

# Clinical Evaluation of the Elecsys $\beta$ -CrossLaps Serum Assay, a New Assay for Degradation Products of Type I Collagen C-Telopeptides

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**Background:** The Elecsys  $\beta$ -CrossLaps serum assay measures type I collagen degradation fragments ( $\beta$ -CTx) that contain the  $\beta$ -isomerized octapeptide EKAHD- $\beta$ -GGR. We investigated the analytical performance of the assay and changes in  $\beta$ -CrossLaps in patients with metabolic bone diseases.

**Methods:** The electrochemiluminescent sandwich immunoassay uses two monoclonal antibodies directed against different regions of the linear EKAHD- $\beta$ -GGR.

**Results:**  $\beta$ -CrossLaps ( $\beta$ -CTx) immunoreactivity was stable in serum and plasma stored at 4 °C for 24 h or at room temperature for 4 h, and it did not decrease appreciably in samples stored at -30 °C for 12 weeks. Nine cycles of repeated freezing-thawing did not affect serum  $\beta$ -CTx. The intra- and interassay imprecision (CVs) for four samples was  $\leq 2.6\%$  (n = 10) and  $\leq 4.1\%$  (n = 10), respectively. The mean day-to-day biological variation (CV) was 20% in 10 postmenopausal women (n = 10 days). Serum  $\beta$ -CTx and osteocalcin were correlated in patients with hyperparathyroidism (r = 0.796; P < 0.0001; n = 28), chronic renal failure on hemodialysis (r = 0.784; P = 0.0003; n = 16), hypoparathyroidism (r = 0.950; P = 0.0001; n = 11), and pseudohypoparathyroidism (r = 0.987; P = 0.130; n = 4). Serum  $\beta$ -CTx decreased by 47.4%  $\pm$  8.8% (mean  $\pm$  SD) and 60.7%  $\pm$  6.5% at 3 and 6 months, respectively, after initiation of estrogen replacement therapy in 34 women. These decreases were greater than the decreases in

urinary excretion of deoxypyridinoline (31.8%  $\pm$  3.9% and 38.1%  $\pm$  4.4%, respectively) or pyridinoline cross-linked C-terminal telopeptide of type I collagen (15.9%  $\pm$  3.9% and 16.9%  $\pm$  4.6%, respectively).

**Conclusions:** The Elecsys  $\beta$ -CrossLaps serum assay provides a potentially useful tool for assessing bone resorption state, including its response to estrogen replacement therapy.

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Although a consensus has been reached that the measurement of bone mineral density is essential for the diagnosis of osteoporosis (1), it does not allow us to evaluate the rate of bone loss at a point in time. As an adjunct of clinical practice in osteoporosis, to monitor the rate of bone loss (2), and thus the risk for osteoporosis, and to identify the major mechanism for bone loss (3), measures of biochemical markers for bone metabolism have been established as clinically valuable. Furthermore, bone markers allow us to determine bone turnover state in patients with various metabolic bone diseases (4, 5) and to effectively assess the effect of drug therapy for osteoporosis by monitoring the acute changes in bone turnover after the initiation of drug therapy (6).

Among the bone resorption markers introduced to date into clinical practice, urinary excretion of deoxypyridinoline (U-DPD)<sup>4</sup> has been used extensively (7). Because U-DPD is corrected for creatinine excretion, it might be affected by various conditions, such as impaired renal function and reduced muscle mass (8). The newly developed Elecsys<sup>®</sup>  $\beta$ -CrossLaps<sup>™</sup> serum assay is proposed as a clinically more useful marker for bone resorption be-

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<sup>4</sup> Nonstandard abbreviations: U-DPD, urinary deoxypyridinoline;  $\beta$ -CTx, degradation products of C-terminal telopeptide of type I collagen that contain the  $\beta$ -isomerized octapeptide EKAHD- $\beta$ -GGR; ERT, estrogen replacement therapy; ICTP, C-terminal telopeptide of type I collagen; PTH, parathyroid hormone; OC, osteocalcin; CRF, chronic renal failure; and HD, hemodialysis.

cause of the improved sensitivity and specificity make measurements of  $\beta$ -CrossLaps in serum possible. The assay measures serum concentrations of the degradation fragments of the C-terminal telopeptide of type I collagen ( $\beta$ -CTx) that contain the  $\beta$ -isomerized octapeptide EK-AHD- $\beta$ -GGR twice (9, 10). The specificity depends on the use of two monoclonal antibodies, each recognizing a different part of linear EKAHD- $\beta$ -GGR.

We investigated (a) the analytical performance of  $\beta$ -CTx in serum obtained from Japanese patients with various metabolic bone diseases to assess the clinical usefulness of this assay as bone resorption marker, and (b) its responsiveness to estrogen replacement therapy (ERT) in Japanese patients with postmenopausal osteoporosis.

### Subjects and Methods

#### INFORMED CONSENT

Blood was drawn after written informed consent was obtained from each subject enrolled in the present study. The study was approved by the ethics committee of the Osaka City Graduate School of Medicine.

#### SAMPLE COLLECTION

Blood, drawn after an overnight fast, was stored in the absence or presence of EDTA at room temperature for no more than 1 h before centrifugation at 1200g for 5 min. The resulting serum and EDTA-plasma samples were kept frozen at  $-30^{\circ}\text{C}$  until assayed. Just before analysis, the frozen sample was thawed, and the measurement was performed immediately after thawing.

#### ELECSYS $\beta$ -CrossLaps SERUM ASSAY

The Elecsys  $\beta$ -CrossLaps serum assay (Roche Diagnostics) is a sandwich immunoassay with two monoclonal antibodies specific for the  $\beta$ -isomerized 8-amino acid sequence (EKAHD- $\beta$ -GGR) of the C-terminal telopeptide of type I collagen (ICTP). A calibrator, control, or unknown serum sample (50  $\mu\text{L}$  each) is incubated with biotinylated antibody for 9 min. After the addition of ruthenium-labeled antibody and streptavidin-coated paramagnetic microbeads, a sandwich complex is formed, which binds to the bead via a biotin-streptavidin interaction. After an additional 9-min incubation, the reaction mixture is aspirated into the measuring cell, where the electrochemiluminescent signal is generated by the ruthenium-labeled sandwich complex. All of the above steps are performed automatically by the Elecsys 2010 analyzer (Roche Diagnostics).

#### SERUM MARKERS FOR CALCIUM AND BONE METABOLISM

Serum intact parathyroid hormone (PTH) was determined by use of an Elecsys PTH assay reagent set (Roche Diagnostics) (11). The intra- and interassay CVs for intact PTH were 1.4–4.6% and 3.6–8.4%, respectively. As bone resorption markers, serum osteocalcin (OC) and ICTP were determined by the Elecsys N-MID<sup>®</sup> OC assay (Roche Diagnostics) (12) and the pyridinoline cross-linked ICTP

<sup>125</sup>I radioimmunoassay (Orion Diagnostica). The intra- and interassay CVs were 1.0–2.5% and 2.7–4.1% for OC and 1.9–2.0% and 3.0–4.9% for ICTP, respectively.

#### STABILITY OF $\beta$ -CTx IMMUNOREACTIVITY

To investigate the stability of  $\beta$ -CTx immunoreactivity during sample preparation, blood samples drawn from 10 healthy subjects were stored in the presence or absence of EDTA at room temperature or  $4^{\circ}\text{C}$  for 1, 2, 4, and 24 h before centrifugation at 1200g for 5 min. The resulting serum and plasma were immediately measured for  $\beta$ -CTx by the Elecsys  $\beta$ -CrossLaps serum assay.

To investigate the influence of storage of blood samples on  $\beta$ -CTx immunoreactivity, samples with various  $\beta$ -CTx concentrations obtained from patients with various metabolic bone diseases were stored at  $-30^{\circ}\text{C}$  for three different time periods between 0 and 12 weeks before measurement by the Elecsys  $\beta$ -CrossLaps serum assay.

#### FREEZE-THAW EXPERIMENTS

Serum samples that were used in the experiments to investigate the effect of storage at  $-30^{\circ}\text{C}$  on  $\beta$ -CTx stability were repeatedly frozen at  $-20^{\circ}\text{C}$  and thawed in a water bath at  $15^{\circ}\text{C}$ . Any one sample was frozen and thawed up to nine times.

#### INTRA- AND INTERASSAY VARIATION OF $\beta$ -CTx

We assessed intraassay precision by measuring four serum samples with different  $\beta$ -CTx concentrations 10 times in the same assay run, and interassay precision with the same samples daily over a 10-day period.

#### VARIATION DURING THE MORNING AND DAY-TO-DAY VARIATION

Variation during the morning and day-to-day variation of  $\beta$ -CTx were determined in 10 postmenopausal women.

**Table 1. Intra- and interassay precision of the Elecsys  $\beta$ -CrossLaps serum assay.**

Mean $\beta$ -CTx, $\mu\text{g/L}$	Intraassay <sup>a</sup> (n = 10)		
	SD, $\mu\text{g/L}$	CV, %	
0.29	0.0077	2.6	
0.68	0.0084	1.3	
0.73	0.0040	0.54	
1.97	0.015	0.76	
	Interassay <sup>b</sup> (n = 10)		
	SD, $\mu\text{g/L}$	CV, %	
	0.30	0.012	4.1
	0.71	0.030	4.1
	0.76	0.023	3.0
2.01	0.037	1.9	

<sup>a</sup> Intraassay CVs for Elecsys  $\beta$ -CrossLaps serum assay were determined by measuring 10 replicates of four serum samples with different serum  $\beta$ -CTx concentrations in the same assay.

<sup>b</sup> Interassay CVs were determined by measuring the same samples daily over a 10-day period.

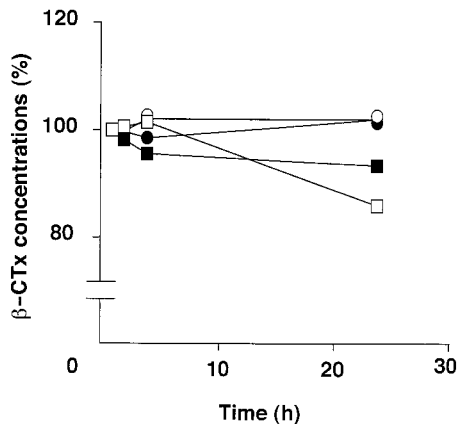


Fig. 1. Stability of  $\beta$ -CTx in serum and EDTA-plasma sample at room temperature and 4 °C.

Blood samples drawn from healthy subjects ( $n = 10$ ) were stored in the presence or absence of EDTA at room temperature or 4 °C for 0, 1, 2, 4, and 24 h before centrifugation. The concentration of  $\beta$ -CTx in samples centrifuged immediately after collection (0 h) was defined as 100%.  $\square$ , serum at room temperature;  $\blacksquare$ , serum at 4 °C;  $\circ$ , plasma at room temperature;  $\bullet$ , plasma at 4 °C.

Variation of serum  $\beta$ -CTx during the morning was assessed by collecting serum samples at 0900, 1030, and 1200, and day-to-day variation was assessed by collecting serum samples at 0900 daily for 10 consecutive days.

#### SERUM $\beta$ -CTx IN PATIENTS WITH VARIOUS METABOLIC BONE DISEASES

$\beta$ -CTx was measured in serum samples obtained from 67 patients: 28 with hyperparathyroidism, 11 with idiopathic hypoparathyroidism, 16 with chronic renal failure (CRF) on hemodialysis (HD), 6 with malignancy, and 4 with pseudohypoparathyroidism. No subject had taken any drug known to affect bone metabolism for at least 1 month before blood collection.

#### RESPONSE OF SERUM $\beta$ -CTx TO ERT IN PATIENTS WITH POSTMENOPAUSAL OSTEOPOROSIS

Thirty-four patients with postmenopausal osteoporosis (mean age,  $57.2 \pm 5.9$  years) were maintained on ERT (conjugated estrogen,  $n = 25$ ; estriol,  $n = 5$ ; estradiol,  $n = 4$ ). Bone resorption markers were measured before and 1 and 3 months after initiation of ERT.

#### Results

##### PRECISION OF ELECSYS $\beta$ -CrossLaps SERUM ASSAY

The intraassay CV for the Elecsys  $\beta$ -CrossLaps serum assay, determined by measuring 10 replicates of four serum samples with different serum  $\beta$ -CTx concentrations in the same assay run, was 0.54–2.6%; the interassay CV, determined by measuring the same samples daily over a 10-day period, was 1.9–4.1%. As shown in Table 1, the precision of the  $\beta$ -CrossLaps assay improved as the  $\beta$ -CTx immunoreactivity increased.

##### STABILITY OF $\beta$ -CTx BEFORE PREPARATION OF SERUM AND EDTA-PLASMA SAMPLES

The stability of  $\beta$ -CTx in serum and plasma at room temperature or 4 °C is shown in Fig. 1.  $\beta$ -CTx did not decrease significantly at 4 °C in either plasma or serum even after a 24-h incubation. However, at room temperature, it decreased by 14% in the serum sample but not in plasma.

The effect of storage at  $-30$  °C on  $\beta$ -CTx in serum and plasma is shown in Fig. 2A.  $\beta$ -CTx in serum and plasma did not decrease significantly by 12 weeks when stored at  $-30$  °C. In addition, the same samples used in the experiments shown in Fig. 2A were repeatedly frozen and thawed up to nine times before their  $\beta$ -CTx concentrations were measured (Fig. 2B). Repeated freezing and thawing did not appreciably affect  $\beta$ -CTx concentrations in any sample.

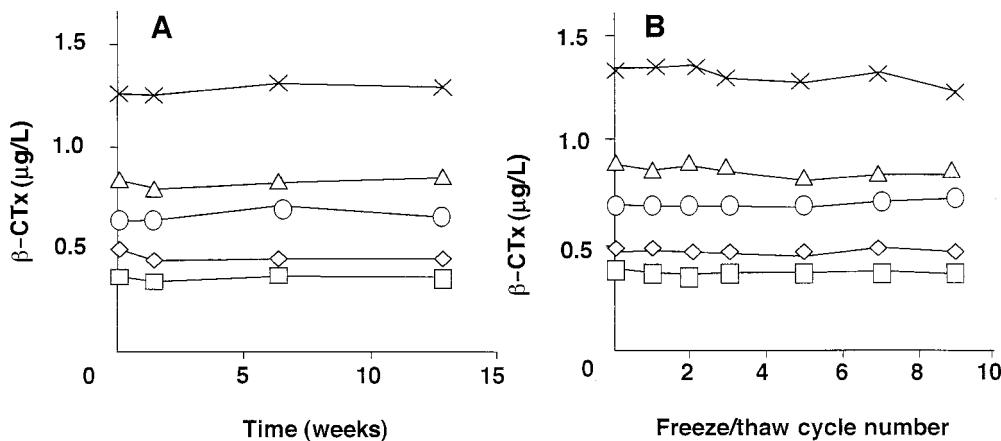


Fig. 2. Stability of  $\beta$ -CTx in serum samples during storage at  $-30$  °C (A) and after repeated cycles of freezing and thawing (B).

(A), serum samples drawn from five subjects with different concentrations of serum  $\beta$ -CTx were measured for  $\beta$ -CTx before and 1, 6, and 12 weeks after storage at  $-30$  °C. (B), the same samples were subjected to repeated freezing-thawing at the indicated times before  $\beta$ -CTx was measured. One cycle of freezing and thawing consisted of freezing at  $-80$  °C and thawing in a water bath at 15 °C.

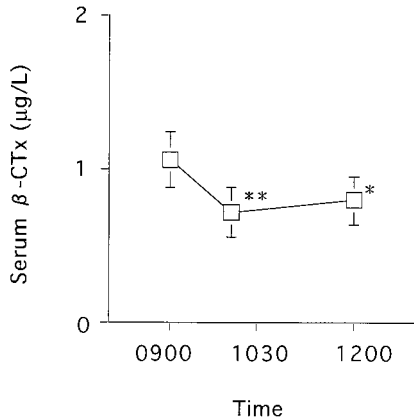


Fig. 3. Variation in serum beta-CTx (□) during the morning. Five postmenopausal women were sampled at 0900, 1030, and 1200. Results are shown as the mean ± SE (bars). \*,  $P < 0.1$ ; \*\*,  $P < 0.01$ .

VARIATIONS IN SERUM beta-CTx DURING THE MORNING AND DAY-TO-DAY IN PATIENTS WITH POSTMENOPAUSAL OSTEOPOROSIS

Variation during the morning and day-to-day variation were determined in eight postmenopausal women. Serum beta-CTx was significantly higher at 0900 than at 1030 or 1200 (Fig. 3), as reported for the other bone resorption markers (13, 14). The mean day-to-day variation of beta-CTx at 0900 was 20%, with a maximum change from the mean of 38%.

SERUM beta-CTx IN PATIENTS WITH VARIOUS METABOLIC BONE DISEASES

Serum beta-CTx was high in the patients with CRF on HD, malignancy, and hyperparathyroidism, whereas it was within the reference interval in those with hypoparathyroidism and pseudohypoparathyroidism (Fig. 4). A significant and positive correlation was observed between

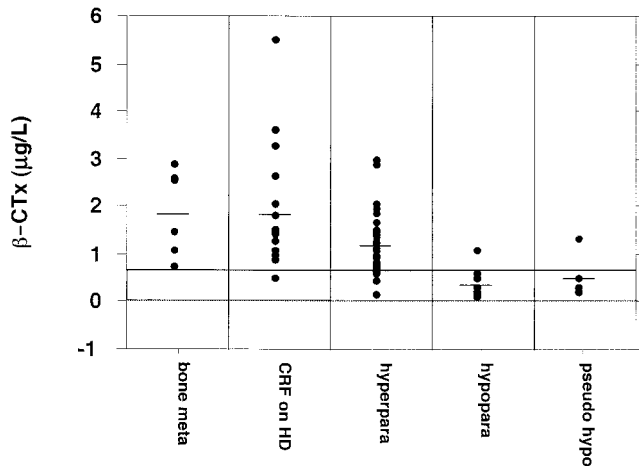


Fig. 4. Serum beta-CTx in patients with various metabolic bone diseases. Serum beta-CTx was measured in patients with pseudohypoparathyroidism (*pseudo hypo*;  $n = 28$ ), hypoparathyroidism (*hypopara*;  $n = 11$ ), hyperparathyroidism (*hyperpara*;  $n = 28$ ), CRF on HD ( $n = 16$ ), and metastatic bone disease (*bone meta*;  $n = 6$ ).

serum beta-CTx and OC in the patients with hyperparathyroidism ( $r = 0.796$ ;  $P < 0.0001$ ;  $n = 28$ ), CRF on HD ( $r = 0.784$ ;  $P = 0.0003$ ;  $n = 16$ ), hypoparathyroidism ( $r = 0.950$ ;  $P = 0.0001$ ;  $n = 11$ ), and pseudohypoparathyroidism ( $r = 0.987$ ;  $P = 0.130$ ;  $n = 4$ ).

CORRELATION OF SERUM beta-CTx WITH SERUM INTACT PTH AND OC

To further validate the measurement of serum beta-CTx as a bone resorption marker, correlation of serum beta-CTx with serum PTH and OC was investigated in the patients with hyper- and hypoparathyroidism. Serum beta-CTx was correlated in a positive manner with either serum PTH ( $r = 0.67$ ;  $P < 0.001$ ) or serum OC ( $r = 0.87$ ;  $P < 0.001$ ; data not shown).

SERUM beta-CTx IN PATIENTS WITH POSTMENOPAUSAL OSTEOPOROSIS AFTER INITIATION OF ERT

Changes in serum beta-CTx in the patients with postmenopausal osteoporosis during ERT are shown in Fig. 5. Serum beta-CTx immunoreactivity decreased by  $13.2\% \pm 9.2\%$ ,  $47.4\% \pm 8.8\%$ , and  $60.7\% \pm 6.5\%$  at 1, 3, and 6 months, respectively, after initiation of ERT. These values at 3 and 6 months were significantly larger than the respective value for the U-DPD assay ( $31.8\% \pm 3.9\%$  and  $38.1 \pm 4.4\%$ , respectively) and the ICTP assay ( $15.9\% \pm 3.9\%$  and  $16.9\% \pm 4.6\%$ , respectively). With regard to the kinetics of the changes in bone resorption markers, the markers did not differ significantly from one another.

Discussion

The present study indicates that clinical application of the Elecsys beta-CrossLaps serum assay is acceptable under routine clinical laboratory conditions from both analytical

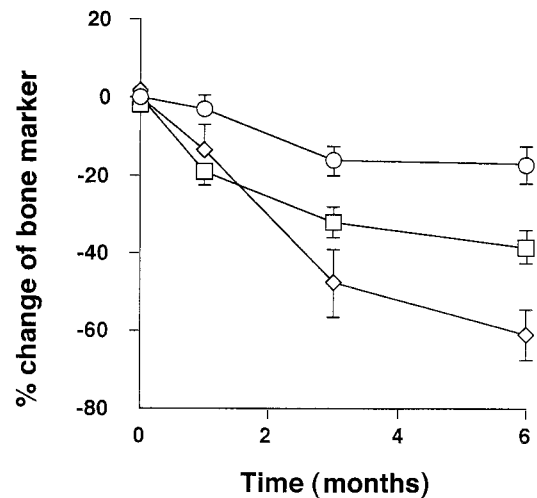


Fig. 5. Time-course changes (%) for serum beta-CTx (◇), U-DPD (□), and serum ICTP (○). Bone resorption markers were measured in patients with postmenopausal osteoporosis at the indicated months after initiation of ERT.

and clinical perspectives for the following reasons: (a) Inter- and intraassay variations were very small throughout the ranges usually used under routine conditions, including various metabolic bone diseases (Table 1 and Fig. 4), indicating that the likelihood of clinically significant error with this assay is very small. (b)  $\beta$ -CTx immunoreactivity is stable enough for easy handling of clinical samples. Although  $\beta$ -CTx immunoreactivity in human serum decreased by 86% after 24 h of incubation at room temperature, at 4 °C it was stable at least for up to 24 h (Fig. 1). When stored at -30 °C,  $\beta$ -CTx immunoreactivity in serum remained unchanged up to 12 weeks (Fig. 2A). (c) Nine repeated cycles of freezing-thawing did not affect serum  $\beta$ -CTx immunoreactivity (Fig. 2B). When these results are taken collectively,  $\beta$ -CTx immunoreactivity is stable enough to allow for easy handling of clinical samples.

The validity of the Elecsys  $\beta$ -CrossLaps serum assay as a bone resorption marker was supported by (a) good reflection of bone resorptive state in various metabolic bone diseases (Fig. 3); (b) good correlation with both PTH and a bone formation marker, serum OC; and (c) ERT-induced changes in serum  $\beta$ -CTx immunoreactivity that were larger than those for U-DPD, the most specific marker for bone resorption to date (Fig. 5). In the patients showing a high bone turnover state resulting from bone metastasis, CRF, and hyperparathyroidism, almost 100% of the patients had serum  $\beta$ -CTx exceeding the upper limit of the reference interval. In contrast, serum  $\beta$ -CTx in patients with hypoparathyroidism was suppressed to below the upper reference limit in 81.8% of the patients, indicating that serum  $\beta$ -CTx is a reliable marker for bone metabolism.

U-DPD has been proposed as a clinically useful markers for bone resorption. However, U-DPD has been reported to have a wide intraindividual variation (7), which has raised questions about its applicability for monitoring the time course of the bone metabolic state in individual patients (7). Serum assessment of biochemical markers offers one advantage when compared with urinary-based measurements in that the confounding errors in association with urinary creatinine measurement are eliminated (7). Considering the stable immunoreactivity of  $\beta$ -CTx in serum and the subsequent easy handling of serum samples, these data may support the clinical usefulness of the Elecsys  $\beta$ -CrossLaps serum assay over the measurement of U-DPD in monitoring the therapeutic effect of osteoporosis. Furthermore, the Elecsys  $\beta$ -CrossLaps serum

assay provides a much more clinically useful assay of bone resorption than the serum ICTP assay, another serum marker commercially available for bone resorption (Fig. 5).

In conclusion, we suggest that the Elecsys  $\beta$ -CrossLaps serum assay may provide a clinically useful tool for assessing bone resorption state in Japanese patients.

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