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Research Article

Automated Measurement of Volumetric Mammographic Density: A Tool for Widespread Breast Cancer Risk Assessment

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Abstract

Introduction: Mammographic density is a strong risk factor for breast cancer and an important determinant of screening sensitivity, but its clinical utility is hampered due to the lack of objective and automated measures. We evaluated the performance of a fully automated volumetric method (Volpara).

Methods: A prospective cohort study included 41,102 women attending mammography screening, of whom 206 were diagnosed with breast cancer after a median follow-up of 15.2 months. Percent and absolute dense volumes were estimated from raw digital mammograms. Genotyping was performed in a subset of the cohort ($N = 2{,}122$). We examined the agreement by side and view and compared density distributions across different mammography systems. We also studied associations with established density determinants and breast cancer risk.

Results: The method showed good agreement by side and view, and distributions of percent and absolute dense volume were similar across mammography systems. Volumetric density was positively associated with nulliparity, age at first birth, hormone use, benign breast disease, and family history of breast cancer, and negatively with age and postmenopausal status. Associations were also observed with rs10995190 in the *ZNF365* gene ($P < 1.0 \times 10^{-6}$) and breast cancer risk [HR for the highest vs. lowest quartile, 2.93; 95% confidence interval, 1.73–4.96 and 1.63 (1.10–2.42) for percent and absolute dense volume, respectively].

Conclusions: In a high-throughput setting, Volpara performs well and in accordance with the behavior of established density measures.

Impact: Automated measurement of volumetric mammographic density is a promising tool for widespread breast cancer risk assessment. *Cancer Epidemiol Biomarkers Prev; 23(9); 1764–72. ©2014 AACR.*

Introduction

Breast cancer is the most common cancer diagnosed in women, accounting for approximately 14% of all female cancer deaths (1). Mammography screening has been subject to intense debate, but an independent expert panel has recently demonstrated its mortality benefits, although room for improvement remains in terms of diagnostic accuracy and cost effectiveness (2, 3). One of the limitations of current screening programs is the lack of consideration of an individual risk profile (4). As of today, screening strategies are based solely on age, ignoring the fact that a woman's lifetime risk is determined by other common risk factors. After age, mammographic density is the strongest breast cancer risk factor with the highest population attributable fraction (5, 6). The impact of mammographic density is 2-fold, as women with dense breasts are not only at increased risk of developing breast cancer (7) but also have a greater chance of a tumor going undetected because of the masking effect of mammographic density (8, 9). As such, mammographic density is a promising tool for optimizing screening programs where resources and extra imaging modalities can be targeted to those with the highest density (10).

Despite its potential clinical applications, current methods for measuring mammographic density are far from ideal. All established methods are reader-dependent, time-consuming, and area-based, not considering the breast as a three-dimensional (3D) object (11, 12). Moreover, most methods are not designed for full-field digital mammography (FFDM), which is now the routine screening method in most clinical practices. To overcome these

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limitations, several automated volumetric methods for FFDM have been developed which could potentially work in high-throughput settings. Volpara is one of these methods with multivendor clearance from the FDA. Volpara has already been validated against breast MRI data and seems to be robust to changes in imaging conditions (13). However, no studies to date have evaluated Volpara based on established properties of mammographic density including the association with breast cancer risk.

Because the widespread application of mammographic density is highly dependent on the accuracy and reliability of its measurement, we studied the performance of Volpara in a large prospective cohort study. The importance of an accurate method is also underlined by recent legislative activities, as several U.S. states have passed laws requiring radiologists to inform patients about their mammographic density (14).

Materials and Methods

Study population

KARMA (KARolinska MAmmography project for risk prediction of breast cancer) is a prospective cohort study initiated in January 2011 and comprises 70,866 women attending mammography screening or clinical mammography at four hospitals in Sweden (15). Upon study entry, participants donated blood and filled out a detailed Web-based questionnaire. In addition, permission was asked for storage of both "for processing" (raw) and "for presentation" (processed) FFDM and linkage to Swedish national registers on inpatient care and cancer. All participants provided written informed consent and the study was approved by the ethical review board at Karolinska Institutet.

For the present analysis, we selected women who attended the mammography screening program (40–74 years) with raw mammograms stored at baseline (N = 50,461). We excluded women with previous cancers other than nonmelanoma skin cancer (N = 3,015), women who underwent breast enlargements/reductions/surgery (N = 2,191), women who did not give information on age and body mass index (BMI; N = 3,908), and women pregnant 12 months before study entry (N = 40), leaving 41,307 women in the study. Of these, 41,102 women had medio-lateral oblique (MLO) mammograms and 40,263 had craniocaudal mammograms. Follow-up on malignant breast cancer was performed through linkage to the Swedish national breast cancer registry, Information Network for Cancer Care (INCA) (16).

Mammographic density assessment

Digital mammography was performed using five different models from three manufacturers [General Electric (GE) Medical Systems, Philips Healthcare, and Sectra Imtec AB]. According to our records, women were not selectively referred to one of these mammography systems except for the GE Senograph DS 53.40 model, which was used for screening women with small breasts.

Volumetric mammographic density was measured using Volpara (version 1.4.3). Technical details of the software have been described elsewhere (13). In brief, the algorithm computes the thickness of dense tissue at each pixel using the X-ray attenuation of an entirely fatty region as an internal reference. The absolute dense volume (cm^3) is measured by integrating the dense thickness at each pixel over the whole mammogram, and the total breast volume (cm^3) is derived by multiplying the breast area by the recorded breast thickness, with an appropriate correction for the breast edge. Percent dense volume (%) is then obtained from the ratio of these two volumetric measures.

To compare Volpara volumetric measures with area-based measures, 77 digital MLO mammograms were processed for measurement with the user-assisted threshold method Cumulus (12). A trained observer (J. Li) set the appropriate gray-scale thresholds to define the breast and dense area (cm^2), after which the percent dense area (%) was calculated. The mammograms were selected from two manufacturers (GE Medical Systems: N = 28 and Sectra Imtec AB: N = 49) in such a way that their density measures covered the full range of possible values. All mammograms were measured in duplicate to assess the intraobserver reliability, which was high (R = 0.93).

Questionnaire data

Participants were asked to report on reproductive history, use of oral contraceptives (OC) and hormone replacement therapy (HRT), previous benign breast disease, and family history of breast cancer. A positive family history was defined as a diagnosis of breast cancer in at least one first-degree relative (i.e., sister, mother, or daughter).

Menopausal status was defined according to information on menstruation status, previous oophorectomy, and age at study entry. Postmenopausal women were defined as those who had no periods during the last year, a history of oophorectomy, or age 55 or older. Women were classified as perimenopausal/unknown when they had menses during the past year but were no longer menstruating during the last 3 months or when they had missing data on menstruation status and were between 46 and 55 years of age. Women were considered premenopausal when they reported having periods during the past 3 months or age <46 years if they had missing data on menstruation status. BMI was calculated based on self-reported height and weight.

Genotyping data

A subset of the study population (N = 2,122) was genotyped using a custom Illumina iSelect genotyping array (iCOGS) as has been described in detail elsewhere (17). For the present analysis, we focused on rs10995190 in the zinc finger protein 365 (*ZNF365*) gene, an SNP that has previously been associated with percent dense area (18).

Statistical analyses

All four standard views were stored, but the main analyses were based on the MLO view, as this is the

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routine view for breast cancer screening in Sweden. For the calculation of mammographic density, both sides (left and right) were considered and the mean was taken.

Before analyses, Volpara mammographic measures were log-transformed and Cumulus measures were square-root transformed to approximate the normal distribution. We first compared distributions of Volpara mammographic measures across different manufacturers, manufacturer models, and X-ray detectors. Analysis of covariance was used to detect differences in age- and BMI-adjusted geometric means of percent and absolute dense volume. We also evaluated the agreement by side and view in correlation analyses including all available mammograms of each woman. To assess the level of agreement with Cumulus area-based measures, Pearson correlation coefficients were calculated.

We then compared the distribution of established density determinants by quintile of percent and absolute dense volume using linear trend tests for continuous variables and χ^2 tests of linear trend for categorical variables. Linear regression models were used to adjust associations for age and BMI. Associations with rs10995190 were assessed using tests for genotype trend effects adjusting for age and BMI.

Finally, we performed Cox proportional hazards analyses to examine the association with breast cancer risk, in which density measures were entered as quartiles and per SD increase. In all analyses, age was used as the underlying time scale with entry and exit time defined as the participant's age at study entry and age at breast cancer diagnosis, death or censoring at the end of follow-up. We constructed 2 models to adjust associations for potential confounders. Age and BMI were included in the first model, after which other breast cancer risk factors were added to create the multivariable model. The proportional hazards assumption was evaluated by visual inspection of the Schoenfeld residual plots, which did not indicate violation of this assumption. We also did a sensitivity analysis to assess potential differential effects by mammogram side by entering the contralateral mammogram for women with incident breast cancer and a randomly selected mammogram for those who remained breast cancer free during follow-up.

Eighteen percent of the women had missing values on one or more covariates. On the basis of the missing data pattern, we assumed the missing values to be "missing at random" (19) and imputed these values using a multiple imputation technique (10 imputation sets; ref. 20). The imputation model was constructed from all potential predictors of missing covariate data (age, BMI, age at menarche, age at first birth, menopausal status, OC use, HRT use, benign breast disease, family history of breast cancer, and mammographic density) including the outcome (Nelson–Aalen estimator for time to event or death and the censoring indicator). Statistical analyses were performed using SAS version 9.2 (SAS Institute Inc.), STATA version 12.0 (Stata Corp.) and PLINK (21).

Results

The mean age at study entry was 55.0 years, and 56.5% of the women were postmenopausal at baseline (Table 1). All mammographic measures showed a positively skewed distribution, and the medians (interquartile range, IQR) for total breast volume, absolute dense volume, and percent dense volume were 760 (493–1,106) cm^3, 57 (42–77) cm^3, and 7.7 (5.3–11.7)%, respectively (Fig. 1). Density distributions were similar across manufacturers, but significant differences in age-and BMI-adjusted geometric means were observed across manufacturer models and X-ray detectors ($P < 0.001$; Table 2; Supplementary Table S1). These differences, however, were mainly driven by the GE Senograph DS 53.40 model (Table 2), due to selective referral of women with small breasts (and thus higher mammographic density) to this specific model. For the other mammography systems, differences were only

Table 1. Baseline characteristics of the study population ($N = 41,102$)

Participant characteristic	
Age (years), mean (SD)	55.0 (9.8)
BMI (kg/m^2), mean (SD)	25.4 (4.2)
Age at menarche (years), mean (SD)	13.1 (1.5)
Missing	1.9 (793)
Parity, % (*N*)	
0	12.1 (4,979)
1	14.1 (5,792)
2	48.4 (19,901)
≥3	25.2 (10,363)
Missing	0.2 (67)
Age at first birth (years), mean (SD)	27.1 (5.2)
Missing, % (*N*)	5.6 (2008)
Menopausal status, % (*N*)	
Premenopausal	40.2 (16,506)
Perimenopausal/unknown	3.3 (1,349)
Postmenopausal	56.5 (23,247)
Ever OC use, % (*N*)	79.3 (32,602)
Missing	0.5 (201)
HRT use, % (*N*)	
Never	74.9 (30,791)
Former	14.5 (5,945)
Current	3.7 (1,521)
Missing	6.9 (2,845)
Benign breast disease, % (*N*)	21.7 (8,919)
Missing	2.0 (811)
Family history breast cancer, % (*N*)	12.7 (5,236)
Missing	3.6 (1,471)
Total breast volume (cm^3), median (IQR)	760 (493–1,106)
Absolute dense volume (cm^3), median (IQR)	57 (42–77)
Percent dense volume (%), median (IQR)	7.7 (5.3–11.7)

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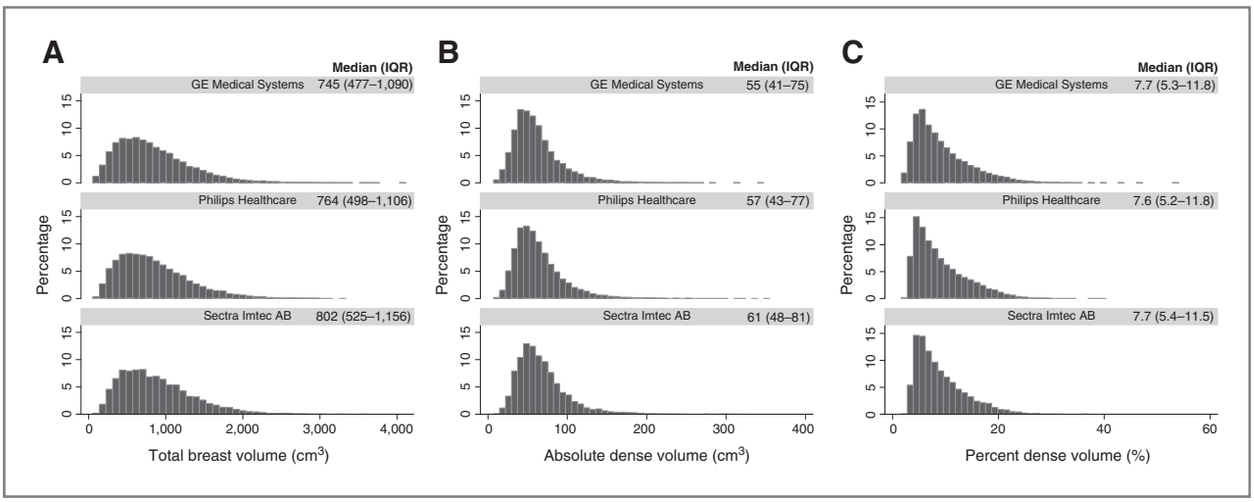

Figure 1. Distribution of volumetric mammographic measures from the mediolateral oblique view per manufacturer: A, total breast volume; B, absolute dense volume; C, percent dense volume.

minor, with the largest percentage deviation being observed for X-ray detectors PL197_02 (GE Senograph Essential 53.40) and KM070400 (Sectra L30). The relative difference in percent dense volume was 3% for these two models when taking the largest category of X-ray detectors (PL678_04) as a reference (Supplementary Table S1).

Agreement by side and view was high in correlation analyses including all mammograms (Supplementary Table S2). The left–right correlations were equally high for the craniocaudal and MLO view, but the craniocaudal/MLO agreement was slightly higher for total breast volume and percent dense volume than for the absolute dense volume.

The correlation between total breast area and total breast volume was high (R, 0.91; 95% confidence interval, CI, 0.87–0.94), as was the correlation between percent dense area and percent dense volume (R, 0.93; 95% CI, 0.89–0.96; Supplementary Fig. S1). The correlation between absolute dense area and absolute dense volume, however, was weak (R, 0.55; 95% CI, 0.38–0.69).

All known determinants of mammographic density were associated with percent and absolute dense volume in a similar way, except for BMI (Table 3 and Supplementary Table S3). Postmenopausal and older women had a lower percent and absolute dense volume, whereas nulliparity, older age at first birth, current HRT use, benign breast disease, and a family history of breast cancer were all associated with a larger amount of dense tissue. By contrast, women with a high BMI had a lower percent dense volume but a larger absolute dense volume. Overall, the strength of associations was comparable between the two volumetric measures, although the effect of parity seemed to be stronger for the absolute dense volume, whereas age and BMI had a larger impact on percent

Table 2. Geometric means of volumetric mammographic measures stratified by manufacturer model

					Geometric mean (95% CI), age and BMI adjusted		
Manufacturer	**Model**	***N***	**Age, mean (SD)**	**BMI, mean (SD)**	**Total breast volume (cm^3)**	**Absolute dense volume (cm^3)**	**Percent dense volume (%)**
GE	Senograph Essential 53.40	15,926	55.0 (9.6)	26.0 (4.4)	729 (724–733)	57.0 (56.6–57.4)	7.8 (7.8–7.9)
	Senograph Essential 53.10.10	397	55.2 (9.6)	25.4 (4.1)	751 (723–781)	60.6 (58.1–63.3)	8.1 (7.8–8.4)
	Senograph DS 53.40	2,743	52.8 (9.6)	22.8 (2.5)	499 (492–506)	44.3 (43.6–45.1)	8.9 (8.8–9.0)
Philips	MammoDiagnost DR	809	55.8 (9.7)	25.3 (4.1)	732 (713–752)	56.9 (55.2–58.6)	7.8 (7.6–8.0)
	L30	14,929	55.2 (10.1)	25.1 (4.1)	744 (740–749)	58.1 (57.7–58.5)	7.8 (7.8–7.9)
Sectra	L30	6,298	55.1 (9.4)	25.4 (4.2)	766 (758–773)	61.3 (60.7–62.0)	8.0 (7.9–8.1)

NOTE: Age- and BMI-adjusted geometric means of volumetric mammographic measures per manufacturer model. All mammographic measures were derived from the mediolateral oblique view.

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Table 3. Distribution of breast cancer risk factors by quintiles of percent and absolute dense volume

	Percent dense volume (%)						Absolute dense volume (cm^3)					
	Q1 (<4.9)	Q2 (4.9–6.5)	Q3 (6.6–9.0)	Q4 (9.1–13.0)	Q5 (>13.0)		Q1 (<39)	Q2 (39–50)	Q3 (51–63)	Q4 (64–83)	Q5 (>83)	
	N = 8,221	*N* = 8,220	*N* = 8,221	*N* = 8,220	*N* = 8,220	*P* trend	*N* = 8,221	*N* = 8,220	*N* = 8,221	*N* = 8,220	*N* = 8,220	*P* trend
Age (years), mean	59.1	57.9	55.9	52.7	49.3	<0.001	56.6	56.8	55.7	54.2	51.6	<0.001
BMI (kg/m^2), mean	29.2	26.6	25.0	23.7	22.4	<0.001	23.2	25.0	25.8	26.3	26.4	<0.001
Age at menarche (years), mean	13.0	13.1	13.1	13.2	13.2	<0.001	13.2	13.2	13.1	13.1	13.0	<0.001
Parity, %						<0.001						<0.001
0	11.8	10.9	11.5	12.1	14.3		8.7	10.0	11.7	13.7	16.6	
1	14.2	13.6	13.9	14.0	14.9		12.6	12.4	13.9	14.9	16.8	
2	47.2	47.6	48.0	50.0	47.7		48.5	50.2	48.8	48.0	47.1	
≥3	26.8	27.9	26.7	23.9	21.0		30.2	27.5	25.7	23.4	19.5	
Age at first birth (years), mean	25.6	26.1	26.9	27.8	28.9	<0.001	26.9	26.7	26.7	27.0	28.0	<0.001
Menopausal status, %						<0.001						<0.001
Premenopausal	21.5	26.3	34.7	50.1	68.3		32.5	31.3	35.9	44.0	57.2	
Perimenopausal/unknown	2.8	3.3	3.5	3.6	3.1		2.7	2.9	3.3	3.6	3.8	
Postmenopausal	75.7	70.4	61.8	46.3	28.6		64.8	65.8	60.8	52.4	39.0	
Ever OC use, %	74.3	78.3	80.4	82.2	83.3	<0.001	78.8	79.4	79.5	79.8	81.2	0.003
HRT use, %[a]						<0.001						0.19
Never	70.1	69.1	68.4	67.7	69.3		69.1	69.0	69.4	69.1	67.9	
Former	26.0	26.2	25.5	24.4	21.6		25.5	25.7	25.1	25.2	24.4	
Current	4.0	4.6	6.1	8.0	9.1	0.001	5.4	5.3	5.5	5.7	7.9	
Benign breast disease, %	17.8	20.6	22.0	24.0	26.4	<0.001	20.7	21.2	20.8	22.9	25.2	<0.001
Family history breast cancer, %	12.7	13.0	13.1	13.6	13.7	0.03	12.3	12.9	13.4	13.3	14.2	0.001

[a]In postmenopausal women only. Percent and absolute dense volumes were derived from the mediolateral oblique view.

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Table 4. Associations of percent and absolute dense volume with breast cancer risk

	N/cases	HR (95% CI)		
		Crude[a]	Model 1[a]	Model 2[a]
Percent dense volume (in quartiles)				
Q1 (<5.3)	10,276/37	Ref. (1.00)	Ref. (1.00)	Ref. (1.00)
Q2 (5.3–7.6)	10,275/66	1.84 (1.23–2.75)	2.17 (1.42–3.31)	2.13 (1.40–3.26)
Q3 (7.7–11.7)	10,276/48	1.53 (0.99–2.36)	2.01 (1.24–3.24)	1.90 (1.17–3.07)
Q4 (>11.7)	10,275/55	2.09 (1.35–3.25)	3.17 (1.89–5.33)	2.93 (1.73–4.96)
P trend		0.01	<0.001	<0.001
Percent dense volume (per SD increase)[b]	41,102/206	1.27 (1.10–1.47)	1.51 (1.26–1.81)	1.46 (1.22–1.76)
Absolute dense volume (in quartiles)				
Q1 (<42)	10,276/47	Ref. (1.00)	Ref. (1.00)	Ref. (1.00)
Q2 (42–56)	10,275/33	0.68 (0.44–1.06)	0.69 (0.44–1.08)	0.68 (0.43–1.07)
Q3 (57–77)	10,276/55	1.19 (0.81–1.77)	1.21 (0.81–1.80)	1.17 (0.78–1.75)
Q4 (>77)	10,275/71	1.66 (1.14–2.42)	1.73 (1.17–2.55)	1.63 (1.10–2.42)
P trend		0.001	<0.001	0.002
Absolute dense volume (per SD increase)[b]	41,102/206	1.30 (1.13–1.50)	1.33 (1.15–1.54)	1.30 (1.12–1.51)

NOTE: Model 1: adjusted for age (years) and BMI (kg/m^2).

Model 2: adjusted for age (years), BMI (kg/m^2), menopausal status (premenopausal, perimenopausal/unknown, postmenopausal), parity and age at first birth (nulliparous, 1 child age at first birth < 25 years, 1 child age at first birth ≥25 years, 2 children age at first birth < 25 years, 2 children age at first birth ≥25 years, ≥3 children age at first birth < 25 years, ≥3 children age at first birth ≥ 25 years), HRT status (never, former, current), benign breast disease (yes vs. no), and family history of breast cancer (yes vs. no). Volumetric percent and absolute dense volumes were derived from the mediolateral oblique view.

[a]Cox proportional hazards models stratified by manufacturer.

[b]Based on log-transformed mammographic measures.

dense volume (Supplementary Table S3). SNP rs10995190 in the *ZNF365* gene was associated with both percent and absolute dense volume (Supplementary Table S4), with betas in the same direction to those reported for percent dense area (18).

After a median follow-up of 15.2 (IQR, 10.4 to 18.9) months, 206 cases of malignant breast cancer were documented. The median (IQR) interval between baseline mammography and breast cancer diagnosis was 22 (13–80) days. A significant association with breast cancer risk was found, with the hazard of breast cancer being highest among those in the upper quartile of volumetric density (Table 4). Adjustment for BMI strengthened the association with percent dense volume, but had no impact on the association with the absolute dense volume. After multivariable adjustment, the HRs were 2.93 (95% CI, 1.73–4.96) for percent dense volume and 1.63 (95% CI, 1.10–2.24) for absolute dense volume when comparing the highest versus lowest quartile. Results were similar when taking the contralateral mammogram for incident breast cancer cases and a randomly assigned view for those without breast cancer at the end of follow-up (data not shown).

Discussion

In this study, we demonstrate the potential of automated volumetric mammographic measures in widespread breast cancer risk assessment. Percent and absolute dense volumes as obtained with Volpara are associated with established determinants of mammographic density as well as breast cancer risk. The method itself showed good agreement by side and view, and distributions of both density measures were similar across mammography systems.

In the past decade, several volumetric methods have been developed for measuring mammographic density in an automated fashion, but only few of these are specifically designed for FFDM (22–27). The main advantage of FFDM-based methods is that they can account for unique acquisition parameters that are automatically stored in the image header. For film mammography, these parameters are not easily retrievable. Another advantage is that pixel values from raw digital mammograms are linearly related to X-ray exposure, resulting in more robust mathematical models. A distinct feature of Volpara is the use of a relative rather than absolute physics model, which makes the method less dependent on accurate physics data provided from the X-ray detector. Therefore, Volpara density distributions are expected to be more or less comparable across mammography systems. In our study, density distributions were similar across manufacturers, but a statistically significant difference was observed between manufacturer models. However, given the size of our study (N > 40,000), any difference can reach statistical significance without being clinically significant. It is therefore important to consider the magnitude of the differences observed. The model that deviated most from the

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others was GE Senograph DS 53.40, but this was due to selective referral of women with small breasts. Differences between other X-ray systems were only minor (for percent dense volume the largest percentage deviation observed was 3%). Moreover, it should be noted that any deviation could also reflect interindividual differences and/or system-related differences (i.e., differences in paddle size). We therefore conclude that the differences are of such a small magnitude that their impact will be minimal in a clinical setting. We also found high correlation coefficients by side and view, which are comparable with those reported for Cumulus area-based measures (28) and higher than those of volumetric measures obtained from film mammograms (29, 30).

Risk factors for breast cancer influence mammographic density in a similar way with only two exceptions (age and menopausal status) for which associations are in the opposite direction (31). In our study, both percent and absolute dense volumes were associated with all known density determinants in the expected direction (Table 3 and Supplementary Table S4). The contrasting effect of BMI on percent and absolute dense volume has been described previously (29, 32–34) and underscores the notion that the inverse association with percent density is mainly due to the positive correlation between BMI and the amount of nondense fatty tissue. Mammographic density is not only influenced by reproductive and hormonal factors, but is also genetically determined. Twin studies estimate that approximately two third of the residual variance in percent dense area is attributable to genetic factors (35, 36), but apart from some nominally significant SNP density associations (37, 38), rs10995190 in the *ZNF365* gene is the only established SNP with genome-wide significance (18). The observed association between rs10995190 and percent and absolute dense volume suggests that volumetric and area-based measures also share genetic determinants.

The underlying distribution of percent dense volume is different from that of percent dense area, being more left-skewed with a smaller range of possible values (0% to 40% vs. 0% to 100% for area-based percent density). Our findings about the correlation with Cumulus agree with those from earlier reports on volumetric methods (29, 30, 32–34, 39), showing a strong correlation for total breast volume and percent dense volume, but a weak correlation for the absolute dense volume. This difference in correlation for the absolute dense tissue is not unexpected as volumetric and area-based methods measure different aspects of mammographic density. Area-based methods reduce mammographic density to projected areas where pixels represent either dense or nondense tissue, whereas volumetric methods account for breast thickness by estimating the relative amount of dense tissue in each individual pixel. The incorporation of breast thickness is also reflected in the differential association with BMI. BMI is inversely related to the absolute dense area, but shows a positive association with absolute dense volume (29, 32–34). Because volumetric methods are better at capturing the actual amount of fiboglandular tissue in the breast, their measures are expected to show a stronger association with breast cancer risk than area-based measures. Nevertheless, results so far have demonstrated the opposite, as volumetric measures from film mammograms are weaker predictors of breast cancer risk than area-based measures (30, 33). This discrepancy is often attributed to the lower accuracy of volumetric methods for film mammograms. Although direct comparisons are difficult to make due to differences in study design and follow-up period, the observed HR for percent dense volume (HR highest vs. lowest quartile = 2.9) is similar to the risk estimate reported by Vachon and colleagues (40) for percent dense area (OR highest vs. lowest quartile = 3.1). When comparing the HRs for percent and absolute dense volume, the HR seemed to be weaker for the latter. This would be in line with results from correlation analyses, suggesting that volumetric and area-based percent densities behave similarly, whereas the absolute dense volume may have some unique features, which in turn explain the different association with breast cancer risk. However, the potential difference in HRs needs to be interpreted with caution, given the relatively small number of incident cases and overlapping CIs.

Our study has several strengths, including the large screening-based cohort, the comprehensive information on breast cancer risk factors, and the availability of SNP data in a subset of the study population. Furthermore, we were able to compare the performance of the method across a wide range of manufacturer models and X-ray detectors. However, analyses were restricted to three manufacturers, and further studies are needed to confirm the generalizability of our findings. Another limitation is that Cumulus measurements were only available for a small subset of the study population, which prevented us from comparing area- versus volumetric-based measures in their association with breast cancer risk.

In conclusion, our results suggest that automated measurement of volumetric mammographic density can be used as part of screening programs to provide risk and masking information that could be used to alter women's clinical management in terms of tumor detection and breast cancer prevention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The funding resources had no role in the study design, data collection, analyses, data interpretation, writing of the article, and/or decision to submit the article for publication.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.S. Brand, J.A. Shepherd, P. Hall

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References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893–917.
2. Marmot MG, Altman DG, Cameron DA, Dewar JA, Thompson SG, Wilcox M. The benefits and harms of breast cancer screening: an independent review. Br J Cancer 2013;108:2205–40.
3. Independent UK Panel on Breast Cancer Screening. The benefits and harms of breast cancer screening: An independent review. Lancet 2012;380:1778–86.
4. Hall P, Easton D. Breast cancer screening: time to target women at risk. Br J Cancer 2013;108:2202–4.
5. Boyd NF, Guo H, Martin LJ, Sun L, Stone J, Fishell E, et al. Mammographic density and the risk and detection of breast cancer. N Engl J Med 2007;356:227–36.
6. Byrne C, Schairer C, Wolfe J, Parekh N, Salane M, Brinton LA, et al. Mammographic features and breast cancer risk: effects with time, age, and menopause status. J Natl Cancer Inst 1995;87:1622–9.
7. McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2006;15:1159–69.
8. Olsen AH, Bihrmann K, Jensen MB, Vejborg I, Lynge E. Breast density and outcome of mammography screening: a cohort study. Br J Cancer 2009;100:1205–8.
9. Buist DS, Porter PL, Lehman C, Taplin SH, White E. Factors contributing to mammography failure in women aged 40–49 years. J Natl Cancer Inst 2004;96:1432–40.
10. Schousboe JT, Kerlikowske K, Loh A, Cummings SR. Personalizing mammography by breast density and other risk factors for breast cancer: analysis of health benefits and cost-effectiveness. Ann Intern Med 2011;155:10–20.
11. ACR (American College of Radiology). Breast imaging and reporting and data system (BI-RADS). 3rd ed. Reston, VA: ACR; 1998.
12. Byng JW, Boyd NF, Fishell E, Jong RA, Yaffe MJ. The quantitative analysis of mammographic densities. Phys Med Biol 1994;39:1629–38.
13. Highnam R, Brady M, Yaffe M, Karssemeijer N, Harvey J. Robust breast composition measurement -Volpara™. In: Digital mammography (Editors: Marté J, Oliver A, Freixenet J, Martí R). ISBN 978-3-642-13665-8. Lectures Notes in Computer Science. Springer Berlin Heidelberg; 2010. p. 342–9.
14. Price ER, Hargreaves J, Lipson JA, Sickles EA, Brenner RJ, Lindfors KK, et al. The California breast density information group: a collaborative response to the issues of breast density, breast cancer risk, and breast density notification legislation. Radiology 2013;269:887–92.
15. KARMA (Karolinska Mammography Project for Risk Prediction of Breast Cancer) [Internet]. Sweden: Karolinska Institutet. [accessed 2013 Nov.] Available from: http://karmastudy.org/sources/.
16. INCA (Information Network for Cancer Care) [Internet]. Sweden: INCA. [accessed 2013 Nov.] Available from: http://www.cancercentrum.se/INCA/kvalitetsregister/Brostcancer/.
17. Michailidou K, Hall P, Gonzalez-Neira A, Ghoussaini M, Dennis J, Milne RL, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet 2013;45:353–61.
18. Lindstrom S, Vachon CM, Li J, Varghese J, Thompson D, Warren R, et al. Common variants in ZNF365 are associated with both mammographic density and breast cancer risk. Nat Genet 2011;43:185–7.
19. Sterne JA, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. BMJ 2009;29:b2393.
20. White IR, Royston P. Imputing missing covariate values for the cox model. Stat Med 2009;28:1982–98.
21. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–75.
22. Highnam R, Pan X, Warren R, Jeffreys M, Davey Smith G, Brady M. Breast composition measurements using retrospective standard mammogram form (SMF). Phys Med Biol 2006;51:2695–713.
23. van Engeland S, Snoeren PR, Huisman H, Boetes C, Karssemeijer N. Volumetric breast density estimation from full-field digital mammograms. IEEE Trans Med Imaging 2006;25:273–82.
24. Pawluczyk O, Augustine BJ, Yaffe MJ, Rico D, Yang J, Mawdsley GE, et al. A volumetric method for estimation of breast density on digitized screen-film mammograms. Med Phys 2003;30:352–64.
25. Malkov S, Wang J, Kerlikowske K, Cummings SR, Shepherd JA. Single x-ray absorptiometry method for the quantitative mammographic measure of fibroglandular tissue volume. Med Phys 2009;36:5525–36.
26. Shepherd JA, Herve L, Landau J, Fan B, Kerlikowske K, Cummings SR. Novel use of single X-ray absorptiometry for measuring breast density. Technol Cancer Res Treat 2005;4:173–82.
27. Ciatto S, Bernardi D, Calabrese M, Durando M, Gentilini MA, Mariscotti G, et al. A first evaluation of breast radiological density assessment by QUANTRA software as compared to visual classification. Breast 2012;21:503–6.
28. Vachon CM, van Gils CH, Sellers TA, Ghosh K, Pruthi S, Brandt KR, et al. Mammographic density, breast cancer risk and risk prediction. Breast Cancer Res 2007;9:217.
29. McCormack VA, Highnam R, Perry N, dos Santos Silva I. Comparison of a new and existing method of mammographic density measurement: intramethod reliability and associations with known risk factors. Cancer Epidemiol Biomarkers Prev 2007;16:1148–54.
30. Boyd N, Martin L, Gunasekara A, Melnichouk O, Maudsley G, Peressotti C, et al. Mammographic density and breast cancer risk: evaluation of a novel method of measuring breast tissue volumes. Cancer Epidemiol Biomarkers Prev 2009;18:1754–62.
31. Assi V, Warwick J, Cuzick J, Duffy SW. Clinical and epidemiological issues in mammographic density. Nat Rev Clin Oncol 2011;9:33–40.
32. Lokate M, Kallenberg MG, Karssemeijer N, Van den Bosch MA, Peeters PH, Van Gils CH. Volumetric breast density from full-field digital mammograms and its association with breast cancer risk factors: a comparison with a threshold method. Cancer Epidemiol Biomarkers Prev 2010;19:3096–105.
33. Aitken Z, McCormack VA, Highnam RP, Martin L, Gunasekara A, Melnichouk O, et al. Screen-film mammographic density and breast cancer risk: a comparison of the volumetric standard mammogram form and the interactive threshold measurement methods. Cancer Epidemiol Biomarkers Prev 2010;19:418–28.

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34. Jeffreys M, Warren R, Highnam R, Davey Smith G. Breast cancer risk factors and a novel measure of volumetric breast density: cross-sectional study. Br J Cancer 2008;98:210–6.
35. Stone J, Dite GS, Gunasekara A, English DR, McCredie MR, Giles GG, et al. The heritability of mammographically dense and non-dense breast tissue. Cancer Epidemiol Biomarkers Prev 2006;15: 612–7.
36. Boyd NF, Dite GS, Stone J, Gunasekara A, English DR, McCredie MR, et al. Heritability of mammographic density, a risk factor for breast cancer. N Engl J Med 2002;347:886–94.
37. Vachon CM, Scott CG, Fasching PA, Hall P, Tamimi RM, Li J, et al. Common breast cancer susceptibility variants in LSP1 and RAD51L1 are associated with mammographic density measures that predict breast cancer risk. Cancer Epidemiol Biomarkers Prev 2012;21: 1156–66.
38. Stevens KN, Lindstrom S, Scott CG, Thompson D, Sellers TA, Wang X, et al. Identification of a novel percent mammographic density locus at 12q24. Hum Mol Genet 2012;21:3299–305.
39. Jeffreys M, Harvey F, Highnam R. Comparing a new volumetric breast density method (Volpara™) to cumulus. In: Digital mammography (Editors: Martí J, Oliver A, Freixenet J, Martí R). ISBN 978-3-642-13665-8. Lectures Notes in Computer Science. Springer Berlin Heidelberg. 2010. p. 408–13.
40. Vachon CM, Brandt KR, Ghosh K, Scott CG, Maloney SD, Carston MJ, et al. Mammographic breast density as a general marker of breast cancer risk. Cancer Epidemiol Biomarkers Prev 2007;16:43–9.

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