

Clinical evaluation of the Serum CrossLaps One Step ELISA, a new assay measuring the serum concentration of bone-derived degradation products of type I collagen C-telopeptides

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The Serum CrossLaps™ One Step ELISA is a sandwich assay using two monoclonal antibodies specific for a β -aspartate form of the epitope EKAHDGGR derived from the carboxy-terminal telopeptide region of type I collagen α_1 -chain. Our objective was to assess the clinical value of the Serum CrossLaps assay for monitoring antiresorptive therapy in osteoporosis treatment. Samples obtained from postmenopausal women treated with different doses of cyclic or continuous hormone replacement therapy (HRT) with an estrogen analog (tibolone) or with a bisphosphonate (ibandronate) were measured in the Serum CrossLaps One Step ELISA at baseline and at various time points during therapy. The corresponding urine samples were measured in the urine CrossLaps™ ELISA and corrected for creatinine excretion. The serum CrossLaps measurements and corresponding urinary CrossLaps measurements were highly correlated ($r > 0.8$ for all studies). The serum and urine CrossLaps measurements showed a significant decrease among the women treated with clinically relevant doses of either of the antiresorptive agents. Furthermore, the annual percentage change in bone mineral density (BMD) correlated with the measured changes in CrossLaps concentration. The serum CrossLaps assay showed a specificity of 83–100% and a sensitivity of 59–83% for assessing BMD changes. The corresponding values for the creatinine-corrected urinary measure-

ments were 83–92% specificity and 68–79% sensitivity. We conclude that performance of the convenient Serum CrossLaps One Step ELISA is at least equivalent to that of the urine test for follow up of antiresorptive treatment in osteoporosis. Further studies are needed to optimize its use in this and other clinical applications.

Prediction of the risk of osteoporosis and accurate assessment of the clinical progression of the disease may be facilitated by measurement of biochemical markers of bone metabolism (1, 2). Degradation products derived from osteoclastic bone resorption of the bone matrix can be used as a specific and sensitive index of the resorption process. The CrossLaps™ ELISA measures molecules in urine derived from the C-terminal telopeptide of collagen type I. Molecules measured in this assay contain the core epitope AHD- β -GGR, where the peptide bond between the aspartate and glycine residues are rearranged from the α - to the β -carboxyl group (3, 4). The CrossLaps assay has been shown to provide an index of bone resorption in women undergoing therapy with antiresorptive agents (5, 6).

Several studies have shown that biochemical markers of bone resorption are of clinical value when used for monitoring the effect of antiresorptive therapy (2, 7). The effect of antiresorptive therapy can also be assessed by measurement of bone mineral density (BMD).³ However, the precision error for bone densitometry is ~1–5% (8); thus extended follow-up periods are generally necessary to assess a statistically significant effect of antiresorptive therapy by BMD measurements. In contrast, a clear re-

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³ Nonstandard abbreviations: BMD, bone mineral density; HRT, hormone replacement therapy; and EP, piperazine estrone sulfate.

sponse measured by biochemical markers of bone resorption has been demonstrated within a short period of time after initiation of the therapy (2, 5, 7). Hence, it has been suggested that biochemical markers such as CrossLaps are valuable in monitoring the effect of antiresorptive therapy (2, 5, 9, 10). We have developed an ELISA for measurement of CrossLaps molecules in serum (11); thus, the C-terminal degradation products of collagen type I measured in the CrossLaps assay can now be measured in both urine and serum. Assessments of the clinical value of the Serum CrossLaps One Step ELISA have not been published before.

Hormone replacement therapy (HRT) has been used for more than two decades to alleviate climacteric symptoms in postmenopausal women, and the ability of this therapy to reduce postmenopausal bone loss is well established (12). However, HRT often has adverse effects (13, 14), which have prompted the development of new therapeutic agents for prevention of bone loss associated with cessation of estrogen production at menopause and for treatment of osteoporosis (15–17).

The present study investigates the clinical utility of both the serum and urine CrossLaps markers for monitoring the effect of HRT and therapy with an estrogen analog (tibolone) or bisphosphonate (ibandronate) and compares these assays to state-of-the-art BMD measurements. Tibolone is a synthetic steroid with a combination of estrogenic, androgenic, and progestogenic properties (18, 19). Ibandronate is highly efficient for reducing bone resorption (7). We assess the ability of the CrossLaps markers to monitor the responses to therapy, and we compare the value of these markers of bone resorption with results obtained by spinal BMD measurements.

Materials and Methods

CLINICAL TRIALS

The clinical trials were performed according to the Helsinki Declaration II and the European standard for good clinical practice after approval by the Copenhagen county research ethics committee. All persons gave written informed consent to participate in the studies. The study populations and baseline demographic data have been described in recent publications (7, 18). Below is an outline of the populations used in the studies.

TIBOLONE STUDY GROUP

The study group comprised 91 healthy, postmenopausal women with BMD values within health-related reference intervals. At baseline they were at least 10 years past natural menopause and <76 years of age (mean, 67 years). The women were randomly allocated to three groups for assessment of the effect of tibolone [(7 α ,17 α)-17-hydroxy-7-methyl-19-norpregn-5(10)-en-20-yn-3-one; livial; Organon]. Thirteen women received only placebo (400 mg of calcium daily), 29 women received 1.25 mg of tibolone in addition to the calcium daily, and 28 women received 2.5

mg of tibolone in addition to calcium daily. The women were followed during a 2-year study period. All women were monitored for spinal BMD, and serum and urine (fasting, second morning void) samples were taken at 3-month intervals during the study period. The baseline demographic data and inclusion criteria for the study-population are described in more detail by Bjarnason et al. (18).

IBANDRONATE STUDY GROUP

The study group originally comprised 180 healthy, postmenopausal women, at least 10 years past natural menopause and <75 years of age (mean, 65 years) who entered a double-blind placebo-controlled dose-finding study of the bisphosphonate ibandronate (ibandronate, Boehringer Mannheim). The participants had a BMD in the distal forearm at least 1.5 SD below the premenopausal mean for healthy white women. One hundred and forty-one women completed the full 1-year study period, and all samples and measurements were available for 131 women. Twenty-three women received placebo; 23 women received 0.25 mg of ibandronate; 21 women received 0.5 mg of ibandronate; 24 women received 1 mg of ibandronate; 23 women received 2.5 mg of ibandronate; and 17 women received 5 mg of ibandronate. All of these women received 1 g of calcium daily. All women were monitored for spinal BMD, and serum and urine (fasting, second morning void) samples were taken at 3-month intervals during the 12-month study period. The study group and baseline demographic data are described by Ravn et al. (7).

HRT STUDY GROUP

The study group originally comprised 200 healthy, Caucasian postmenopausal women, at least 12 months past natural menopause and <65 years of age (mean, 59 years) with a BMD within health-related reference intervals. Two hundred women were enrolled and randomly allocated into one of four groups of 50 each. One hundred and fourteen women completed the full 2-year study period and had samples available for all measuring times. Thirty-four women received placebo treatment; 33 women received continuous HRT with 2 mg of estradiol and 1 mg of norethisterone acetate daily (kliogest); 38 women received a cyclic regimen of 0.75 mg/day piperazine estrone sulfate (EP) plus five periods of 3 days/month with norethisterone (low EP); and 31 women received 1.5 mg/day EP plus five periods of 3 days with 0.7 mg/day norethisterone (high EP). All women were monitored for spinal BMD, and serum and urine (fasting, second morning void) samples were taken at 6-month intervals (0, 6, 12, 18, and 24 months) during the 2-year study period. The study group and baseline demographic data are described in detail by Alexandersen et al. (manuscript submitted for publication).

SERUM CrossLaps ONE-STEP ELISA

The Serum CrossLaps One Step ELISA is performed as a monoclonal sandwich assay in a one-step procedure. Calibrators, controls, or unknown serum samples are added into microtiter wells coated with streptavidin, followed by a mixture of a biotinylated antibody and a peroxidase-conjugated antibody. A complex between CrossLaps antigens, biotinylated antibody, and peroxidase-conjugated antibody will be generated, and this complex will bind to the streptavidin surface via the biotinylated antibody. The amount of bound antigen is quantified by the use of a chromogenic peroxidase substrate. A more thorough description and characterization of the assay is presented by Rosenquist et al. (11) in this issue.

URINE CrossLaps ELISA

Urinary excreted degradation products of type I collagen were measured by the CrossLaps ELISA (Osteometer Biotech). This assay uses a polyclonal antiserum specific for the CrossLaps antigens in a competitive ELISA format (20).

BMD MEASUREMENTS

BMD in the lumbar spine was measured at baseline and every 3 or 6 months thereafter. BMD was measured by dual energy x-ray absorptiometry (Hologic, Inc., model QDR-2000 or QDR-1000) in the lumbar spine anterior posterior projection (L1–L4) according to the manufacturer's instructions (7, 18).

STATISTICAL ANALYSIS

The comparability of the study groups at baseline was assessed as appropriate by one-way ANOVA. The serial measurements of the biochemical markers were calculated as percentage of baseline values, which were set at 100% for each woman and used as summary measures in the response groups to account for individual differences at baseline and to get a harmonized way of presentation. The individual responses in BMD at the different regions were calculated as the slopes of the lines estimated by linear regression, using serial BMD measurements and used as a summary measure (percentage of change per year was designated α -BMD). Single and multiple regressions were used to examine the relationship between the response in BMD and the change from baseline in serum CrossLaps measurements. The diagnostic validity of the serum CrossLaps marker was analyzed by ROC analysis. The number of "true positives" were plotted against the number of "true negatives" at different cutoff values for change in CrossLaps concentration. The cutoff value used to calculate the sensitivity and specificity was a spinal α -BMD of -1% per year. This cutoff value was used to take into account the precision error of the spinal BMD measurements (1% per year).

Results

EFFECT OF TREATMENT WITH TIBOLONE

We evaluated the effect of this estrogen analog on the serum CrossLaps concentration by measuring samples from 91 women who were subjected to a double-blind placebo-controlled clinical study as described previously (18, 19). Fig. 1 shows the dose responses measured in each of the three groups in the two CrossLaps assays, where the values for each woman are expressed in percentage of change from the baseline values. The overall correlation between serum and urinary CrossLaps measurements for all samples measured in the study was 0.80.

BMD measurements of the spine demonstrated a dose-dependent increase as a result of tibolone treatment (19). The annual percentage of change in spinal BMD (α -BMD) showed an increase significantly different from the pla-

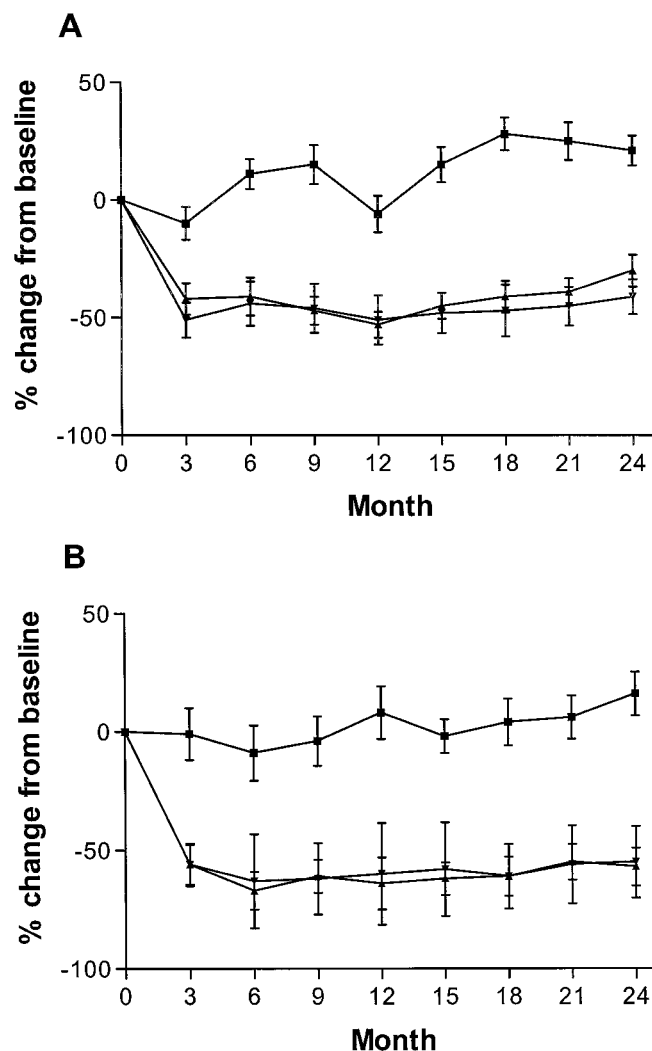


Fig. 1. Serial measurement of dose response in the tibolone study.

(A) Serial measurements of Serum CrossLaps concentration; (B) corresponding creatinine-corrected urine CrossLaps measurements. Results are expressed in percentage of change from baseline and are given as the mean (%) with SE (bars) within each group. Urinary CrossLaps measurements have been reported previously (18). Average SE of the serum measurements was 7.5% and 11.4% for the urinary measurements. ■, 0 mg/day; ▲, 1.25 mg/day; ▼, 2.5 mg/day.

Table 1. ROC analysis of sensitivity and specificity of changes in serum and urinary CrossLaps measurement for prediction of α -BMD response.^a

Study	Correlation to α -BMD	Cutoff	Specificity	Sensitivity	Area under the curve	95% confidence interval
Tibolone						
Serum	$r = -0.62$	-25.4%	83%	83%	0.833	0.719-0.915
Urine	$r = -0.56$	-36.8%	83%	79%	0.797	0.678-0.888
Ibandronate						
Serum	$r = -0.53$	-54.9%	92%	59%	0.788	0.688-0.868
Urine	$r = -0.52$	-52.8%	92%	68%	0.811	0.714-0.887
HRT						
Serum	$r = -0.54$	-27.8%	100%	82%	0.869	0.800-0.921
Urine	$r = -0.43$	-41.2%	92%	72%	0.821	0.746-0.882

^a The difference in CrossLaps measurement at baseline and after 12 months was calculated (% change from baseline) and used in ROC analysis to predict the BMD response (significant net BMD loss: α -BMD $< -1\%$; no significant BMD change and BMD gain: α -BMD $\geq -1\%$) in all three study populations.

cebo group among the women treated with any one of the three tibolone regimens. In the placebo group no significant change in spinal BMD was observed. When α -BMD was correlated to the change in serum CrossLaps concentration observed over 12 months, $r = -0.62$ was obtained (Table 1). The change over 3, 6, and 9 months correlated, with $r = -0.53$, -0.48 , and -0.59 , respectively. Likewise the changes in urinary CrossLaps concentration observed after 3, 6, 9, and 12 months correlated to α -BMD with $r = -0.54$, -0.49 , -0.59 , and -0.56 , respectively.

The diagnostic sensitivity and specificity of changes in serum and urinary CrossLaps concentration observed after 12 months for predicting changes in spine BMD was calculated by ROC (Table 1). Both assays showed a specificity of 83%, whereas the sensitivity was 83% for the serum assay and 79% for the urinary CrossLaps assay for prediction of BMD response. The odds ratio for significant bone loss (α -BMD $< -1\%$) was 24.0 for the serum and 7.7 for the urinary CrossLaps measurements when the women showing an insignificant decrease or an increase in CrossLaps concentration were compared with the women showing a significant decrease (Table 2). The odds

ratio of significant bone gain (α -BMD $> 1\%$) was 33.3 and 41.0, respectively, for serum and urinary measurements when the women showing a larger change in the biochemical markers were compared with women showing an insignificant decrease or an increase.

EFFECT OF IBANDRONATE TREATMENT OF POSTMENOPAUSAL WOMEN

The baseline demographic data and other data about the study population have been reported previously (7). Urine and serum samples, taken at 3-month intervals, were available for 131 women who had completed a 1-year dose-finding study of the effect of ibandronate. The measurements were expressed in percentage of change from the baseline values for each woman, and the average responses within each of the five treatment groups are shown in Fig. 2. The effect of the treatment could be detected by serum CrossLaps measurements after 3 months of treatment, where the women receiving the clinically relevant doses of 1, 2.5, or 5 mg daily showed an average decrease of 67.6%, 77.4%, and 79.4%, respectively, from the baseline values. A similar effect was also seen in

Table 2. Odds ratio analysis of the risk of significant bone loss (α -BMD $< -1\%$) or chance of significant gain in BMD (α -BMD $> 1\%$) calculated for the three study populations.^a

Study	Cutoff	Odds ratio of α -BMD $< -1\%$ when change is lower than cutoff	95% confidence interval	Odds ratio of α -BMD $> 1\%$ when change is higher than cutoff	95% confidence interval
Tibolone					
Serum	-25.4%	24.0	2.5-228	33.3	6.3-177
Urine	-36.8%	7.7	1.3-46.9	41.0	7.6-220
Ibandronate					
Serum	-54.9%	6.3	1.3-31.2	3.8	1.4-10.1
Urine	-52.8%	22.1	2.7-182	6.7	2.4-19.0
HRT					
Serum	-27.8%	>100		12.4	5.0-31.2
Urine	-41.2%	22.7	3.4-222	11.0	4.4-27.9

^a The women were separated into two groups showing either a significant decrease in CrossLaps concentration or an insignificant decrease or an increase in concentration, using the cutoff values for change in the markers as determined by ROC analysis (Table 1). The odds ratios with 95% confidence intervals are calculated for the difference in risk of significant bone loss in the two groups of women, classified according to change in CrossLaps concentration. The odds ratios between the chances of BMD gain in the two groups of women in each study population are also given. In the HRT study, all of the women with a significant bone loss (α -BMD $< -1\%$) had an increase or a decrease in serum CrossLaps concentration $< -27.8\%$ (see Fig. 6), which renders exact calculation of an odds ratio impossible.

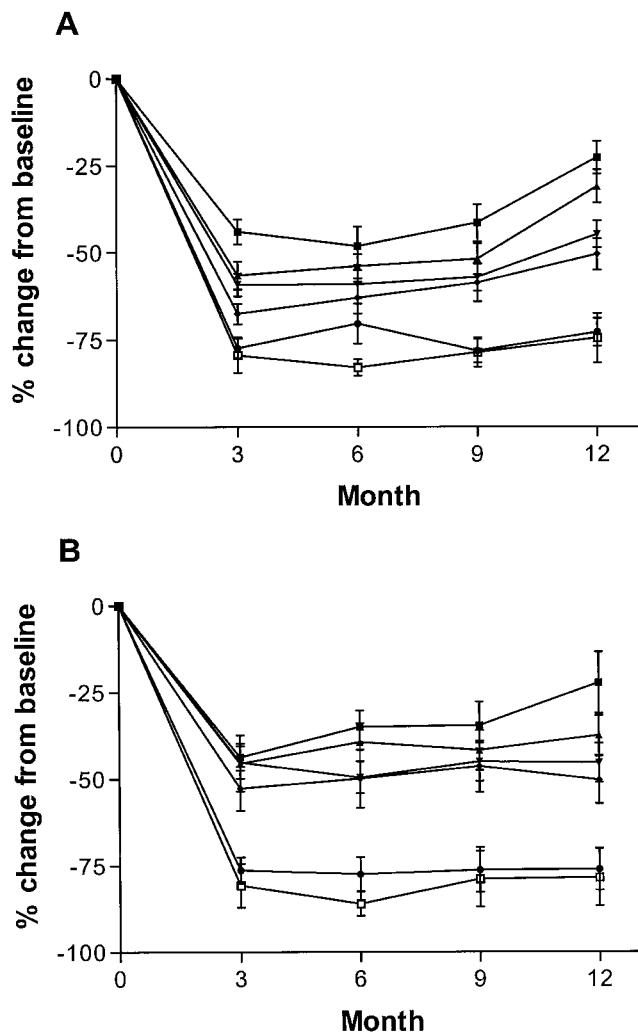


Fig. 2. Serial measurements of Serum CrossLaps concentration (A) and creatinine urine CrossLaps concentration (B) in the six groups of women given placebo or ibandronate.

Results are expressed in percentage of change from baseline and given as the mean (%) with SE (bars) within each group. Urinary CrossLaps measurements have been reported previously (7). Average SE was 4.3% and 6.1% for the serum and urinary measurements, respectively. ■, 0 mg/day (placebo); ▲, 0.25 mg/day; ▼, 0.5 mg/day; ◆, 1 mg/day; ●, 2.5 mg/day; □, 5 mg/day.

urinary CrossLaps measurements, with decreases after 3 months of 52.7%, 76.3%, and 80.7%, respectively, in the groups receiving 1, 2.5, and 5 mg ibandronate. Measurements of urinary and serum CrossLaps concentrations correlated well, with an overall $r = 0.86$ for all measurements in the ibandronate study.

The changes observed over 6 and 12 months in serum CrossLaps concentration as a result of ibandronate therapy correlated with the changes observed in spinal BMD ($r = -0.38$ and -0.53 , respectively; Table 1). In addition, the changes observed in urinary CrossLaps correlated with α -BMD ($r = -0.36$ and -0.52 for changes observed over 6 and 12 months, respectively; Table 1). The diagnostic sensitivity and specificity for the change in CrossLaps concentration over 12 months for predicting the

α -BMD response was calculated by ROC analysis (Table 1). Odds ratios for the risk of significant bone loss (α -BMD $< -1\%$) or gain (α -BMD $> 1\%$) were calculated for women in the two groups showing either a significant decrease in CrossLaps concentration or an insignificant decrease or an increase in the concentration (Table 2).

EFFECT OF HRT

The effect of HRT on serum CrossLaps concentration was assessed by measurements of samples from 136 women who had participated in a 2-year study of the effect of cyclic and continuous HRT (Alexandersen et al., manuscript submitted for publication). Urine and serum samples were measured in the urine CrossLaps ELISA and Serum CrossLaps One Step ELISA, respectively (Fig. 3). The correlation between serum and corresponding creatinine-corrected urine CrossLaps measurements was 0.86. Both the serum and creatinine-corrected urine CrossLaps measurements showed a significant decrease among the three HRT-treated groups, whereas the placebo group showed no overall change during the study period (Fig. 3). Fig. 4 shows the response in serum and urine CrossLaps over the first 6 months of the study period in each individual in the four treatment groups. The changes were significantly different in the three groups of women receiving HRT compared with the placebo group ($P < 0.001$).

Spine α -BMD correlated with the change in serum CrossLaps concentration over 6, 12, 18, and 24 months with the following coefficients: $r = -0.56$, -0.54 , -0.66 , and -0.55 (Fig. 5). The corresponding values for the creatinine-corrected urinary measurements were $r = -0.27$, -0.43 , -0.62 , and -0.44 (Table 1 and Fig. 5).

ROC analysis was used to calculate the sensitivity and specificity for the changes measured over 12 months in the CrossLaps assays to predict α -BMD in individual patients (Table 1). The change in serum CrossLaps concentration observed after 12 months showed a sensitivity of 82% and a specificity of 100% for predicting the BMD response using a cutoff value of α -BMD = -1% /year and a calculated cutoff value for significant change in serum CrossLaps concentration of 27.8% decrease from baseline (Fig. 6, A and C). The diagnostic sensitivity and specificity of changes in urine CrossLaps concentration to predict changes in spinal BMD were 72% and 92%, respectively, at 12 months, using the same cutoff value of -1% for the α -BMD measurements and a calculated cutoff value of significant change in the concentration of the urinary CrossLaps of 41.2% (Fig. 6, B and D).

When the women were stratified in quartiles according average on-study concentration of either urinary or serum CrossLaps concentration, a correlation to α -BMD was apparent as the highly significant differences in average α -BMD concentration between the first and the last quartile ($P < 0.0001$; data not shown).

Odds ratios for the risk of significant bone loss (α -BMD $< -1\%$) or gain (α -BMD $> 1\%$) were calculated for women

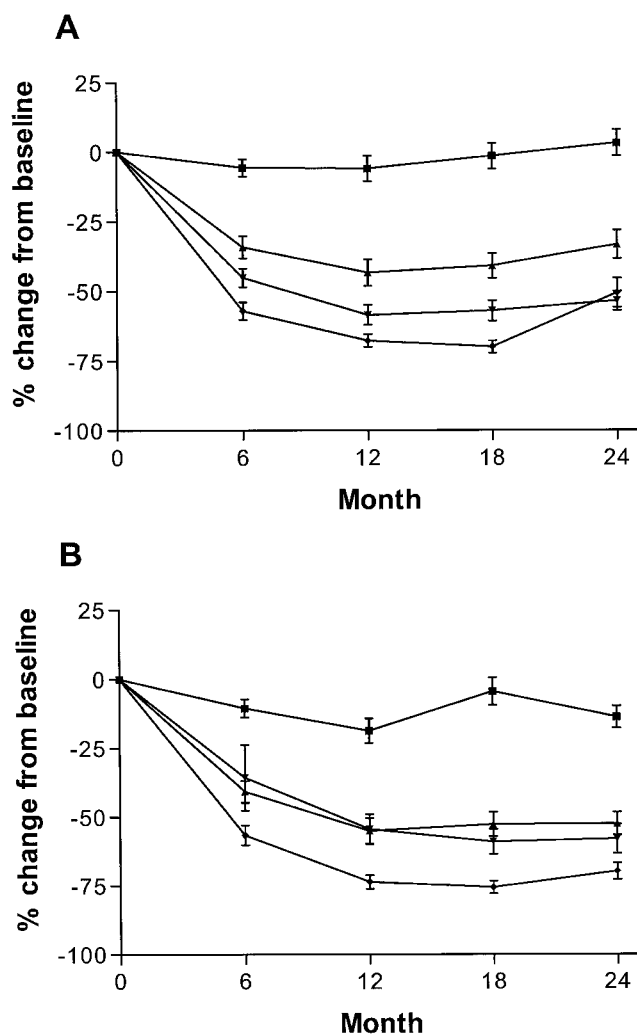


Fig. 3. Serial measurements of Serum CrossLaps concentration (A) and creatinine-corrected urine CrossLaps concentration (B) in the four groups of women given placebo, low EP, high EP, or kligest.

Results are expressed in percentage of change from baseline and given as the mean (%) with SE (bars) within each group. Average SE of the serum measurements was 3.9% and 4.5% for the urinary measurements. ■, placebo; ▲, 0.75 mg EP daily + cyclic norethisterone (0.35 mg; low EP); ▼, 1.5 mg daily EP + cyclic norethisterone (0.7 mg; high EP); ◆, continuous HRT therapy with 1 mg of 17β -estradiol + norethisterone acetate daily (kligest).

in the two groups showing either a significant decrease in CrossLaps concentration or an insignificant decrease or an increase in the concentration (Table 2).

We assessed whether baseline CrossLaps concentrations was also associated with BMD changes, and whether this marker was predictive for the outcome of antiresorptive therapy (Table 3). The women in either the placebo group or the combined HRT-treated group were stratified into quartiles according to baseline serum CrossLaps concentration or according to creatinine-corrected urine CrossLaps concentration. The average α -BMD in each quartile was calculated. In the placebo group the women in the lowest quartile of serum CrossLaps had a net gain in spinal BMD of 0.66% per year, whereas the women in

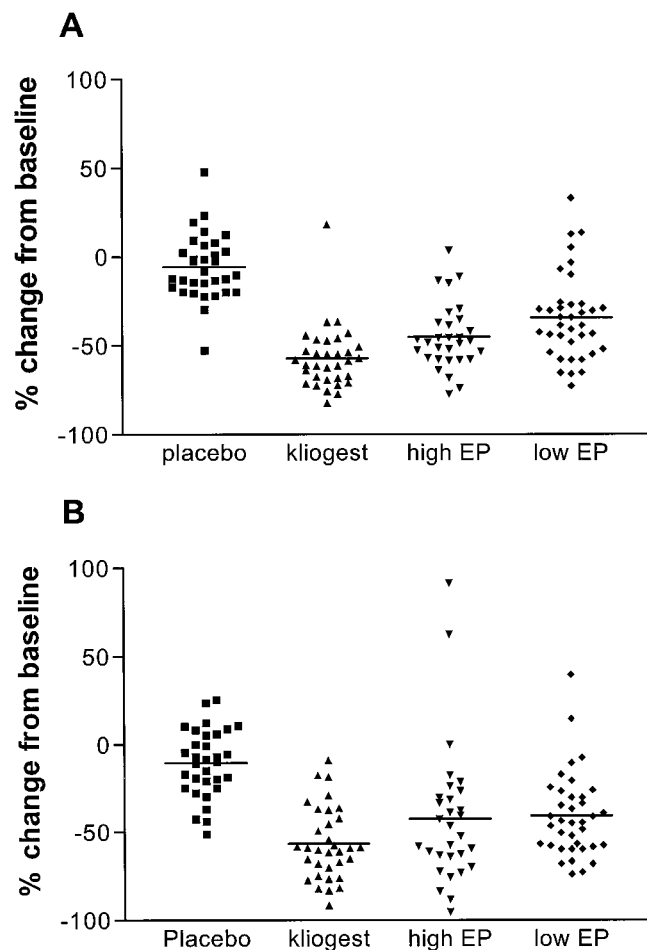


Fig. 4. Change in serum (A) and urinary (B) CrossLaps concentration during the first 6 months in each individual in the four groups of women receiving placebo or HRT treatment.

The mean and 95% confidence intervals of the serum measurements are -5.6% (1.0 to -12.3%), -57.0% (-50.7 to -63.4%), -45.16% (-38.3 to -52.1%), and -34.2% (-26.2 to -42.3%) for the placebo, kligest, high EP, and low EP groups, respectively. The corresponding values for the urinary measurements are -10.4% (-3.5 to -17.3%), -56.6% (-49.3 to -63.9%), -42.4% (-27.6 to -57.2%), and -40.8% (-32.8 to -48.8%). Symbols and dosages are as in Fig. 3.

the highest quartile of serum CrossLaps concentration had an average annual BMD loss of 0.26%. When the stratification was carried out according to the urinary CrossLaps measurements, the lowest quartile among the placebo group was found to have a net gain in spine BMD of 0.42% per year; the highest quartile had an average annual loss of 0.23% spine BMD. Because of the small number of individuals, these differences were not statistically significant. Among the HRT-treated women, the lowest quartile showed a significantly lower response to the therapy than the women in the highest quartile (average α -BMD, 2.5 for the lowest quartile and 4.53 for the highest quartile; $P = 0.0051$) when stratified according to serum CrossLaps measurements, whereas the difference between the highest and lowest quartile stratified according to urine CrossLaps was statistically insignificant (average α -BMD, 3.06 for the lowest quartile and 3.95 for the highest quartile).

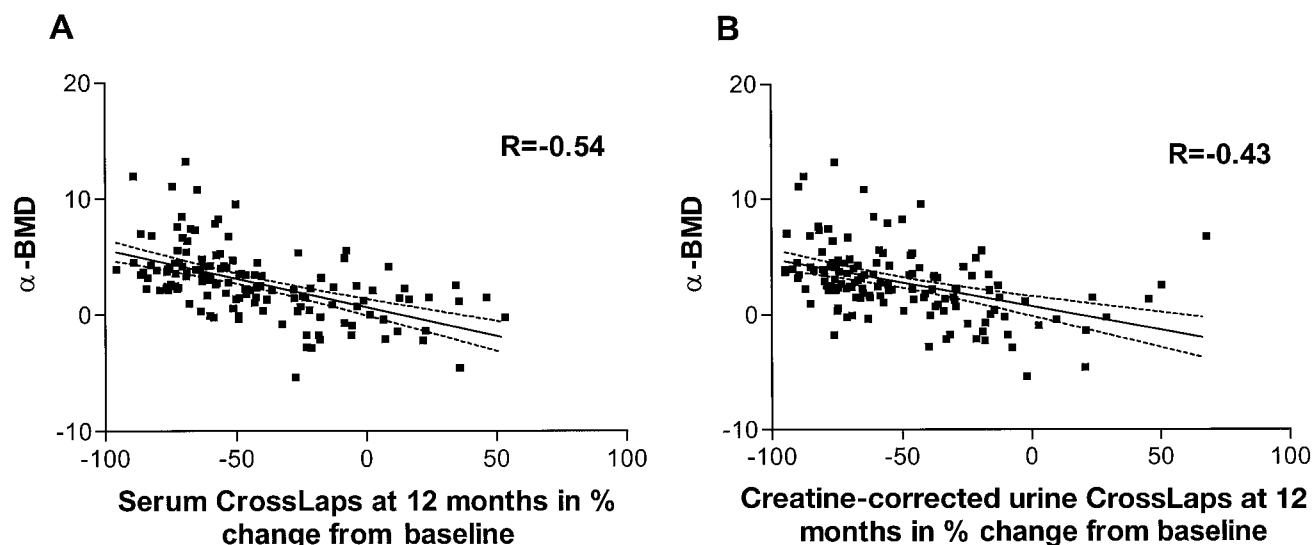


Fig. 5. The correlation between the response in spinal BMD (% change per year) and the change from baseline in serum (A) and creatinine-corrected urine (B) CrossLaps for all women in the HRT study.

The change in BMD was calculated as the average annual percentage of change (α -BMD). The change in CrossLaps concentration was calculated as percentage of change from the baseline measurements after 12 months.

The relative risk of losing bone if not treated with HRT was significantly larger among the women in the highest quartile of baseline serum CrossLaps concentration (2.7-fold relative risk; 95% confidence interval, 1.3–5.4) compared with the women in the lowest quartile (1.2-fold relative risk; 95% confidence interval, 0.7–2.0). Similarly, when the women were stratified according to baseline urine CrossLaps measurements, the women in the highest quartile had a significantly higher relative risk of losing bone if not treated with HRT compared with the women in the lowest quartile [relative risk, 2.5 (1.3–5.0) vs 1.5 (0.9–2.6)].

Discussion

The main objective of the present study has been to assess the clinical value of the Serum CrossLaps One Step ELISA compared with the urine CrossLaps ELISA and spinal

BMD measurements for evaluation of antiresorptive therapy in prevention and treatment of osteoporosis.

The Serum CrossLaps One Step ELISA measures collagen type I C-telopeptide fragments containing two cross-linked EKAHD- β -GGR epitopes. The serum assay exclusively measures molecules where the peptide bond between the aspartate (D) and the glycine residue (G) has undergone a spontaneous rearrangement from the α - to the β -carboxyl group of the aspartate side chain (3), and the lysine residue (K) participates in an intermolecular covalent cross-link (21). The urinary CrossLaps assay is performed as a competitive assay measuring both cross-linked and non-cross-linked fragments containing the EKAHD- β -GGR epitope (3). In spite of this difference in assay formats, the correlation between the serum and urine concentrations of the molecules measured in

Table 3. Stratification of women from the HRT study according to baseline CrossLaps concentration.^a

	Quartile			
	1	2	3	4
Baseline CrossLaps conc. cutoff values				
Serum, pmol/L	2295	2967	4006	7021
Urine, mg/mol	235	314	428	899
Average α -BMD (% change per year) within each quartile				
Placebo				
Serum	0.66	-0.29	-0.56	-0.29
Urine	0.42	-0.34	-0.41	-0.23
HRT-treated				
Serum	2.50	3.88	3.97	4.53
Urine	3.06	3.83	3.80	3.95

^a The placebo or HRT-treated women were stratified into quartiles according to either baseline serum or urine CrossLaps concentration, and the average α -BMD (% change per year) was calculated for each quartile. The cutoff values (upper limits in each quartile) used for separation in the four quartiles are given in the upper portion of the table.

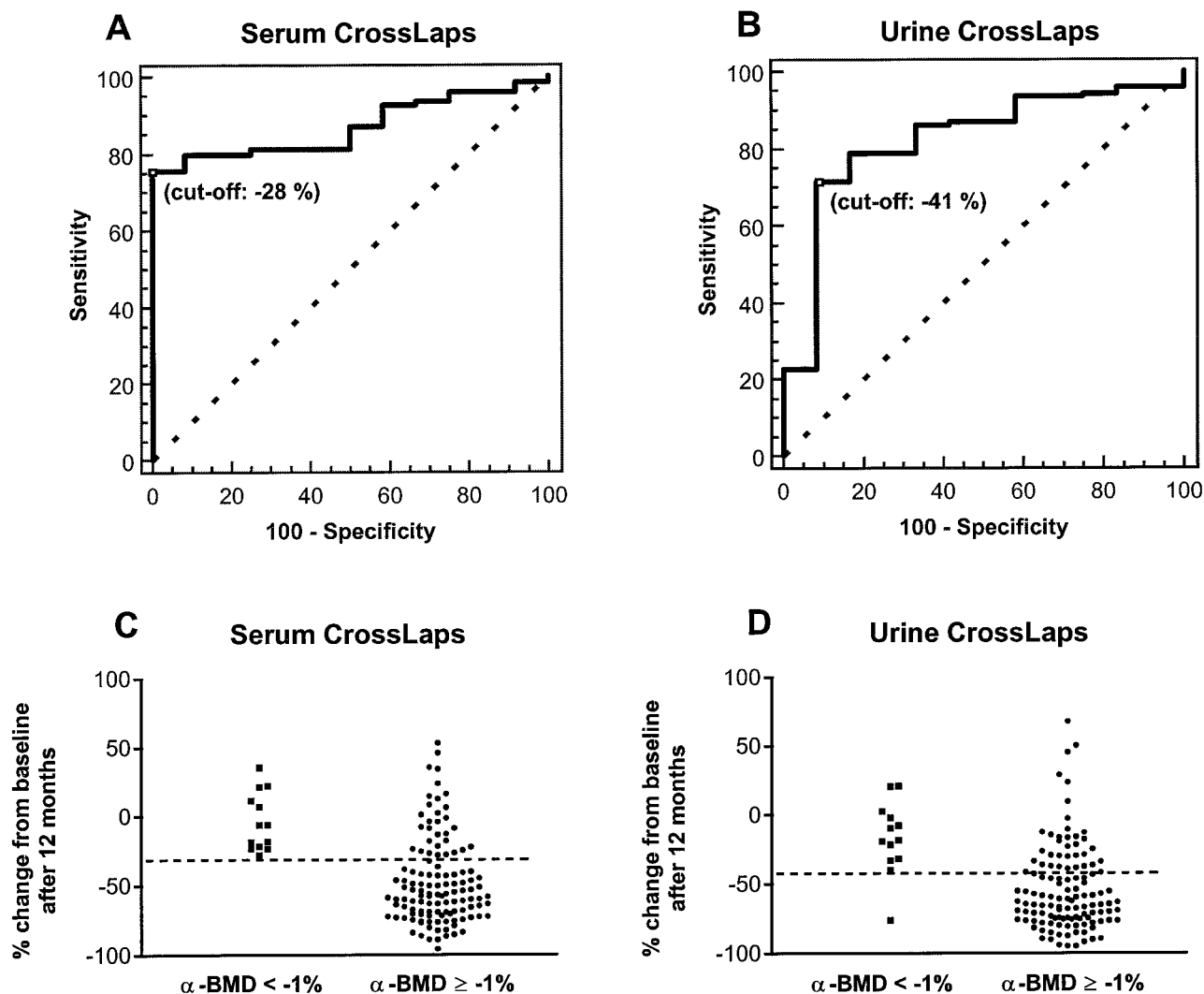


Fig. 6. ROC analysis of the predictive ability of changes in serum (A and C) and urinary (B and D) CrossLaps measurements observed over 12 months for prediction of spine BMD response.

The change from baseline (%) was calculated for each woman participating in the HRT study, and ROC analysis (A and B) was used for calculation of sensitivity and specificity for predicting significant net bone loss ($\alpha\text{-BMD} < -1\%$) or no significant loss or gain of BMD ($\alpha\text{-BMD} \geq -1\%$) over the 24-month study period. The women were grouped according to their $\alpha\text{-BMD}$ responses into two groups ($\alpha\text{-BMD} < -1\%$ or $\alpha\text{-BMD} \geq -1\%$), and changes in serum (C) and urine (D) CrossLaps concentrations for each woman in the groups were plotted.

the two CrossLaps assays was above $r = 0.8$ for all studies reported here. This suggests that the serum and urinary CrossLaps assays measure similar populations of antigens (11).

The restriction of the serum CrossLaps assay to measurement of fragments containing two posttranslational modifications (β -isomerization and intermolecular cross-links) may be important for assuring that the majority of the molecules measured in the assay are derived from resorption of mature collagen and not generated during synthesis of new collagen during bone formation (4). Hence, the measurements are likely to represent a true indicator for the resorptive process, with no or very little

contribution from bone formation. However, this will need to be established in future studies.

The C-telopeptide fragments of type I collagen measured in the Serum CrossLaps One Step ELISA may be generated by several tissues unrelated to bone because type I collagen is widespread throughout the body (22). However, the pronounced effects of all tested antiresorptive therapies suggest that the large majority of the molecules measured in the serum CrossLaps assay are bone-derived. The decreases of $>50\%$ in the serum CrossLaps concentration observed in all women as a result of treatment with the two highest doses of ibandronate are especially noteworthy. Ibandronate and other bisphos-

phonates have not been reported to have any effect on collagen metabolism in tissues other than bone (15).

Another serum assay for a C-telopeptide fragment of type I collagen, the ICTP assay (23, 24), has been described previously. This marker has shown only a very limited response to antiresorptive bisphosphonate therapy (25, 26). Furthermore, ICTP does not reflect the decreased bone resorption obtained by HRT or other antiresorptive therapies (10, 11). The epitope recognized by the ICTP assay is believed to comprise a larger part of the cross-linked telopeptide closer to the N terminus than the EKAHDGGR epitope recognized in the CrossLaps assay, as well as parts of the α -helix (27). It has been demonstrated in vitro that CrossLaps reactive fragments are generated by osteoclasts (28). Whether the larger ICTP reactive fragments are generated during osteoclastic bone resorption or during collagen synthesis is not known. In fact, it has been suggested that the ICTP marker predominantly reflects collagen type I synthesis in bone and other tissues (29).

We evaluated the Serum CrossLaps One Step ELISA clinically in three studies of the antiresorptive effects of conventional HRT, tibolone, or ibandronate. The three investigated therapies have different methods of action and efficacy for reducing bone turnover (14, 15, 18). However, each drug clearly reduced bone resorption in a dose-dependent manner, as shown by the pronounced dose-dependent decreases in serum CrossLaps concentration occurring in all three studies (Figs. 1–3). A similar decrease was also observed in corresponding creatinine-corrected urinary CrossLaps measurements in accordance with previous reports on the clinical value of measurements of this biochemical marker of bone resorption (2, 5–7, 18). It is also noteworthy that in the tibolone and ibandronate studies, the antiresorptive effect of treatment assessed by the CrossLaps measurements could be demonstrated after the first 3 months (Figs. 1 and 2). In the HRT study, the response measured in either of the two CrossLaps assays over the first 6 months was significantly different in all three treated groups compared with the placebo group ($P < 0.001$; Figs. 3 and 4).

The antiresorptive effects could be monitored as an increase in spine BMD. The correlation between the average changes in spinal BMD over 1 year (α -BMD) and the change in serum concentration over 3 to 12 months varied from -0.53 to -0.62 in the three studies. Thus, the measurement of changes in the CrossLaps concentration in serum reflected the changes in BMD. The change in the urinary CrossLaps correlated with α -BMD in all studies, with correlation coefficients of the same magnitude or slightly lower than the change in the serum CrossLaps concentration (Fig. 5 and Table 1).

The sensitivity and specificity of the serum CrossLaps measurements for predicting BMD loss was calculated by ROC analysis using a cutoff value of α -BMD = -1% (Table 1). This value was chosen to take into account the precision error of the spine BMD measurements, which is 1%. Thus, only women with α -BMD $< -1\%$ can be said to

have significant bone loss. The sensitivity varied from 59% to 83% in the serum assay and from 68% to 79% in the urinary assay in the three study populations, depending on the follow-up time. The specificity varied from 83% to 100% in the serum and from 83% to 90% in the urine CrossLaps ELISA. This suggests that both CrossLaps assays are of high value for monitoring the response in individual patients, by performing a measurement at baseline and after 3–12 months of therapy, and that the change observed in the CrossLaps concentration may be used as an indicator of BMD response. When the ROC analysis was performed using other cutoff values for α -BMD (0% or 1%), similar values for specificity and sensitivity of the change in the marker for prediction of BMD response were obtained (not shown).

The ROC analysis used for calculation of the assay sensitivity and specificity also yields an assessment of the cutoff values of the biochemical markers for evaluating a significant response to the therapies (Table 1). In both the HRT and tibolone studies, a change from baseline after 12 months of ~ 25 –30% in the serum and 35–40% in the urinary CrossLaps measurements was calculated as giving the optimal sensitivity and specificity of the test when α -BMD = -1% was used as a cutoff value. For the bisphosphonate study, a change of 50–55% after 12 months therapy was found to give the best sensitivity and specificity.

The odds ratio of significant bone loss (α -BMD $< -1\%$) for women where no significant decrease in CrossLaps was observed after 12 months compared with women showing a significant decrease was 6.3 to >100 for the serum assay and 7.7–22.7 for the urine assay in the three studies (Table 2). The odds ratio for significant bone mass gain (α -BMD $>1\%$) for women showing a substantial decrease in CrossLaps concentration compared with women with a small decrease or an increase in CrossLaps concentration was 3.8–33.3 for the serum measurements, and 6.7–41.0 for the urinary CrossLaps measurements (Table 2). All odds ratios were calculated using cutoff values for significant changes in CrossLaps concentration as determined by ROC analysis (Table 1), and all odds ratios were highly significant. This again supports the observation that changes measured in the CrossLaps assays may be used as a reliable measure of BMD response.

Stratification of women according to either change in serum CrossLaps concentration or average on-study serum CrossLaps concentration demonstrated a significant difference between average α -BMD in the quartiles (not shown) in accordance with the good correlation between the change in serum CrossLaps concentration and α -BMD (Table 1 and Fig. 5). Similar results were obtained when the stratification was based on the response in urinary CrossLaps measurements. This is in accordance with results reported for another urinary marker of bone resorption measuring N-telopeptides of type I collagen (30).

Baseline CrossLaps concentrations were also associ-

ated with BMD changes and response to therapy. Among the HRT-treated women stratified according to baseline serum CrossLaps concentration, the lowest quartile showed a significantly lower response to the therapy than the women in the highest quartile (Table 2). When the stratification was performed on the basis of the baseline urinary CrossLaps measurements, the highest quartile did show a more pronounced effect of the therapy compared with the lowest quartile, but the difference was not statistically significant (Table 2). When the relative risk of losing bone if not treated with HRT was calculated for women in the highest quartile of baseline CrossLaps concentration, stratification according to both serum and urinary measurements showed a significantly increased relative risk of bone loss (2.7- and 2.5-fold, respectively), compared with the women in the lowest quartile of baseline CrossLaps concentration, who had no significantly increased risk of losing bone if left untreated.

A similar stratification was not possible for the tibolone and ibandronate studies because of the limited number of individuals in each group. However, the pronounced and highly significant response to therapy, the relatively high correlation to spine BMD measurements, and high sensitivity and specificity for predicting net gain or loss in spine BMD observed in all three studies with both urinary and serum CrossLaps measurements suggest that both these assays are suitable for monitoring antiresorptive therapy and that they may also be used for selecting individuals with high bone resorption who are at increased risk of developing osteoporosis.

In conclusion, the studies reported here suggest that follow up of therapy by measurement of CrossLaps concentration provides a rapid and equally predictive measure of the response in the individual patients to the antiresorptive therapy compared with traditional BMD measurements. The data demonstrate that serum CrossLaps measurements have a clinical value for assessment of the outcome of antiresorptive therapy of postmenopausal women.

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