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Circulating Receptor Activator of Nuclear Factor-κB (RANK), RANK ligand (RANKL) and Mammographic Density in Premenopausal Women

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Abstract

The receptor activator of nuclear factor-κB (RANK) pathway plays essential roles in breast development. Mammographic density is a strong risk factor for breast cancer, especially in premenopausal women. We, therefore, investigated the associations of circulating RANK, and soluble RANK ligand (sRANKL) with mammographic density in premenopausal women. Mammographic density was measured as volumetric percent density in 365 cancer-free premenopausal women (mean age, 47.5 years) attending screening mammogram at the Washington University School of Medicine, St. Louis, MO. We used linear regression models adjusted for confounders, to compare the least-square means of volumetric percent density across tertiles of circulating RANK and sRANKL. Further, because RANKL levels in mammary tissue are modulated by progesterone, we stratified analyses by progesterone levels. The mean volumetric percent density increased across tertiles of circulating RANK from 8.6% in tertile 1, to 8.8% in tertile 2, and 9.5% in tertile 3 (p-trend=0.02). For sRANKL, the mean volumetric percent density was 8.5% in tertile 1, 9.4% in tertile 2, and 9.0% in tertile 3 (p-trend=0.30). However, when restricted to women with higher progesterone levels, the mean volumetric percent density increased from 9.1% in sRANKL tertile 1 to 9.5% in tertile 2, and 10.1% in tertile 3 (p-trend=0.01). Circulating RANK was positively associated with volumetric percent density, while circulating sRANKL was positively associated with volumetric percent density among women

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with higher progesterone levels. These findings support the inhibition of RANKL signaling as a pathway to reduce mammographic density and possibly breast cancer incidence in high-risk women with dense breasts.

Keywords

Breast cancer; Mammographic density; Premenopausal; Prevention; RANK; sRANKL

Introduction

Mammographic breast density is one of the strongest risk factors for breast cancer (1). Almost 2.4 million premenopausal women in the United States have extremely dense breasts (2), hence, providing targeted prevention interventions to these women could have a substantial impact on reducing breast cancer incidence. However, the biological drivers of mammographic breast density are poorly understood. In addition, mammographic density and breast cancer share similar biological and genetic pathways (3, 4). Therefore, ascertaining biological pathways that are associated with mammographic density may identify potential targets for reducing breast density, opening up novel approaches to breast cancer prevention in premenopausal women.

The receptor activator of nuclear factor-κB (RANK) pathway is essential for bone health (5, 6). RANK is the signaling receptor for RANK ligand (RANKL), while osteoprotegerin (OPG) acts as the decoy receptor for RANK (7). This pathway also plays functional roles in mice mammary gland development (8–10). In particular, RANK/RANKL are essential for hormone-driven mammary epithelial proliferation (11, 12). The RANK pathway has, therefore, been suggested as a target for breast cancer chemoprevention in high-risk women (13–15). Two recent studies indicate that serum soluble RANKL (sRANKL) levels are positively associated with breast cancer risk among women with high progesterone levels (16), and women with estrogen receptor positive (ER+) breast cancer (17).

We recently demonstrated that higher breast tissue RANKL gene expression is positively associated with mammographic density in premenopausal women (18). This study was limited to 48 women and mammographic density was based on the radiologists' assessment using the Breast Imaging Reporting and Data (BI-RADS®) quartile system. Many research studies now evaluate mammographic density using quantitative measures, with percent density (equivalent to volumetric percent density when using volumetric measures) being the strongest predictor of breast cancer risk (19). In addition, RANK and RANKL are expressed as membrane bound in tissues or in soluble form within the circulation (20) but to the best of our knowledge, there are no data yet on the associations of circulating RANK and sRANKL with mammographic density. A recent small study (N=100) reported that OPG was associated with mammographic density in postmenopausal women, but not in premenopausal women (21). We therefore investigated the associations of circulating RANK and sRANKL with volumetric measures of mammographic density in premenopausal women. Further, because RANKL levels in mammary tissue are modulated by progesterone in mice studies, we also investigated associations stratified by progesterone levels.

Materials and Methods

Study Population:

We recruited 383 premenopausal women who were scheduled for an annual screening mammography at the Joanne Knight Breast Health Center at the Washington University School of Medicine, and Siteman Cancer Center, St. Louis, MO in 2016. Detailed description of the study population has been provided previously (22). Complete mammographic density data and blood samples were available for 375 women. Eligibility criteria included (i) premenopausal at the time of mammogram (defined as having had regular menstrual periods over the preceding 12 months, no prior history of bilateral oophorectomy, and no use of menopausal hormone therapy), (ii) no serious medical condition that would prevent the participant from returning for her annual mammogram in 12 months, (iii) not pregnant. Women were excluded from the study if they had (i) history of any cancer, (ii) history of breast augmentation, reduction, or implants, (iii) history of selective estrogen receptor modulators (SERM) use during the previous 6 months. Eligible participants were asked to fast on the day of their screening mammogram appointment. On the day of screening mammogram, trained phlebotomists collected blood samples from all study participants. Hence, biomarker analyses were done on blood samples that were collected on the same day the women had their mammograms. Blood samples were processed and stored at −80°C at the Tissue Procurement Core (TPC), Siteman Cancer Center, St. Louis within 30 minutes of collection. We measured height (using a stadiometer) and current weight (OMRON Full Body Sensor Body Composition Monitor and Scale model HBF-514C) in all study participants on the same day they had their screening mammogram. Participants also completed a questionnaire (a modified version of the Predicting Risk of Breast Cancer at Screening questionnaire) with information on breast cancer risk factors and determinants of mammographic density. We did not collect data on SERM use. Study approval was granted by the Institutional Review Board of the Washington University School of Medicine, Saint Louis, MO. All study participants provided informed consent.

Circulating RANK Biomarkers:

Circulating RANK, and sRANKL levels were assayed at the Research Laboratory Service, Maine Medical Center Research Institute using commercially available enzyme-linked immunosorbent assays (ELISA) kits according to manufacturers' instructions (BioVendor, Modrice, Czech Republic for sRANKL and Abnova, Taipei 114, Taiwan for RANK). Laboratory precision was monitored by the inclusion of blinded pooled quality control samples. Inter-assay coefficients of variation were 5.7% for RANK and 12.0% for RANKL, based on blind replicates in our samples. Intra-assay coefficients of variation were 4.4% for RANK and 9.4% for sRANKL.

Circulating Progesterone levels:

Circulating Progesterone levels were assayed at the Department of Laboratory Medicine, Boston Children's Hospital. Progesterone was measured by a competitive electrochemiluminescence immunoassay on the Roche E Modular system (Roche Diagnostics, Indianapolis, IN). The assay is FDA-approved for clinical use and has been

used in previous studies (23, 24). The intra-assay coefficients of variation were 2.9% at 0.73 ng/mL and 0.9% at 32.4 ng/mL based on blind replicates in our samples. The inter-assay coefficients of variation were 4.8% at 0.73 ng/mL and 2.0% at 35.3 ng/mL. Sensitivity was 0.15 ng/mL and specificity was 0.81.

Mammographic Density Assessment:

We used Volpara (version 1.5, (Matakina Technology Ltd, Wellington, New Zealand) to determine volumetric percent density, dense volume, and non-dense volume (25, 26). Dense volume is the volume of fibroglandular tissue in the breast (cm^3). Volumetric percent density is the ratio of the volume of fibroglandular tissue (i.e. dense volume) to the total breast volume, expressed as a percentage. Corresponding to the four categories (a)~(d) of the breast imaging reporting and data system (BI-RADS®) (5th edition), Volpara volumetric percent density measures translate to: <3.5 (a- almost entirely fatty breasts); ≥3.5 and <7.5 (b- scattered areas of fibroglandular density); ≥7.5 and <15.5 (c- heterogeneously dense breasts); ≥15.5%−34.5% (d- extremely dense breasts) (27).

Statistical Analyses

We summarized continuous variables using means and standard deviations and categorical variables using counts and percentages. We performed square-root transformations on volumetric percent density, dense volume, and non-dense volume for conformation to normality. We categorized study participants into tertiles of RANK and sRANKL based on the empirical distribution of the biomarkers in our study population. We used linear regression models with adjustment for confounders, to evaluate the associations between the tertiles of circulating RANK, sRANKL and mammographic density by comparing least-square means of volumetric percent density, dense volume, and non-dense volume within tertiles of circulating RANK and sRANKL concentrations. We also estimated the difference from the lowest tertile of volumetric percent density for each tertile of circulating RANK and sRANKL. In the minimally adjusted model 1, we adjusted the analyses for age (continuous) and body mass index (continuous) derived as weight (kg)/height (m^2) (kg/m^2). In model 2, we additionally adjusted for family history of breast cancer in a first-degree relative (no, yes, and unknown), race (Non-Hispanic White, Black/African American, Others), age at menarche (continuous), parity (0, 1, 2, ≥3) and current alcohol intake (no, yes). We also adjusted the statistical analyses for quantity of alcohol consumed, but alcohol intake was low in our study population and the findings were similar to when we categorized alcohol intake as yes or no. We also tested the following confounders: phase of menstrual cycle, oral contraceptive use, circulating progesterone levels, and breastfeeding but they did not change the estimates by up to 10%. Phase of menstrual cycle was derived from information provided by the participants; i.e. average menstrual cycle length, date of onset of their last menstrual period and the date of the predicted onset of their next menses. We additionally evaluated the impact of other potential breast cancer risk factors such as physical activity, vitamin D intake etc., but these had no impact on mammographic density. We tested for linear trends using Wald tests by ordinally modeling the median of the tertiles. We further evaluated if the associations of circulating RANK, sRANKL and mammographic density varied by progesterone levels by assessing the Wald tests on the interaction term between the ordinal median biomarkers levels and progesterone levels. We then categorized

study participants into 2 groups based on median circulating progesterone levels in our study population. We classified women who had progesterone levels below the median value as having lower progesterone and women with progesterone levels above the median value as having higher progesterone. We repeated linear regression analyses stratified by lower and higher progesterone. Some women (N=36) had circulating RANK levels reported as <2 pg/mL. We assumed that these participants had missing values and imputed their circulating RANK values by chained equation, which is based on the conditional probability of a variable, with the other covariables serving as predictors. (28). Nevertheless, we performed sensitivity analyses excluding women whose RANK values were reported as <2 pg/mL. All statistical tests were two-sided, and p-values < 0.05 were considered statistically significant. All analyses were done with the Statistical Analyses Systems (SAS) version 9.4 (SAS Institute, Cary, NC).

Results

The mean age of the study participants was 47.5 years (Table 1). The mean body mass index (BMI) was 30.8 kg/m^2, consistent with the BMI of women attending screening mammogram at the Breast Health Center. Many were Non-Hispanic White (65.6%), and African American (29.3%). Most participants (42.1%) had volumetric percent density between 3.5–7.5%, equivalent to BI-RADS® category b.

We observed positive associations of circulating RANK with volumetric percent density (Table 2). In the age and BMI adjusted model, the mean volumetric percent density increased across tertiles of circulating RANK from 8.1% in tertile 1, to 8.4% in tertile 2, and 9.2% in tertile 3 (p-trend=0.01). In the full multivariable adjusted model, the mean volumetric percent density increased from 8.6% in tertile 1, to 8.8% in tertile 2, and 9.5% in tertile 3 (p-trend=0.02). Further adjustment for phase of menstrual cycle did not change the results. We also estimated the difference from the lowest tertile of volumetric percent density for each tertile of circulating RANK and sRANKL. Women in the highest tertile of RANK had 1.1% points higher volumetric percent density that women in the lowest tertile (95% CI −0.2, 2.4, p-trend=0.02) (Supplementary Table 1). We observed no significant associations between circulating sRANKL and volumetric percent density. In the full multivariable adjusted model, the mean volumetric percent density was 8.5% in tertile 1, 9.4% in tertile 2, and 9.0% in tertile 3 (p-trend=0.30). We then investigated the associations of circulating RANK, and sRANKL with dense volume. As expected, the findings were in the same direction as those for volumetric percent density, but not as apparent. For RANK, the mean dense volume increased from 75.1cm^3 in tertile 1 to 76.5cm^3 in tertile 2 to 81.2cm^3 in tertile 3 (p-trend=0.05). For sRANKL, the mean dense volume was 75.8cm^3 in tertile 1, 75.1cm^3 in tertile 2, and 81.8cm^3 in tertile 3 (p-trend=0.35).

Next, we investigated the associations of circulating RANK, sRANKL with mammographic density stratified by progesterone levels. We observed an interaction between circulating sRANKL and progesterone on volumetric percent density (p-interaction =0.045). Circulating sRANKL was positively associated with volumetric percent density among women with higher progesterone levels (above median levels in our study population), but not among women with lower progesterone levels (Figure 1). Among women with higher progesterone

levels, the mean volumetric percent density increased from 9.1%, in tertile 1 to 9.5% in tertile 2, and 10.1% in tertile 3 (p-trend=0.01) in the full multivariable adjusted model. Women in the highest tertile of sRANKL had 1.3% points higher volumetric percent density than women in the lowest tertile (95% CI −0.6, 3.2, p-trend=0.01) (Supplementary Table 1). Among women with lower progesterone levels, the mean volumetric percent density was 7.9% in tertile 1, 9.4% in tertile 2 and 7.9% in tertile 3 (p-trend=0.72). Likewise, the associations of RANK with volumetric percent density was more evident among women with higher progesterone levels (p-trend=0.03) than those with lower progesterone levels (p-trend=0.13) (Figure 2), but there was no interaction.

There were no statistically significant associations between RANKL, sRANKL and breast cancer risk factors (Table 3), although BMI at age 18 appeared to decrease with increasing sRANKL concentrations. Also, 64% of women within the lowest tertile of sRANKL concentrations had a history of breast feeding compared to 68% among women within the second tertile and 74% among women within the highest tertiles.

There were no associations between both circulating RANK and sRANKL and non-dense volume (Supplementary Table 2). In sensitivity analyses, we excluded women with RANK values <2 pg/mL and the findings were similar to those reported using the full data (Supplementary Table 3).

Discussion

To the best of our knowledge, this is the first large study on the associations of circulating RANK and sRANKL with mammographic density in premenopausal women. Circulating RANK was positively associated with volumetric percent density. Circulating sRANKL was positively associated with volumetric percent density among women with higher progesterone levels only.

Data on the associations of RANK pathway with mammographic density and breast cancer risk are emerging. We previously reported that higher breast tissue RANKL gene expression is positively associated with mammographic density, assessed using BI-RADS®, in premenopausal women (18). In the current study, we evaluated RANK within the circulation and measured mammographic density using Volpara, which enabled automated volumetric measures. Therefore, this current study provides new, and important insights into the associations of RANK pathway with mammographic density in premenopausal women.

It is intriguing that circulating RANK was positively associated with volumetric percent density in the overall analyses but circulating sRANKL was not. Unlike sRANKL, there is very limited data on factors that influence circulating RANK concentrations, and there are no published studies on circulating RANK and breast cancer. In a previous study, we measured circulating RANK and sRANKL using the same methods in the same lab (29). RANKL functions as a major paracrine effector via RANK. Both RANK and RANKL induce differentiation and proliferation of mammary epithelial cells via the NF-kB and cyclin D1 axis (7, 12), which are key pathways involved in breast cancer development. The differential associations may be due to how these biomarkers are expressed in human tissues

and factors determining such expression. RANK is constitutively expressed on mammary epithelial cells, whereas RANKL expression in mammary epithelial cells, as well as within the circulation is induced primarily by progesterone (30). Thus, progesterone levels might influence the associations of sRANKL with mammographic density and our findings that circulating sRANKL was positively associated with volumetric percent density only among women with higher progesterone levels fits with this biological observation. Consistent with our observation for volumetric percent density, Penninger's group analyzed data from the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) trial and reported that circulating sRANKL was positively associated with an increased risk of breast cancer only among women with higher progesterone levels (16). Another study from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort reported that higher sRANKL concentrations were associated with increased risk of ER+ breast cancer, but there was also evidence suggestive of an inverse association with ER-/PR- breast cancer (17). RANKL signaling activates NF-κB and canonical NF-κB pathways are most active in ER+ breast cancers (31). Taken together, these findings suggest that the positive associations of sRANKL with mammographic density and breast cancer risk appear limited to women with higher hormone concentrations.

Further, RANKL signaling is the major mediator of progesterone induced mammary epithelial proliferation (10–12, 32, 33), and progesterone-driven expansion of mammary stem cells (6, 34, 35). Progesterone is a proliferative hormone in the breast, independent of estrogen (36–43). Combined estrogen+progestin hormone therapy is classified as carcinogenic to human by the International Agency for Research on Cancer (43). Clinical trials, including the Women's Health Initiative have demonstrated that 12 months of estrogen+progestin is associated with a 5% increase in mammographic density (44, 45) and increased breast cancer risk (40).

Higher OPG levels have also been associated with lower mammographic density in high-risk postmenopausal women (21), which is biologically plausible since OPG blocks RANK activation and RANKL signaling. Nevertheless, findings from the 2 studies on circulating OPG and breast cancer risk are mixed. Among patients with BRCA mutations, higher OPG levels were associated with lower risk of breast cancer (46). The other study reported that higher OPG levels are associated with an increased risk of ER-ve disease, but a decreased risk of ER+ve disease (47).

Our study has the following limitations. It is observational. We measured RANK and sRANKL at the time of enrollment only. Longitudinal assessments may provide a more detailed insight on how mammographic density changes with RANKL and sRANKL over time. Nevertheless, studies have reported moderate correlations between sRANKL measurements in samples taken one year (17), and five years apart (48), suggesting that a single measurement of sRANKL is a good reflection of circulating levels over the short-term. We evaluated circulating sRANKL measured in the blood. We are unaware of studies that have related breast tissue RANKL levels to circulating sRANKL levels, hence, it is not clear to what extent circulating sRANKL reflects breast tissue levels. Our previous study showing that breast tissue RANKL gene expression is positively associated with

mammographic density indicates circulating sRANKL concentrations could be a good surrogate for the biomarker in the breast.

Strengths of our study include the following. Study participants were recruited among women attending annual routine screening mammogram, which enhances generalizability. We adjusted for many potential confounders in statistical analyses. We evaluated associations stratified by progesterone levels, which modulates circulating sRANKL concentrations. We collected fasting samples from study participants and biomarker measurements were done on aliquots that had never been thawed. We assessed mammographic density using Volpara, which provides automated and robust volumetric measures of density, and has been found to be highly reproducible (25, 26, 49, 50).

In conclusion, circulating RANK concentrations were positively associated with volumetric percent density, and circulating sRANKL concentrations were positively associated with volumetric percent density among women with higher progesterone levels only. Our findings offer new insights on the biological determinants of mammographic density in premenopausal women and support the inhibition of RANKL signaling as a pathway to reduce mammographic density and possibly breast cancer incidence in high-risk women with dense breasts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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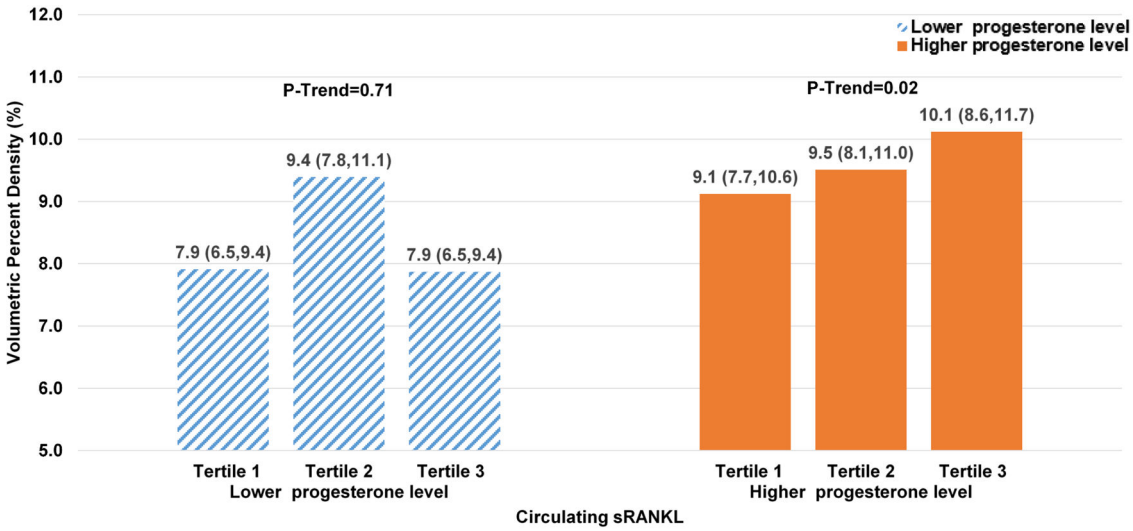

Figure 1.

Least Square Means of Volumetric Percent Density (%) by Tertiles of Circulating sRANKL Level stratified by Progesterone Levels

Footnotes: Multivariable model adjusted for age (continuous), age at menarche (continuous), body mass index (continuous), family history of breast cancer (Yes/No/Unknown), parity (0, 1, 2, ≥3), race (White/African American/Other) and recent alcohol intake (Yes/No).

Least Square Means and 95% Confidence Intervals were calculated

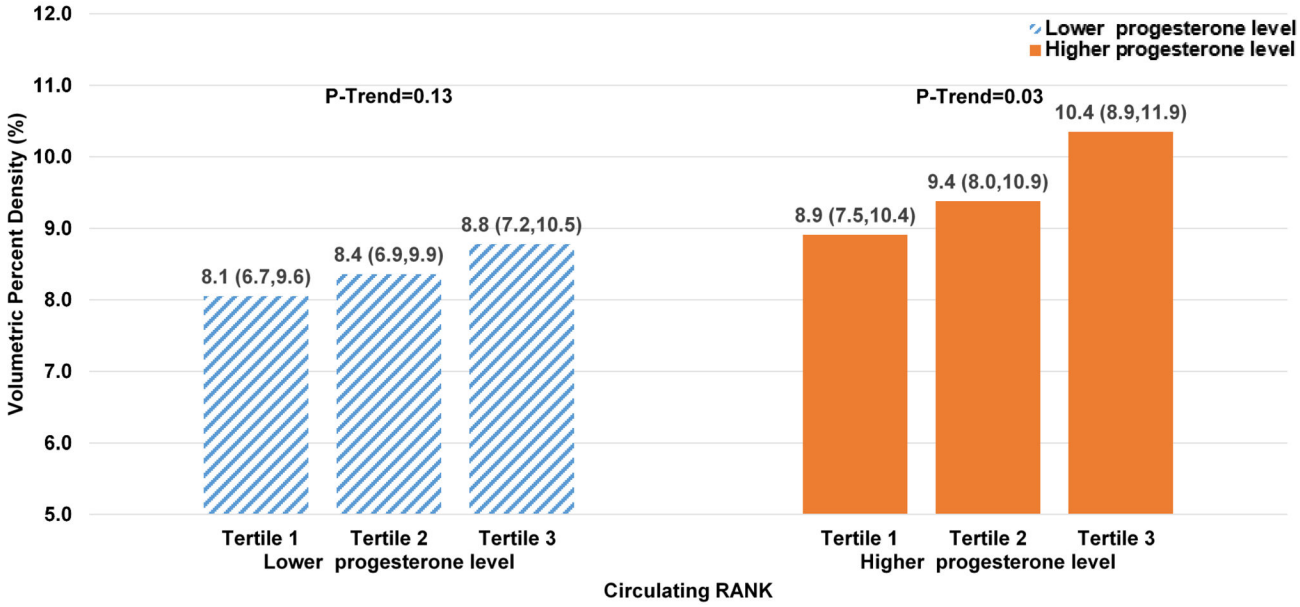

Figure 2.

Least Square Means of Volumetric Percent Density (%) by Tertiles of Circulating RANK Level stratified by Progesterone Levels

Footnotes: Multivariable model adjusted for age (continuous), age at menarche (continuous), body mass index (continuous), family history of breast cancer (Yes/No/Unknown), parity (0, 1, 2, ≥3), race (White/African American/Other) and recent alcohol intake (Yes/No).

Least Square Means and 95% Confidence Intervals were calculated

Table 1.

Characteristics of 375 Premenopausal Women Recruited During Annual Screening Mammogram at the Joanne Knight Breast Health Center, Washington University School of Medicine, St. Louis, MO.

Characteristic	Number	Mean (Range) and Percentage
Age (years)	375	47.5 (32–58)
Age at Menarche (years)	373	12.8 (9–18)
Age at first birth (years)	302	26.0 (13–41)
Parity		
Nulliparous	70	19.7%
Parous	305	81.3%
Ever used oral contraceptive		
Yes	333	88.8%
No	42	11.2%
Current alcohol intake		
Yes	244	65.1%
No	130	34.6%
Missing	1	0.3%
Family history of breast cancer		
Yes	88	23.5%
No	275	73.3%
Missing	12	3.2%
Race		
Non-Hispanic White	246	65.6%
Black or African American	110	29.3%
Others/Unknown	19	5.1%
Education level		
High school or less	36	9.6%
Post high school/some college	75	20.0%
College graduate	144	38.4%
Postgraduate	118	31.5%
Missing	2	0.5%
Adiposity measures		
Current body mass index (BMI) (kg/m^2)	375	30.8 (17.9–63.1)
BMI at age 30 (kg/m^2)	374	25.7 (13.2–39.6)
BMI at age 18 (kg/m^2)	374	21.7 (17.0–50.8)
Biomarkers		
RANK (pg/mL)	339	310.0 (0.9–3969.3)
sRANKL (pmol/L)	375	277.0 (2.0–2948.1)
Progesterone (ng/mL)	375	2.9 (0.1–24.3)
Mammogram density		
Volumetric Percent Density (%)[1]	375	9.5 (2.5–31.9)
<3.5%	34	9.10%

Characteristic	Number	Mean (Range) and Percentage
3.5–7.5%	158	42.10%
≥7.5%−15.5%	119	31.70%
≥15.5%	64	17.10%
Dense Volume (cm^3)	375	80.7 (19.5–396.2)
Non-Dense Volume (cm^3)	375	1079.0 (55.3–4081.2)

[1]Volumetric Percent Density. Volpara volumetric percent density ranges from 0.5%−34.5%. Corresponding to the four categories (a)~(d) of the breast imaging reporting and data system (BI-RADS®) (5th edition), Volpara volumetric percent density measures translate to: <3.5 (a – almost entirely fatty breasts); ≥3.5 and <7.5 (b- scattered areas of fibroglandular density); ≥7.5 and <15.5 (c- heterogeneously dense breasts); ≥15.5% (d – extremely dense breasts).

Table 2.

Least Square Means of Volumetric Percentage Density and Dense Volume by Tertiles of Circulating RANK and sRANKL.

	Volumetric Percentage Density (%)			P-trend
Biomarkers	**Tertile 1**	**Tertile 2**	**Tertile 3**	
RANK, range (pg/mL)	0.2–8.3	8.3–47.6	≥47.6	
Adjusted for age and BMI[1]	8.1	8.4	9.2	0.01
Multivariable adjusted[2]	8.6	8.8	9.5	0.02
sRANKL, range (pmol/L)	2.1–142.2	142.3–255.7	≥255.7	
Adjusted for age and BMI[1]	8.2	9.1	8.5	0.27
Multivariable adjusted[2]	8.5	9.4	9.0	0.30
	Dense Volume (cm^3)			
RANK (pg/mL)	0.2–8.3	8.3–47.6	≥47.6	
Adjusted for age and BMI[1]	72.9	75.5	80.6	0.04
Multivariable adjusted[2]	75.1	76.5	81.2	0.05
sRANKL (pmol/L)	2.1–142.2	142.3–255.7	≥255.7	
Adjusted for age and BMI[1]	76.0	73.3	79.8	0.31
Multivariable adjusted[2]	75.8	75.1	81.9	0.35

[1]Adjusted for age and body mass index (BMI)

[2]Adjusted for age (continuous), age at menarche (continuous), body mass index (continuous), family history of breast cancer (Yes/No/Unknown), parity (0, 1, 2, ≥3), race (White/African American/Other) and current alcohol intake (Yes/No).

Table 3.

Characteristics of 375 Premenopausal Women by Tertiles of Circulating RANK and sRANKL

Characteristics	RANK Mean and Percentage			P-value	sRANKL Mean and Percentage			P-value
	Tertile 1	Tertile 2	Tertile 3		Tertile 1	Tertile 2	Tertile 3	
Age (years)	47.5	47.0	48.1	0.23	48.0	46.9	47.6	0.2
Age at Menarche (years)	12.8	12.9	12.8	0.96	12.8	12.8	12.9	0.97
Age at first birth (years)	25.3	26.8	26.4	0.28	26.0	26.3	25.6	0.68
Parity				0.27				0.07
Parous	88 (83.3)	89 (83.9)	80 (75.44)		95 (75.6)	109 (87.8)	101 (79.7)	
Nulliparous	18 (16.7)	18 (16.1)	26 (24.56)		30 (24.4)	16 (12.2)	24 (20.3)	
Ever breastfed				0.45				0.5
Yes	63 (71.9)	60 (66.9)	61 (76.5)		63 (63.9)	79 (68.4)	74 (74.0)	
No	25 (28.1)	29 (33.1)	19 (23.5)		32 (36.1)	30 (31.6)	27 (26.0)	
Ever used oral contraceptive				0.32				0.73
Yes	90 (84.5)	97 (90.0)	96 (90.5)		109 (86.0)	111 (89.2)	113 (87.7)	
No	16 (15.5)	10 (10.0)	10 (9.5)		16 (14.0)	14 (10.8)	12 (12.4)	
Current alcohol intake				0.25				0.44
Yes	66 (61.8)	76 (70.6)	64 (58.8)		77 (62.1)	86 (71.5)	81 (61.6)	
No	40 (38.2)	31 (29.4)	41 (41.2)		48 (37.9)	38 (28.5)	44 (38.4)	
Family history of breast cancer				0.51				0.17
Yes	21 (19.5)	26 (26.4)	28 (27.8)		33 (26.1)	33 (27.9)	22 (18.9)	
No	85 (80.5)	81 (73.6)	78 (72.2)		92 (73.9)	92 (72.1)	103 (81.1)	
Race				0.64				0.26
Non-Hispanic White	67 (62.9)	73 (68.2)	72 (67.3)		75 (59.7)	88 (70.8)	83 (65.6)	
Black or African American	35 (34.1)	27 (24.9)	30 (27.7)		45 (35.3)	29 (23.7)	36 (30.3)	
Others / Unknown	4 (3.1)	7 (6.9)	4 (5.1)		5 (4.9)	8 (5.5)	6 (4.1)	
Education level				0.29				0.21
High school or less	12 (10.3)	7 (6.9)	11 (11.7)		15 (11.9)	8 (5.7)	13 (11.3)	
Post high school/some college	24 (24.1)	24 (23.3)	18 (16.3)		21 (19.1)	21 (18.2)	33 (25.7)	
College graduate	43 (39.1)	42 (40.5)	33 (34.1)		46 (37.4)	55 (43.7)	43 (33.3)	
Postgraduate	27 (26.5)	34 (29.4)	42 (37.9)		42 (31.6)	41 (32.5)	35 (29.8)	

Characteristics	RANK Mean and Percentage			P-value	sRANKL Mean and Percentage			P-value
	Tertile 1	Tertile 2	Tertile 3		Tertile 1	Tertile 2	Tertile 3	
Adiposity measures								
Current BMI (kg/m^2)	30.9	30.6	31.6	0.7	31.4	31.4	30.0	0.38
BMI at age 18 (kg/m^2)	21.9	21.5	22.0	0.58	22.1	21.8	21.3	0.27
Body fat (%)	40.7	39.6	39.2	0.61	41.6	39.5	39.1	0.05