



Serum bone markers and risk of osteoporosis and fragility fractures in women who received endocrine therapy for breast cancer: a prospective study

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Abstract

Purpose Osteoporosis and fragility fracture are major bone toxicities of aromatase inhibitors (AIs) for postmenopausal hormone receptor-positive breast cancer. Except for a few small studies on bone turnover markers and reduced bone mineral density after AI treatment, data on the associations of bone markers and risk of osteoporosis or fracture from prospective studies are lacking.

Methods In a prospective study of 1709 women on AIs, two bone turnover markers, BALP and TRACP, and two bone regulatory markers, RANKL and OPG, were measured and examined in relation to risk of osteoporosis and fragility fractures during a median follow-up time of 6.1 years.

Results Higher levels of BALP and TRACP were both associated with increased risk of osteoporosis and higher BALP/TRACP ratios were associated with lower risk of osteoporosis, but no associations were observed for fracture risk. Higher levels of OPG were associated with increased risk of fracture, whereas higher levels of RANKL were associated with lower risk. As a result, OPG/RANKL ratios were positively associated with fracture risk [hazard ratio (HR) = 2.49, 95% confidence interval (CI) 1.34–4.61]. After controlling for age and fracture history, the associations became non-significant but a suggestive trend remained (HR = 1.80, 95% CI 0.96–3.37).

Conclusion Our study provides suggestive evidence for the potential utility of OPG/RANKL ratios in predicting risk of fracture in women treated with AIs for breast cancer. Further validation may be warranted.

Keywords Breast cancer · Osteoporosis · Fracture · Bone markers

Introduction

Adjuvant endocrine therapy is effective in lowering the risk of recurrence for women diagnosed with hormone receptor-positive breast cancer. Its importance in the clinical management of breast cancer has become even more prominent in the context of the recent findings from the TAILORx

clinical trial reporting that most patients with an intermediate recurrence score from the 21-gene test would benefit from endocrine therapy alone, forgoing the need of chemotherapy [1]. However, endocrine therapy is not free of side effects. Some long-term complications for postmenopausal patients, particularly from aromatase inhibitors (AIs), pose challenges to patients' quality of life. A major side effect related to AI use is bone weakening. AIs almost completely block the peripheral conversion of androgens to estrogens in adipose tissue, which is a major source of estrogens in postmenopausal women. The resulting estrogen deficiency puts patients at high risk of osteoporosis and fractures. In a recent meta-analysis, it was shown that patients treated with AIs had a 35% higher fracture risk than those treated with tamoxifen [2]. Even the steroidal exemestane, the AI that was bone sparing in animal models due to its androgenic

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structure, caused a similar number of fragility fractures as the non-steroidal anastrozole in the MA-27 trial [3].

Clinical management of AI-associated bone loss in postmenopausal women with hormone receptor-positive breast cancer usually involves recommendations for exercise and calcium/vitamin D supplementation, and therapeutic treatment such as bisphosphonates and denosumab. These intervention strategies are developed by considering bone mineral density (BMD) and conventional fracture risk factors, which are largely extrapolated from the literature on bone health in the general population without cancer. It is thus important to evaluate known and novel predictors of fracture risk in breast cancer patients treated with AIs.

In an ongoing observational study, we are investigating lifestyle, molecular markers and genetic factors as potential predictors for the risk of osteoporosis and fractures in a large population of patients who received AIs for their endocrine therapy for breast cancer in an integrated healthcare clinical setting [4]. This community-based clinical setting is different from most previous studies of bone health in the clinical trial setting. In this current study, we hypothesized that bone markers in circulation may provide important information about the baseline state of bone turnover and regulation, which cannot be directly assessed by dual energy x-ray absorptiometry (DXA) scans or surveying of other risk factors. Indeed, a significant proportion of fractures occur in postmenopausal women with apparently normal BMD, supporting the need of bone biomarkers in addition to DXA scans.

We measured four markers in 1709 patients shortly after breast cancer diagnosis, including two bone turnover markers and two bone regulatory markers. In a previous study, we reported findings of these markers with bone health history before breast cancer diagnosis [5]. The present study focuses on these bone markers in relation to risk of osteopenia, osteoporosis and fractures identified prospectively after the initiation of AI therapy.

Patient population and methods

Patient population

This bone marker study was nested in the Pathways Study, a prospective cohort of breast cancer survivors at Kaiser Permanente Northern California (KPNC). Both the parent study and this ancillary study have been described in detail previously [4–6]. In brief, women newly diagnosed with invasive breast cancer at KPNC were enrolled, on average, 2 months post-diagnosis after written consent was obtained. Between January 2006 and April 2013, a total of 4505 patients were enrolled by completing a baseline in-person interview after informed consent. Non-fasting blood samples were obtained

from 90% of participants after the baseline interview, approximately 2 months after diagnosis, and shipped on dry ice overnight to the Data Bank and Biorepository (DBBR) laboratories at Roswell Park Comprehensive Cancer Center for processing. Serum samples were aliquoted into 0.5 straws and stored in liquid nitrogen until analysis. For this ancillary study, 1709 patients who were treated with AIs and had blood samples collected for bone marker measurement were included. In this subcohort, blood samples were collected at a median 50 days before the start of AI treatment, whereas 515 patients had samples collected shortly after the start of AI treatment (median = 32 days). The study was approved by Institutional Review Boards of Roswell Park Comprehensive Cancer Center and Kaiser Permanente Northern California for human subject protection.

Measurement of bone markers

Four bone markers were selected for measurement using serum samples and commercially available kits. These include two bone turnover markers, bone-specific alkaline phosphatase (BALP) for bone formation and tartrate-resistant acid phosphatase 5b (TRACP) for bone resorption (both from Quidel, San Diego, CA); and two bone regulatory markers, receptor activator of nuclear factor kappa-B ligand (RANKL) (Biovender, Ashville, NC) secreted by osteoclasts and osteoprotegerin (OPG) secreted by osteoblasts (R&D System, Minneapolis, MN). The assays were performed according to manufacturers' protocols and each sample was tested in duplicates. When the coefficient of variation (CV) for a sample exceeded 15%, the assay was repeated. The average CV was 1.9% for BALP assay, 2.3% for TRACP assay, 5.8% for RANKL assay, and 5.5% for OPG assay. No significant differences were noted in the levels of any of the bone markers between those with blood samples collected before and after the start of AI therapy.

Bone mineral density, osteoporosis, and fracture

To identify patients with osteopenia or osteoporosis after breast cancer diagnosis, bone mineral density (BMD) values for the femoral neck, total hip and lumbar spine were extracted from the radiology reports of DXA scans, which were performed at the discretion of physicians for usual clinical care, in the KPNC electronic medical record (EMR). Algorithms were developed for this purpose, with the performance validated by comparison to manual review of 239 patients with 532 BMD values as previously reported [4]. Among 1709 patients included in this study, 1509 (87.1%) had at least one DXA after breast cancer diagnosis, whereas 220 (12.9%) had no DXA scan after cancer diagnosis. T-scores were calculated based on BMD values, and osteopenia and osteoporosis were classified as previously

described [5]. A patient was determined to be osteopenia or osteoporotic if the *T*-score was at any point after cancer diagnosis within the specified range. Similarly, any osteopenia/osteoporosis was defined as yes if at least one of the three sites measured (femur, hip and spine) fell within the specified range.

Fractures occurring after breast cancer diagnosis at the humerus, wrist, hip, and spine were obtained from the EMR using ICD-9 codes. All encounter data were then manually reviewed by a medical record abstractor and subsequently validated by the study endocrinologist (J. Lo). Traumatic fractures, prevalent fractures and pathologic fractures including bone metastases were flagged and removed from the analysis. Major fragility fractures were defined as those at the humerus, wrist, hip, or spine. Previous history of osteoporosis and fractures before breast cancer diagnosis were obtained from the EMR, and bisphosphonate use was also used as an indicator of history of osteoporosis as previously described [5].

Statistical analysis

Mean and standard deviation for continuous variables and count and percentage for categorical variables were used to summarize the patient population. The values of all four markers fit a normal distribution and thus were used without transformation. Two ratios, BALP/TRACP and OPG/RANKL, were derived and log-transformed. For fracture outcomes, cumulative incidence curves were generated according to the quartile levels of the individual bone biomarkers and the two derived ratios for bone turnover and bone resorption. Cox proportional hazards models were developed with and without adjustment for age and prior fracture history, and hazards ratios (HRs) and 95% confidence intervals (CIs) were reported. The proportional hazards assumption was examined by the scaled Schoenfeld residuals and no violation was identified in any of the models tested. Because the incidence of frailty fractures was sparse in separate anatomic sites for patients treated with AIs, the analysis focused on major fractures as a combined outcome. In addition, osteopenia and osteoporosis were analyzed separately in comparison to patients without abnormal BMD results using multinomial logistic regression models. Odds ratios (ORs) and 95% CIs were reported for the quartiles of the levels of biomarkers and the two derived ratios. The analyses were conducted using R version 3.6.1.

Results

Table 1 describes the characteristics of the study population. As expected, most of the patients were Whites, diagnosed after menopause, with stage I/II, estrogen receptor-positive

Table 1 Descriptive characteristics of Pathways Study patient population who received aromatase inhibitor treatment

Characteristic	N (%)
Age at diagnosis (years)	
< 50	54 (3.2)
50–59	498 (29.1)
60–69	717 (42.0)
≥ 70	440 (25.7)
Menopausal status	
Premenopausal	99 (5.8)
Postmenopausal	1610 (94.2)
Race/ethnicity	
White	1269 (74.3)
Black	92 (5.4)
Asian	159 (9.3)
Hispanic	158 (9.2)
American Indian/Pacific Islander	31 (1.8)
AJCC stage	
I	1031 (58.1)
II	579 (33.9)
III	151 (8.8)
IV	21 (1.2)
Estrogen receptor status	
Positive	1695 (99.2)
Negative	14 (0.8)
HER2 status	
Negative	1484 (86.8)
Positive	162 (9.5)
Not done	63 (3.7)
Osteoporosis prior to diagnosis	
No	1576 (92.2)
< 5 years	91 (5.3)
≥ 5 years	42 (2.5)
Any fracture prior to diagnosis	
No	1426 (83.4)
< 5 years	153 (9.0)
≥ 5 years	130 (7.6)

AJCC American Joint Cancer Committee, *HER2* human epithelial growth factor 2

and HER2-negative disease. A small percentage of the patients had a history of osteoporosis (7.8%) and any fracture (16.6%).

Almost half (48.9%) of the patients on AIs with were classified as osteopenia based on available DXA scans performed after breast cancer diagnosis, and another 15.8% were osteoporotic (Table 2). Spine was the most frequent anatomic site diagnosed with osteoporosis, followed by femur and hip. During a median follow-up time of 6.1 years (range 0.2–9.8 years), 117 (6.8%) incident, non-traumatic, non-pathologic fractures were ascertained, which are

Table 2 Bone health outcomes among breast cancer patients after treatment with aromatase inhibitors

	N (%)
Any osteopenia/osteoporosis	
Osteopenia	835 (48.9)
Osteoporosis	270 (15.8)
None	604 (35.3)
Hip osteopenia/osteoporosis	
Osteopenia	564 (33.0)
Osteoporosis	51 (3.0)
None	1094 (64.0)
Femur osteopenia/osteoporosis	
Osteopenia	715 (41.8)
Osteoporosis	132 (7.7)
None	862 (50.4)
Spine osteopenia/osteoporosis	
Osteopenia	582 (34.1)
Osteoporosis	174 (10.2)
None	953 (55.8)
Any fracture	
Yes	117 (6.8)
No	1592 (93.2)
Hip fracture	
Yes	17 (1.0)
No	1692 (99.0)
Humerus fracture	
Yes	31 (1.8)
No	1678 (98.2)
Wrist fracture	
Yes	47 (2.8)
No	1662 (97.2)
Spine fracture	
Yes	28 (1.6)
No	1681 (98.4)

summarized in Table 2. Wrist was the most frequent anatomic site with fractures, followed by humerus, spine, and hip.

Higher levels of BALP and TRACP were associated with higher risk of both osteopenia (Q4 BALP, age adjusted OR = 1.33, 95% CI 0.97–1.81; Q4 TRACP, age adjusted OR = 2.07; 95% CI 1.52–2.82) and osteoporosis (Q4 BALP, age adjusted OR = 1.63, 95% CI 1.08–2.45; Q4 TRACP, age adjusted OR = 2.07; 95% CI 1.34–3.19) with age adjustment; yet higher BALP/TRACP ratios were associated with lower risk of osteopenia (Q4, age adjusted OR = 0.61; 95% CI 0.44–0.83) and osteoporosis (Q4, age adjusted OR = 0.67; 95% CI 0.44–1.02) (Table 3). Higher OPG levels were also associated with lower risk of osteopenia (Q4, age adjusted OR = 0.54, 95% CI 0.39–0.75) and osteoporosis (Q4, age adjusted OR = 0.64, 95% CI 0.41–1.02), but no associations

were found with RANKL levels and OPG/RANKL ratios. In analyses of osteopenia and osteoporosis at the femur, hip, and spine separately, the associations with osteopenia at these sites remained largely the same. Similar associations were also found with osteoporosis at the spine but not at the femur or the hip (data not shown). Further adjustment for prior osteoporosis history did not noticeably change the results, and in some cases, resulted in stronger associations (data not shown).

In unadjusted models, the levels of BALP, TRACP, or BALP/TRACP ratios were not associated with fracture risk (Table 4), whereas the fourth quartile of OPG and RANKL levels were associated with increased (HR = 2.42, 95% CI 1.41–4.15) and decreased (HR = 0.50, 95% CI 0.28–0.90) risks of fracture, respectively. As a result, the third and fourth quartiles of OPG/RANKL ratios were associated with increased risk of fracture (Q4 HR = 2.49, 95% CI 1.34–4.61; Fig. 1). After adjusting for age at diagnosis, the results became attenuated and non-significant, although the trends remained the same (Q4 OPG/RANKL ratios, adjusted HR = 1.74, 95% CI 0.93–3.26). Further adjustment for prior fracture history did not noticeably change the risk estimates (HR = 1.80, 95% CI 0.96–3.37).

Discussion

In this large population of breast cancer patients in an integrated healthcare setting who received AI therapy for breast cancer, we found that bone turnover markers at diagnosis, including BALP and TRACP, were associated with risk of osteopenia and osteoporosis but not fragility fractures, whereas the bone regulatory markers, OPG and RANKL, were associated with risk of fracture. In comparison to single markers, the relative ratios between OPG and RANKL reflective of bone resorption and bone formation appeared to be more closely related to risk of osteoporosis or fracture.

Bone-damaging effects of AIs for breast cancer are well established in many randomized clinical trials comparing AIs to tamoxifen or placebo where fracture events were recorded [7–15]. The role of bone biomarkers in either assessing fracture risk or monitoring response to antiresorptive therapy, however, is much less established. The limited existing evidence comes mostly from ancillary studies to clinical trials based on relatively small sample sizes [9, 16, 17]. Because those trials were not designed to evaluate bone outcomes as primary events, to the best of our knowledge, no studies have examined the associations of bone turnover markers with osteoporosis or fractures in AI users. In addition, despite their central role in coupling the processes of bone resorption and formation, we are unaware of any studies on OPG and RANKL as two bone regulatory markers in breast cancer patients. Thus, this is the first and largest

Table 3 Odds ratio of osteopenia and osteoporosis associated with bone biomarkers with at any site in patients treated with aromatase inhibitors

	Age adjusted		Age and prior osteoporosis adjusted	
	Osteopenia	Osteoporosis	Osteopenia	Osteoporosis
BALP				
Q1	1.00	1.00	1.00	1.00
Q2	0.88 (0.64–1.19)	0.79 (0.52–1.22)	0.92 (0.67–1.25)	1.01 (0.64–1.61)
Q3	1.36 (1–1.84)	1.03 (0.67–1.58)	1.44 (1.06–1.95)	1.45 (0.92–2.3)
Q4	1.33 (0.97–1.81)	1.63 (1.08–2.45)	1.42 (1.04–1.94)	2.44 (1.56–3.8)
TRACP				
Q1	1.00	1.00	1.00	1.00
Q2	1.53 (1.13–2.07)	1.53 (0.99–2.36)	1.59 (1.17–2.15)	1.84 (1.15–2.94)
Q3	1.53 (1.13–2.07)	1.7 (1.11–2.61)	1.62 (1.19–2.21)	2.33 (1.47–3.7)
Q4	2.07 (1.52–2.82)	2.07 (1.34–3.19)	2.23 (1.63–3.04)	3.07 (1.93–4.89)
BALP/TRACP ratio				
Q1	1.00	1.00	1.00	1.00
Q2	0.73 (0.54–0.99)	0.74 (0.49–1.1)	0.74 (0.55–1)	0.8 (0.53–1.22)
Q3	0.71 (0.53–0.96)	0.66 (0.43–0.99)	0.72 (0.53–0.97)	0.68 (0.44–1.04)
vQ4	0.61 (0.44–0.83)	0.67 (0.44–1.02)	0.6 (0.44–0.82)	0.63 (0.41–0.98)
OPG				
Q1	1.00	1.00	1.00	1.00
Q2	0.95 (0.69–1.32)	1.12 (0.71–1.75)	0.93 (0.67–1.3)	1.01 (0.63–1.61)
Q3	0.69 (0.5–0.95)	0.87 (0.56–1.36)	0.69 (0.5–0.94)	0.86 (0.54–1.37)
Q4	0.54 (0.39–0.75)	0.64 (0.41–1.02)	0.54 (0.39–0.75)	0.63 (0.39–1.01)
RANKL				
Q1	1.00	1.00	1.00	1.00
Q2	0.89 (0.66–1.19)	0.85 (0.56–1.3)	0.89 (0.66–1.2)	0.86 (0.55–1.33)
Q3	0.9 (0.67–1.22)	0.94 (0.61–1.44)	0.89 (0.66–1.21)	0.89 (0.57–1.4)
Q4	1.04 (0.76–1.41)	1.34 (0.88–2.02)	1.04 (0.76–1.41)	1.31 (0.85–2.03)
OPG/RANKL ratio				
Q1	1.00	1.00	1.00	1.00
Q2	0.78 (0.56–1.08)	0.96 (0.61–1.5)	0.78 (0.56–1.08)	0.98 (0.61–1.57)
Q3	0.83 (0.6–1.15)	0.83 (0.52–1.3)	0.85 (0.61–1.18)	0.93 (0.58–1.5)
Q4	0.74 (0.54–1.02)	0.72 (0.46–1.13)	0.74 (0.54–1.02)	0.74 (0.46–1.18)

prospective study evaluating the relationships of bone turnover and regulatory markers in relation to AI-related osteoporosis and fracture.

In postmenopausal women without breast cancer, some studies showed that elevated levels of bone turnover markers were associated with increased fracture risk independent of BMD or prior fracture [18–21]; however, a few other studies showed no such associations [22, 23]. In our breast cancer patient population, higher levels of bone turnover markers were associated with higher risk of osteopenia and osteoporosis, i.e., low BMD, but not fracture. Thus, our findings suggest that patients with relatively high activity of bone turnover at cancer diagnosis may be at higher risk of BMD loss due to AIs, but do not support the utility of the two bone turnover markers for assessment of fracture risk in postmenopausal breast cancer patients undergoing AI therapy. Of note, biomarkers for bone formation and resorption were both associated with osteoporosis in the same direction.

This suggests that both processes become more active in the event of accelerated bone turnover, as shown in previous studies evaluating changes in bone turnover markers after AI therapy [9, 16, 17]. It is the balance between the two contrasting processes as evaluated by BALP/TRACP ratio that may determine the net effect on BMD. These findings support future studies to evaluate the ratio instead of either marker separately to better assess the state and direction of bone turnover.

The RANKL/RANK/OPG pathway is the central signaling axis regulating the activities of osteoblasts and osteoclasts in bone turnover [24]. While RANKL binds to RANK receptor expressed on the precursor cells of osteoclasts to activate its differentiation and the receptor on mature osteoclasts to activate its functions in bone resorption, OPG works as a decoy receptor for RANKL and disrupts its activity before binding to RANK. Thus, the suppression of RANKL by OPG shifts the balance towards osteoblasts and

Table 4 Hazard ratio of fracture associated with bone biomarker levels in patients treated with aromatase inhibitors

Outcome	Unadjusted HR (95% CI)	Age adjusted HR (95% CI)	Age and prior Fx adjusted HR (95% CI)
BALP			
Q1	1.00	1.00	1.00
Q2	1.87 (1.07–3.27)	2.01 (1.15–3.52)	2.05 (1.17–3.59)
Q3	1.62 (0.92–2.85)	1.77 (1.00–3.11)	1.80 (1.02–3.18)
Q4	1.02 (0.55–1.89)	1.20 (0.65–2.23)	1.27 (0.68–2.36)
TRACP			
Q1	1.00	1.00	1.00
Q2	1.34 (0.73–2.45)	1.30 (0.71–2.37)	1.34 (0.73–2.44)
Q3	1.82 (1.03–3.24)	1.67 (0.94–2.97)	1.75 (0.98–3.12)
Q4	1.65 (0.92–2.94)	1.40 (0.78–2.51)	1.47 (0.82–2.63)
BALP/TRACP ratio			
Q1	1.00	1.00	1.00
Q2	1.46 (0.92–2.32)	1.57 (0.99–2.50)	1.56 (0.98–2.48)
Q3	0.83 (0.49–1.41)	1.03 (0.60–1.75)	1.01 (0.59–1.72)
Q4	0.59 (0.32–1.09)	0.83 (0.45–1.54)	0.82 (0.44–1.53)
OPG			
Q1	1.00	1.00	1.00
Q2	0.90 (0.47–1.74)	0.77 (0.40–1.49)	0.80 (0.41–1.54)
Q3	0.94 (0.50–1.77)	0.72 (0.38–1.38)	0.75 (0.39–1.42)
Q4	2.42 (1.41–4.15)	1.52 (0.86–2.70)	1.61 (0.91–2.85)
RANKL			
Q1	1.00	1.00	1.00
Q2	0.88 (0.54–1.42)	1.00 (0.62–1.61)	1.02 (0.63–1.64)
Q3	0.82 (0.50–1.35)	1.09 (0.66–1.81)	1.09 (0.66–1.81)
Q4	0.50 (0.28–0.90)	0.57 (0.32–1.02)	0.57 (0.32–1.02)
OPG/RANKL ratio			
Q1	1.00	1.00	1.00
Q2	1.13 (0.55–2.31)	1.05 (0.51–2.13)	1.06 (0.52–2.16)
Q3	2.13 (1.13–4.02)	1.78 (0.94–3.38)	1.81 (0.96–3.44)
Q4	2.49 (1.34–4.61)	1.74 (0.93–3.26)	1.80 (0.96–3.37)

bone formation. Our finding of higher OPG levels associated with lower risk of osteopenia and osteoporosis is consistent with its role in bone formation; yet the finding of higher OPG/RANKL ratio associated with higher risk of fracture contradicts this balance between osteoblasts and osteoclasts in favor of bone formation. A possible explanation is that the OPG/RANKL ratio reflects less the current state of bone turnover, but the direction to which the regulatory signaling axis attempts to adjust the current imbalance between the activities of osteoblasts and osteoclasts. Under this speculation, a higher OPG/RANKL ratio may be interpreted as a signal for stronger bone formation over resorption, in response to a current imbalance towards bone resorption. This hypothesis requires validation in future studies. The findings of the significant relationships of the two bone

regulatory markers but not the bone turnover markers with AI-induced fracture also call for more research on OPG and RANKL in AI-induced bone loss among postmenopausal women.

Currently, there is no clear role of bone turnover markers in predicting risk of fractures or selecting candidates for BMD testing or anti-resorption therapy in either the general or postmenopausal breast cancer patients who undergo AI treatment, and guideline committees generally do not recommend its routine use [25, 26]. This lack of clinical utility is primarily due to large within-individual variability in the rate of bone loss and fracture given a certain bone biomarker value, which makes the interpretation of the test results difficult in individual patients. The potential clinical utility of bone markers is further complicated by their biological variability and lack of standardization of the assays [27]. Some studies support the use of bone turnover markers to identify non-responders to antiresorptive therapies such as bisphosphonates in osteoporotic patients [28–30], which may be applicable to the breast cancer setting. In addition to prospective studies to establish an optimal strategy to incorporate bone biomarkers into clinical practice, continued research efforts to identify novel markers beyond the few commonly used bone turnover markers, such as the two bone regulatory markers, OPG and RANKL, are warranted.

Our study has several strengths, including a large contemporary breast cancer patient population, vigorous efforts to identify and confirm fragility fractures and to exclude traumatic and pathologic fractures, inclusion of the two bone regulatory markers OPG and RANKL, and the evaluation of the ratios of markers constructed based on biological rationale. Our study also has several limitations. The median follow-up time of 6.1 years is still relatively short, and thus the number of fragility fractures is limited given the relatively low rate of fracture, especially after stratification by fracture site. We were missing BMD data on 12.9% of the cohort due to lack of a clinically available DXA scan after breast cancer diagnosis. Further, DXA scans were conducted at the clinician's discretion and not uniformly at predefined intervals, possibly resulting in under diagnosis of osteoporosis. In our analysis, a number of pre-analytic variables, including time of day (circadian rhythm), season, and fasting status, were not well controlled during the time of blood collection, which could lead to possible confounding effects. Moreover, we only included two bone turnover markers, whereas several other commonly tested markers, including *N*-terminal propeptide of type I procollagen (PINP) for bone formation and *C*-terminal telopeptide of type I collagen (CTX) for bone resorption, were not measured. We chose BALP and TRACP for their low intra-individual variability, low circadian variability, high thermostability, and robustness to non-fasting blood samples [31], whereas CTX is very sensitive to pre-analytic variations and might not be compatible to

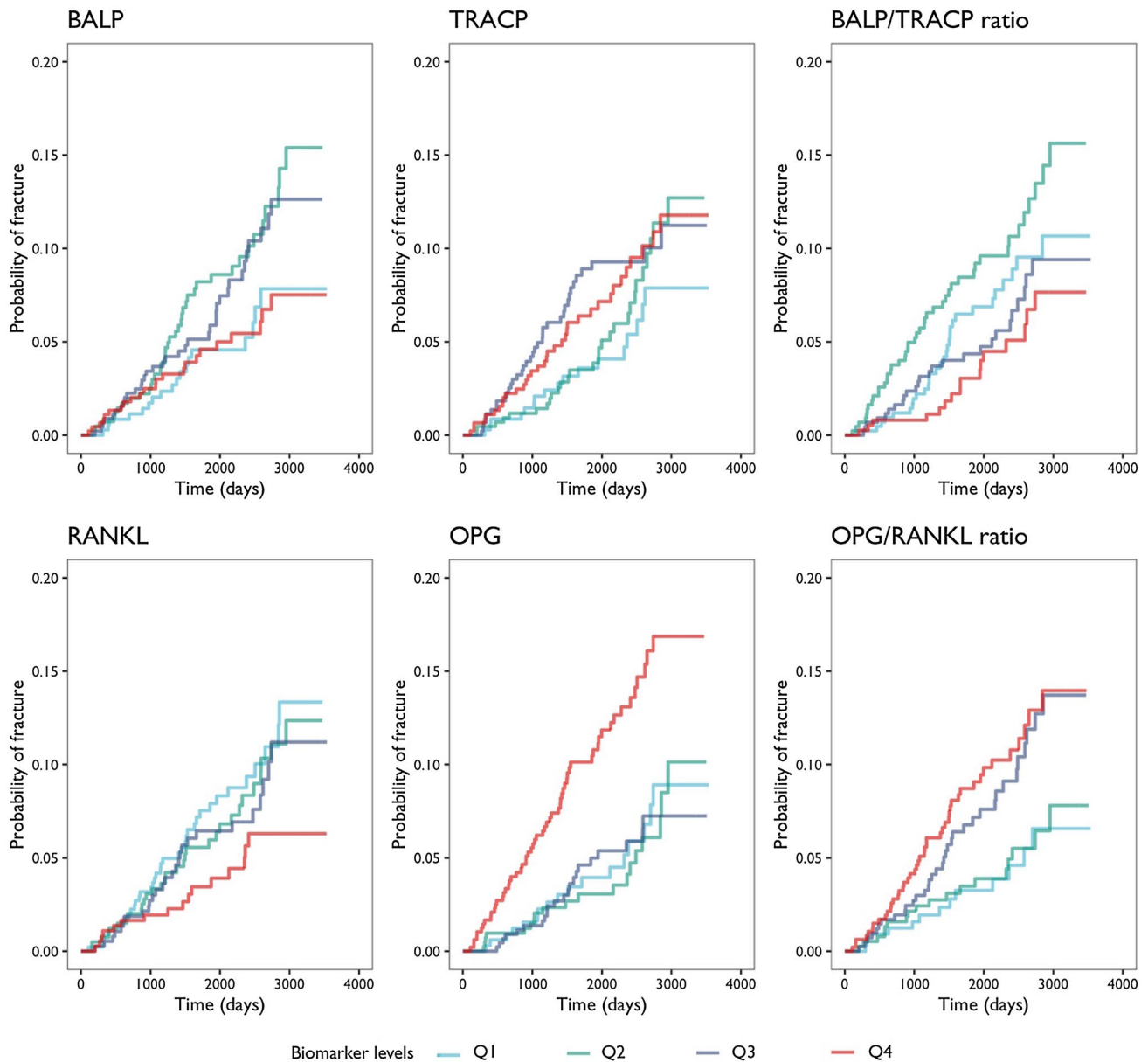


Fig. 1 Cumulative incidences of major fragility fractures are plotted according to quartiles of each of the four biomarkers and the two contrived ratios. The curves are colored to indicate the first to the fourth quartile of each biomarker

the conditions under which the blood samples were collected in this study. While BALP is considered one of the most useful clinical markers for bone formation, TRACP is relatively less studied than serum CTX or urinary N-terminal telopeptide crosslink (NTX) as markers for bone resorption. It remains to be determined which bone turnover markers are most predictive for osteoporosis and fracture in breast cancer patients. As a future direction, it will be important to confirm our findings of OPG/RANKL under more stringently controlled condition, with predefined fasting and time of day for blood collection and repeated sampling over time of

follow-up, with DXA scans regularly performed at a similar time frame.

In conclusion, our large prospective study in an integrated healthcare setting provides suggestive evidence for the potential utility of bone regulatory markers, OPG and RANKL, in predicting risk of fracture in women after AI treatment for breast cancer. Future studies are warranted to validate the findings and to potentially inform the development of a comprehensive risk assessment tool of clinical risk factors, BMD and bone biomarkers specifically for this patient population at high risk of fracture.

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Compliance with Ethical Standards

Conflict of interest The authors declare no potential conflicts of interest.

Ethical approval The study was approved by Institutional Review Boards of Roswell Park Comprehensive Cancer Center and Kaiser Permanente Northern California for human subject protection.

Informed consent Written consents were obtained from all study participants.

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