

Serum-Based Test of the Pathologic Breakdown of Type I Collagen Fibers

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Featured Article: Risteli J, Elomaa I, Niemi S, Novamo A, Risteli L. Radioimmunoassay for the pyridinoline cross-linked carboxy-terminal telopeptide of type-I collagen: a new serum marker of bone-collagen degradation. *Clin Chem* 1993;39:635–40.²

Our 1993 report described the *in vitro* production of a breakdown product of type I collagen that could be measured in human serum, which we called cross-linked carboxy-terminal telopeptide of type I collagen (ICTP),³ and characterized an immunoassay for monitoring it. We isolated the antigen from collagenase-treated demineralized femoral bone, identified it as the carboxy-terminal telopeptide of the collagen molecule, and showed that it contained an intermolecular cross-link. The corresponding antigen in the blood was homogeneous and cleared by the kidneys. We also referred to concomitant publications that suggested ICTP to be a good predictor of the clinical course of multiple myeloma and rheumatoid arthritis.

The project leading to the report was part of a research program that our group had designed more than 10 years earlier. There were 2 historical roots for our approach: the insight into protein chemistry and immunochemistry of collagens that we had gained as postdocs in Rupert Timpl's laboratory in Martinsried, Germany, and the understanding of clinical chemistry that we acquired during residencies in the Laboratory of Oulu University Hospital, Finland. Our idea was to find a means for measuring the biosynthesis and breakdown rates of each of the major collagen types in the human body through the use of serum samples, so that the need for repeated tissue biopsies could be reduced. Considering that collagens are among the most abundant human proteins, it may seem strange that no such methods were available at the time. The only collagen-

related method then generally known was the assay of urinary 4-hydroxyproline, which is specific for neither collagen nor collagen type. We first concentrated on the biosynthesis side to develop and commercialize assays for propeptide antigens that are freed from the biosynthetic precursors of type I collagen [carboxy-terminal propeptide of type I procollagen (PICP) (1) and amino-terminal propeptide of type I procollagen (PINP) (2)] and type III collagen [amino-terminal propeptide of type III procollagen (PIIINP) (3)]. Measurement of these propeptides allows estimation of synthesis rates in the same manner that C-peptide functions as an indicator of endogenous insulin production.

After obtaining a permanent position as consultant of clinical chemistry, Juha started the work on the degradation side of type I collagen metabolism that led to the ICTP publication. Because this research was a high-risk project, we did not involve any PhD students in the work. The starting point was the fact that collagen fibers are stabilized by covalent cross-links between the molecules. We reasoned that the presence of a cross-link would render the corresponding parts of the polypeptide chains relatively resistant to the action of degrading enzymes. The cross-linking process is complex and tends to lead to different end products in different tissues. It was therefore surprising to find only one peptide peak when we ran the bone-digestion mixture through HPLC and monitored the pyridinoline cross-link-specific fluorescence. The material in this peak was immediately used to raise polyclonal antibodies.

The newly established ICTP RIA was tested in diseases that involve bone breakdown. For rheumatoid arthritis, we collaborated with Markku Hakala, a friend from medical school, and for testing with malignancies, we collaborated with Inkeri Elomaa, a Helsinki-based oncologist who had encouraged us to develop something like the ICTP test to monitor bone metastases or multiple myeloma. The results from the initial studies were quite promising. The ICTP assay was patented and commercialized by Orion Diagnostica; 2 of the coauthors worked in the company.

The response from the general bone-research community was mixed. At that time, it was close to a dogma to monitor bone degradation by studying urine, rather than blood. Furthermore, it soon became clear

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² This paper has been cited more than 505 times since publication.

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³ Nonstandard abbreviations: ICTP, cross-linked carboxy-terminal telopeptide of type I collagen; PICP, carboxy-terminal propeptide of type I procollagen; PINP, amino-terminal propeptide of type I procollagen; PIIINP, amino-terminal propeptide of type III procollagen.

that the ICTP test is not sensitive to the effect of estrogen on bones. It took several years to discover the explanation, which was that 2 distinct pathways exist for bone collagen breakdown and that the ICTP antigen is generated by the matrix metalloproteinase pathway, which is typically involved in pathologic bone degradation (4). The other, the cathepsin K-related pathway, functions in physiological bone turnover and destroys the ICTP epitope (5). Nowadays, serum-based methods also exist for assessing the latter process.

More than 700 reports in the Web of Science database now mention ICTP. Of these reports, all that are related to medicine use the abbreviation to refer to the degradation product of type I collagen. These reports, as well as those that explicitly cite our 1993 publication, come mostly from clinical fields such as endocrinology, oncology, and rheumatology. ICTP in serum has proved useful as an indicator of the clinical course of diseases involving pathologic collagen breakdown. The assay has also found a use in detecting type I collagen breakdown in soft tissues and in cell culture systems.

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