Beta-CrossLaps (β-CTx)

Beta-CrossLaps (β-CTx), also known as beta C-terminal telopeptides, are degradation products of Type I collagen. Bone tissue undergoes continuous remodeling through the process of bone resorption followed by replacement by new bone. In young people, the processes of resorption and formation of bone tissue are balanced. However, this balance is often disrupted during menopause in females, increasing the resorption process and leading to bone loss [1].

Age-related bone loss is also accelerated by insufficient calcium intake and low levels of 25-hydroxyvitamin D (25(OH)D). Decreased production of the main precursor of vitamin D in the skin and reduced synthesis of 1,25(OH)2D, the active metabolite of vitamin D, contribute to decreased calcium absorption in the kidneys, which leads to increased secretion of parathyroid hormone (PTH) and, consequently, increased bone resorption.

During the synthesis of the main matrix protein, type I collagen, polypeptide chains (α1 and α2) are first formed, which, combining in a 2:1 ratio to form the three-helix structure of procollagen molecules. Procollagen is secreted into the extracellular environment where the terminal propeptides are split off, resulting in immature collagen that is incorporated into fibrils. The maturation of collagen occurs as a result of a number of modifications of its molecules in the composition of fibrils and their connection with pyridinoline cross-links.

During bone resorption, osteoclasts attach to the bone surface and release acids and enzymes that degrade collagen fibrils into molecular fragments.

The main degradation products of type I collagen C-telopeptides are structures that consist of two octapeptides (8 amino acid residues, EKAH(beta-D)GGR) linked by a pyridinoline cross-link. In newly formed bone, the octapeptide sequences contain α-aspartic (Asp) acid, but as the bone ages, this amino acid isomerizes to the β-form (i.e., the degree of isomerization is proportional to the age of the bone). Consequently, C-terminal telopeptide fragments, including cross-linked carboxyl-terminal telopeptide of type I collagen (β-CTx), are released into the bloodstream. This release is indicative of bone resorption activity and serves as a commonly used biomarker for bone turnover (BTM) and osteoporosis [2].

β-CTx and Procollagen Type I N-propeptide (PINP) in the blood have been designated as reference BTMs in osteoporosis by the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). In situations where other diagnostic methods, such as bone density scanning, might not yield definitive results, these biomarkers assist in providing a clearer picture. Moreover, for patients undergoing treatment for osteoporosis, the β-CTx assay can be used to monitor the effectiveness of the treatment. Conditions like hyperthyroidism, hyperparathyroidism, or certain medications can lead to secondary osteoporosis, where β-CTx can help determine the extent of bone metabolism. Some cancers that metastasize to bones lead to increased bone resorption; in these cases, elevated β-CTx levels can serve as an indicator, complementing imaging methods.

CLINICAL UTILITY

- **Assessment and monitoring of osteoporosis treatment**
- **Diagnostic support and management of various bone-related disorders**

A method for measurement of β-CTx in human serum has been developed [1]. Hytest developed antibodies specific to β-CTx octapeptides as well as β-CTx sandwich immunoassays.

Monoclonal antibodies for developing β-CTx immunoassays

Hytest offers eight new monoclonal antibodies (MAbs) for the development of highly sensitive and quantitative β-CTx immunoassays. β-CTx antibodies were derived from mice and rabbits by using synthetic EKAH(betaD)GGR peptide conjugated with bovine serum albumin as the immunogen. Rabbit antibodies were obtained in recombinant format with rabbit IgG constant domains. Mouse antibodies were produced from hybridoma cell lines *in vitro*. These MAbs are specific to human β-CTx, and the recommended MAb pairs (as shown in Table 1) are capable of detecting β-CTx in human plasma/serum samples. The performance of these MAbs was evaluated using a chemiluminescent sandwich immunoassay.

Table 1.

Recommended MAb combinations for the detection of human β-CTx. Sensitivity measurements: a mixture of capture antibodies labelled with biotin, serial dilutions of reference EDTA-plasma sample, and detection antibodies labelled with alkaline phosphatase was incubated for 15 minutes at 37°C. β-CTx concentration in the reference EDTA-plasma sample was pre-measured by using Elecsys® β-CrossLaps/serum electrochemiluminescence immunoassay (ECLIA).

Capture MAb	Detection MAb	LoD (pg/ml)
CX39	CX21	13.4
CX80	CX21	33.1
CX50	CX21	11,3
CX50	CX23	17,5
CX50	CX26	63,5

Correlation with Roche Elecsys® β-Cross-Laps/serum

Assay prototypes that utilize new MAbs as capture or detection antibodies can effectively detect β-CTx in serum and plasma samples from donated blood with high sensitivity. Assays utilizing these MAb pairs have demonstrated a strong correlation with the commercially available Elecsys® β-CrossLaps/serum ECLIA. β-CTx concentrations were measured in twenty EDTA-plasma samples using assay prototypes in comparison with the Elecsys® β-CrossLaps/serum assay (Fig. 1).

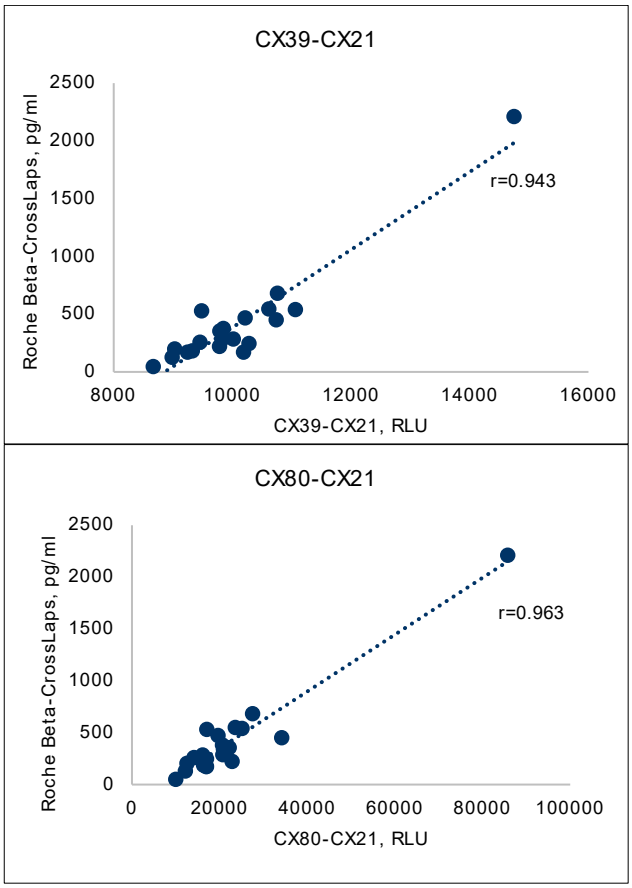

Figure 1.

Correlation of β-CTx levels measured by Hytest assay prototypes CX39-CX21 and CX80-CX21 with Elecsys® β-CrossLaps/serum β-CTx levels were measured in twenty EDTA-plasma samples and ranged from 129 to 2210 pg/ml according to Roche Beta-CrossLaps/serum assay. Assay prototypes CX39-CX21 and CX80-CX21 were performed as follows: a mixture of capture antibodies labelled with biotin, tested samples, and detection antibodies labelled with alkaline phosphatase was incubated for 15 minutes at 37°C. The luminescent signals are expressed in RLU.

Cross-reactivity with α-CTx

α-CTx is isomer of β-CTx. As age grows, the Asp amino acid in CTX sequence undergo β–Isomerization and transform into β-Asp, the ratio of α-CTx/β-CTx is important in assessing bone metabolism. Cross-reactivity levels of anti-β-CTx antibodies with α-CTx peptide are shown in Table 2.

Epitopes of anti-β-CTx MAbs

Precise epitopes of anti-β-CTx MAbs were determined by using elongated or truncated β-CTx peptides as indicated in Table 3.

Table 2.

Cross-reactivity of anti-β-CTx antibodies. The EKAH(α-D)GGR peptide (α-CTx) was conjugated with ovalbumin via an additional cysteine amino acid residue at the N-terminus of the peptide. The cross-reactivity of anti-β-CTx antibodies was tested in an indirect ELISA with the preabsorbed α-CTx-ovalbumin conjugate and expressed as a percentage (%) of β-CTx immunoreactivity.

MAb name	Cross-reactivity with α-CTx
CX14	6%
CX21	10%
CX23	11%
CX26	11%
CX39	15%
CX50	5%
CX52	5%
CX80	1%

Table 3.

Epitopes of anti-β-CTx antibodies. Elongated or truncated β-CTx peptides were conjugated with ovalbumin via additional cysteine amino acid residue at the N-terminus of the peptide. Immunoreactivity of anti-β-CTx antibodies was tested in indirect ELISA with preabsorbed peptide-ovalbumin conjugates. (*) - free carboxyl terminus of the peptide is crucial for recognition by corresponding antibody.

MAb name	Epitope
CX14	AH(betaD)G
CX21	AH(betaD)G
CX23	AH(betaD)GG/ AH(betaD)G
CX26	AH(betaD)GG
CX39	AH(betaD)GGR-COOH*
CX50	AH(betaD)GGR
CX52	AH(betaD)GGR
CX80	AH(betaD)GGR-COOH*

REFERENCES

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2. **Nagy, Előd Ernő et al.** Soluble Biomarkers of Osteoporosis and Osteoarthritis, from Pathway Mapping to Clinical Trials: An Update. Clinical interventions in aging vol. 15 501-518. 8 Apr. 2020, doi:10.2147/CIA.S242288Papa, L. et al.

ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Monoclonal anti-beta-C-terminal telopeptide of collagen I (bCTX)	4BT1	CX14	IgG1	*In vitro*, EIA, ECLIA
		CX21	IgG	Recombinant, EIA, ECLIA
		CX23	IgG	Recombinant, EIA, ECLIA
		CX26	IgG	Recombinant, EIA, ECLIA
		CX39	IgG	Recombinant, EIA, ECLIA
		CX50	IgG1	*In vitro*, EIA, ECLIA
		CX52	IgG2B	*In vitro*, EIA, ECLIA
		CX80	IgG	Recombinant, EIA, ECLIA

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