Mammographic Density and Breast Cancer Risk: Evaluation of a Novel Method of Measuring Breast Tissue Volumes

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Abstract

Background: **Mammographic density has been found to be strongly associated with risk of breast cancer. We have assessed a novel method of assessing breast tissue that is fully automated, does not require an observer, and measures the volume, rather than the projected area, of the relevant tissues in digitized screen-film mammogram.**
Methods: **Sixteen mammography machines in seven locations in Toronto were calibrated to allow the estimation of the proportion of radiologically dense (stromal and epithelial tissue) and nondense (fatty) tissue represented in each pixel of the mammographic image. This information was combined with a measurement of breast thickness to calculate the volumes of these tissues. Women with newly diagnosed breast cancer (cases) identified on these mammography machines during the years 2000 to 2003 were compared with other women of the same age who did not have breast cancer (controls).**
Results: **Three hundred sixty-four cases and 656 controls were recruited, epidemiologic data were collected, screen-film mammograms were digitized and measured using both a computer-assisted thresholding method, and the new measure of the volume of density. After adjustment for other risk factors, the odds ratio for those in the 5th quintile compared with the 1st quintile was 1.98 (95% confidence interval, 1.3-3.1) for the volume measure and 1.86 (95% CI, 1.1-3.0) for the area measurement. After inclusion of the volume and area measures in a predictive model, the volume measure lost significance, whereas the area measure remained significant.**
Conclusions: **Contrary to our expectations, measurement of the volume of breast tissue did not improve prediction of breast cancer risk.** (Cancer Epidemiol Biomarkers Prev 2009;18(6):1754–62)

Introduction

Mammographic density has been shown repeatedly to be associated with risk of breast cancer (1, 2). However, current methods of measuring mammographic density, either by radiologist's estimation or by computer-assisted measurement, have two major limitations (3). The first is the subjective nature of the assessment in both radiologist-dependant and computer-assisted approaches, and although both methods are highly reproducible, they require training. The second limitation arises from characterizing the three dimensions of breast tissue by measuring only the projected area of the mammogram. Mammographically dense breast tissue in different individuals may have similar projected areas but differ substantially in thickness and thus in quantity. Because the risk of breast cancer is likely to be more directly related to quantity of the relevant tissue than to the projected area, measurement of breast tissue volume is expected to be more strongly associated with breast cancer risk than projected area.

To assess the volume of radiologically dense breast tissue as a risk factor for breast cancer, we have acquired images prospectively under controlled conditions in a case-control study that has generated estimates of the relative risk of breast cancer associated with both the volume of mammographically dense breast tissue measured using a novel fully automated method, and the projected area of breast tissue (4). This new measurement may reduce error in the measurement of an important risk factor and enhance our ability to use this marker of risk in research into the etiology and prevention of breast cancer.

Materials and Methods

General Method. Using a matched case-control design, we have compared measurements of the volume and the projected area of mammographically dense breast tissue in screen-film mammograms from cases with histologically verified invasive breast cancer and matched controls. Two controls were matched to each case. Controls were of the same age as the case and selected from the same mammography departments as the cases. One control was examined on the same mammography machine as the case, the other on a different machine. Ethics approval for the study was obtained from the University Health Network, Mount Sinai Hospital, Sunnybrook and Women's College Hospital and from Cancer Care Ontario (for the Ontario Breast Screening Programme).

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Recruitment of Subjects. We recruited cases and controls who had been examined on mammography units in the clinics of Mount Sinai Hospital, Women's College Hospital, Princess Margaret Hospital/The Toronto Hospital (University Health Network), Sunnybrook Health Sciences Centre, and the North York and Scarborough sites of the Ontario Breast Screening Programme, all in Toronto, Canada. The selection of cases and controls was from all subjects having mammography in these sites during the period 13 March, 2000 and 7 July 7, 2003. All mammography units in these clinics were calibrated using the methods described below.

Identification and Selection of Cases. Potentially eligible cases were all incident cases diagnosed between 13 March, 2000 and 7 July 2003 in hospitals where the machines had been calibrated and with at least one screen-film mammogram done before diagnosis. Cases were identified from lists of subjects with invasive breast cancer that were generated monthly by the pathology departments of the participating hospitals. Cases with bilateral synchronous breast cancer, in whom a screen-film mammogram without radiological signs of cancer was not available, were excluded. Subjects who had breast implants, or reduction mammoplasty were also excluded.

Identification and Selection of Controls. Controls were selected from the same study population as cases. We attempted to identify two controls for each case, one examined on the same mammography machine as the case and other from a different machine. However, some mammography clinics had only one machine, and for these, we recruited only controls examined on the same machine as the cases.

At each participating mammography center, a list was generated of all women examined by mammography in the weeks before and after each case. Separate lists were prepared for potential controls examined on the same and different mammography machines (where available) as the cases. We then selected randomly from these lists three potential controls for each case, using the following matching criteria: potential controls were examined within a week of the case, were of the same age in years as the case at the time of diagnosis of the case, had no previous or present history of breast cancer, and had not had breast implants or reduction mammoplasty. The time intervals between the dates of mammography for potential controls and cases were extended as necessary to find a control that met all of the matching criteria. Three potential controls from the same machine, and an additional three from a different machine, were selected for each case and contacted in turn, and the first eligible control that agreed to participate was selected. If all three potential controls declined participation, a further set of potential controls was selected. For mammography sites with only one machine, only one control per case was recruited.

Recruitment and Data Collection. With the agreement of their physician, potentially eligible case and control subjects were contacted by mail. The study was explained and they were asked for consent to the use of their mammogram and to participate in a telephone interview to provide information about risk factors and other exposures relevant to mammographic density. Information on demographic and ethnic characteristics, and on risk factors for breast cancer, was obtained by telephone interview including age, body weight before diagnosis, height, parity, alcohol use, hormone use, both past and present, menopausal status, and previous history of mammograms and biopsies. We mailed letters to 919 cases, and 534 (58%) gave consent, and to 3,297 controls, of whom 1,375 (42%) gave consent.

Measurement of Mammographic Density. After consent had been obtained, screen-film mammograms for the case and control subjects selected were obtained from the participating mammography units. All were cranial-caudal screen-film mammograms. Where more than one previous mammogram was available for cases, we selected the mammogram taken closest to the date of diagnosis. To ''blind'' the process of measurement to case or control status, we selected the image of the breast contralateral to the cancer, and the corresponding mammograms in the matched controls. Two methods of measurement were applied to the mammograms.

Computer-Assisted Measurement of Breast Tissue Areas. The methods of digitization and measurement of screen-film mammograms has been described previously (5). Computer-assisted measurement of mammographic density was carried out by one reader (NFB) using *Cumulus 4* software, in sets of ~120 images, composed of triplets of randomly ordered cases and their matched controls. Measurements of the areas of dense tissue and total area were generated and percent density calculated. The reliability of these measurements was assessed by re-reading within each reading set a randomly selected 10% sample of images, randomly distributed among the images being read, and a further 10% of images were re-read between sessions. The reliability of measurement of percent density within and between reads was 0.96.

Measurement of Breast Tissue Volumes. Volumetric measurements of breast tissue and dense breast tissue can be made from a screen-film mammogram if a simple model is assumed in which the breast is composed of only two components, fibroglandular tissue and fat. Each mammography machine from which we recruited was calibrated to determine the relationship between the image signal (absorbance or blackness of the processed film value) in each pixel, the exposure factors (kilovoltage, average milliampere second, tube target, and beam filter), and the amount of radiation transmitted by the breast. The latter can then be related to the combination of breast thickness and composition by imaging a ''phantom'' composed of steps of tissue-equivalent plastics of different thicknesses and representing a range of combinations of fat/fibroglandular (4). Therefore, under specified exposure conditions, for a given measured image signal, the tissue composition corresponding to each pixel can be estimated from the screen-film mammogram if the breast thickness is known. The total volume of dense (fibroglandular) tissue was obtained by multiplying the fibroglandular fraction for each pixel by the area of the pixel and the thickness of the compressed breast and then summing over all pixels. Similarly, the total breast volume was simply the sum of the areas of all pixels in the image of the breast, each multiplied by the corresponding breast thickness.

Thickness Correction. Compressed breast thickness is the distance between the compression paddles of a mammography machine and the breast supporting tabletop under the conditions used for acquiring the mammographic exposure. The readout device provided with the mammography system is not designed to provide an accurate measure of compressed breast thickness. Furthermore, because the compression paddle is not necessarily parallel to the breast support table when the breast is compressed, breast thickness is not constant across the breast area; we thus need a breast thickness map for each X-ray image to calculate the total volume and dense volume of the breast using *Cumulus V* (volume) software. Equations to predict a thickness map for each image were developed from the readout thickness reported by each mammography machine, coordinates in the plane parallel to the breast support table, and the compression force reported by a mammography machine. Details of the methods used to develop a formula for thickness correction have been reported previously (6), and are given in the Appendix.

Statistical Methods. We calculated mean and SDs for continuous variables for selected characteristics of the case and control subjects, and percentages for categorical variables. We used the two-sample t test to compare continuous variables, and the χ^2 test for categorical variables. All P values were calculated from two-tailed tests of statistical significance.

The association of mammographic density with risk of breast cancer was examined using unconditional and conditional logistic regression models (7). The linear trend of increasing risk with increasing density was tested by the Cochran-Armitage test. Percent mammographic density and absolute dense area were modeled both as continuous and categorical variables, with categories defined by quintiles of the distribution of each variable in control subjects. All analyses were carried out before and after adjustment for other risk factors for breast cancer.

Results

Characteristics of Subjects. Figure 1 illustrates the recruitment of cases and controls and the process of selection for inclusion in the analyses shown here.

Selected characteristics of cases and controls are shown in Table 1. The average age of the subjects studied was 59 years, and most were parous and postmenopausal. Cases had a slightly later age at menopause than either group of controls but the distribution of other menstrual, reproductive, and anthropometric risk factors for breast cancer was similar in cases and in both groups of controls. The median interval between the mammogram dates for cases and controls was 0 day, and the interquartile range was −5 to +6 days.

The imaging parameters of kilovoltage peak, compression force, and breast thickness were similar in cases and both groups of controls. The average milliampere seconds in cases was similar to controls examined on the same machine but significantly greater than in controls examined on different machines. This difference could not be explained by differences in the characteristics of subject, or by differences in machine manufacturer. The differences were confined to one mammography site

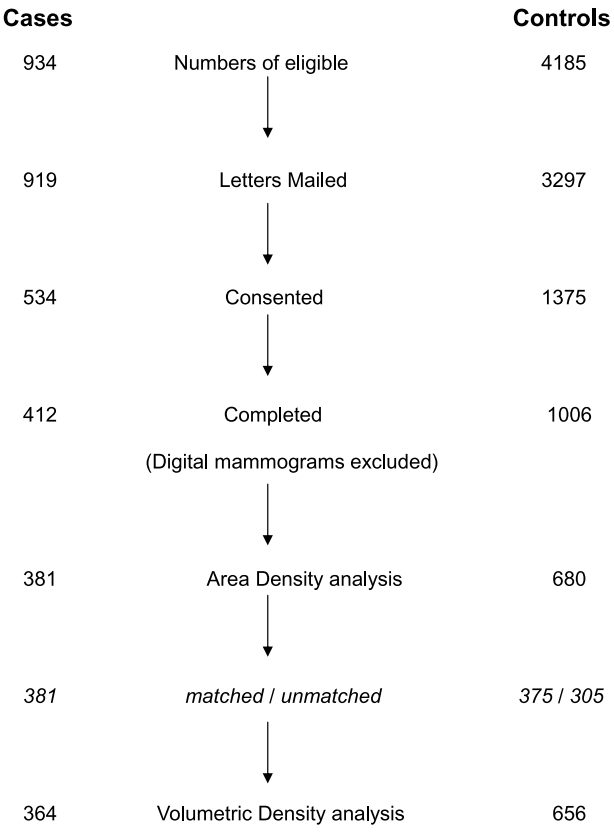

Figure 1. Flowchart of recruitment of cases and controls.

(data not shown), and were not seen in controls not matched by machine in other mammography sites. The difference seems to reflect differences in the practices of individual technologists at one location.

Average measures of percent mammographic density area and volume were both greater in cases than in either control group, and these differences were greater between cases and controls not examined on the same machines. As shown in Table 1, these differences in mammographic measures were not associated with differences in age, parity, number of births, body mass index (BMI), or hormone use between the control groups. Average measures of total area and volume were both smaller in cases than in either group of controls.

Distributions of Mammographic Measurements. Figure 2 shows the distributions of volume and area measurements in the combined case-control triplets, and the correlations between selected area and volume measurements.

Compared with the area measures, volumetric measures of percent density and dense tissue were skewed more toward the left side of the distributions. The distributions of total breast volume and area were similar in shape and were strongly correlated (r = 0.93). Area and volume measurements of percent density (r = 0.68) and of dense tissue (r = 0.55) were moderately correlated.

Association of Mammographic Measures with other Risk Factors. The area measure of percent mammographic density has repeatedly been shown to be

Table 1. Selected characteristics of all subjects (n = 1,020) by case-control status

	Cases (n = 364)	Controls			
		Matched by machine (n = 359)	P*	Not matched by machine (n = 297)	P*
Risk factors					
Height (cm)	162.6 (6.9)	163.1 (6.6)	0.32	163.4 (6.2)	0.12
Weight (kg)	68.3 (14.4) n = 362	68.0 (14.6) n = 358	0.75	68.3 (14.7)	0.96
BMI (kg/m^2)	25.8 (5.2) n = 362	25.6 (5.5) n = 358	0.50	25.6 (5.2)	0.47
Age at mammogram (y)	59.6 (11.0)	59.5 (11.0)	0.90	58.4 (11.1)	0.17
Age at menarche (y)	12.7 (1.4) n = 361	12.8 (1.5)	0.51	12.7 (1.4) n = 295	0.93
Parity (% parous)	70.9	76.0	0.12	69.7	0.74
Age at birth of 1st child (y)	26.3 (5.0) n = 258	26.6 (5.4) n = 274	0.53	26.6 (5.6) n = 206	0.54
No. of live births	1.7 (1.4)	1.8 (1.4)	0.20	1.6 (1.4)	0.76
Menopausal status (% postmenopausal)	68.0 (n = 363)	69.9	0.59	69.6 (n = 296)	0.67
Age at menopause (y)	49.4 (6.2) n = 211	47.7 (6.4) n = 220	0.01	48.1 (6.5) n = 178	0.05
HRT ever used (% yes)	45.1	45.5 (n = 358)	0.90	44.4	0.88
Years HRT used (y)	4.0 (6.9)	4.2 (7.5) n = 358	0.69	3.8 (7.0)	0.73
Breast cancer in 1st degree relatives (% yes)	21.6 (n = 361)	26.3 (n = 357)	0.14	23.0 (n = 296)	0.68
Imaging parameters					
Breast thickness (cm)	5.4 (1.2)	5.4 (1.4)	0.92	5.4 (1.4)	0.57
KVP	26.8 (2.0)	26.7 (2.0)	0.62	26.8 (2.1)	0.81
MAS	155.3 (48.7)	156.0 (57.9)	0.85	144.9 (52.4)	0.01
Compression force (N)	104.7 (32.6)	103.9 (31.8)	0.74	101.3 (33.0)	0.20
Volume breast measurements					
Percent dense volume (%)	11.3 (16.1)	9.9 (14.6)	0.23	7.7 (12.9)	0.001
Dense volume (cm^3)	57.9 (76.6)	53.6 (86.5)	0.25	38.9 (60.4)	0.0001
Total volume (cm^3)	725.2 (360.8)	761.6 (417.2)	0.44	752.7 (406.8)	0.58
Area breast measurements					
Percent dense area (%)	33.3 (20.5)	30.3 (19.7)	0.07	30.1 (19.9)	0.05
Dense area (cm^2)	40.8 (26.9)	38.4 (27.3)	0.21	36.3 (23.4)	0.04
Total area (cm^2)	141.5 (61.0)	146.4 (64.2)	0.31	144.6 (63.4)	0.55

NOTE: Shown are mean (SD) and percentage for, respectively, continuous and categorical variables.
HRT, hormone replacement therapy; KVP, kilovoltage peak; MAS, milliampere seconds.
*Two-sided two-sample t test for continuous variables, and χ^2 test for categorical variables. Cubic root and square root transformations were applied to, respectively, volume and area breast measurements.

associated with age, age at first birth, parity, BMI, and menopausal status (8). In the present data, all of these expected associations were observed with both area and volume measures.

In all subjects, the area measure of percent density showed inverse associations with age (r = −0.42), BMI (r = −0.51), a lower mean level in postmenopausal (mean, 26.3; SD, 18.3) than in premenopausal women (mean, 42.4; SD, 19.6), and a lower mean level in parous (mean, 29.7; SD, 19.9) than in nulliparous (mean, 35.5; SD, 20.1) women.

The corresponding associations for the volume measure of percent density were for age (r = −0.35), BMI (r = −0.38), a lower mean level in postmenopausal women (mean, 6.2; SD, 11.3) than in premenopausal women (mean, 15.0; SD, 17.2), and a lower mean level in parous (mean, 7.2; SD, 12.7) than in nulliparous women (mean, 11.6; SD, 16.5).

Mammographic Measures and Risk of Breast Cancer. Tables 2 and 3 show the results of unconditional logistic regression analyses in which case or control status was the dependant variable, and the mammographic measures of percent density and absolute dense area and volume the predictor variables. Results are shown before and after the inclusion of other risk factors for breast cancer listed in the table footnotes according to quintiles of each measure. Each set of results is shown separately in Table 2 for all cases and controls (n = 1,020) and then in Table 3 for the subset of subjects for the matched triplets of cases and 2 controls (n = 813).

For both the volume and area measurements, there were more cases in the highest quintile of percent density and dense tissue than in the lowest quintile, and more controls in the lowest quintile than in the highest. For all subjects (Table 2), the adjusted odds ratio for percent density comparing the 5th quintile to the 1st was 1.98 [95% confidence interval (95% CI), 1.3, 3.1] for the volume measure and 1.86 (95% CI, 1.1-3.0) for the area measure. In the subset of matched triplets (Table 3), the corresponding adjusted odds ratios were 2.21 (95% CI, 1.3-3.7) for the volume measure and 2.40 (95% CI, 1.3-4.3) for the area measure. In both analyses and for both volume and area measures, tests for trend of increasing risk with increasing percent density were strongly statistically significant.

Similar results were seen for the absolute measure of density. The gradient in risk was slightly greater for the volume measures of absolute density than for the area measurements for all subjects, but the gradients in odds ratios with the area measures were stronger than the volume measures in the matched triplets. Similar results were obtained using conditional logistic regression (data not shown).

Contributions of Volume and Area Measures to Risk Prediction. To assess the independent contributions of

the volume and area measures of mammographic density to the prediction of breast cancer risk, we carried out unconditional and conditional logistic regression analyses with both measures treated in analysis as continuous variables, and with and without additional risk factors included in the analyses. Unadjusted and adjusted analyses were carried out first with the volume and area measures included separately and then together. The results shown in Tables 4 and 5 are for unconditional regression analysis. Conditional regression analysis gave similar results (data not shown).

For percent density, the volume measure separately was significantly associated with risk of breast cancer before (P = 0.01) and after adjustment for other risk factors in all subjects (P = 0.001; Table 4), and in the subset of case-control triplets (P = 0.005 and P = 0.003, respectively; Table 5). The area measure separately was also statistically significantly associated with breast cancer risk in all subjects (P = 0.0005; Table 4) and in case-control triplets (P < 0.0001; Table 5). When the volume and area measures of percent density were both included in the model as continuous variables, the volume measure lost significance, both in all subjects (P = 0.08) and in matched triplets (P = 0.29), whereas the area measure remained significant in both analyses (P = 0.03 and 0.002, respectively).

Similar results were seen for the absolute measure of density. The volume and area measures of absolute density were both significantly associated with risk of breast cancer when considered separately, before and after adjustment for other risk factors. When both were included in the model, the volume measure of density remained significant in the analysis of all subjects but lost significance in the analysis of matched triplets. The area measure of density lost significance in the first of these analyses but retained significance in the second.

Discussion

Percent mammographic density, as assessed here in the area measurement, reflects variations in breast tissue composition (9) and has repeatedly been shown to be strongly associated with risk of breast cancer, with 4- to 5-fold differences in risk of the disease between women with >75% percent density compared with those with

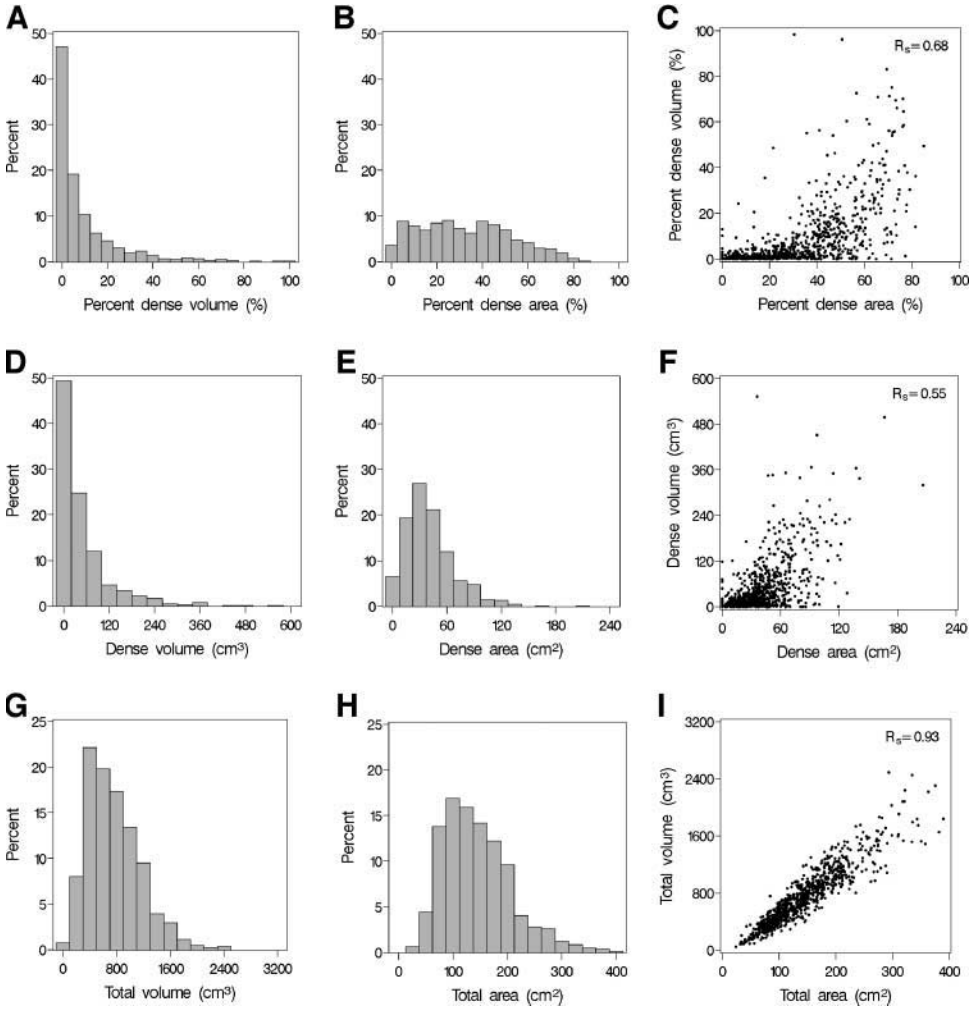

Figure 2. Observed distributions and correlations between volume and area breast measurements for case-control triplets (n = 813). R_s, a Spearman correlation coefficient.

Table 2. Risk of breast cancer according to quintiles of percent and absolute density for all subjects (*n* = 1,020)

	Quintile of percent density					*P**	Quintile of absolute density					*P**
	1	2	3	4	5		1	2	3	4	5	
	Percent dense volume (%)						Dense volume (cm^3)					
No. of cases	62	64	75	78	85		64	68	61	86	85	
No. of controls	142	140	129	126	119		140	136	143	118	119	
OR unadjusted (95% CI)	Reference	1.05 (0.7, 1.6)	1.33 (0.9, 2.0)	1.42 (0.9, 2.1)	1.64 (1.1, 2.5)	0.01	Reference	1.09 (0.7, 1.7)	0.93 (0.6, 1.4)	1.59 (1.1, 2.4)	1.56 (1.0, 2.3)	0.1
OR adjusted for risk factors† (95% CI)	Reference	1.08 (0.7, 1.6)	1.39 (0.9, 2.1)	1.60 (1.0, 2.5)	1.98 (1.3, 3.1)	0.001	Reference	1.10 (0.7, 1.7)	0.98 (0.6, 1.5)	1.69 (1.1, 2.6)	1.68 (1.1, 2.6)	0.003
	Percent dense area (%)						Dense area (cm^2)					
No. of cases	70	60	76	76	82		71	62	69	78	84	
No. of controls	134	144	128	128	122		133	142	135	126	120	
OR unadjusted (95% CI)	Reference	0.80 (0.5, 1.2)	1.14 (0.8, 1.8)	1.14 (0.8, 1.7)	1.29 (0.9, 1.9)	0.06	Reference	0.82 (0.5, 1.2)	0.96 (0.6, 1.4)	1.16 (0.8, 1.7)	1.31 (0.9, 2.0)	0.05
OR adjusted for risk factors† (95% CI)	Reference	0.92 (0.6, 1.4)	1.41 (0.9, 2.2)	1.49 (0.9, 2.3)	1.86 (1.1, 3.0)	0.003	Reference	0.90 (0.6, 1.4)	1.07 (0.7, 1.6)	1.29 (0.8, 2.0)	1.48 (1.0, 2.3)	0.02

NOTE: Shown are mean (SD) and percentage for, respectively, continuous and categorical variables.
*Unconditional logistic regression analysis. *P* value is from a test of linear trend with quintiles as an ordinary variable in the model.
† Age at mammogram (y), age at first birth (y), weight (kg), height (cm), menopausal status (premenopausal, postmenopausal), and parity (parous, nonparous).

Table 3. Risk of breast cancer according to quintiles of percent and absolute density for case-control triplets (*n* = 813)

	Quintile of percent density					*P**	Quintile of absolute density					*P**
	1	2	3	4	5		1	2	3	4	5	
	Percent dense volume (%)						Dense volume (cm^3)					
No. of cases	44	45	56	59	67		45	49	46	65	66	
No. of controls	119	117	107	103	96		118	113	117	97	97	
OR unadjusted (95% CI)	Reference	1.04 (0.6, 1.7)	1.42 (0.9, 2.3)	1.55 (1.0, 2.5)	1.89 (1.2, 3.0)	0.002	Reference	1.14 (0.7, 1.8)	1.03 (0.6, 1.7)	1.76 (1.1, 2.8)	1.78 (1.1, 2.8)	0.002
OR adjusted for risk factors† (95% CI)	Reference	1.06 (0.6, 1.7)	1.48 (0.9, 2.4)	1.72 (1.0, 2.8)	2.21 (1.3, 3.7)	0.001	Reference	1.15 (0.7, 1.9)	1.04 (0.6, 1.7)	1.82 (1.1, 2.9)	1.86 (1.1, 3.0)	0.003
	Percent dense area (%)						Dense area (cm^2)					
No. of cases	41	45	68	56	61		40	46	55	67	63	
No. of controls	122	117	95	106	102		123	116	108	95	100	
OR unadjusted (95% CI)	Reference	1.14 (0.7, 1.9)	2.13 (1.3, 3.4)	1.57 (1.0, 2.5)	1.78 (1.1, 2.9)	0.01	Reference	1.22 (0.7, 2.0)	1.57 (1.0, 2.5)	2.17 (1.3, 3.5)	1.94 (1.2, 3.1)	0.0005
OR adjusted for risk factors† (95% CI)	Reference	1.34 (0.8, 2.2)	2.60 (1.6, 4.3)	2.04 (1.2, 3.5)	2.40 (1.3, 4.3)	0.002	Reference	1.35 (0.8, 2.3)	1.74 (1.0, 2.9)	2.35 (1.4, 3.9)	2.11 (1.3, 3.5)	0.0005

NOTE: Shown are mean (SD) and percentage for, respectively, continuous and categorical variables.
*Unconditional logistic regression analysis. *P* value is from a test of linear trend with quintiles as an ordinary variable in the model.
† Age at mammogram (y), age at first birth (y), weight (kg), height (cm), menopausal status (premenopausal, postmenopausal), and parity (parous, nonparous).

Table 4. Risk of breast cancer according to continuous percent and absolute density for all subjects (*n* = 1,020)

		Regression coefficient	*P*	Regression coefficient	*P*
		Percent dense volume* (%)		Dense volume* (cm^3)	
Unadjusted	Separate predictors	0.1814	0.01	0.1197	0.005
	Both in the model	0.1427	0.11	0.1071	0.04
Adjusted for risk factors†	Separate predictors	0.2549	0.001	0.1372	0.003
	Both in the model	0.1573	0.08	0.1106	0.03
		Percent dense area‡ (%)		Dense area‡ (cm^2)	
Unadjusted	Separate predictors	0.0703	0.03	0.0568	0.06
	Both in the model	0.0286	0.49	0.0160	0.65
Adjusted for risk factors†	Separate predictors	0.1478	0.0005	0.0760	0.02
	Both in the model	0.1079	0.03	0.0382	0.30

NOTE: Unconditional logistic regression analysis.
*Cubic root transformed.
†Age at mammogram (y), age at first birth (y), weight (kg), height (cm), menopausal status (premenopausal, postmenopausal), and parity (parous, nonparous).
‡Square root transformed.

<10% (1, 2). The smaller gradient in risk associated with the area measure of mammographic density in the present study is attributable partly to the use of quintiles rather than the aforementioned categories, and the methods used to recruit subjects. Our method of recruitment selected for breast cancers detected by mammography, where the gradient in risk associated with density is less than for all breast cancers (2).

It is however recognized that current approaches to measurement have a number of limitations and that the true underlying risk of breast cancer associated with variations in breast tissue composition may be much stronger. All of the existing methods of assessing mammographic density have limitations. None takes into account the thickness of the breast, and are all thus based on the area rather than the volume of breast tissue. Current computer-assisted methods of measurement require that a dichotomous threshold be placed between dense and nondense tissue, and do not allow a gradual transition from one to the other, as is likely to exist in reality. Mammographically dense breast tissue in different individuals may vary substantially in thickness and thus in quantity, and because we expect the quantity of the relevant tissue to be more directly related to risk of breast cancer than the projected area, we predicted that the automated measurement of the volume of percent mammographic density would create larger gradients in risk than measurement of the projected area, and that the volume of mammographically dense breast tissue would be more strongly associated with other risk factors for breast cancer than the projected area (3, 4).

Notwithstanding these limitations, the relative risks of breast cancer associated with extensive mammographic density generated by these studies are larger than for most other risk factors for breast cancer, and have been shown to persist after adjustment for other risk factors. Although larger relative risks apply to the small proportion of the population who carry mutations in *BRCA1* and *BRCA2* mutations (10, 11), the attributable risk associated with these mutations is only about 5%, substantially smaller than the attributable risks of about 30% for density in >50% of the breast.

The research described here sought to address some of the limitations of measurements based on the image of the breast projected in a mammogram. The volume method is fully automated and does not require an observer. We acquired images prospectively under controlled conditions from a defined group of mammography units that had been calibrated to allow estimation of the proportion of fat and fibro-glandular tissue in each pixel of the mammographic image. Together with estimates of breast thickness, these data were used to

Table 5. Risk of breast cancer according to continuous percent and absolute density for all case-control triplets (*n* = 813)

		Regression coefficient	*P*	Regression coefficient	*P*
		Percent dense volume* (%)		Dense volume* (cm^3)	
Unadjusted	Separate predictors	0.2252	0.005	0.1597	0.001
	Both in the model	0.0873	0.41	0.0979	0.10
Adjusted for risk factors†	Separate predictors	0.2797	0.003	0.1705	0.002
	Both in the model	0.1150	0.29	0.1062	0.09
		Percent dense area‡ (%)		Dense area‡ (cm^2)	
Unadjusted	Separate predictors	0.1262	0.001	0.1148	0.001
	Both in the model	0.0993	0.05	0.0759	0.07
Adjusted for risk factors†	Separate predictors	0.2041	<0.0001	0.1227	0.001
	Both in the model	0.1742	0.002	0.0858	0.05

NOTE: Unconditional logistic regression analysis.
*Cubic root transformed.
†Age at mammogram (y), age at first birth (y), weight (kg), height (cm), menopausal status (premenopausal, postmenopausal), and parity (parous, nonparous).
‡Square root transformed.

calculate the volumes of fat and fibro-glandular tissue per pixel that were then summed over the entire image of the breast. Additional corrections for exposure and processing were made using a step wedge included in each image.

Our results show that a novel method of measuring the volumes of total and dense breast tissue, and a previously used method of measuring the projected areas of these tissues, both provided information about risk of breast cancer that was independent of other risk factors for the disease. The area and volume measures were moderately correlated with each other and had different distributions but showed similar associations with age and other risk factors for breast cancer. Contrary to our expectations however, the volume measure did not improve on the risk predictions made by the area measure, but the automated volume method did provide similar risk information that of the area measure. There were similar gradients in risk of breast cancer over quintiles of each measure, which were slightly greater for volume than area in the analysis of all subjects, and slightly greater for area than volume in the analysis of case-control triplets. When analyzed as continuous variables, the volume measures gave no additional information about risk after the area measures had been taken into account.

Other investigators have compared the computer-assisted thresholding measurement of area used in the present study with alternative approaches to measuring tissue volumes. Ding et al. (12) carried out a large case-control study that used standard mammography form (SMF) to assess the association of the volume of breast density with risk of breast cancer and compare these measurements with other methods of assessing density including the computer-assisted threshold method used here. SMF uses information about the nonfat tissue in the breast, in conjunction with the thickness of the compressed breast and the breast imaging variables of tube voltage and exposure time, to generate estimates of breast tissue volumes (12). The volume measures of percent density generated by SMF were associated with breast cancer risk but less strongly than the threshold measures of percent density by area. After adjustment for the threshold measure of percent density, the SMF-derived measures were no longer significantly associated with breast cancer risk (12). Other methods of measuring breast density volumes have been described, but there are as yet no published data on their ability to predict breast cancer risk (13–16).

Knowledge of the thickness of the compressed breast was essential for the calculation of tissue volumes in the present study, and the volumes calculated were very sensitive to small variations in measured thickness. The retrospective application of corrections for the thickness measurements made on multiple machines may have attenuated the breast cancer risk associated with the volume measures, as factors that varied among individual patients, technologists, and in the flexibility of compression paddles, could not be taken into account. Attention to these aspects of measurement may allow improved prediction of breast cancer risk using breast tissues volumes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Appendix. Thickness Prediction Equations and Volume Breast Measurements

Breast thickness is a vertical distance between the compression plates of a mammography machine. The top and the bottom plates are not perfectly parallel when the breast is compressed. As a result, the breast thickness is not constant across the breast area, and the breast thickness reported by a mammography machine needs correction. We collected data and developed machine-specific prediction equations to determine thickness of the compressed breast above any point of the breast area for subjects included in the case-control study.

The data to develop thickness prediction equations were collected using three phantoms with sizes corresponding to 10th, 50th, and 90th percentiles of total breast area. The following experiment was conducted using one of the Lorad MIV mammography machines at Sunnybrook hospital in Toronto. Each phantom was compressed, and, in addition to taking its X-ray image, we measured thickness of the phantom above a grid of points spread ~20 mm apart in the horizontal plane, using an optical method developed by Dr Yaffe and his colleagues (6). The optical method allowed measuring directly the nonconstant thickness of the compressed phantom. For each phantom, the experiment was repeated at three levels of the compression force, and the values of thickness, measured by the optical method, and readout thickness and compression force as reported by the mammography machine were recorded.

Prediction equations had the following general form:

$$Z_p = Z_r + f(x, y, F),$$

where Z_p is the predicted thickness, Z_r is the readout thickness reported by a mammography machine, x and y are coordinates in the horizontal plane, F is the compression force reported by a mammography machine, and $f(x, y, F)$ is a linear function of x, y, and F, and is a correction factor for the constant readout thickness.

For the functional form of $f(x, y, F)$, we considered 1st, 2nd, 3rd, and 4th degree polynomials, and the following three functions:

$$f(x,y,F) = \beta_0 + \beta_1|x| + \beta_2 y + \beta_3|x|y + \beta_4 F + \beta_5 F|x| + \beta_6 Fy + \beta_7 F|x|y \quad (1)$$

$$f(x,y,F) = \beta_0 + \beta_1 F + \beta_2 F|x| + \beta_3 F|y-20| + \beta_4 F|x|^2 + \beta_5 F|x||y-20|^2 + \beta_6 F|y-20|^2 \quad (2)$$

$$f(x,y,F) = \beta_0 + \beta_1 F + \beta_2 F|x| + \beta_3 F|y-20| \quad (3)$$

We fitted the polynomial models and models (1–3) to the difference between the thickness of the compressed phantom measured by the optical method and the readout thickness using SAS PROC MIXED (SAS V8.2, SAS Institute, Inc.).

To decide which model did the best, we collected mammographic and optical data for 176 women. Women

had an X-ray image taken at the mammography machine used in the phantom experiment, and thickness of the compressed breast was measured using the optical method described above. Readout thickness and compression force reported by the machine were recorded. For each subject, we calculated breast volume measurements such as total volume, dense volume, and percent volumetric density using the optical thickness map and thickness map predicted by each of the seven equations described above. Volume measurements were computed using Cumulus V software (6). We used Bland-Altman analysis of agreement (17, 18) to compare volume measurements based on optical and predicted thickness maps. The best agreement was achieved with model (3) as it showed minimum mean difference and minimum SD of the difference between two methods, with respect to total volume, dense volume, and percent volumetric density. Furthermore, total volume of the phantoms used in the experiment was determined by a water-displacement method, and agreed the best with the predicted total volume based on model (3).

We repeated the phantom experiment for all mammographic units and plate sizes included in the case-control study to estimate coefficients in model (3). Then we used these machine- and plate size-specific prediction equations to compute the volume breast measurements for study subjects.

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