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Invited critical review

The amino- and carboxyterminal cross-linked telopeptides of collagen type I, NTX-I and CTX-I: A comparative review

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ABSTRACT

Bone diseases such as osteoporosis or bone metastases are a continuously growing problem in the ageing populations across the world. In recent years, great efforts have been made to develop specific and sensitive biochemical markers of bone turnover that could help in the assessment and monitoring of bone turnover. The amino- and carboxyterminal cross-linked telopeptides of type I collagen (NTX-I and CTX-I, respectively) are two widely used bone resorption markers that attracted great attention due to their relatively high sensitivity and specificity for the degradation of type I collagen, and their rapid adaptation to automated analyzers. However, the clinical performance of both markers differs significantly depending on the clinical situation. These differences have caused considerable confusion and uncertainty. If used correctly, both markers have great potential to improve the management of many bone diseases. We here review the biochemistry, analytical background and clinical performance of NTX-I and CTX-I, as documented in the accessible literature until March 2008.

Contents

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Abbreviations: BMD, bone mineral density; CTX-I, carboxyterminal cross-linked telopeptides of type I collagen; HRT, hormone replacement therapy; ICTP, carboxyterminal cross-linked telopeptides of type I collagen; NTX-I, aminoterminal cross-linked telopeptides of type I collagen.

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1. Introduction

Bone diseases such as osteoporosis or bone metastases are a continuously growing problem in the ageing populations across the world. For example, osteoporosis represents one of the most common age-related diseases worldwide, affecting about 75 million people in Europe, the USA and Japan alone [1,2]. At present, the diagnosis of osteoporosis is mainly based upon a history of a previous fragility fracture and the measurement of bone mineral density (BMD) [3,4]. However, the onset of most bone diseases precedes measurable changes in BMD, or the occurrence of fractures by years if not decades.

Beside BMD, other factors, such as bone remodelling, are major determinants of bone strength [5–10]. In recent years, great efforts have been made to develop specific and sensitive biochemical markers of bone turnover that could help in the assessment and monitoring of bone turnover. At present, there are more than 10 different bone turnover markers commercially available. The three major macro-molecular products of collagen degradation, namely the aminoterminal (NTX) and the carboxyterminal (CTX-I, ICTP) cross-linked telopeptides of type I collagen can be measured by specific immunoassays, some of which have been adapted to automated analyzers. While the assay for ICTP in serum was the first of the telopeptide markers to be developed, the assays for NTX-I and CTX-I have become the most commonly used methods to measure bone resorption rates. This review will therefore concentrate on NTX-I and CTX-I. While both these markers may be measured in serum and urine, their clinical performance differs significantly depending on the clinical situation [11–13]. These differences have caused considerable confusion and uncertainty. However, if used correctly, both markers have great potential to improve the management of many bone diseases.

We here review the biochemistry, analytical background and clinical performance of NTX-I and CTX-I, as documented in the accessible literature until November 2007.

2. Biochemistry of aminoterminal cross-linked telopeptides of type I collagen (NTX-1) and carboxyterminal cross-linked telopeptides of type I collagen (CTX-1)

NTX-I and CTX-I are degradation products of type I collagen (Fig. 1). Collagen type I is the major organic component of the extracellular matrix and is present as a triple helix. The cross-links covalently link individual collagen molecules within the triple helix. The main molecular sites involved in collagen cross-linking are the short non-helical peptides at both ends of the collagen molecule, termed amino- (N) and carboxy- (C) terminal telopeptides. In normal collagen, these telopeptides are each linked via pyridinium or pyrrole compounds to the helical portion of neighbouring collagen molecules (Fig. 1) [14–17]. During collagen breakdown, N- and C-terminal telopeptide fragments of various sizes, still attached to the helical portions of a nearby molecule by a pyridinium or pyrrole cross-link, are released into the circulation.

NTX-I and CTX-I are small enough to be readily cleared by the kidneys. Hence, they can be measured in both serum and urine. Of note, NTX-I and CTX-I are also present in tissues other than bone and non-skeletal processes may therefore influence their circulating or urinary levels [18–23]. Consequently, both markers are not disease specific but reflect, as an integral measure, alterations in bone metabolism independently of the underlying cause. Hence, results of CTX-I and NTX-I measurement should always be interpreted against the background of their basic science and the clinical picture.

3. Analytical background

3.1. Cross-linked N-terminal telopeptide of type I collagen (NTX-1) in urine

The NTX-I urine assay is based on a monoclonal antibody that specifically recognizes an epitope embedded in the α2-chain of the N-telopeptide fragment. The peptide has the sequence QYDGKGVG, where K is involved in a trivalent cross-linking site. The compound still contains the pyridinium cross-link, but the antibody does not recognize the pyridinoline or deoxypyridinoline *per se* [24]. Collagen must be broken down to small cross-linked peptides that contain the exact sequence before antibody binding occurs with the NTX-I antigen. The antibody recognizes peptides in culture medium conditioned by osteoclasts resorbing human bone particles [25,26]. These data suggest that the NTX-I peptide is a direct product of osteoclastic proteolysis and appears not to be metabolized further [24,25]. However, cross-reaction of the antibody with peptides derived from skin has also been reported [15,18].

The NTX-I assay is calibrated using standard amounts of human bone collagen digested with bacterial collagenases, or a synthetic sequence of the NTX-I epitope fs™, Ostex International, Seattle, WA) [27]. Generally, measurement is performed in second morning spot urine. Results are reported as bone collagen equivalents (BCE), in nM, corrected for creatinine excretion to compensate for differences in urine dilution. For information regarding sensitivity, intra- and interassay coefficients of variation see Table 1.

As the α2-chain of the N-terminal telopeptide of the collagen I molecule contains an Asp-Gly bond, isomerization of the α2-chain may occur [28,29]. Preliminary data indicate that the α-NTX-I represents native or newly formed type I collagen, while the isomerized form, β-NTX-I, appears to correspond to older collagen molecules. A lower β to α-peptide ratio was observed in the urine of growing children, indicative of a higher rate of bone metabolism allowing less time for the isomerization to occur [28]. No significant differences were found between postmenopausal healthy and osteoporotic women. However, the currently available NTX-I assays do not account for isomerization and its clinical relevance needs further exploration.

3.2. Cross-linked N-terminal telopeptide of type I collagen (NTX-I) in serum

An assay for the measurement of NTX-I in serum had been developed in the late 1990s [30]. Experimental and clinical data demonstrated this assay to provide a useful index of bone resorption, however, mostly for technical reasons, such as assay stability and variability, the serum assay has not become as widely used as the assay for NTX-I in urine [12,27,31–38].