Published in final edited form as:

Breast Cancer Res Treat. 2017 February ; 161(3): 501–513. doi:10.1007/s10549-016-4068-5.

Bone remodeling and regulating biomarkers in women at the time of breast cancer diagnosis

Song Yao[1], **Yali Zhang**[1,2], **Li Tang**[1], **Janise M. Roh**[3], **Cecile A. Laurent**[3], **Chi-Chen Hong**[1], **Theresa Hahn**[2], **Joan C. Lo**[3], **Christine B. Ambrosone**[1], **Lawrence H. Kushi**[3], and **Marilyn L. Kwan**[3]

[1]Department of Cancer Prevention and Control, Roswell Park Cancer Institute, Buffalo, NY

[2]Department of Medicine, Roswell Park Cancer Institute, Buffalo, NY

[3]Division of Research, Kaiser Permanente Northern California, Oakland, CA

Abstract

The majority of breast cancer patients receive endocrine therapy, including aromatase inhibitors known to cause increased bone resorption. Bone-related biomarkers at the time of breast cancer diagnosis may predict future risk of osteoporosis and fracture after endocrine therapy. In a large population of 2,401 female breast cancer patients who later underwent endocrine therapy, we measured two bone remodeling biomarkers, TRAP5b and BAP, and two bone regulating biomarkers, RANKL and OPG, in serum samples collected at the time of breast cancer diagnosis. We analyzed these biomarkers and their ratios with patients' demographic, lifestyle, clinical tumor characteristics, as well as bone health history. The presence of bone metastases, prior bisphosphonate (BP) treatment and blood collection after chemotherapy had a significant impact on biomarker levels. After excluding these cases and controlling for blood collection time, age, race/ethnicity, body mass index, physical activity, alcohol consumption, smoking, and hormonal replacement therapy were significantly associated with bone biomarkers, while vitamin D or calcium supplements and tumor characteristics did not. When prior BP users were included in, recent history of osteoporosis and fracture was also associated. These findings support further investigation of these biomarkers with bone health outcomes after endocrine therapy initiation in women with breast cancer.

Keywords

breast cancer; aromatase inhibitor; tamoxifen; bone; biomarker

Corresponding author: Song Yao, PhD, Department of Cancer Prevention and Control, Roswell Park Cancer Institute, Buffalo, NY 14263, Phone: 716-845-4968, song.yao@roswellpark.org.

Compliance with Ethnical Standards:

Funding: This study was funded by National Institute of Health (R01 CA105274; U24 CA171524; P30 CA16056; R01 CA166701)

Conflict of interest: J.L. and/or an immediate family member have received past or current research funding from Amgen, Sanofi, AstraZeneca, GlaxoSmithKline, Novartis, CSL Behring and Milestone Pharmaceuticals, all unrelated to the current study. The other authors declare that they have no conflict of interest.

Ethnical approval: The study was approved by institutional review boards of Roswell Park Cancer Institute and Kaiser Permanente Northern California for human subject protection.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Introduction

Despite its rigid appearance, bone is metabolically active and undergoes constant remodeling. The counteracting processes of bone formation and resorption reflect the activities of two types of osteocytes, osteoblasts and osteoclasts, both of which are regulated by a central signaling axis consisting of three molecules: receptor activator of nuclear factor kappa-B (RANK), RANK ligand (RANKL), and osteoprotegrin (OPG) [1]. RANKL produced by osteoblasts binds to RANK, the receptor expressed on the surface of osteoclasts and essential for the differentiation and maturation of osteoclasts, thus favoring bone resorption. OPG, also produced by osteoblasts, is a decoy receptor for RANKL, and its binding by RANKL blocks osteoclast formation and suppresses bone resorption.

When bone resorption is more active, it leads to a loss of bone mass and weakened bone microarchitecture, predisposing patients to osteoporotic fractures. While bone mineral density (BMD) measured by dual energy X-ray absorptiometry (DXA) remains the standard for diagnosis of osteoporosis [2], biochemical markers of bone resorption and formation may also be useful as adjunctive measures for predicting future bone loss and bone fragility [3].

In recent years, a number of bone biomarkers have been studied with BMD, osteoporosis or fractures [4]. A selective combination of formation and resorption biomarkers in blood or urine samples are usually measured in parallel; yet bone regulating markers, including RANKL and OPG, have only been occasionally examined [4]. Furthermore, these studies were conducted largely in non-cancer populations, except for women diagnosed with breast cancer where several studies have examined bone remodeling biomarkers before and after endocrine therapy, including tamoxifen and aromatase inhibitors (AIs) [5–8]. However, to our knowledge, no previous studies have examined bone biomarkers with patients' demographic, lifestyle, or tumor characteristics at the time of breast cancer diagnosis. A better understanding of bone biomarkers and their relationships with other clinical characteristics at baseline and with prior history of bone health may be particularly relevant to patients treated with AIs, for predicting future bone health outcomes. Because it has been hypothesized that women diagnosed with breast cancer are likely to have higher BMD than those without breast cancer due to stronger estrogen exposures [9–11], it would be important to characterize these relationships which may differ from those in non-cancer populations.

The present study addresses current research gaps using data from a large contemporary breast cancer survivor cohort. Serum samples collected soon after cancer diagnosis were used for measuring the levels of bone biomarkers. In addition to RANKL and OPG, the two central bone regulating molecules indicative of osteocyte activity, we also selected two bone remodeling biomarkers, tartrate-resistant acid phosphatase (TRAP5b), an osteoclastic enzyme for bone resorption, and bone alkaline phosphatase (BAP), an osteoblastic enzyme for bone formation. Both BAP and TRAP5b have been commonly used in bone metabolism studies, given their relatively low intra-individual variability, low circadian variability, and high thermostability [4]. We examined the relationship of these biomarkers with patient characteristics and prior bone health history at the time of breast cancer diagnosis.

Methods

Patient population

The study population was drawn from the Pathways Study, a prospective cohort study of breast cancer survivors at Kaiser Permanente Northern California (KPNC). Details of the study have been published elsewhere [12]. In brief, a total of 4,505 eligible patients were identified through rapid case ascertainment procedures and enrolled in the study from January 2006 to April 2013 by completion of a baseline in-person interview. Women were enrolled on average two months post-diagnosis. Extensive information on sociodemographic and lifestyle factors, established breast cancer risk factors, and health and medical history was collected by interviewer- and self-administered questionnaires at baseline. Anthropometric measures were also obtained at baseline. Blood samples were obtained after the baseline interview from 90% of participants. Non-fasting blood was drawn by phlebotomy and shipped on dry ice overnight to Roswell Park Cancer Institute (RPCI) for processing through the auspices of the Data Bank and Biorepository (DBBR) [13]. Red blood cells, buffy coat, plasma, and sera were aliquoted into 0.5 ml straws using the MAPI Cryobiosystem (IMV Tech., Paris, France), slow frozen to −80°C, and transferred to liquid nitrogen for long-term storage until analysis.

A total of 3,315 Pathways Study participants treated with tamoxifen or aromatase inhibitor (AI) were included in an ancillary study to investigate lifestyle, molecular and genetic factors for bone health among breast cancer patients [14]. For the measurement of bone biomarkers, patients who had blood samples collected and were treated with either tamoxifen or AI but not both were included (n=2,401).

Measurement of bone biomarkers in serum samples

The four bone biomarkers were measured using serum samples collected at a median time of 73 days after breast cancer diagnosis (range: 28–321 days). ELISA assays for each biomarker were performed using the following commercially available kits: TRAP5b and BAP from Quidel (San Diego, CA), OPG from R&D System (Minneapolis, MN), and total soluble RANKL from Biovendor (Asheville, NC). All assays were performed according to manufacturers' protocols and each sample tested in duplicates, and those with a coefficient of variation (CV) exceeding 15% were repeated. The average CV was 2.3% for TRAP5b assay, 1.9% for BAP assay, 5.8% for RANKL assay, and 5.5% for OPG assay.

Collection of clinical data, bone mineral density, and patient history of osteoporosis and fracture

Breast tumor characteristics were obtained from the KPNC Cancer Registry approximately 6 months post-diagnosis. Bone mineral density data were obtained from women who underwent dual energy x-ray absorptiometry (DXA) at KPNC medical facilities using a Hologic densitometer, the near exclusive type of scanner used at KPNC except for central California medical centers which did not include Pathways participants. A small proportion of scans done using a GE Lunar densitometer were excluded from the BMD analysis. Algorithms were developed to extract BMD values for the femoral neck, total hip and lumbar spine from the radiology reports of DXA scans in the KPNC electronic medical

record (EMR) using key text string searches. The performance of the algorithm was validated by manual review of a random subset of patients (n=239 with 532 BMD values) and comparison to electronic data obtained directly from Hologic machines prior to 2008 [15], which showed a 96.2% concordance rate. Baseline BMD at the time of breast cancer diagnosis was determined from DXA scans obtained within 3 years prior or within 1 year after the breast cancer diagnosis; for patients with multiple DXA scans within this time interval, the one closest to the time of breast cancer diagnosis was used. T-scores were calculated from BMD values for osteoporosis classification, using the following formula: T-score = (observed BMD – peak BMD)/standard deviation of peak BMD, with peak BMD values obtained from Hologic (for lumbar spine) or Hologic NHANES III (for femoral neck or total hip) Caucasian reference populations. For these analyses, we used calculated t-scores rather than those provided in the report due to challenges with T-score extraction using key text string searches. Osteoporosis was defined by a BMD T-score of −2.5 or below, osteopenia by BMD T-score between −1 and −2.5 and normal BMD [16] by T-score of −1 or greater [17]. History of diagnosed osteoporosis and fracture before breast cancer diagnosis was identified based on appropriate ICD-9 codes [14] and prescription data of bisphosphonate (BP) use from the KPNC EMR and categorized based on years before cancer diagnosis (<5 years and ≥5 years). Traumatic and pathologic fractures were excluded. In addition to any clinical fracture, major osteoporotic fracture was defined as those to the spine, hip, humerus and wrist.

Statistical analysis

Descriptive characteristics of the patient population were summarized using mean and standard deviation for continuous variables and count and percentage for categorical variables. Distributions of the biomarker levels were examined, and no evidence of deviation from a normal distribution was found. The ratios of BAP/TRAP5b and OPG/RANKL were log-transformed to improve distribution normality. Correlations between bone biomarker levels and BMD and T-scores were assessed using Pearson correlation test.

Associations of bone biomarker levels with factors that might markedly affect these levels were first tested using a generalized linear model, controlling for age at diagnosis and menopausal status. These factors included bone metastasis, prior BP treatment, time of blood collection relative to chemotherapy initiation, and time of blood collection relative to endocrine therapy initiation. Least squared means and corresponding confidence intervals (CIs) of bone biomarkers for each factor assessed are presented. Because our analyses demonstrated a significant impact on biomarker levels from bone metastasis, prior BP treatment and timing of blood collection after chemotherapy infusion, we excluded the small number of patients with bone metastasis (n=20) and prior BP treatment (n=200) from subsequent analyses examining the association with demographic, lifestyle and clinical factors. However, because BP treatment was an indication of osteoporosis, when analyzing prior bone health history, we included the prior BP users. Additional analyses were also performed after excluding them. We also controlled for time of blood collection relative to chemotherapy [mean (SD)=8 (96) days] in multivariable linear models. Sensitivity analyses were also performed by excluding patients with blood collected after the chemotherapy initiation (n=554).

Associations of bone biomarker levels with cancer clinical characteristics and bone health history were tested using the same generalized linear model approach described above. In addition to the 4 measured biomarkers, we also assessed the ratios of bone formation and resorption markers, BAP/TRAP5b and OPG/RANKL. All analyses were also stratified by menopausal status at the time of breast cancer diagnosis. The Bonferroni method was applied to correct for study-wide multiple comparison errors (6 markers times 20 variables).

Results

Descriptive characteristics of the patient population

A total of 2,401 breast cancer patients were included in the biomarker analysis. Table 1 summarizes the demographic, lifestyle and clinical characteristics, as well as bone health history before breast cancer diagnosis of the patient population, overall and by menopausal status. The average age at diagnosis was 60.4 years, with a majority diagnosed after menopause (n=1,775, or 74%). The majority of patients were white (67%), overweight (31%) or obese (36%), engaged in some physical activity (96%), never smoked (56%), drank alcohol (73%), did not use supplements (64%), previously used birth control (75%) and, among postmenopausal women, took hormone replacement therapy (59%). Most patients were diagnosed with early stage breast cancer, with 29 stage IV cancer patients, including 20 patients with metastatic disease to the bone. As expected, almost all patients had hormone receptor positive tumors; however, 4 patients had HER2-enriched tumors (ER−, PR− and HER2+) and 7 had triple-negative tumors (ER−, PR−, and HER2−), which were subsequently confirmed by chart review.

Only 7% of patients had a history of osteoporosis prior to breast cancer diagnosis, while 13% had a prior history of any fracture and 4% had a prior history of any major fracture. Most of the prior osteoporosis or fracture diagnoses occurred among postmenopausal women, and the first fracture occurred at or after age 55 years.

Impact of bone metastasis, prior bisphosphonate treatment, and time of blood collection on the measured biomarker levels

As shown in Supplementary Table 1, 20 patients with bone metastasis had much higher levels of BAP and TRAP5b levels than those with non-bone metastasis or no metastasis. Patients previously treated with BP (n=200, 8%) had lower levels of BAP and TRAP5b levels than those who did not, but there was no difference in the levels of RANKL or OPG. Although blood collection after endocrine therapy initiation (n=1,695, 71%) had no impact on any of the measured biomarker levels, samples collected after chemotherapy initiation (n=554, 23%) had higher levels of TRAP5b and lower levels of RANKL compared to samples collected prior to chemotherapy or from patients not treated with chemotherapy.

Correlations between bone biomarkers and BMD

After excluding patients with bone metastasis and prior BP treatment, we examined correlations between BMD (spine, hip, and femur) at time close to breast cancer diagnosis (within 3 years prior and 1 year post) and levels of the four biomarkers, as well as BAP/TRAP5b and OPG/RANKL ratios. The median time interval between DXA scan and blood

draw was −63 days (range: −1188 to 260 days). As shown in Supplementary Table 2, among the four biomarkers, the only correlation was between TRAP5b and BAP (r=0.37, p<0.001), while RANKL and OPG were not correlated (r=−0.01). Supplementary Table 2 also displays the correlations between biomarker levels and BMD and T-scores. Both TRAP5b and BAP were negatively correlated with BMD at hip and femur, with the strongest correlation between TRAP5b and femur BMD (r=−0.22, p<0.001). RANKL and OPG levels were not correlated with BMD, except for a weak correlation between OPG level and the OPG/RANKL ratio with spine BMD.

Associations of bone biomarker levels with demographic and lifestyle factors

A number of demographic and lifestyle factors were associated with bone remodeling biomarkers (Table 2) and bone regulating biomarkers (Table 3). Patients diagnosed at an older age had higher levels of TRAP5b, lower BAP/TRAP5b ratio, lower levels of RANKL, higher levels of OPG, and higher OPG/RANKL ratio (p<0.001). Black women had the highest levels of TRAP5b and OPG and OPG/RANKL ratio (p<0.001). Women with a higher body mass index had lower levels of TRAP5b, higher levels of BAP, and thus higher BAP/TRAP5b ratio; and lower levels of RANKL, higher levels of OPG, and thus higher OPG/RANKL ratio (p≤0.007). Higher physical activity was associated with higher levels of TRAP5b, lower BAP/TRAP5b ratio, and lower levels of OPG (p≤0.001). Higher alcohol intake was associated with lower levels of BAP, lower BAP/TRAP5b ratio, lower levels of OPG, and lower OPG/RANKL ratio (p≤0.003). In addition, current smoking and hormone replacement therapy use were associated with higher and lower levels of OPG, respectively, but not with the other biomarkers measured. Supplement use (either calcium, vitamin D, or both), or birth control use had little impact on bone biomarker levels. The results remained unchanged after stratifying by menopausal status at the time of breast cancer diagnosis, or excluding samples collected after the chemotherapy initiation (data not shown).

Associations of bone biomarker levels with tumor characteristics

Overall, we did not observe strong associations of bone biomarker levels with tumor characteristics (Tables 4 and 5). The only two exceptions were higher TRAP5b levels in PR negative than in PR positive patients (p<0.001), and patients with higher grade tumors had higher levels of BAP (p=0.02). The results remained unchanged after stratifying by menopausal status at the time of breast cancer diagnosis, or excluding samples collected after chemotherapy initiation (data not shown).

Associations of bone biomarker levels with history of osteoporosis and fracture

Patients with a history of osteoporosis (including those with prior BP use), particularly those with a more recent history within 5 years before breast cancer diagnosis, had lower levels of TRAP5b, BAP and RANKL (Tables 4 and 5). Similarly, those with a recent history of any fracture also had lower levels of TRAP5b and BAP, although the difference in BAP was not statistically significant. The results were similar after excluding samples collected after chemotherapy initiation (data not shown). Upon stratification by menopausal status, the associations existed only among postmenopausal women (data not shown). However, when excluding patients with prior BP treatment, we did not observe any strong associations of bone biomarker levels with history of osteoporosis, any fracture, any major fracture, and age

at first fracture, with the exception of RANKL levels being the highest and OPG/RANKL ratio being the lowest among those with any fracture more than 5 years before breast cancer diagnosis, compared to those with any fracture within 5 years or with no fracture history.

Discussion

In a large cohort of breast cancer patients, we found that bone metastasis, BP treatment and chemotherapy treatment had a strong impact on serum levels of bone remodeling biomarkers, TRAP5b and BAP, and bone regulating biomarkers, RANKL and OPG. In addition, age at diagnosis, self-reported race/ethnicity, body mass index, physical activity, alcohol intake, smoking and hormone replacement therapy were also associated with the levels of these biomarkers. Nevertheless, there were few noteworthy relationships between these bone biomarkers with breast cancer clinical characteristics. BMD around breast cancer diagnosis was only weakly and negatively related with TRAP5b and BAP levels, but not with RANKL or OPG levels. Lastly, women with a recent history of osteoporosis or any fracture within 5 years of breast cancer diagnosis had lower levels of TRAP5b, BAP and RANKL.

Our finding of higher levels of bone remodeling biomarkers in patients with bone metastasis is expected. The growth of bone metastatic lesions causes increased bone resorption, and factors released from bone resorption stimulate cancer cell growth, forming a vicious cycle of bone destruction and elevated levels of degradation products in circulation [18]. Although RANKL has been hypothesized as a therapeutic target for bone metastasis [19], our analyses in the small sample of bone metastatic patients in our study (n=20) revealed no statistically significant difference in the serum levels of RANKL or OPG. These findings are consistent with most of the literature on using bone biomarkers for early diagnosis of bone metastasis [20]. Previous studies have shown that the levels of bone remodeling biomarkers, but not RANKL or OPG, were elevated in bone metastatic patients [21–23]; however, the sensitivity and specificity for using these biomarkers to diagnose bone metastasis were limited [20].

Few studies have characterized the associations of bone biomarkers with demographic, lifestyle or clinical factors among women diagnosed with breast cancer. In our patient population, we found that older patients tend to have higher levels of bone resorption marker TRAP5b and lower BAP/TRAP5b ratio, indicating a balance shifted towards bone resorption. However, they also had lower levels of RANKL and higher levels of OPG, and a higher OPG/RANKL ratio, indicating a balance favoring bone formation. This discordance was also reflected in the lack of correlation between RANKL/OPG and TRAP5b/BAP in our data. The higher levels of OPG in older patients were consistent with a study in postmenopausal women in the Women’s Health Initiative (WHI), which did not find an association of RANKL levels with age [24]. Because BMD gradually declines after peaking in early adulthood [25], the higher levels of TRAP5b and lower BAP/TRAP5b ratio found in our study better reflect this trend than RANKL or OPG levels, and thus may be more appropriate biomarkers for the bone aging process.

High BMI as a measure of obesity was associated with lower levels of bone resorption biomarkers (TRAP5b and RANKL) and higher levels of bone formation biomarkers (BAP,

OPG, BAP/TRAP5b, OPG/RANKL). The associations may be explained by higher estrogen levels among women with high BMI, which are known to be critical in maintaining bone density in women [26].

Alcohol consumption has been suggested to be protective for bone health among postmenopausal women, possibly by suppressing bone turnover [27, 28]. One earlier study showed that alcohol consumption was associated with reduced levels of both bone resorption and formation biomarkers [28]. In our study, we found lower levels of bone formation markers (BAP and OPG) among alcohol drinkers, particularly among postmenopausal women (data not shown), but no differences in bone resorption markers. Interestingly, in a rat animal model, bone formation was reduced in alcohol-fed animals [29], consistent with our findings. The associations might be due to reduced parathyroid hormone or increased estrogen concentrations caused by alcohol consumption [27].

Being physically active has, in general, been associated with higher BMD [30]. However, in our study, women with above median levels of physical activity had higher levels of TRAP5b and lower levels of OPG than those with no regular or below the median physical activity, thus indicating a balance shifted towards bone resorption. Published data on physical activity and bone biomarkers in adult women are sparse. The aforementioned WHI study did not find any association between energy expenditure from physical activity and RANKL or OPG levels [24]. Our findings may need validation in future studies among women with breast cancer.

Interestingly, calcium or vitamin D supplementation had no impact on any biomarker levels measured in our study, in contrast to their well-established roles in bone metabolism. Although our analysis was observational, the null findings are consistent with those from several prospective trials of vitamin D and/or calcium supplementation [31–33], which evaluated changes in bone remodeling biomarkers after intervention. The lack of an impact of supplementation on bone biomarkers might be due to the tight control of 1,25α-dihydroxyvitamin D levels, the active vitamin D metabolite in calcium homeostasis, which may be in a normal range even in individuals with vitamin D deficiency [34]. As a result, the impact of vitamin D or calcium supplementation may not be reflected in bone biomarkers.

The majority of breast cancers are estrogen receptor (ER) positive and/or progesterone receptor (PR) positive, making them eligible for hormonal therapy consisting of single or sequential use of tamoxifen or AIs. Because of superior efficacy compared with tamoxifen, AIs have largely replaced tamoxifen in the setting of postmenopausal breast cancer [35]. Nevertheless, AIs have a distinct profile of toxicities. Compared to osteo-protective effects of tamoxifen, the third-generation AIs can be damaging to bones by essentially cutting off estrogen synthesis from adipose tissues [36]. This significantly elevates the risk of osteoporosis and fragility fracture among postmenopausal women, who are already at high risk due to markedly lower estrogen levels after menopause. Several clinical trials reported a decrease in BMD and increase in bone turnover after AI treatment as measured by bone resorption and formation biomarkers [5–8]. While these previous studies were focused on changes in bone remodeling biomarkers after AI treatment, to our knowledge, no studies have evaluated the predictive value of incorporating bone biomarker levels prior to treatment

into a risk prediction model for AI-related fractures. We have now characterized in detail the associations of bone biomarkers measured before treatment with baseline patient characteristics, thus setting the stage for our future work to study these biomarkers with bone health outcomes after hormonal therapy.

In conclusion, in a large breast cancer survivor cohort, we found that serum levels of bone regulating and remodeling biomarkers at the time of breast cancer diagnosis were associated with several patient characteristics and lifestyle factors, but not with tumor characteristics, except for bone metastasis. We plan to investigate the association of these baseline biomarkers with the risk of osteoporosis and fracture after hormonal therapy in our future work.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Pathways Study was supported by the National Cancer Institute at the National Institutes of Health (R01 CA105274, PI: Kushi LH; R01 CA166701, PIs: Kwan ML, Yao S). Electronic clinical data abstraction and integration was supported in part by Cancer Research Network (CRN) (U19 CA079689, U24 CA171524, PI: Kushi LH). RPCI DBBR is CCSG Shared Resource supported by P30 CA16056 (PI: Ambrosone CB). The authors thank office and field staff for data collection, processing, and preparation, and DBBR staff for biospecimen processing. We thank all Pathways Study participants for their numerous contributions to this study. The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of the funding agencies.

References

1. Datta HK, Ng WF, Walker JA, Tuck SP, Varanasi SS. The cell biology of bone metabolism. J Clin Pathol. 2008; 61(5):577–587. [PubMed: 18441154]
2. Kanis JA, McCloskey EV, Johansson H, Oden A, Melton LJ 3rd, Khaltaev N. A reference standard for the description of osteoporosis. Bone. 2008; 42(3):467–475. [PubMed: 18180210]
3. Rabenda V, Bruyere O, Reginster JY. Relationship between bone mineral density changes and risk of fractures among patients receiving calcium with or without vitamin D supplementation: a meta-regression. Osteoporos Int. 2011; 22(3):893–901. [PubMed: 21060990]
4. Wheater G, Elshahaly M, Tuck SP, Datta HK, van Laar JM. The clinical utility of bone marker measurements in osteoporosis. J Transl Med. 2013; 11:201. [PubMed: 23984630]
5. Eastell R, Hannon RA, Cuzick J, Dowsett M, Clack G, Adams JE. Effect of an aromatase inhibitor on bmd and bone turnover markers: 2-year results of the Anastrozole, Tamoxifen, Alone or in Combination (ATAC) trial (18233230). J Bone Miner Res. 2006; 21(8):1215–1223. [PubMed: 16869719]
6. Perez EA, Josse RG, Pritchard KI, Ingle JN, Martino S, Findlay BP, Shenkier TN, Tozer RG, Palmer MJ, Shepherd LE, et al. Effect of letrozole versus placebo on bone mineral density in women with primary breast cancer completing 5 or more years of adjuvant tamoxifen: a companion study to NCIC CTG MA.17. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2006; 24(22):3629–3635. [PubMed: 16822845]
7. Lonning PE, Geisler J, Krag LE, Erikstein B, Bremnes Y, Hagen AI, Schlichting E, Lien EA, Ofjord ES, Paolini J, et al. Effects of exemestane administered for 2 years versus placebo on bone mineral density, bone biomarkers, and plasma lipids in patients with surgically resected early breast cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005; 23(22):5126–5137. [PubMed: 15983390]

8. McCaig FM, Renshaw L, Williams L, Young O, Murray J, Macaskill EJ, McHugh M, Hannon R, Dixon JM. A study of the effects of the aromatase inhibitors anastrozole and letrozole on bone metabolism in postmenopausal women with estrogen receptor-positive breast cancer. Breast Cancer Res Treat. 2010; 119(3):643–651. [PubMed: 19941160]
9. Zhang Y, Kiel DP, Kreger BE, Cupples LA, Ellison RC, Dorgan JF, Schatzkin A, Levy D, Felson DT. Bone mass and the risk of breast cancer among postmenopausal women. N Engl J Med. 1997; 336(9):611–617. [PubMed: 9032046]
10. Chen Z, Arendell L, Aickin M, Cauley J, Lewis CE, Chlebowski R. Hip bone density predicts breast cancer risk independently of Gail score: results from the Women's Health Initiative. Cancer. 2008; 113(5):907–915. [PubMed: 18666209]
11. Cauley JA, Lucas FL, Kuller LH, Vogt MT, Browner WS, Cummings SR. Bone mineral density and risk of breast cancer in older women: the study of osteoporotic fractures. Study of Osteoporotic Fractures Research Group. JAMA. 1996; 276(17):1404–1408. [PubMed: 8892715]
12. Kwan ML, Ambrosone CB, Lee MM, Barlow J, Krathwohl SE, Ergas IJ, Ashley CH, Bittner JR, Darbinian J, Stronach K, et al. The Pathways Study: a prospective study of breast cancer survivorship within Kaiser Permanente Northern California. Cancer causes & control : CCC. 2008; 19(10):1065–1076. [PubMed: 18478338]
13. Ambrosone CB, Nesline MK, Davis W. Establishing a cancer center data bank and biorepository for multidisciplinary research. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive. Oncology. 2006; 15(9):1575–1577.
14. Kwan ML, Lo JC, Tang L, Laurent CA, Roh JM, Chandra M, Hahn TE, Hong CC, Sucheston-Campbell L, Hershman DL, et al. Bone health history in breast cancer patients on aromatase inhibitors. PloS one. 2014; 9(10):e111477. [PubMed: 25354083]
15. Lo JC, Pressman AR, Chandra M, Ettinger B. Fracture risk tool validation in an integrated healthcare delivery system. Am J Manag Care. 2011; 17(3):188–194. [PubMed: 21504255]
16. Bonnick, SL. Bone Densitometry in Clinical Practice: Application and Interpretation (Current Clinical Practice). Humana Press; 2009.
17. Schousboe JT, Shepherd JA, Bilezikian JP, Baim S. Executive summary of the 2013 International Society for Clinical Densitometry Position Development Conference on bone densitometry. J Clin Densitom. 2013; 16(4):455–466. [PubMed: 24183638]
18. Guise TA, Mohammad KS, Clines G, Stebbins EG, Wong DH, Higgins LS, Vessella R, Corey E, Padalecki S, Suva L, et al. Basic mechanisms responsible for osteolytic and osteoblastic bone metastases. Clin Cancer Res. 2006; 12(20 Pt 2):6213s–6216s. [PubMed: 17062703]
19. Dougall WC, Holen I, Gonzalez Suarez E. Targeting RANKL in metastasis. Bonekey Rep. 2014; 3:519. [PubMed: 24795813]
20. Jung K, Lein M. Bone turnover markers in serum and urine as diagnostic, prognostic and monitoring biomarkers of bone metastasis. Biochim Biophys Acta. 2014; 1846(2):425–438. [PubMed: 25220832]
21. Shimozuma K, Sonoo H, Fukunaga M, Ichihara K, Aoyama T, Tanaka K. Biochemical markers of bone turnover in breast cancer patients with bone metastases: a preliminary report. Jpn J Clin Oncol. 1999; 29(1):16–22. [PubMed: 10073146]
22. Leeming DJ, Koizumi M, Byrjalsen I, Li B, Qvist P, Tanko LB. The relative use of eight collagenous and noncollagenous markers for diagnosis of skeletal metastases in breast, prostate, or lung cancer patients. Cancer Epidemiol Biomarkers Prev. 2006; 15(1):32–38. [PubMed: 16434583]
23. Leeming DJ, Delling G, Koizumi M, Henriksen K, Karsdal MA, Li B, Qvist P, Tanko LB, Byrjalsen I. Alpha CTX as a biomarker of skeletal invasion of breast cancer: immunolocalization and the load dependency of urinary excretion. Cancer Epidemiol Biomarkers Prev. 2006; 15(7): 1392–1395. [PubMed: 16835341]
24. LaCroix AZ, Jackson RD, Aragaki A, Kooperberg C, Cauley JA, Chen Z, Leboff MS, Duggan D, Wactawski-Wende J. OPG and sRANKL serum levels and incident hip fracture in postmenopausal Caucasian women in the Women's Health Initiative Observational Study. Bone. 2013; 56(2):474–481. [PubMed: 23735608]

25. Warming L, Hassager C, Christiansen C. Changes in bone mineral density with age in men and women: a longitudinal study. Osteoporos Int. 2002; 13(2):105–112. [PubMed: 11905520]

26. Imai Y, Youn MY, Kondoh S, Nakamura T, Kouzmenko A, Matsumoto T, Takada I, Takaoka K, Kato S. Estrogens maintain bone mass by regulating expression of genes controlling function and life span in mature osteoclasts. Ann N Y Acad Sci. 2009; 1173(Suppl 1):E31–39. [PubMed: 19751412]

27. Rapuri PB, Gallagher JC, Balhorn KE, Ryschon KL. Alcohol intake and bone metabolism in elderly women. Am J Clin Nutr. 2000; 72(5):1206–1213. [PubMed: 11063451]

28. Marrone JA, Maddalozzo GF, Branscum AJ, Hardin K, Cialdella-Kam L, Philbrick KA, Breggia AC, Rosen CJ, Turner RT, Iwaniec UT. Moderate alcohol intake lowers biochemical markers of bone turnover in postmenopausal women. Menopause. 2012; 19(9):974–979. [PubMed: 22922514]

29. Sampson HW. Effect of alcohol consumption on adult and aged bone: a histomorphometric study of the rat animal model. Alcohol Clin Exp Res. 1998; 22(9):2029–2034. [PubMed: 9884147]

30. Hagberg JM, Zmuda JM, McCole SD, Rodgers KS, Ferrell RE, Wilund KR, Moore GE. Moderate physical activity is associated with higher bone mineral density in postmenopausal women. J Am Geriatr Soc. 2001; 49(11):1411–1417. [PubMed: 11890577]

31. Trautvetter U, Neef N, Leiterer M, Kiehntopf M, Kratzsch J, Jahreis G. Effect of calcium phosphate and vitamin D(3) supplementation on bone remodelling and metabolism of calcium, phosphorus, magnesium and iron. Nutr J. 2014; 13:6. [PubMed: 24438153]

32. Seamans KM, Hill TR, Wallace JM, Horigan G, Lucey AJ, Barnes MS, Taylor N, Bonham MP, Muldowney S, Duffy EM, et al. Cholecalciferol supplementation throughout winter does not affect markers of bone turnover in healthy young and elderly adults. J Nutr. 2010; 140(3):454–460. [PubMed: 20089776]

33. Aloia J, Bojadzievski T, Yusupov E, Shahzad G, Pollack S, Mikhail M, Yeh J. The relative influence of calcium intake and vitamin D status on serum parathyroid hormone and bone turnover biomarkers in a double-blind, placebo-controlled parallel group, longitudinal factorial design. J Clin Endocrinol Metab. 2010; 95(7):3216–3224. [PubMed: 20463100]

34. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. Chemistry & biology. 2014; 21(3):319–329. [PubMed: 24529992]

35. Burstein HJ, Temin S, Anderson H, Buchholz TA, Davidson NE, Gelmon KE, Giordano SH, Hudis CA, Rowden D, Solky AJ, et al. Adjuvant endocrine therapy for women with hormone receptor-positive breast cancer: american society of clinical oncology clinical practice guideline focused update. J Clin Oncol. 2014; 32(21):2255–2269. [PubMed: 24868023]

36. Geisler J, Haynes B, Anker G, Dowsett M, Lonning PE. Influence of letrozole and anastrozole on total body aromatization and plasma estrogen levels in postmenopausal breast cancer patients evaluated in a randomized, cross-over study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2002; 20(3):751–757. [PubMed: 11821457]

Table 1

Descriptive characteristics of Pathways Study patient population

Characteristic	Overall (n=2400) N (%)	Premenopausal women (n=625) N (%)	Postmenopausal women (n=1775) N (%)
Demographic and Lifestyle Factors			
Age at diagnosis, years			
<50	463 (19.3)	427 (68.3)	36 (2)
50–59	673 (28)	191 (30.5)	482 (27.2)
60–69	774 (32.2)	7 (1.1)	767 (43.2)
≥70	490 (20.4)		490 (27.6)
Race/ethnicity			
White	1611 (67.1)	303 (48.5)	1308 (73.7)
Black	151 (6.3)	59 (9.4)	92 (5.2)
Hispanic	292 (12.2)	106 (16.9)	186 (10.5)
Asian	297 (12.4)	142 (22.7)	155 (8.7)
Other	49 (2)	15 (2.4)	34 (1.9)
Body mass index at diagnosis, kg/m^2			
<25	797 (33.5)	274 (44.2)	523 (29.8)
25–29.9	728 (30.6)	166 (26.8)	562 (32)
≥30	854 (35.9)	180 (29)	674 (38.3)
Physical activity at diagnosis			
None	98 (4.1)	12 (1.9)	86 (4.9)
Below median	1097 (45.9)	268 (43)	829 (46.9)
Above median	1194 (50)	343 (55)	851 (48.2)
Smoking at diagnosis			
Never	1329 (55.6)	412 (66.2)	917 (51.8)
Former	933 (39.1)	173 (27.9)	760 (43)
Current	129 (5.4)	36 (5.8)	93 (5.3)
Alcohol intake at diagnosis			
None	544 (26.6)	148 (29.4)	396 (25.7)
Below median	749 (36.7)	171 (34.2)	578 (37.5)
Above median	749 (36.7)	183 (36.3)	566 (36.8)
Supplement use at diagnosis			
None	1536 (64.3)	453 (72.9)	1083 (61.3)
Calcium intake only	381 (16)	82 (13.2)	299 (16.9)
Vitamin D intake only	285 (11.9)	54 (8.7)	231 (13.1)
Both	186 (7.8)	33 (5.3)	153 (8.7)
Birth control use			
None	583 (24.9)	108 (17.7)	475 (27.4)
≤3 year	597 (25.5)	161 (26.4)	436 (25.2)
3–10 years	615 (26.3)	159 (26.1)	456 (26.3)
>10 years	545 (23.3)	180 (29.7)	365 (21.1)

Characteristic	Overall (n=2400) N (%)	Premenopausal women (n=625) N (%)	Postmenopausal women (n=1775) N (%)
Hormone replacement therapy use among postmenopausal women			
None	--	--	709 (40.1)
<10 years	--	--	507 (28.6)
≥10 years	--	--	554 (31.3)
Clinical Factors			
AJCC stage			
I	1328 (55.3)	297 (47.4)	1031 (58.1)
II	833 (34.7)	256 (41)	577 (32.5)
III	210 (8.8)	64 (10.2)	146 (8.2)
IV	29 (1.2)	8 (1.3)	21 (1.2)
Tumor grade			
Grade 1 (Well differentiated)	711 (31.4)	150 (25.6)	561 (33.5)
Grade 2 (Moderately differentiated)	1141 (50.4)	291 (49.7)	850 (50.7)
Grade 3 (Poorly differentiated)	410 (18.2)	144 (24.7)	266 (15.9)
Estrogen receptor status			
Positive	2384 (99.3)	621 (99.4)	1763 (99.3)
Negative	16 (0.7)	4 (0.6)	12 (0.7)
Progesterone receptor status			
Positive	1824 (76)	492 (78.7)	1332 (75)
Negative	576 (24)	133 (21.3)	443 (25)
HER2 status			
Negative	2061 (85.9)	511 (81.8)	1550 (87.3)
Positive	251 (10.5)	95 (15.2)	156 (8.8)
Not done	88 (3.7)	19 (3.0)	69 (3.9)
IHC subtype			
Luminal A	2054 (88.8)	509 (83.9)	1545 (90.5)
Luminal B	247 (10.7)	94 (15.5)	153 (8.9)
Her2 positive	4 (0.2)	1 (0.2)	3 (0.2)
Triple negative	7 (0.3)	2 (0.3)	5 (0.4)
Bone Health History			
Osteoporosis prior to diagnosis			
No	2224 (92.7)	622 (99.5)	1602 (90.3)
<5 years	113 (4.7)	2 (0.3)	111 (6.3)
≥5 years	63 (2.6)	1 (0.2)	62 (3.5)
Any fracture prior to diagnosis			
No	2063 (86)	584 (93.4)	1479 (83.3)
<5 years	181 (7.5)	23 (3.7)	158 (8.9)
≥5 years	156 (6.5)	18 (2.9)	138 (7.8)
Any major fracture prior to diagnosis			

Characteristic	Overall (n=2400) N (%)	Premenopausal women (n=625) N (%)	Postmenopausal women (n=1775) N (%)
No	2306 (96.1)	620 (99.2)	1686 (95)
<5 years	60 (2.5)	3 (0.5)	57 (3.2)
≥5 years	34 (1.4)	2 (0.3)	32 (1.8)
Age at first fracture			
No	2063 (86)	584 (93.5)	1479 (83.3)
<55 years	97 (4)	40 (6.4)	57 (3.2)
≥55 years	240 (10)	1 (0.2)	239 (13.5)

Table 2

Associations of bone metabolizing biomarker levels and demographic and lifestyle factors and bone health history

Characteristic	TRAP5b, U/L			BAP, U/L			BAP/TRAP5b ratio†		
	N	LS mean (95% CI)	P	N	LS mean (95% CI)	P	N	LS mean (95% CI)	P
Age at diagnosis, years			<0.001*			<0.001*			<0.001*
<50	459	2.7 (2.6–2.9)		460	19.2 (18.5–20)		458	2 (1.9–2)	
50–59	642	3 (2.9–3.2)		642	20.4 (19.9–21)		640	2 (1.9–2)	
60–69	694	3.1 (3–3.3)		691	18.9 (18.2–19.6)		691	1.9 (1.8–1.9)	
≥70	379	3.4 (3.2–3.6)		380	19.1 (18.3–20)		379	1.8 (1.7–1.8)	
Menopausal status at diagnosis			0.18			<0.001*			0.08
Premenopausal	614	3 (2.9–3.2)		616	18.2 (17.5–18.9)		613	1.9 (1.8–1.9)	
Postmenopausal	1560	3.1 (3.1–3.2)		1557	20.7 (20.3–21.2)		1555	1.9 (1.9–1.9)	
Race/ethnicity			0.04			<0.001*			0.18
White	1439	3.1 (3–3.1)		1436	19.1 (18.6–19.6)		1434	1.9 (1.9–1.9)	
Black	147	3.4 (3.2–3.6)		147	20 (19–21.1)		147	1.8 (1.8–1.9)	
Hispanic	271	3.1 (2.9–3.3)		273	20.6 (19.8–21.4)		270	1.9 (1.9–2)	
Asian	273	3.1 (2.9–3.3)		273	19.9 (19.1–20.7)		273	1.9 (1.9–2)	
Other	44	2.8 (2.4–3.2)		44	17.7 (15.8–19.5)		44	1.9 (1.8–2)	
Body mass index at diagnosis, kg/m^2			<0.001*			0.01			<0.001*
<25	694	3.5 (3.3–3.6)		693	18.9 (18.3–19.4)		690	1.7 (1.7–1.8)	
25–29.9	650	3.1 (3–3.2)		651	19.7 (19.2–20.3)		649	1.9 (1.9–1.9)	
≥30	810	2.7 (2.6–2.8)		809	19.8 (19.3–20.3)		809	2.1 (2–2.1)	
Physical activity at diagnosis			<0.001*			0.34			<0.001*
None	84	3 (2.7–3.3)		84	20.4 (19–21.7)		84	1.9 (1.9–2)	
Below median	999	3 (2.9–3.1)		997	19.4 (18.9–19.9)		995	1.9 (1.9–2)	
Above median	1082	3.2 (3.1–3.3)		1083	19.3 (18.8–19.8)		1080	1.8 (1.8–1.9)	
Smoking at diagnosis			0.84			0.40			0.15
Never	1207	3.1 (3–3.2)		1207	19.5 (19.1–20)		1203	1.9 (1.9–1.9)	
Former	836	3.1 (3–3.2)		836	19.2 (18.7–19.8)		835	1.9 (1.8–1.9)	
Current	122	3 (2.8–3.3)		121	19.9 (18.8–21.1)		121	1.9 (1.9–2)	

Characteristic	TRAP5b, U/L			BAP, U/L			BAP/TRAP5b ratio†		
	N	LS mean (95% CI)	P	N	LS mean (95% CI)	P	N	LS mean (95% CI)	P
Alcohol intake at diagnosis			0.90			<0.001*			0.003
None	472	3.1 (3–3.3)		472	19.8 (19.1–20.4)		471	1.9 (1.9–1.9)	
Below median	678	3.1 (3–3.2)		677	19.8 (19.3–20.4)		675	1.9 (1.9–1.9)	
Above median	685	3.1 (3–3.2)		685	18.5 (18–19.1)		683	1.8 (1.8–1.9)	
Supplement use at diagnosis			0.60			0.42			0.95
None	1406	3.1 (3–3.2)		1403	19.3 (18.8–19.7)		1400	1.9 (1.9–1.9)	
Calcium intake only	346	3.1 (3–3.3)		347	19.5 (18.8–20.2)		346	1.9 (1.8–1.9)	
Vitamin D intake only	249	3.1 (2.9–3.3)		249	19.9 (19.1–20.7)		249	1.9 (1.8–2)	
Both	162	3.2 (3–3.4)		163	19.7 (18.7–20.8)		162	1.9 (1.8–1.9)	
Birth control use			0.09			0.40			0.04
None	509	3.2 (3–3.3)		510	19.6 (19–20.2)		509	1.9 (1.8–1.9)	
≤3 year	544	3 (2.9–3.2)		541	19.4 (18.8–20.1)		540	1.9 (1.9–2)	
3–10 years	566	3 (2.8–3.1)		566	19.4 (18.7–20)		565	1.9 (1.9–2)	
>10 years	498	3.1 (3–3.2)		499	19 (18.3–19.6)		497	1.9 (1.8–1.9)	
Hormone replacement therapy use among postmenopausal women			0.59			0.003			0.02
None	629	3.2 (3.1–3.3)		630	21.2 (20.6–21.8)		628	1.9 (1.9–2)	
<10 years	440	3.2 (3–3.3)		436	20.3 (19.6–20.9)		436	1.9 (1.9–1.9)	
≥10 years	487	3.3 (3.1–3.4)		487	20 (19.3–20.6)		487	1.8 (1.8–1.9)	

Note: Overall models adjusted for age at diagnosis (continuous, not for the analysis of age at diagnosis), menopausal status at baseline (Pre/Post, not for the analysis of menopausal status), and time of blood collection relative to chemotherapy (before/after).

*p-values remain significant after correcting for a total of 120 tests (6 markers × 20 variables, unadjusetd p <0.0004).

†The BAP/TRAP5b ratio was log-transformed.

Table 3

Associations of bone regulating biomarker levels and demographic and lifestyle factors and bone health history

Characteristic	RANKL, pmol/L			OPG, pg/ml			OPG/RANKL ratio†		
	N	LS mean (95% CI)	P	N	LS mean (95% CI)	P	N	LS mean (95% CI)	P
Age at diagnosis, years			<0.001*			<0.001*			<0.001*
<50	443	127.8 (118.6–137)		461	1327 (1256–1398)		443	2.4 (2.3–2.5)	
50–59	610	122.9 (115.7–130.2)		644	1348 (1292–1404)		610	2.5 (2.5–2.6)	
60–69	663	106.4 (97.5–115.2)		694	1530 (1462–1599)		663	2.8 (2.7–2.9)	
≥70	357	104.3 (93.6–115.1)		375	1901 (1818–1984)		353	3.1 (3–3.2)	
Menopausal status at diagnosis			0.10			0.02			0.62
Premenopausal	590	119 (110.2–127.7)		617	1590 (1523–1658)		590	2.7 (2.6–2.8)	
Postmenopausal	1483	110.1 (104.7–115.6)		1557	1498 (1456–1540)		1479	2.8 (2.7–2.8)	
Race/ethnicity			0.53			<0.001*			<0.001*
White	1375	116.6 (110.8–122.4)		1438	1485 (1441–1529)		1371	2.7 (2.6–2.7)	
Black	135	110 (96.5–123.4)		146	1839 (1738–1940)		135	3 (2.9–3.1)	
Hispanic	260	113.9 (103.8–124)		274	1592 (1515–1669)		260	2.8 (2.7–2.9)	
Asian	260	110 (100–120.1)		272	1619 (1542–1696)		260	2.8 (2.7–2.9)	
Other	43	103.3 (80.1–126.6)		44	1468 (1289–1647)		43	2.8 (2.6–3.1)	
Body mass index at diagnosis, kg/m^2			0.005			<0.001*			<0.001*
<25	661	118.9 (111.9–125.8)		697	1500 (1446–1553)		660	2.7 (2.6–2.8)	
25–29.9	626	118.3 (111.1–125.4)		649	1523 (1468–1578)		624	2.7 (2.6–2.7)	
≥30	767	107.2 (100.3–114)		808	1614 (1562–1667)		766	2.9 (2.8–2.9)	
Physical activity at diagnosis			0.25			0.001			0.05
None	79	127.5 (110.2–144.9)		83	1679 (1546–1812)		78	2.7 (2.6–2.9)	
Below median	947	112.8 (106.6–119)		996	1578 (1530–16252)		944	2.8 (2.7–2.8)	
Above median	1039	114.9 (108.7–121.1)		1086	1497 (1449–1545)		1039	2.7 (2.6–2.8)	
Smoking at diagnosis			0.09			0.03			0.81
Never	1163	113.1 (107.3–118.9)		1209	1526 (1481–1571)		1161	2.7 (2.7–2.8)	
Former	787	115 (108.2–121.7)		835	1562 (1511–1614)		785	2.7 (2.7–2.8)	
Current	116	129.4 (114.8–144)		122	1666 (1554–1779)		116	2.8 (2.6–2.9)	

Characteristic	RANKL, pmol/L			OPG, pg/ml			OPG/RANKL ratio†		
	N	LS mean (95% CI)	P	N	LS mean (95% CI)	P	N	LS mean (95% CI)	P
Alcohol intake at diagnosis			0.55			<0.001*			0.001
None	443	117 (108.9–125.1)		470	1626 (1563–1689)		441	2.8 (2.7–2.9)	
Below median	650	112.7 (105.4–119.9)		680	1564 (1508–1620)		650	2.8 (2.7–2.9)	
Above median	660	116.6 (109.2–124.1)		686	1463 (1406–1521)		658	2.6 (2.6–2.7)	
Supplement use at diagnosis			0.59			0.16			0.12
None	1339	113.1 (107.4–118.7)		1407	1562 (1518–1606)		1337	2.8 (2.7–2.8)	
Calcium intake only	329	117.6 (108.3–126.9)		346	1507 (1435–1578)		329	2.7 (2.6–2.8)	
Vitamin D intake only	235	119.2 (108.7–129.7)		247	1484 (1404–1565)		233	2.7 (2.6–2.8)	
Both	160	113.2 (100.7–125.8)		163	1569 (1471–1666)		160	2.8 (2.6–2.9)	
Birth control use			0.45			0.04			0.11
None	482	114.4 (106.6–122.3)		506	1544 (1483–1604)		480	2.8 (2.7–2.8)	
≤3 year	518	114.8 (106.9–122.6)		546	1512 (1452–1572)		518	2.7 (2.6–2.8)	
3–10 years	537	119.4 (111.5–127.2)		565	1524 (1463–1584)		535	2.7 (2.6–2.8)	
>10 years	481	111.8 (103.9–119.6)		500	1610 (1550–1671)		481	2.8 (2.7–2.9)	
Hormone replacement therapy use among postmenopausal women			0.33			0.89			0.48
None	600	104.6 (97.4–111.8)		627	1617 (1559–1675)		598	2.9 (2.8–3)	
<10 years	418	102.5 (94.2–110.7)		440	1598 (1533–1664)		417	2.9 (2.8–3)	
≥10 years	461	109.9 (101.8–118)		486	1610 (1545–1674)		460	2.8 (2.8–2.9)	

Note: Overall models adjusted for age at diagnosis (continuous, not for the analysis of age at diagnosis), menopausal status at baseline (Pre/Post, not for the analysis of menopausal status), and time of blood collection relative to chemotherapy (before/after).

*p-values remain significant after correcting for a total of 120 tests (6 markers × 20 variables, unadjusetd p <0.0004).

†The BAP/TRAP5b ratio was log-transformed.

Table 4

Associations of bone metabolizing biomarker levels and patient clinical characteristics and bone health history

Characteristic	TRAP5b, U/L			BAP, U/L			BAP/TRAP5b ratio†		
	N	LS mean (95% CI)	P	N	LS mean (95% CI)	P	N	LS mean (95% CI)	P
AJCC stage		0.32				0.40			0.35
I	1199	3 (2.9–3.1)		1200	19.3 (18.7–19.8)		1197	1.9 (1.9–1.9)	
II	769	3.2 (3–3.3)		767	19.7 (19.1–20.2)		765	1.9 (1.8–1.9)	
III	197	3 (2.8–3.2)		197	19.5 (18.5–20.4)		197	1.9 (1.8–2)	
IV	9	2.9 (2.1–3.8)		9	16.9 (12.9–21)		9	1.7 (1.5–2)	
Tumor grade			0.88			0.02			0.17
Grade 1 (Well differentiated)	645	3.1 (2.9–3.2)		646	18.9 (18.3–19.6)		645	1.9 (1.8–1.9)	
Grade 2 (Moderately differentiated)	1029	3.1 (3–3.2)		1028	19.4 (19–19.9)		1025	1.9 (1.9–1.9)	
Grade 3 (Poorly differentiated)	376	3.1 (3–3.3)		376	20.1 (19.4–20.8)		375	1.9 (1.9–2)	
Estrogen receptor status			0.36			0.14			0.16
Positive	2160	3.1 (3–3.2)		2159	19.5 (19.1–19.9)		2154	1.9 (1.9–1.9)	
Negative	14	3.4 (2.8–4)		14	17.3 (14.4–20.2)		14	1.8 (1.6–1.9)	
Progesterone receptor status			<0.001*			0.14			0.002
Positive	1666	3 (2.9–3.1)		1665	19.3 (18.9–19.8)		1662	1.9 (1.9–1.9)	
Negative	508	3.3 (3.2–3.4)		508	19.8 (19.2–20.4)		506	1.8 (1.8–1.9)	
HER2 status			0.49			0.25			0.74
Negative	1866	3.1 (3–3.2)		1865	19.6 (19.1–20)		1860	1.9 (1.9–1.9)	
Positive	233	3 (2.8–3.2)		233	19.1 (18.3–20)		233	1.9 (1.8–2)	
Not done	75	3.1 (2.8–3.4)		75	18.5 (17.1–20)		75	1.9 (1.8–2)	
IHC subtype			0.28			0.07			0.82
Luminal A	1860	3.1 (3–3.2)		1859	19.6 (19.1–20)		1854	1.9 (1.9–1.9)	
Luminal B	224	3 (2.8–3.2)		229	19.2 (18.4–20.1)		229	1.9 (1.8–2)	
Her2 positive	4	2.1 (1–3.3)		4	13 (7.6–18.5)		4	1.9 (1.5–2.3)	
Triple negative	6	3.2 (2.4–4.1)		6	17.4 (13.3–21.5)		6	1.8 (1.5–2)	
Osteoporosis prior to diagnosis			<0.001*			<0.001*			0.61
No	2198	3.1 (3–3.2)		2198	19.3 (18.9–19.7)		2193	1.9 (1.9–1.9)	

Characteristic	TRAP5b, U/L			BAP, U/L			BAP/TRAP5b ratio†		
	N	LS mean (95% CI)	P	N	LS mean (95% CI)	P	N	LS mean (95% CI)	P
<5 years	112	2.6 (2.3–2.8)		113	16.4 (15.2–17.6)		112	1.9 (1.8–2)	
≥5 years	63	2.7 (2.4–3.1)		60	16.9 (15.3–18.5)		60	1.9 (1.8–2)	
Any fracture prior to diagnosis			0.05			0.11			0.32
No	2039	3.1 (3–3.2)		2038	19.2 (18.8–19.6)		2033	1.9 (1.9–1.9)	
<5 years	181	2.8 (2.6–3)		180	18.3 (17.3–19.3)		180	1.9 (1.9–2)	
≥5 years	153	3 (2.8–3.2)		153	18.7 (17.6–19.7)		152	1.9 (1.8–1.9)	
Any major fracture prior to diagnosis			0.37			0.95			0.37
No	2280	3.1 (3–3.1)		2278	19.1 (18.7–19.5)		2273	1.9 (1.9–1.9)	
<5 years	60	2.9 (2.5–3.2)		60	18.9 (17.2–20.5)		60	1.9 (1.8–2)	
≥5 years	33	2.8 (2.4–3.3)		33	19 (16.9–21.2)		32	2 (1.8–2.1)	
Age at first fracture			0.10			0.05			0.10
No	2039	3.1 (3–3.2)		2038	19.2 (18.8–19.6)		2033	1.9 (1.9–1.9)	
<55 years	95	2.8 (2.5–3.1)		96	19.2 (17.9–20.5)		95	2 (1.9–2.1)	
≥55 years	239	3 (2.8–3.1)		237	18.1 (17.3–19)		237	1.9 (1.8–1.9)	

Note: Overall models adjusted for age at diagnosis (continuous), menopausal status at baseline (Pre/Post), and time of blood collection relative to chemotherapy (before/after). For clinical characteristics, patients with bone metastasis and prior bisphosphonate treatment were excluded; for bone health history, only patients with bone metastasis were excluded.

*p-values remain significant after correcting for a total of 120 tests (6 markers × 20 variables, unadjusetd p <0.0004).

†The BAP/TRAP5b ratio was log-transformed.

Table 5

Associations of bone regulating biomarker levels and patient clinical characteristics and bone health history

Characteristic	RANKL, pmol/L			OPG, pg/ml			OPG/RANKL ratio†		
	N	LS mean (95% CI)	P	N	LS mean (95% CI)	P	N	LS mean (95% CI)	P
AJCC stage			0.69			0.04			0.45
I	1142	114.7 (107.9–121.6)		1198	1515 (1462–1568)		1139	2.7 (2.7–2.8)	
II	735	116.2 (109.6–122.7)		770	1540 (1490–1591)		734	2.7 (2.7–2.8)	
III	188	108.6 (96.8–120.4)		197	1632 (1542–1724)		188	2.8 (2.7–2.9)	
IV	8	108.9 (55.9–162)		9	1905 (1511.2–2298)		8	2.9 (2.3–3.4)	
Tumor grade		112.6	0.85			0.45			0.54
Grade 1 (Well differentiated)	618	(104.7–120.5)		645	1526 (1466–1587)		617	2.7 (2.6–2.8)	
Grade 2 (Moderately differentiated)	980	114.6 (108.4–120.8)		1031	1557 (1509–1604)		979	2.8 (2.7–2.8)	
Grade 3 (Poorly differentiated)	356	112.6 (103.8–121.3)		375	1576 (1509–1643)		355	2.8 (2.7–2.9)	
Estrogen receptor status			0.45			0.24			0.53
Positive	2059	114.4 (109.3–119.5)		2160	1542 (1503–1582)		2055	2.7 (2.7–2.8)	
Negative	14	128 (92.5–163.5)		14	1712 (1432–1991)		14	2.6 (2.2–3)	
Progesterone receptor status			0.22			0.47			0.52
Positive	1588	113.4 (107.9–118.8)		1663	1539 (1497–1581)		1584	2.7 (2.7–2.8)	
Negative	485	118.3 (110.4–126.1)		511	1561 (1501–1621)		485	2.7 (2.6–2.8)	
HER2 status			0.42			0.29			0.21
Negative	1779	113.3 (107.9–118.8)		1867	1547 (1505–1589)		1775	2.8 (2.7–2.8)	
Positive	222	119.6 (108.9–130.2)		232	1553 (1470.8–1635)		222	2.7 (2.6–2.8)	
Not done	72	120.7 (102.6–138.8)		75	1436 (1296–1576)		72	2.6 (2.4–2.8)	
IHC subtype			0.004			0.73			0.16
Luminal A	1773	113.9 (108.4–119.4)		1861	1544 (1502–1587)		1769	2.7 (2.7–2.8)	
Luminal B	218	116.9 (106.2–127.7)		228	1548 (1465–1631)		218	2.7 (2.6–2.8)	
Her2 positive	4	234.5 (167.7–301.3)		4	1677 (1147–2207)		4	2.1 (1.4–2.8)	
Triple negative	6	92.3 (42.4–142.1)		6	1754 (1358–2149)		6	3.1 (2.6–3.6)	
Osteoporosis prior to diagnosis			0.45			0.47			0.24
No	2099	114.1 (108.9–119.2)		2198	1570.7 (1531–1610.5)		2095	2.8 (2.7–2.8)	
<5 years	107	120.6 (105.3–135.9)		113	1514.6 (1396.5–1632.6)		107	2.7 (2.5–2.8)	

Characteristic	RANKL, pmol/L			OPG, pg/ml			OPG/RANKL ratio†		
	N	LS mean (95% CI)	P	N	LS mean (95% CI)	P	N	LS mean (95% CI)	P
≥5 years	59	124.2 (104.1–144.4)		63	1628.4 (1473.4–1783.5)		59	2.7 (2.5–2.9)	
Any fracture prior to diagnosis			0.01			0.67			0.02
No	1946	113.2 (108–118.5)		2043	1572.4 (1531.8–1613.1)		1944	2.8 (2.7–2.8)	
<5 years	173	113.2 (101.1–125.3)		179	1578.5 (1484.3–1672.7)		172	2.8 (2.6–2.9)	
≥5 years	146	133 (119.9–146)		152	1527 (1425.3–1628.6)		145	2.6 (2.4–2.7)	
Any major fracture prior to diagnosis			0.67			0.33			0.32
No	2177	114.8 (109.7–119.9)		2281	1572.8 (1533.3–1612.3)		2174	2.8 (2.7–2.8)	
<5 years	57	106.5 (86.1–126.8)		60	1556 (1398.3–1713.6)		57	2.8 (2.6–3)	
≥5 years	31	120 (92.6–147.3)		33	1413.1 (1202.6–1623.5)		30	2.6 (2.3–2.8)	
Age at first fracture			0.16			0.55			0.18
No	1946	113.2 (108–118.5)		2043	1572 (1531.4–1612.7)		1944	2.8 (2.7–2.8)	
<55 years	92	121.2 (105–137.4)		96	1503.2 (1377.2–1629.2)		92	2.7 (2.5–2.8)	
≥55 years	227	122.7 (111.7–133.7)		235	1578.1 (1492.2–1664)		225	2.7 (2.6–2.8)	

Note: Overall models adjusted for age at diagnosis (continuous), menopausal status at baseline (Pre/Post), and time of blood collection relative to chemotherapy (before/after). For clinical characteristics, patients with bone metastasis and prior bisphosphonate treatment were excluded; for bone health history, only patients with bone metastasis were excluded.

*p-values remain significant after correcting for a total of 120 tests (6 markers × 20 variables, unadjusetd p <0.0004).

†The BAP/TRAP5b ratio was log-transformed.