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Low bone turnover levels predict increased risk of cancer

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ABSTRACT

Background: Several epidemiological studies have shown an association between bone mineral density (BMD) and risk of breast cancer in postmenopausal women, but it remains unknown whether bone turnover is associated with increased risk of cancer. The aim of this study was to investigate if markers of bone formation and resorption are associated with increased risk of cancer.

Material and methods: The study population included 5855 postmenopausal Danish women enrolled in the Prospective Epidemiologic Risk Factor (PERF) study. Cancer diagnosis was obtained from the Danish Cancer Registry. Baseline spine, femur, and whole-body BMD were evaluated by DXA-scanners. Baseline bone turnover (CTX-1 and osteocalcin) were measured in serum. Multivariate Cox analysis was performed with 3, 6 and 12 years of follow-up. All continuous variables were transformed into z-score for the cox analyses.

Results: 252 developed cancer after 3 years, 462 developed cancer after 6 years, and 881 developed cancer with 12 years of follow-up. CTX-1, osteocalcin and spine BMD were all predictors of cancer at all time points (3 years of follow-up: Spine BMD, HR = 1.20, $p = 0.003$; CTX-1, HR = 0.82, $p = 0.005$; osteocalcin, HR = 0.75, $p < 0.001$). After adjusting for cancer risk factors and other bone measures CTX-1 and osteocalcin remained independent predictors of cancer (3 years of follow-up: CTX-1, HR = 0.82, $p = 0.02$; osteocalcin, HR = 0.75, $p = 0.002$).

Conclusions: We found that levels of the bone turnover markers CTX-1 and osteocalcin were inversely associated with risk of cancer independent of BMD and other known cancer risk factors in postmenopausal women.

1. Introduction

The need for identifying risk factors for the prevention of cancer is becoming more evident as the western world are aging and the incidence of cancer is rising. Several epidemiological studies have shown an association between bone mineral density (BMD) and risk of breast cancer in postmenopausal women [1–6]. The underlying link is however still unknown with several possible explanations including lifetime estrogen exposure, physical activity, smoking habits and obesity [1–9].

BMD is an essential component in the assessment of bone quality and is used to determine the risk of osteoporotic fractures and to monitor the natural progression and maintenance of bone mass. To measure bone turnover, serological markers of bone resorption and bone formation are used as the preferred choice. Such markers have been developed over the past 20 years [10–12], and include the c-terminal telopeptide (CTX-1) that reflect bone resorption and osteocalcin that reflect bone formation [13,14].

Osteocalcin has recently been linked to regulation of glucose and fat metabolism possibly through stimulation of the adipocyte-derived hormone adiponectin [15–17]. Both osteocalcin and adiponectin are inversely associated with obesity and diabetes and they have been linked to mammographic breast density in postmenopausal women [18]. Several studies have also shown that adiponectin is inversely associated with breast cancer risk [19–25], but it remains unknown whether osteocalcin and CTX-1 are associated with risk of cancer.

The aim of this study was to investigate if markers of bone turnover (CTX-1 and osteocalcin) are associated with risk of overall and site-specific cancer independently of other bone measures and known cancer risk factors in a large cohort of elderly women.

2. Methods

2.1. Study design

The PERF I study was established in 1999–2001 (baseline) to identify risk factors for age-related diseases [26]. Women who previously participated in clinical prevention trials or had been screened for inclusion in the clinical prevention trials at Center for Clinical and

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Basic Research (CCBR) were invited to participate in PERF I. A total of 5855 Danish postmenopausal women (aged 48–89) were enrolled in the PERF I.

The study was carried out in accordance with the protocol approved by the Research Ethics Committee of Copenhagen County, of Viborg and Northern Jutland, Denmark (KA 99070 g) and written informed consent was obtained from all participants.

2.2. Linkage to the Danish national registries

In Denmark, every citizen has a unique personal identification number (CPR number) that enables matching of individuals to health registers. Cancer diagnosis, osteoporosis diagnosis, and mortality information were extracted from the Danish Cancer Registry, the Danish National Patient Registry and the Danish National Death Registry at 31-12-2014 corresponding to end of study. The Danish Cancer registry was last updated in 31-12-2012, making the median follow up of this study 12 years (median). All diagnosis in the Danish health registries has been classified using the Danish version of the International Classification of Diseases (ICD8 and ICD10).

2.3. Inclusion criteria

Women with a diagnosis of solid cancer after baseline (cases, $n = 881$) and women not present in the Danish Cancer Registry (non-cases, $n = 3870$) were included in the analysis. A solid cancer diagnosis after baseline was defined as breast cancer: ICD10 C50, D05, respiratory cancer: C30-C39, D02, gastrointestinal cancers: C15-C26, D00-D01, and other cancers: C00-C14, C40-C43, C45-C49, C51-81, C97, D03-D04, D06-D09. Women with only non-solid cancer or non-malignant tumors specified as benign tumors, non-melanoma skin cancer, hematologic cancer, desmoplasia and neoplasms of uncertain behavior and women with only a solid cancer diagnosis before baseline were exclude from the analysis ($n = 1104$). In case of a woman having more than one solid cancer diagnosis, the diagnosis closest to baseline was chosen for the analysis.

2.4. Baseline investigations

At baseline, the participants completed an interview with a doctor or a nurse covering questions related to physical health, lifestyle and medical history, including smoking status (daily precious/current/never smokers), use of alcohol (≥7 drinks/week), physical activity (≥1 h/week), level of education (elementary/high school/university), age at menarche, age at menopause and prior bisphosphonate treatment (yes/no). Height and weight were measured at baseline and body mass index (BMI, kg/m^2) was calculated. Reproductive life span was calculated by dividing age at menopause with age at menarche.

From the questionnaires and the Danish National Patient Registry, it was determined whether the subjects had been diagnosed with osteoporosis before baseline. Osteoporosis was defined as a positive self-reported osteoporosis diagnosis and/or one of the following codes from the Danish National Patient Registry: 723 (ICD8), M80, M81, M82 or M844 (ICD10).

Bone mineral density was assessed by spine, femur, and whole body DXA-scans using Hologic 4500, Hologic 2000, and LunarProdigy machines. Data was corrected to Hologic 2000 level using cross-calibration from 20 subjects.

All subjects provided fasting blood samples at baseline and serum was stored at −80 °C. Serum calcium levels were measured on ADVIA 1800. Serum osteocalcin (N-MID, ng/mL) and CTX-1 (beta-crosslaps, ng/mL) were both measured on an automatic analyzer (Roche Cobas). White blood cell count was measured using an automated blood cell analyzer (Sysmex). All biomarkers were measured blinded in a CAP-certified laboratory.

2.5. Statistical analysis

Baseline demographic characteristics and medical information were compared by a two-tailed *t*-test for continuous variables and chi-square test for categorical variables.

The associations between all-cause cancer and site-specific cancer and the different bone measures were evaluated by univariate proportional Cox hazard regression with age as timescale. The relationship between the bone measures and cancer were found to be linear. Age was used as timescale in the Cox analysis to ensure that the estimation was based on comparisons of individuals of the same age to prevent confounding by age [27]. Non-cases were censored with the age between baseline and end of the study or date of death, whichever came first. To adjust for confounders, multivariate Cox regression was applied. The bone measures were transformed into z-scores (subtracting each person's value from the sample mean and dividing by the sample standard deviation). Hazard Ratios (HR) were calculated for the bone measures adjusted for BMI (kg/m^2), previous/current smoker (yes/no), alcohol (≥7 drinks/week), exercise (≥1 h/week), education (elementary/high school/university), reproductive life span (years), and white blood cell count (cells/μL). Subjects with missing values were excluded from the analysis. Variance inflation factor (VIF) values were estimated for each regression model. None of the VIF values were larger than 2.5 indicating no existence of collinearity.

To visualize the HR for the univariate Cox analysis, the biomarker levels were entered into the model as a spline with four knots and plotted with restricted cubic splines.

The associations between all-cause mortality and cancer-specific mortality and the different bone measures were evaluated by multivariate proportional Cox hazard regression with age as timescale among the participants that were diagnosed with cancer after baseline.

The statistical analyses were performed by R software (3.4.1 version, R Development Core Team, 2017). The coxph function from the survival package was used with default settings to fit the proportional hazard regression model. The plot.Predict function from the rms package was used to fit and plot the proportional hazard regression models. *P*-values < 0.05 was chosen for statistical significance.

3. Results

3.1. Cohort characteristics

A total of 5855 postmenopausal women were included in the PERF I study. Of the women enrolled 252 developed cancer after 3 years of follow-up, 462 developed cancer after 6 years of follow-up and 881 developed cancer with 12 years of follow-up.

Table 1 summarizes the baseline characteristics of the entire cohort stratified by women with no history of cancer (non-cases) and women who developed cancer after up to 12 years after baseline (cases). The mean age for the non-cases was 70 years at baseline, with the cases being significantly older (years, 71.09 vs 70.02, $p < 0.001$). The cases were also characterized by a significantly higher percentage of smokers (current, 26.6% vs 22.1%, $p < 0.001$), and significantly fewer women being physically active (exercise, 91.9% vs 89.8%, $p = 0.045$) compared to the non-cases. Alcohol consumption, educational level, BMI, HOMA-IR and reproductive life span were non-significant between the two groups.

When looking at different bone measurements, bone turnover (osteocalcin and CTX-1) were significantly lower in the cases compared to the non-cases (osteocalcin, 30.77 ng/mL vs 29.40 ng/mL, $p = 0.004$, CTX-1, 0.45 ng/mL vs 0.42 ng/mL, $p = 0.001$). Spine BMD was significantly higher in the cases compared to the non-cases (BMD, 0.91 g/cm^3 vs 0.93 g/cm^3, $p = 0.002$). Calcium levels, femur BMD, whole body BMD, number of osteoporosis patient and patients receiving bisphosphonate (BP) treatment were non-significant between the two groups.

Table 1
Patient demographics.

	Cases (n = 881)		Non-cases (n = 3870)		P-value
	Mean (SD)	n	Mean (SD)	n	
Age at BL (years)	71.09 (6.14)	881	70.02 (6.60)	3870	< 0.001
Education (n (%))					0.37
Elementary	646/880 (73.4)		2766/3862 (71.6)		
High School	171/880 (19.4)		833/3862 (21.6)		
University	63/880 (7.2)		263/3862 (6.8)		
Smoking (n (%))					< 0.001
Never	353/881 (40.1)		1890/3870 (48.8)		
Current	234/881 (26.6)		856/3870 (22.1)		
Previous	294/881 (33.4)		1124/3870 (29.0)		
Alcohol > 7 units/week (n (% yes))	302/877 (34.4)		1240/3848 (32.2)		0.22
Excercise > 1 h/week (n (% yes))	790/880 (89.8)		3551/3863 (91.9)		0.045
BMI (kg/cm^2)	26.26 (4.24)	843	26.15 (4.24)	3728	0.50
HOMA-IR	2.43 (2.93)	839	2.25 (2.46)	3710	0.065
Reproductive life span	35.12 (5.17)	867	35.24 (5.10)	3824	0.554
White blood cells (cells/μL)	6.08 (1.71)	844	5.83 (1.62)	3748	< 0.001
Calcium (mmol/L)	2.38 (0.10)	846	2.38 (0.10)	3749	0.72
CTX-1 (ng/mL)	0.42 (0.22)	834	0.45 (0.24)	3719	0.001
Osteocalcin (ng/mL)	29.40 (12.03)	840	30.77 (12.64)	3733	0.004
Bone mineral density					
Femur (g/cm^3)	0.79 (0.13)	825	0.79 (0.12)	3686	0.95
Spine (g/cm^3)	0.93 (0.17)	843	0.91 (0.16)	3728	0.002
Whole body (g/cm^3)	0.97 (0.11)	787	0.96 (0.10)	3530	0.07
Osteoporosis at BL (n (% yes))	106/876 (12.1)		467/3839 (12.2)		1
Bisphosphonate treatment (n (% yes))	56/881 (6.4)		305/3870 (7.9)		0.14

3.2. Bone measures and overall-cancer risk

To investigate the relationship between cancer diagnosis and the different bone measurements we conducted uni- and multivariate Cox regression analysis with age as timescale. In the univariable analysis CTX-1, osteocalcin, spine BMD and whole body BMD were all predictors of cancer, but the strongest association were observed with 3 years of follow-up (Table 2). In detail, an increase in one standard deviation (SD) in spine BMD were associated with 120% increased risk of cancer at 3 years of follow-up (Spine, HR = 1.20, $p = 0.00$), whereas an increase in one SD CTX-1 and osteocalcin levels were associated with 22% and 33% decreased risk of cancer (CTX-1, HR = 0.82, $p = 0.005$; osteocalcin, HR = 0.75, $p < 0.001$).

To test if the variables were independent predictors of cancer, we performed a multivariate Cox regression analysis (Table 3, multivariate test 1). After adjusting for covariates (BMI, HOMA-IR, education, smoking, alcohol, walking, exercise, reproductive life span and white blood cells) CTX-1, osteocalcin, Spine BMD and whole body BMD remained independent predictors of cancer (CTX-1, HR = 0.83, $p = 0.01$; osteocalcin, HR = 0.77, p < 0.001, spine BMD, HR = 1.24, p = 0.001; WB BMD, HR = 1.19, $p = 0.03$) (Table 3).

We also tested if CTX-1 and osteocalcin were predictors of cancer independent of the other bone measures and cancer risk factors (Table 2, multivariate test 2). After adjusting for calcium, whole body BMD, osteoporosis, and bisphosphonate treatment, BMI, HOMA-IR, education, smoking, alcohol, walking, exercise, reproductive life span, and white blood cells, CTX-1, and osteocalcin remained independent predictors of cancer (CTX-1, HR = 0.82, p = 0.02; osteocalcin, HR = 0.75, p = 0.002).

3.3. Bone measures and site-specific cancer risk

Of the women who developed cancer after baseline 240 women developed breast cancer, 230 women developed gastrointestinal cancer, 123 women developed respiratory cancer and 288 women develop other cancers. To determine whether the results were applicable to all type of cancer we used restricted cubic splines to evaluate HRs calculated from a univariate Cox regression analysis with age as timescale and 12 years of follow-up (Fig. 1).

The results showed that levels of bone turnover (CTX-1 and osteocalcin) were associated with increased risk of all types of cancer. For whole body, femur and spine BMD high levels were associated with increased risk of breast and other cancers, whereas high levels were associated with a decreased risk of respiratory cancers. GI cancers were

Table 2
Univariate Cox regression analysis with age as timescale.

	3 years				6 years				12 years			
	HR	CI	P	n[b]	HR	CI	P	n[b]	HR	CI	P	n[b]
CTX-1 (ng/mL)[a]	0.82	0.71–0.94	0.005	3955/236	0.85	0.77–0.94	0.002	4155/436	0.89	0.82–0.96	0.001	4553/834
Osteocalcin (ng/mL)[a]	0.75	0.64–0.77	< 0.001	3972/239	0.83	0.74–0.92	< 0.001	4172/439	0.89	0.82–0.98	0.002	4573/840
Calcium (mmol/L)[a]	0.98	0.87–1.14	0.77	3991/242	0.98	0.89–1.07	0.61	4193/444	1.01	0.94–1.08	0.77	4595/846
Bone mineral density (g/cm^3)												
Femur[a]	1.09	0.95–1.24	0.22	3928/242	1.07	0.97–1.18	0.17	4119/433	1.03	0.90–1.11	0.37	4511/825
Spine[a]	1.20	1.07–1.36	0.003	3973/245	1.15	1.06–1.27	0.002	4170/442	1.12	1.105–1.20	< 0.001	4571/843
Whole body[a]	1.14	0.98–1.32	0.08	3757/227	2.10	1.08–4.07	0.03	3939/409	1.11	1.03–1.20	0.008	4317/787
Osteoporosis at BL (yes/no)	0.85	0.57–1.28	0.44	4089/250	0.913	0.68–1.22	0.53	4298/459	0.995	0.81–1.22	0.96	4715/876
Bisphosphonate treatment	0.65	0.38–1.12	0.12	4122/252	0.681	0.46–1.01	0.05	4332/462	0.798	0.61–1.05	0.10	4751/881

[a] Transformed into *z*-scores.
[b] Subjects/events.

Table 3
Multivariate Cox regression analysis with 3 years of follow-up and age as timescale.

	Multivariable model 1[b]				Multivariable model 2[c]			
	HR	CI	P	n[d]	HR	CI	P	n[d]
CTX-1 (ng/mL)[a]	0.83	0.72–0.96	0.01	3838/228	0.82	0.76–0.96	0.02	3599/211
Osteocalcin (ng/mL)[a]	0.77	0.66–0.90	< 0.001	3855/231	0.75	0.62–0.9 +	0.002	3615/213
Calcium (mmol/L)[a]	0.96	0.85–1.10	0.59	3871/232	–			
Bone mineral density (g/cm^3)								
Femur[a]	1.16	1.00–1.35	0.06	3824/228	–			
Spine[a]	1.24	1.09–1.42	0.001	3865/231	–			
Whole body[a]	1.19	1.01–1.740	0.03	3658/215	–			
Osteoporosis at BL (yes/no)	0.45	0.55–1.29	0.43	3849/231	–			
Bisphosphonate treatment (yes/no)	0.70	0.40–1.20	0.20	3873/232	–			

[a] Transformed into z-scores.
[b] Model 1: All variables are adjusted for HOMA-IR, BMI, smoking, education, alcohol, exercise, reproductive life span, and white blood cells.
[c] Model 2: All variables are adjusted for co-variates included in model 1 + calcium, WB BMD, osteoporosis at BL and biphosphate treatment.
[d] Subjects/events.

generally not associated with either of the BMD measures.

To validate the graphs, we made a multiple Cox regression analysis adjusted for HOMA-IR, BMI, education, smoking, alcohol, walking, exercise, reproductive life span and white blood cells. The multivariate Cox regression analysis confirmed the trends of the cubic splines, but only CTX-1, Osteocalcin, spine BMD, femur BMD, and whole body BMD were significantly associated with breast cancer (CTX-1; HR = 0.84, p = 0.03; osteocalcin HR = 0.04, p = 0.03; femur, HR = 1.24, *p* = 0.008; spine, HR = 1.33, p < 0.001; WB, HR = 1.34, p < 0.001) and calcium was significantly associated with GI cancer (HR = 0.106, p = 0.04) (Table 4).

4. Discussion

In this study, we found that women with low bone turnover levels, measured by CTX-1 and osteocalcin, had an increased risk of cancer. This was independent of BMD and other cancer risk factors such as smoking, BMI, and insulin resistance (HOMA-IR). To our knowledge, the current study is the first to investigate the relationship between bone turnover and cancer risk in postmenopausal women.

How bone turnover is associated with cancer risk requires further investigation, but several potential mechanisms are worthy of consideration. A minimal remodeling is necessary to repair old and damaged cells, release growth factors and maintain tissue integrity. We have previously shown that a balanced turnover of both bone and soft tissue is associated with a decreased risk of mortality [28]. Women in the lowest and highest quintile of CTX-1 and osteocalcin had a two times increased risk of mortality compared to women in the middle quintile, highlighting the importance of a healthy ECM balance. In light of these results, it is possible that a decline in bone turnover is a sign of a decline in tissue regenerative potential and a mirror of general health [29,30].

Our results are also in line with several previous studies from large databases such as the Rotterdam, Women's Health Initiative, and Framingham, which found a higher incidence of breast cancer in postmenopausal women with high BMD [1,2,31]. The Rotterdam study found that women in the highest tertile of spine BMD had a twofold higher incidence of breast cancer compared to women in the middle tertile and in the in the Framingham study women in the highest quartile had 3.5 times increased risk of breast cancer compared to the lowest quartile [1,2]. Studies have also shown that both CTX-1 and osteocalcin correlate negatively with BMD and therefore could reflect BMD [32,33]. In this study, however, CTX-1 and osteocalcin were associated with cancer risk independent of BMD. It should also be noted that, although not significant, both low bone turnover and low BMD were associated with increased risk of respiratory cancer. Other studies have found that low BMD are associated increased risk of lung cancer and it is well known that patients with chronic obstructive pulmonary diseases (COPD) have a higher risk of osteoporosis [34–36]. These results are in line with ours and stresses that bone turnover may not merely be a proxy variable for BMD. Further studies are needed to elucidate the biological relationship between bone turnover, BMD and cancer risk.

It has also been hypothesized that bone turnover is linked to the regulation of energy metabolism [37]. Ferron et al. showed that osteocalcin in wildtype mice directly regulates glucose metabolism and thereby affect insulin sensitivity and fat mass [38]. Furthermore, a meta-analysis of 52 observational studies concluded that high osteocalcin levels were associated with a reduction in glucose levels, HOMA-IR and BMI [39]. It has been speculated that osteocalcin is linked to energy metabolism through stimulation of the adipocyte-derived hormone adiponectin [15–17]; a hormone that in several meta-studies have been shown to be inversely associated with cancer risk [19–25]. It is, therefore, possible that bone turnover markers are linked to cancer risk through the regulation of energy metabolism. It should, however, be mentioned that both CTX-1 and osteocalcin in this study was associated with increased risk of cancer independent of HOMA-IR and BMI.

Several strength and limitations should be noted. The data is restricted to postmenopausal Danish Caucasian women and may therefore not be generalizable to the general population. The design of this study does also not allow for evaluation of the causative relationship between bone measures and risk of cancer. Furthermore, in this study, serum free estradiol and estradiol metabolites were not measured, and we were not able to discriminate between different forms of osteocalcin. Lastly, as CTX-1 and osteocalcin has a high variation they are not suited to predict cancer on a patient level. The strength of this study is the prospective design, the long follow-up period, the access to the Danish patient registries, the standardized protocol and the amount of obtained variables.

In conclusion, in this large study of postmenopausal women, we found low levels of bone turnover markers were predictors of increased risk of cancer independent of BMD and other known cancer risk factors. Further studies are needed to investigate the biological relationship between bone turnover markers and cancer risk.

Declaration of Competing Interest

Cecilie Liv Bager and Frederikke Bay are employed at ProScion. Morten Karsdal is employed. Claus Christiansen and Morten Karsdal hold stock at Nordic Bioscience.

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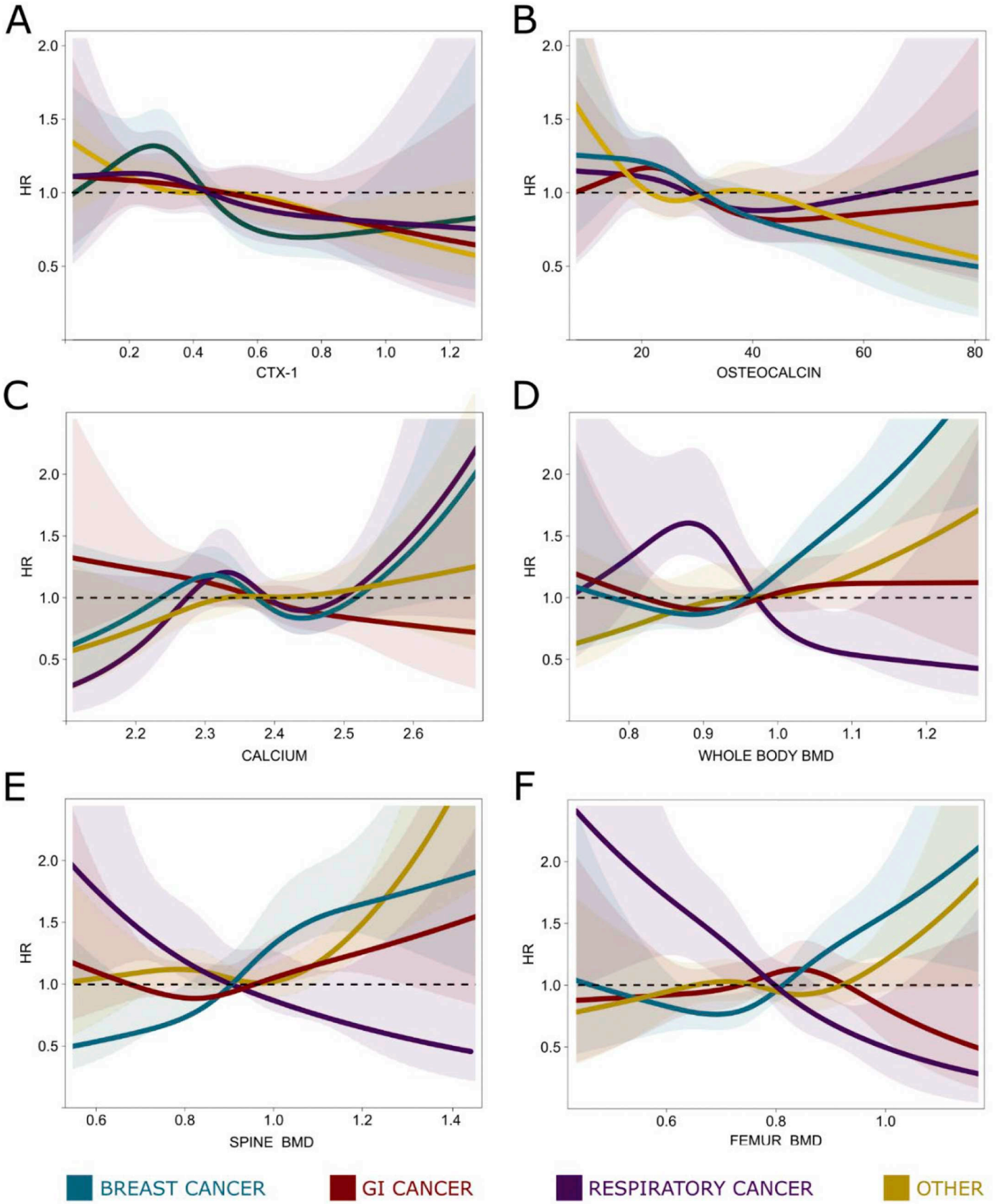

Fig. 1. Hazard ratios (HR) of breast cancer (green with light grey 95% CI), GI cancers (pink with grey 95% CI), respiratory cancer and other cancers (blue with dark grey 95% CI) for (A) CTX-1, (B) osteocalcin, (C) calcium, (D) Whole body BMD, (E) Spine BMD and (F) Femur BMD. The HR are calculated by Cox-analysis with age as timescale and plotted as linear tail-restricted cubic splines with 4 knots. The horizontal dashed line correspond to the normal reference HR of 1.0, values above are associated with increase cancer risk, and values below are associated with decreased cancer risk.

Table 4
Multivariate cox regression analysis with 12 years of follow up and age as timescale.

	Breast[a]				Respiratory[a]			
	HR	CI	P	n[c]	HR	CI	P	n[c]
CTX-1 (ng/mL)[b]	0.84	0.72–0.98	0.03	3824/214	0.89	0.73–1.10	0.28	3721/111
Osteocalcin (ng/mL)[b]	0.84	0.71–0.99	0.03	3842/218	0.94	0.76–1.16	0.56	3736/112
Calcium[b]	1.03	0.90–1.17	0.72	3858/219	1.08	0.89–1.31	0.40	3752/113
Bone mineral density (g/cm^3)								
Femur[b]	1.24	1.06–1.45	0.008	3803/207	0.85	0.68–1.06	0.14	3708/112
Spine[b]	1.33	1.16–1.54	< 0.001	3851/217	0.88	0.71–1.09	0.23	3747/113
Whole body[b]	1.34	1.14–1.57	< 0.001	3645/202	0.87	0.169–1.10	0.25	3550/107
Osteoporosis at BL (yes/no)	1.21	0.80–1.82	0.37	3838/220	1.04	0.60–1.82	0.88	3730/112
Bisphosphonate treatment	0.74	0.40–1.36	0.33	3861/220	0.98	0.51–1.89	0.94	3754/113

	Gastrointestinal[a]				Other[a]			
	HR	CI	P	n[c]	HR	CI	P	n[c]
CTX-1 (ng/mL)[b]	0.93	0.81–1.08	0.35	3830/220	0.92	0.80–1.04	0.19	3874/264
Osteocalcin (ng/mL)[b]	0.93	0.80–1.08	0.34	3844/220	0.92	0.80–1.05	0.19	3889/265
Calcium[b]	0.88	0.77–1.00	0.05	3859/220	1.07	0.95–1.21	0.24	3905/266
Bone mineral density (g/cm^3)								
Femur[b]	1.01	0.86–1.19	0.87	3811/215	1.05	0.91–1.20	0.50	3859/263
Spine[b]	1.11	0.97–1.28	0.13	3854/220	1.14	1.00–1.29	0.05	3899/265
Whole body[b]	1.04	0.88–1.23	0.62	3650/207	1.27	1.01–1.36	0.03	3689/246
Osteoporosis at BL (yes/no)	1.12	0.76–1.66	0.59	3838/220	0.88	0.59–1.30	0.52	3881/263
Bisphosphonate treatment	0.86	0.51–1.45	0.58	3861/220	0.92	0.57–1.47	0.71	3907/266

[a] All variables are adjusted for HOMA-IR, BMI, smoking, alcohol, exercise, education, reproductive life span and white blood cells.
[b] Transformed into z-scores.
[c] Subjects/events.

PERF I study.

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