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Circulating cathepsin K and cystatin C in patients with cancer related bone disease: Clinical and therapeutic implications☆

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Abstract

The clinical significance of serum cathepsin K and cystatin C was assessed in patients with breast cancer (BCa) or prostate cancer (PCa) with confined disease (M0) or bone metastasis (BM). Cathepsin K and cystatin C circulating levels were determined by ELISAs in 63 cancer patients, in 35 patients with nonmalignant diseases and in 42 healthy blood donors (control group). In BCa patients, cathepsin K serum levels were significantly lower than in sex matched control group (HS; $p = 0.0008$) or in patients with primary osteoporosis (OP; $p = 0.0009$). On the contrary, cystatin C levels were significantly higher in BCa patients than in HS ($p = 0.0001$) or OP ($p = 0.017$). In PCa patients, cathepsin K concentrations did not significantly differ from those measured in sex matched HS or in patients with benign prostatic hyperplasia (BPH). Conversely, cystatin C was more elevated in cancer patients than in controls ($p = 0.0001$) or BPH patients ($p = 0.0078$). Furthermore, in PCa patients, a positive correlation was observed between cystatin C and cathepsin K ($r_S = 0.34$; $p = 0.047$). No further relationship was highlighted between these molecules and the clinicobiological parameters of BCa or PCa progression including the number of bone lesions. Moreover, ROC curve analysis showed a poor diagnostic performance of cathepsin K and cystatin C in the detection of BM patients. Interestingly, the administration of zoledronic acid (ZA), a bisphosphonate derivative endowed with a potent antiosteoclastic activity, induced in BM patients a marked increase of cathepsin K and cystatin C serum levels compared to baseline values. However, this phenomenon was statistically significant only in the PCa group. In conclusion Cystatin C and cathepsin K may be regarded as possible markers to monitor the therapeutic response to bisphosphonate treatments. Nevertheless, their clinical value as specific gauges of skeletal metastasis remains questionable.
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1. Introduction

Cathepsin K (Cath K) is a member of the cysteine proteinase family predominantly expressed in osteoclasts [1,2].

However, recent observations report its presence also in osteoblasts [3]. Current studies indicate that this proteinase appears to play a key role in osteoclast-mediated bone resorption as, unlike other cathepsins, it has been shown to degrade specifically bone resident type I collagen which constitutes 90% of the organic bone matrix [1,2]. The intracellular activity of Cath K is regulated by specific endogenous cysteine proteinase inhibitors such as cystatin C (Cyst C) [4,5]. Interestingly, recent findings highlight that this inhibitor, besides its enzyme inhibiting activity, may also decrease osteoclast formation and antagonize transforming growth factor-beta (TGF-β) signalling in normal and tumor cells [4,6,7]. These observations suggest that Cyst C may be implicated in the modulation of osteoclast and osteoblast activity during bone

remodelling [3,5–7]. As a consequence, any disregulation in the expression level of Cath K and/or Cyst C may result in disturbances of the normal metabolic turnover of bone tissue, which may lead to the onset of severe bone diseases. This hypothesis is corroborated by several clinical studies which highlighted that the expression levels of Cath K and/or Cyst C are altered in a number of nonmalignant and malignant diseases associated with an excessive bone resorption such as muscoloskeletal disorders, primary bone tumors or bone metastasis [4,8–18]. These findings suggest that Cath K and Cyst C may be regarded as suitable targets for appropriate treatments of these pathological processes and may be of value as additional circulating markers for the therapeutic monitoring and follow-up of patients with these diseases [4,9]. On the basis of these considerations we have undertaken some investigations to assess the role and the clinical significance of the circulating levels of Cath K and Cyst C in patients with bone metastasis from breast cancer (BCa) or prostate cancer (PCa). These tumors were chosen as (i) they metastasize preferentially to the bone and (ii) may induce different types of bone lesions, namely, lytic (breast cancer) or blastic (prostate cancer) [19]. Finally, in order to assess the role of these molecules as additional markers for the therapeutic monitoring of BM patients undergoing palliative treatments with bisphosphonates, parallel studies were undertaken to evaluate the effects of zoledronic acid (ZA), a third generation bisphosphonate derivative endowed with a potent antiosteoclastic activity, [20] on the circulating levels of Cath K and Cyst C.

2. Patients and methods

The study included 42 registered healthy blood donors (HS) of both sexes (21 female and 21 male) who served as a control group, 15 female patients with age-related osteoporosis (OP; mean age 74.1 ± 10.5 years), 20 patients with histologically confirmed benign prostatic hyperplasia (BPH; mean age 62.4 ± 6.3 years), 28 patients with breast cancer (BCa; mean age 59.3 ± 9.8 years) and 35 patients with prostate cancer (PCa; mean age 72.1 ± 8.3 years). Both BCa and PCa groups comprised patients with localized disease (M0) and patients with bone metastases (BM) and no clinical evidence of extraskeletal involvement. BM patients underwent serial treatments with the bisphosphonate derivative zoledronic acid (ZA) (Zometa® Novartis, Basel, Switzerland, 4 mg i.v. by a 15-min infusion every 21 days) as described in detail elsewhere [21]. The study was approved by the local ethical committee and carried out in accordance with the Declaration of Helsinki [22].

2.1. Cathepsin K and Cystatin C assay

Blood specimens from cancer patients, OP patients or BPH patients were obtained before starting any therapy. An additional blood sample was obtained from patients treated with ZA, prior to the second drug administration (day 21). Samples were drawn into polycarbonate tubes, allowed to clot at room temperature and then centrifuged at 3500 rpm for 15 min

(Hereus Omnifuge 2.0 RS, Hereus Sepatech). Serum aliquots were stored at -80 °C until assays. Cath K and Cyst C serum concentrations were determined by commercially available two-step sandwich enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instruction (Cathepsin K ELISA kit Biomedica, Wien, Austria; Cystatin C (human) ELISA kit, Biovendor, Modrice, Czech Republic). The detection limits reported by the manufacturers were 1.1 pmol/L for cathepsin K and 80 mg/L for cystatin C.

2.2. Statistical analysis

Statistical analysis was carried out, where required, by the nonparametric Mann–Whitney U test, the Wilcoxon signed rank test and the Kruskal–Wallis test. Linear regression analysis was used to assess the correlation between circulating Cath K and Cyst C in BCa and PCa patients. The diagnostic sensitivity and the specificity of Cath K and Cyst C to discriminate between M0 and BM patients were determined by the receiver operating characteristic curve (ROC) [23]. The significance of the difference between the areas under the ROC curves (AUCs) was assessed according to Hanley and McNeil [24]. p-Values ≤ 0.05 were considered statistically significant. Data analysis was performed by using the Medcalc 7.4 statistical software package (MEDCALC version 7.4, Mariakerke, Belgium).

3. Results

Cath K serum levels were significantly higher in HS (mean 11.3 ± 14.0 pmol/L) or OP patients (mean 3.7 ± 2.9 pmol/L) than in BCa patients (mean 1.2 ± 1.9 pmol/L) (Fig. 1 and Table 1). Conversely, Cyst C levels were significantly higher in BCa patients (mean 1.5 ± 0.59 mg/L) than in sex matched HS (mean 0.85 ± 0.16 mg/L) or OP patients (mean 1.1 ± 0.24 mg/L) (Fig. 1 and Table 1). However, in cancer patients no significant association was observed between serum Cath K and Cyst C, nor Cath K or Cyst C levels were correlated with any of the clinicobiological parameters of BCa progression considered in the present study, namely, tumor grade (Cath K, $p = 0.70$; Cyst C, $p = 0.24$), estrogen receptors (Cath K, $p = 0.54$; Cyst C, $p = 0.50$), progesterone receptors (Cath K, $p = 0.92$; Cyst C, $p = 0.66$), serum CA15.3 (Cath K, $p = 0.82$; Cyst C, $p = 0.18$) and number of bone lesions (Cath K, $p = 0.088$; Cyst C, $p = 0.5$). In PCa patients, Cath K levels (mean 20.7 ± 50.5 pmol/L) did not significantly differ from those measured in sex matched HS (mean 9.6 ± 20.7 pmol/L) or in BPH patients (mean 1.6 ± 2.3 pmol/L) (Fig. 1 and Table 1). Furthermore, no relationship was highlighted between this proteinase and some clinicobiological parameters of progression, such as Gleason score ($p = 0.4$), number of skeletal metastases ($p = 0.44$) or prostate specific antigen (PSA) levels ($p = 0.32$) while a positive correlation was highlighted between Cyst C and Cath K (Fig. 2) . On the other hand, the circulating levels of Cyst C were significantly increased in PCa patients (mean 1.7 ± 0.65 mg/L) as compared to HS (mean 1.1 ± 0.24 mg/L) or BPH patients (mean
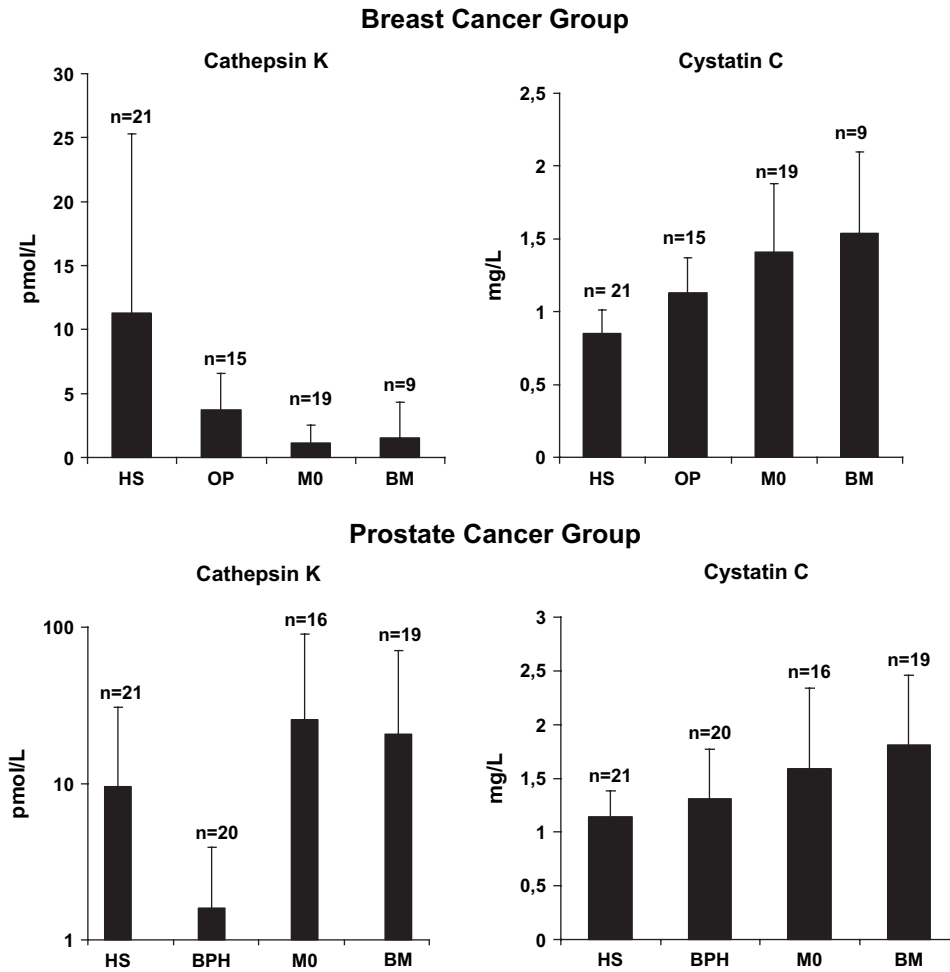
Fig. 1. Cathepsin K and cystatin C serum levels in healthy subjects (HS), in patients with primary osteoporosis (OP) or benign prostate hyperplasia (BPH) and in breast cancer or prostate cancer patients with (BM) or without (M0) bone metastases.

1.31 ± 0.46 mg/L) (Fig. 1 and Table 1). However, the serum concentrations of this inhibitor did not correlate with the Gleason score ($p = 0.88$), PSA serum levels ($p = 0.26$) or the number of bone lesions ($p = 0.34$) in this case too (Fig. 1 and Table 1). Furthermore, ROC curve analysis generated to assess the effectiveness of serum Cath K and Cyst C to discriminate between M0 and BM patients, showed a poor diagnostic performance of these molecules in this respect (Fig. 3 and Table 2). Interestingly, the administration of ZA to patients with bone metastasis induced a marked increase of cathepsin K and cystatin C serum levels compared to pre-treatment serum levels (Fig. 4). However, statistically significant differences between pre- and post-treatment values were noted only in PCa patients (Cath K, $p = 0.018$; Cyst C, $p = 0.0001$) (Fig. 4).

4. Discussion

Cathepsin K and Cystatin C are highly expressed in a number of malignant diseases associated with an altered metabolic turnover of bone tissue [4,11,15–18,25–27]. These observations are suggestive for an active involvement of the cathepsin

Table 1
Statistical evaluation of the data reported in Fig. 1

	p-Value[a]	
	Cathepsin K	Cystatin C
Breast cancer group[b]		
HS vs OP	0.28 n.s.	0.0021
HS vs M0	0.0021	<0.0001
HS vs BM	0.016	0.0001
OP vs M0	0.0013	0.033
OP vs BM	0.017	0.044
M0 vs BM	0.94 n.s.	0.82 n.s.
Prostate cancer group[b]		
HS vs BPH	0.085 n.s.	0.41 n.s.
HS vs M0	0.62 n.s.	0.01
HS vs BM	0.2 n.s.	0.0001
BPH vs M0	0.33 n.s.	0.13 n.s.
BPH vs BM	0.92 n.s.	0.0032
M0 vs BM	0.97 n.s.	0.15 n.s.

[a] Data analysis was computed by the non parametric Mann–Whitney *U* test; n.s. = not significant.

[b] HS = healthy subjects; OP = primary osteoporosis; BPH = benign prostatic hyperplasia; M0 = primary tumor; BM = bone metastasis.
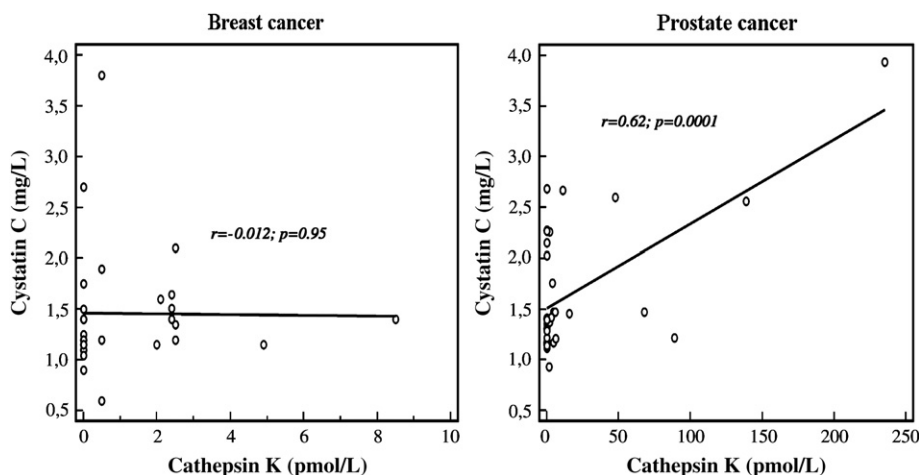
Fig. 2. Linear regression analysis between cathepsin K and cystatin C serum levels in patients with breast cancer or prostate cancer.

K/cystatin C system also in the pathogenesis of metastatic bone disease. Therefore, the role and the clinical significance of the circulating levels of these molecules were investigated in patients with bone metastasis from breast cancer or prostate cancer. Furthermore, because zoledronic acid, a bisphosphonate derivative used in the palliative treatment of bone metastasis [20], strongly inhibits osteoclast activity, parallel studies were carried out to assess the effect of this drug on the serum levels of cathepsin K and cystatin C. These investigations show that cystatin C is significantly increased in cancer patients as compared to healthy subjects or to patients with non-malignant diseases. The increased levels of this inhibitor in cancer patients does not seem to be due to an impaired kidney function as none of these patients showed clinically evident alteration in renal function. Therefore, these data are consistent with those from other studies reporting that cystatin C levels are enhanced in malignant tissues or in body fluids of patients with neoplastic diseases including patients with breast cancer or prostate cancer and that this phenomenon is associated with more aggressive forms of these tumors [4,11,26−30]. These findings support the concept that cystatin C may facilitate the malignant progression of these tumors [4,26,27,30]. On the other hand, recent experimental evidence showing that this inhibitor may directly promote tumor cell proliferation and dissemination by other mechanisms not related to its enzyme inhibiting activity, further corroborates this hypothesis [4,7,26,31,32]. Therefore, cystatin C may be regarded as a novel target for cancer therapy and as a possible marker of cancer progression [4,30]. However, its clinical value as specific gauge of metastatic bone disease remains controversial. In fact, the lack of significant differences in the serum concentrations of cystatin C between M0 and BM patients either in BCa or in PCa and the poor diagnostic accuracy of this inhibitor to detect BM patients, seems to rule out a direct involvement of this molecule in bone metastasis formation and its usefulness as specific marker of metastatic spread to the bone. Surprisingly, the present data show that cathepsin K blood levels are markedly lower (mean 9.4-fold) in BCa patients than in HS or in OP patients (mean 3.1-fold), while in PCa patients the serum concentrations of this proteinase are
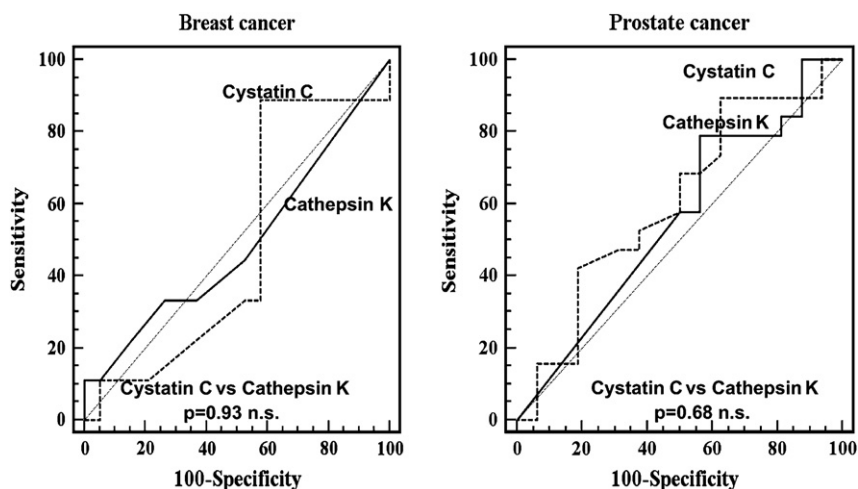

Fig. 3. ROC curves for cathepsin K and cystatin C in patients with breast cancer or prostate cancer with and without bone metastases.

Table 2
Sensitivity, specificity and diagnostic accuracy of cathepsin K and cystatin C in the detection of patients with bone metastases

	AUC (95% C.I.)	Cut-off values[a]	Sensitivity (95% C.I.)	Specificity (95% C.I.)	Accuracy (%)
Breast cancer					
Cathepsin K	0.49 ± 0.12 (0.30–0.67) $p = 0.94$ n.s.	4.9 pmol/L	11.1 (1.8–48.3)	100 (82.2–100)	67.8
Cystatin C	0.47 ± 0.11(0.29–0.67) $p = 0.82$ n.s.	1.41 mg/L	90 (55.5–98.3)	42.1 (20.3–66.6)	39.3
Prostate cancer					
Cathepsin K	0.56 ± 0.10 (0.38–0.72) $p = 0.96$ n.s.	3.7 pmol/L	78.9 (54.4–93.8)	43.7(19.8–70.1)	45.7
Cystatin C	0.64 ± 0.13(0.46–0.79) $p = 0.13$ n.s.	1.36 mg/L	90.0 (55.5–98.3)	37.5(15.3–64.5)	65.7

AUC = area under the curve.

[a] Cut-off values were determined by ROC curve analysis.

not significantly different from those measured in HS or BPH patients. Furthermore, no significant difference in the serum content of this enzyme was observed between M0 and BM patients. These results seem to question an involvement of cathepsin K in bone metastasis formation. On the other hand, recent experimental observations by Garnero et al. [33] indicate that this proteinase appears to be mainly implicated in the degradation of collagen in osteoporosis, while matrix metalloproteinases seem to play a major role in the degradation of bone collagen in metastatic bone disease. These findings may fit well with the results showing significantly higher circulating levels of cathepsin K (mean 2.5-fold) in OP patients than in BM patients. However, it cannot be ruled out that this proteinase, which has its optimum activity in an acid microenvironment as encountered in the resorption lacunae, may act locally and for this reason may not be secreted in excess into the extracellular space or into the bloodstream [1,2,34]. Although these observations do not rule out a direct involvement of cathepsin K in bone metastasis formation, they suggest that this proteinase does not appear suitable as additional serum marker of metastatic spread to the bone.

Interestingly, our results show that the administration of ZA induces a statistically significant increase of serum cathepsin K and cystatin C only in patients with bone metastasis from prostate cancer, a tumor which is known to induce mainly osteoblastic or mixed osteolytic/osteoblastic metastasis [19]. As recent studies show that this proteinase is secreted also by osteoblasts [3] and that cystatin C appears to modulate osteoblastic activity and decrease osteoclastic activity [4,6,7], it is conceivable to speculate that the changes in the circulating levels of these molecules induced by ZA administration may reflect an enhanced osteoblastic activity related to bone remodelling processes elicited by the drug. These observations are consistent with other studies which show that ZA stimulates in vitro the proliferation and differentiation of bone trabecular osteoblastic cells [35]. Therefore, the Cathepsin K/Cystatin C system may be regarded as possible, as additional marker of osteoblastic activity associated with the therapeutic response of BM patients to bisphosphonate treatments. Nevertheless, their clinical value as specific gauges of skeletal metastasis remains questionable. Further investigations with a wide number of patients may better define the clinical role
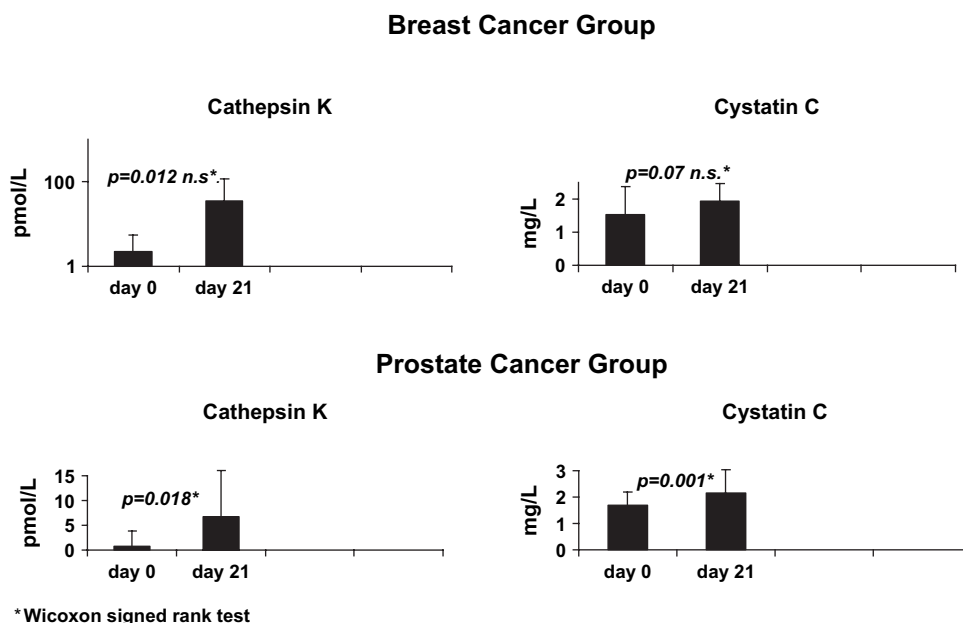

Fig. 4. Effects of zoledronic acid administration (4 mg i.v.) on cathepsin K and cystatin C circulating levels in patients with bone metastases from breast cancer or prostate cancer.

of these molecules in the diagnosis and clinical management of patients with cancer related bone disease.

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