated with increased risk of hip, vertebral, and non-hip and non-vertebral fractures over follow-up periods ranging from 1.8 to 5 years (18, 29–31), independent of bone mass. Indeed, increased bone resorption may induce microarchitectural deterioration of bone tissue, such as perforation of trabeculae, a major component of bone strength (32). Consequently, use of a combination of BMD assessment and bone resorption markers has been suggested for improving risk assessment (33).

In this study, we found that higher physical activity was slightly associated with increased serum CTX concentrations, suggesting that increased physical activity may be associated with increased bone resorption. However, the association was modest, the physical activity score explaining <2% of the interindividual variability in serum CTX concentrations. The relationship between physical activity and bone resorption is actually unclear. Exercise has indeed been reported to be associated with increased (34, 35), decreased (36), or unchanged (37, 38) bone resorption. This discrepancy could be related to the type of exercise (anaerobic vs aerobic) (36) and/or its duration and intensity. Women with serum automated-CTX greater than the mean + 2 SD values in premenopausal women lost bone at the mid radius 8 times more rapidly over the subsequent 4 years and had an ~1.8-fold higher risk of fracture than individuals with CTX concentrations below the mean + 2 SD cutoff after adjustment for physical activity. The predictive value of bone loss and fracture risk is similar to that of serum CTX by ELISA and to that reported previously in the same population for urinary CTX (17, 18). Timing of sample collection and fasting status of the subjects, however, seem to be important for the clinical value of serum CTX measurements. In nonfasting conditions, the maximum concentration of serum CTX is reached during the night, followed by a

marked decrease during the morning until 1100, then by a nadir stable from 1100 to ~1500, after which serum CTX increases (14). Interestingly, this circadian rhythm is markedly decreased when women are fasting throughout the day, producing a day-to-day variation that is smaller in fasting than nonfasting women (7% vs 14%) (14). In the EPIDOS cohort, a large prospective study of elderly women, we found that serum automated-CTX measured in the morning in nonfasting subjects was not predictive of hip fracture risk (39). However, when the analysis was restricted to the patients for whom serum samples had been collected during the afternoon (1300–1500) after lunch, a significant relationship between increased concentrations of serum CTX and the risk of hip fracture was found (39). In nonfasting women from the EPIDOS study, when samples were taken at different times during the morning, variability of the measurement was high, which probably explains the absence of a predictive value. In the afternoon, women were at the nadir of the rhythm with stable concentrations. The data we obtained in this study lend support to that hypothesis because we found that when sampling is performed in the morning, but at a standardized time (between 0730 and 0930) and in a fasting state, serum automated-CTX is predictive of fracture risk.

In conclusion, we have validated a new automated assay for serum CTX. This assay is fast, convenient to use, precise, and was able to detect the increase of bone resorption in perimenopausal and postmenopausal women. Serum automated-CTX predicted rapid bone loss and fracture risk in a large population of postmenopausal women with a value similar to those of serum and urinary resorption markers measured by manual ELISA. This serum-based resorption marker is likely to be useful for the investigation of patients with osteoporosis.

This study was partly supported by Contract INSERM-MSD Chibret (Ofely study). We thank Roche Diagnostics for providing with β -Crosslaps/serum reagents.

References

- Garnero P, Delmas PD. Measurements of biochemical markers: methods and limitations. In: Bilezikian JP, Raisz LG, Rodan GA, eds. Principles of bone biology. San Diego, CA: Academic Press, 1996:1277–91.
- Garnero P, Delmas PD. Laboratory assessment of postmenopausal osteoporosis. In: Seibel M J, Robins SP, Bilezikian JP, eds. Dynamics of bone and cartilage metabolism. Principles and clinical applications. San Diego, CA: Academic Press, 1998:465–77.
- Eyre DR, Knoob TJ, Van Ness KP. Quantitation of hydroxypyridinium cross-links in collagen by high performance liquid chromatography. *Ann Biochem* 1984;137:380–8.
- Fujimoto D, Suzuki M, Uchiyama A, Miyamoto S, Inoue T. Analysis of pyridinoline, a cross-linking compound of collagen fibers, in human urine. *J Biochem* 1983;94:1133–6.
- Seyedin S, Zuk R, Kung V, Daniloff Y, Shepard K. An immunoassay to urinary pyridinoline: the new marker of bone resorption. *J Bone Miner Res* 1993;8:635–42.
- Robins SP, Woitge H, Hesley R, Ju J, Seyedin S, Seibel MJ. Direct, enzyme-linked immunoassay for urinary deoxypyridinoline as a specific marker for measuring bone resorption. *J Bone Miner Res* 1994;9:1643–9.
- Hanson DA, Weiss MAE, Bollen AM, Maslan SL, Singer FR, Eyre DR. A specific immunoassay for monitoring human bone resorption: quantitation of type I collagen cross-linked N-telopeptides in urine. *J Bone Miner Res* 1992;7:1251–8.
- Bonde M, Qvist P, Fledelius C, Riss BJ, Christiansen C. Immunoassay for quantifying type I collagen degradation products in urine evaluated. *Clin Chem* 1994;40:2022–5.
- Hannon RA, Bluhmson A, Naylor KE, Eastell R. Response of biochemical markers of bone turnover to hormone replacement therapy: impact of biological variability. *J Bone Miner Res* 1998;13:1124–33.
- Garnero P, Delmas PD. Variability and response of urinary resorption markers to hormone replacement therapy. *J Bone Miner Res* 1999;14:470–1.
- Rosenquist C, Fledelius C, Christgau S, Pedersen BJ, Bonde M, Qvist P, Christiansen C. Serum CrossLaps One Step ELISA. First application of monoclonal antibodies for measurement in serum of bone-related degradation products from C-terminal telopeptides of type I collagen. *Clin Chem* 1998;44:2281–9.
- Clemens JD, Herrick MV, Singer FR, Eyre DR. Evidence that serum NTX (collagen I N-telopeptides) can act as an immunochemical marker of bone resorption. *Clin Chem* 1997;43:2058–63.
- Halleen JM, Alatalo SL, Suominen H, Cheng S, Janckila AJ, Väänänen HK. Tartrate-resistant acid phosphatase 5b: a novel serum marker of bone resorption. *J Bone Miner Res* 2000;15:1337–45.
- Christgau S, Bitsch-Jensen O, Bjarnason NH, Henriksen E, Qvist P, Alexandersen P, Henriksen D. Serum Crosslaps for monitoring the response in individuals undergoing antiresorptive therapy. *Bone* 2000;26:505–11.
- Eastell R, Mallinak N, Weiss S, Ettinger M, Pettinger M, Cain D, et al. Biological variability of serum and urinary N-telopeptides of type I collagen in postmenopausal women. *J Bone Miner Res* 2000;15:594–8.
- Garnero P, Sornay-Rendu E, Chapuy MC, Delmas PD. Increased bone turnover in late postmenopausal women is a major determinant of osteoporosis. *J Bone Miner Res* 1996;11:827–34.
- Garnero P, Sornay-Rendu E, Duboeuf F, Delmas PD. Markers of bone turnover predict postmenopausal forearm bone loss over 4 years: the Ofely Study. *J Bone Miner Res* 1999;14:1614–21.
- Garnero P, Sornay-Rendu E, Claustrat B, Delmas P. Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the Ofely Study. *J Bone Miner Res* 2000;15:1526–36.
- Garnero P, Sornay-Rendu E, Delmas PD. Low serum IGF-1 is a strong predictor of osteoporotic fracture in postmenopausal women: the Ofely Study. *Lancet* 2000;355:898–9.
- Black DM, Palermo L, Nevitt MC, Genant KK, Christensen L, Cummings SR. Defining incident vertebral deformity: a prospective comparison of several approaches. *J Bone Miner Res* 1999;14:90–101.
- Sherman BM, West JH, Korenman SG. The menopausal transition: analysis of LH, FSH, estradiol and progesterone concentration during the menstrual cycles of older women. *J Clin Endocrinol Metab* 1976;42:629–36.
- Chapurlat RD, Garnero P, Sornay-Rendu E, Arlot ME, Delmas PD. Longitudinal study of bone loss in pre and perimenopausal women: evidence for bone loss in perimenopausal women. *Osteoporos Int* 2000;11:493–8.

23. Heany RP, Recker RR, Saville RP. Menopausal changes in bone remodeling. *J Lab Clin Med* 1978;92:964–70.
24. Delmas PD, Stenner D, Wahner HW, Mann KG, Riggs BL. Increase in serum γ -carboxyglutamic acid protein with aging in women. *J Clin Invest* 1983;71:1316–21.
25. Melton LJ III, Khosla S, Atkinson EJ, O'Fallon WM, Riggs BL. Relationship of bone turnover to bone density and fractures. *J Bone Miner Res* 1997;12:1083–91.
26. Johansen JS, Riis BJ, Delmas PD, Christiansen C. Plasma BGP: an indicator of spontaneous bone loss and effect of estrogen therapy in postmenopausal women. *Eur J Clin Invest* 1988;18:191–5.
27. Ross PD, Knowlton W. Rapid bone loss is associated with increased levels of biochemical markers. *J Bone Miner Res* 1998;13:297–302.
28. Osamu C, Yoshikata I, Kikuchi R, Nakayama M, Uchiyama Y, Hirahara F, Gorai I. The predictive value of bone mineral density in postmenopausal Japanese women. *J Bone Miner Res* 2000;15:1537–44.
29. van Daele PLA, Seibel MJ, Burger H, Hofman A, Grobbee DE, van Leeuwen JPTM, et al. Case-control analysis of bone resorption markers, disability, and hip fracture risk: the Rotterdam study. *BMJ* 1996;312:482–3.
30. Garnero P, Hausher E, Chapuy MC, Marcelli C, Grandjean H, Muller C, et al. Markers of bone resorption predict hip fracture in elderly women: the Epidos prospective study. *J Bone Miner Res* 1996;11:1531–8.
31. Ross PD, Kress BC, Parson RE, Wasnich RD, Armour KA, Mizrahi IA. Serum bone alkaline phosphatase and calcaneus bone density predict fractures: a prospective study. *Osteoporos Int* 2000;11:76–82.
32. Parfitt AM, Mathews CHE, Villanueva AR, Kleerekoper M, Frame B, Rao DS. Relationship between surface, volume and thickness of iliac trabecular bone in aging and in osteoporosis. *J Clin Invest* 1983;72:1396–409.
33. Garnero P, Dargent-Molina P, Hans D, Schott AM, Bréart G, Meunier PJ, Delmas PD. Do markers of bone resorption add to bone mineral density and ultrasonographic heel measurement for the prediction of hip fracture in elderly women? The EPIDOS prospective study. *Osteoporos Int* 1998;8:563–9.
34. Hetland M, Haarbo J, Christiansen C. Low bone mass and high bone turnover in male long distance runners. *J Clin Endocrinol Metab* 1993;77:656–62.
35. Welsh L, Rutherford OM, James I, Crowley C, Comer M, Wolman R. The acute effects of exercise on bone turnover. *Int J Sports Med* 1997;18:247–51.
36. Voitge HW, Friedmann B, Suttner S, Farahmand I, Müller M, Schmidt-Gayk H, et al. Changes in bone turnover induced by aerobic and anaerobic exercise in young males. *J Bone Miner Res* 1998;12:1797–804.
37. Fujimura R, Ashizawa N, Watanabe M, Mukai N, Amagai H, Fukubayashi T. Effect of resistance exercise training on bone formation and resorption in young male subjects assessed by biomarkers of bone metabolism. *J Bone Miner Res* 1997;12:656–62.
38. Eliakim A, Raizis L, Brasel A, Cooper D. Evidence for increased bone formation following a brief endurance-type training intervention in adolescent males. *J Bone Miner Res* 1997;12:1708–13.
39. Chapurlat D, Garnero P, Bréart G, Meunier P, Delmas PD. Serum type I collagen breakdown product (serum CTX) predicts hip fracture in elderly women: the Epidos study. *Bone* 2000;27:283–6.