# ORIGINAL ARTICLES

## Soluble cathepsin K: A novel marker for the prediction of nontraumatic fractures?

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**We sought to evaluate serum concentrations of cathepsin K in peripheral blood and to determine whether they correlated with bone-mineral density (BMD) and the incidence of nontraumatic fractures. We took blood samples from 162 patients (101 with osteoporosis, 48 with osteopenia) and 13 healthy controls, then conducted quantitative measurements of cathepsin K using a commercially available enzyme-linked immunosorbent assay. Cathepsin K concentrations were correlated with the incidence of nontraumatic fracture, BMD, markers of bone turnover (alkaline phosphatase, bone-specific alkaline phosphatase, osteocalcin, parathyroid hormone, and C-telopeptide). The correlations between cathepsin K concentrations in subjects without fractures and in those with multiple nontraumatic fractures were statistically significant ($t = -2.1$, degrees of freedom = 107, $P = .036$). The cathepsin K levels of controls and patients with osteoporosis were significantly different ($t = -3.7$, degrees of freedom = 58.9, $p > 0.0001$) These results suggest that the serum level of cathepsin K could serve as a marker for fracture prediction and BMD. (J Lab Clin Med 2005;146:13–17)**

**Abbreviations:** BMD = bone-mineral density; DEXA = dual-energy x-ray absorptimetry; df = degrees of freedom; FN = femoral neck; LS = lumbar spine; PF = proximal femur

Throughout the life span, bone-mass homeostasis is balanced by an intimate coupling of osteoclastic bone resorption and osteoblastic bone formation; in older individuals, resorption outstrips formation. Resorption is accomplished by hematopoietically derived osteoclasts, large specialized multinucleated cells. Osteoclasts synthesize cathepsin K[1] and secrete it into the extracellular microcompartment at the attachment site between osteoclasts and the bone surface. The resorption hemivacuole provides an optimal pH for certain proteolytic enzymes, and acidity induces demineralization of the matrix.[2]

Cathepsin K, a highly expressed member of the papain cystein protease family, is produced as a 329-amino-acid precursor that is cleaved to its active form with a length of 215 amino acids (23495 Da).[3] Cathepsin K has a low optimum pH and degrades many bone-matrix proteins—not only type I collagen, but also osteopontin and osteonectin.[4]

Current data confirm the predominant role of cathepsin K in bone resorption: (1) Cathepsin K messenger RNA and protein are expressed in human and murine osteoclasts. (2) Cathepsin K is localized at the ruffled border of actively resorbing osteoclasts and is absent from osteoblasts and osteocytes.[5,6] (3) Cathepsin K expression is enhanced by osteoprotegerin ligand, which stimulates osteoclastic bone resorption.[7]

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A rare human genetic disorder, pycnodysostosis, an autosomal recessive osteochondrodysplasia resulting from mutations in the cathepsin K gene,[8] is characterized clinically by short stature, osteosclerosis, and bone fragility. Cathepsin K causes a profound deterioration of bone quality with respect to trabecular architecture and lamellar arrangement; this may be the reason for the bone fragility seen in pycnodysostosis.[9]

Osteoporosis, one of the main causes of illness in older individuals, is characterized by a persistent excess of osteoclastic bone resorption leading to progressive bone loss. Cathepsin K is an attractive target for therapeutic intervention to prevent and mitigate the significant harmful impact of osteoporosis.[10,11] Recently pharmaceutical interest has been focused on inhibitors of cathepsin K as a means of preventing bone loss.[12–16] One of them, SB357114, was shown to potently reduce bone resorption in nonhuman primates.[17]

Established biochemical serum markers in osteoporosis are bone-specific alkaline phosphatase, osteocalcin (signaling bone formation), and C-terminal crosslinks of type I collagen (C-telopeptid crosslaps) (signaling bone rsorption). Recently our group was able to shown that the serum concentration of cathepsin L (also secreted by osteoclasts) is a marker for BMD.[18]

Cathepsin K is actually the most abundantly synthesized protein of the active, resorbing osteoclast. In this study we sought to investigate the value of cathepsin K serum concentrations with respect to bone density and nontraumatic fractures.

## METHODS

**Subjects.** We recruited 162 consecutive individuals and examined them at our clinic for the diagnosis and treatment of osteoporosis. Controls and patients who had diseases or treatments that might interfere with bone metabolism, such as estrogen-replacement therapy (or some other hormone-replacement treatment) or calcium, vitamin D, bisphosphonates treatment, were excluded from this study. All subjects underwent the same diagnostic procedures; healthy controls failed to exhibit osteopenic or osteoporotic BMD or nontraumatic fractures. Individuals detailed their history of fractures and their lifestyle (smoking, alcohol, etc) on a questionnaire. Clinical data were taken from the patients' files. The study protocol was reviewed and approved by the ethics committee, and informed consent was received from all individuals included in this study.

All peripheral-blood samples were collected before the start of osteoporosis therapy. Immediately after collection, samples were centrifuged at 4000*g* for 10 minutes; sera were stored at −80°C until examination.

We monitored the serum cathepsin K concentrations of 18 patients for a 14.24 ± 11,57 months (range 1–48 months) after the start of osteoporosis treatment (oral bisphosphonate plus a combination of calcium and vitamin D).

**Nontraumatic fractures.** We recorded nontraumatic fractures with the use of a questionnaire; the fractures were verified by means of radiography. Subjects were classified into 3 groups on the basis of fracture status (no nontraumatic fractures, a single nontraumatic fracture, multiple nontraumatic fractures). Fractures of the spine were classified semiquantitatively with the use of the method described by Genant.[19]

**DEXA.** All individuals underwent DEXA of the LS, and most underwent DEXA of the PF as well. Whole-body DEXA was not possible because several patients had some sort of metal implant. Subjects were classified into 3 groups on the basis of the T-score of the LS and the FN as recommended by the World Health Organization guidelines. The overall BMD score was defined as the lower of the LS or FN scores. Patients with an overall BMD score of 2.5 or less were described as having from osteoporosis. An overall BMD score between −2.5 and −1.0 was defined as evidence of osteopenia, and an overall BMD score of 1 or more was regarded as normal.[20]

**Laboratory testing.** All parameters such as blood count, creatinine, blood urea nitrogen, aspartate aminotransferase, alanine aminotransferase, and the thyroid hormones, plus serum alkaline phosphatase, bone-specific alkaline phosphatase, osteocalcin, parathyroid hormone, calcitonin, C-telopeptide, luteinizing hormone, follicle-stimulating hormone, estradiol, androstendion, and testosterone were measured routinely in our laboratory. Cathepsin L serum levels were measured as described previously.[18]

**Cathepsin K serum assay.** For a quantitative measurement of cathepsin K in serum, we used a commercially available enzyme-linked immunoassay (BI-20432; Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria). In brief, 50-$\mu$L samples and 200 $\mu$L of horseradish peroxidase–labeled detection antibody were added simultaneously to microtiter plates in duplicate. Cathepsin K in the sample binds with the precoated capture antibody and forms a sandwich with the detection antibody. After overnight incubation at room temperature (18°–26°C), the plate was washed 5 times with 0.1 mol/L phosphate-buffered saline solution (pH 7.5) with 1% Triton X-100 to remove all nonspecifically bound material. As a substrate, tetramethylbenzidine was added to the wells. We stopped color development after 30 minutes at room temperature and immediately read the intensity of color at 450 nm (reference wavelength 620 nm) using a Elx808 microwell reader (Bio-Tek, Winooski, Vt). The color intensity developed is directly proportional to the amount of cathepsin K present in the sample. Results were calculated from a standard curve (Cathepsin K standard: range 0–300 pmol/L) generated from a 4 parameter logistic curve-fit (algorithm) and expressed in picomoles per liter of serum. The assay has been reported to recognize human cathepsin K and does not cross-react with structurally related cathepsins E, D, B, or L or with rheumatoid factors. The manufacturer sets the sensitivity of the assay at 1.1 pmol/L and says that the intra- and interassay variations range from 4% to 8%.

**Statistical analysis.** We assessed the correlations between cathepsin K on one hand and age, sex, nontraumatic fractures, T-scores of the LS and FN on the other using the Pearson