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Mammographic breast density and breast cancer risk in a Mediterranean population: a nested case–control study in the EPIC Florence cohort

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Abstract
Purpose Mammographic breast density (MBD) has been consistently associated with breast cancer (BC) risk, and at the same time it is modulated by established BC risk factors related to reproductive and hormonal history and to lifestyle. We aimed to evaluate the association between the clinical breast imaging reporting and data system (BI-RADS), a qualitative MBD classification used in clinical setting, and BC risk through a case–control nested in the EPIC Florence cohort where baseline information on reproductive history, lifestyle and anthropometry were collected.
Methods The study includes 136 newly diagnosed BC cases and 635 controls from the 10,083 healthy women enroled in the cohort between 1993 and 1998 and followed for 6 years on average. MBD was assessed on a negative mammogram performed at least one year before diagnosis in cases and on a mammogram performed in the same period for controls matched for age, enrolment date and menopausal status. Multivariate analyses adjusted for education, body mass index, parity, number of children, breastfeeding, BC family history, history of breast biopsies and Hormone Replacement Therapy use were performed.
Results An increase in BC risk across BI-RADS categories emerged with adjusted odds ratios (OR) 1.79 (95% CI 1.06–3.01), OR 2.09 (95% CI 1.17–3.74) and OR 2.67 (95% CI 1.08–6.62) for categories 2, 3 and 4 in comparison with the reference category (p for trend = 0.008).
Conclusions We confirm in this Mediterranean population the association of increasing MBD, classified according to BI-RADS with BC risk also taking into account other well-known risk factors for this neoplasm.

Keywords Breast cancer · Breast density · BI-RADS · Nested case–control · Prospective study

Abbreviations

BC	Breast cancer
BI-RADS	Breast imaging reporting and data system
BMI	Body mass index
EPIC	European prospective investigation into cancer and nutrition
HRT	Hormone replacement therapy
MBD	Mammographic breast density
OR	Odds ratio

Introduction

The extent of mammographically detected fibroglandular breast tissue, known as mammographic breast density (MBD) has emerged in the last few decades as one of the strongest risk factors for breast cancer (BC) and this association seems to persist after adjustment for other factors related to BC risk [1]. High MBD has been consistently associated with increased BC in studies using different methods of MBD evaluation ranging from subjective MBD evaluation of radiologists aimed to classify subjects in broad categories to fully automated methods allowing to obtain quantitative measures of breast density

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and in studies based both on films and digital mammograms [2, 3].

Among the qualitative systems of MBD classification based on the radiologist's visual evaluation, the breast imaging reporting and data system (BI-RADS) is the most widely used in clinical settings and it has been reported to be able to classify women at different BC risks [2, 4, 5].

MBD is influenced by many factors that in turns modulate BC risk. Most of these factors including reproductive variables, hormonal aspects and possibly diet and physical exercise modulate in the same direction MBD and BC risk, however, MBD decreases with age and more strongly with increasing BMI. Therefore the availability of accurate information on the above-mentioned variables is mandatory in the evaluation of the relationship between MBD and BC risk [6].

Moreover, the interplay between MBD and other strong risk factors for BC such as history of breast biopsies has been investigated and these aspects have been included in models for the prediction of absolute risk of BC development [7]. We aimed to investigate the association between increased MBD as assessed according to BI-RADS classification and BC risk through a case–control study nested in a cohort of Mediterranean women, taking into account several factors known to influence MBD and in turns to modulate BC risk.

Materials and methods

Study cohort

The European Prospective Investigation into Cancer and nutrition (EPIC) Florence cohort has been set up as a part of the EPIC Europe prospective study and enroled (between 1993 and 1998) 10,083 clinically healthy women aged 35–64 years residing in the Florence area (Tuscany, Central Italy). All study participants signed an informed consent and gave permission to use the data collected during the study. The study was approved by the local Ethics Committee.

At enrolment, weight, height, waist and hip circumferences were measured by trained nurses according to an international standard protocol. Data on frequency of consumption of over 160 foods and drinks and usual portion size were obtained through a validated self-administered Food Frequency Questionnaire specifically developed to capture the Italian dietary habits. A standardized lifestyle questionnaire collected detailed information on reproductive history, smoking and alcohol drinking history, exposure to environmental tobacco, medical history including history of breast biopsies, educational level and other socio-economic and lifestyle variables including physical activity. Information on drug use including use of hormone replacement therapy (HRT), occupation and family history of cancer was also collected [8].

Standardized follow-up procedures have been periodically implemented for the identification of cancer cases diagnosed after enrolment. The ascertainment of vital status was carried out through the linkage with the local town offices and the local Mortality Registry, thereby identifying the deceased subjects and the date and cause of death. The identification of BC cases, was obtained through linkages with hospital discharge system and other sources such as Pathology Department registries [8].

We also linked periodically the EPIC Florence female cohort with the mammographic archives of the population-based mammographic screening in the Florence area of which the Cancer Research and Prevention Institute—ISPO (formerly CSPO)—is in charge and with the archive of the mammograms performed in a clinical setting at our Institution, in order to update the mammographic examination history of the EPIC female participants [9].

Design of the nested case–control study

Cases

All newly diagnosed invasive breast cancer cases (code C50 according to ICD-O-2 classification) after the date of recruitment until 31 December 2001 for which it was possible to identify and retrieve one negative mammogram at least 1 year before the diagnosis.

Controls

For each case, a maximum of six controls, individually matched by age (±12 months), date of enrolment (±12 months), menopausal status and date of mammographic examination (±6 months) were randomly selected among those still at risk of BC at the time of diagnosis of each case.

Mammographic breast density assessment

The assessment of MBD was performed by an experienced radiologist (D.A.), blind to case–control status, according to BI-RADS classification (4th edition) in the following four categories: 1—the breast is entirely fatty (<25% fibroglandular tissue); 2—there are scattered fibroglandular densities (25–50%); 3—the breast tissue is heterogeneously dense which may obscure small masses (51–75%) and 4—the breasts are extremely dense, which lowers the sensitivity of mammography (>75%) [10].

We retrieved most of the identified mammographic examinations (screen film mammograms) directly from the

ISPO archives, but we also invited study participants to send us the films if these were kept at home. The oblique medio-lateral view of both breasts was used for mammographic assessment. Only this view was utilized to assess the MBD even in the group of subjects with two films because in the mammographic population screening programme in Florence, the two views (oblique medio- lateral and cranio-caudal) were performed at the subsequent screening test after the first screening mammogram only in women with dense breast until 2000. In order to avoid reader being aware of the previous classification, for all subjects only the oblique medio-lateral view was used. We have already evaluated the effect of using only the oblique medio-lateral view to assess parenchymal patterns versus both views in a subset of 50 MEs utilized in a previous study and we found a very high concordance [9].

Statistical analysis

Distribution of the main baseline characteristics and of BI-RADS categories was reported separately for BC cases and controls. Tests of heterogeneity between categories were performed.

The association of MBD, classified according to BI-RADS categories, with BC risk was evaluated by conditional logistic regression, which takes into account the matching of controls to cases. Crude and adjusted odds ratios (OR) and 95% confidence intervals (CI) were estimated.

Adjustments were performed by variables that were reported to be associated with both MBD and BC risk in previous studies [9, 11]: education (primary/secondary school, high school/university), BMI (continuous), number of children (0, 1–2, ≥3), breast feeding(≤6 months, >6 months), first-degree BC family history (yes/no), previous breast biopsy(yes/no) and current HRT use (yes/no). To calculate the p value for trend, categories of BI-RADS were entered as an ordinal term in the model.

The analyses were also carried out separately according to menopausal status.

Interaction of MBD with previous history of breast biopsies, BMI, first-degree BC family history and HRT use were also investigated.

All analyses were performed using the SAS statistical software (SAS/STAT version 9.2); a p value <0.05 was considered statistically significant.

Results

For 140 (78.2%) out of the 179 BC cases occurred in the follow-up period, we were able to identify and retrieve a negative mammographic examination suitable for the study. The remaining 39 BC cases were younger (45.2 vs. 54.0 years $p < 0.0001$), more educated (66.7% reported to have obtained at least a high school degree vs. 55.0% $p = 0.002$), with a lower proportion of previous breast biopsies (2.6 vs. 17.9% $p = 0.001$) and a lower proportion of reported BC first-degree family history (5.1 vs. 14.3% $p = 0.07$). The assessment of MBD was not possible for the mammograms of four cases due to technical problems, thus leaving 136 cases for the analysis. We identified 635 matched controls for which the mammograms were available for the MBD assessment. Most cases (n 76) had five matched controls while one case had only one matched control.

The mammograms used for the definition of the breast density were performed on average 2.7 years (SD 1.96) before BC diagnosis.

In Table 1, the distribution of BI-RADS categories and of selected characteristics is reported separately for cases and controls. Overall, 28.7% (n 39) of BC cases and 43.0% (n 273) of controls were categorized to have a "entirely fatty breast" (category 1) while the proportion of BC cases and controls categorized in the category 4 "extremely dense breast" was 8.8% (n 12) and 6.5% (n 41), respectively (overall $p = 0.02$). Cases and controls also differed with respect to the proportion of previous breast biopsies and first-degree BC family history as reported at enrolment ($p = 0.02$).

Both in crude and adjusted analyses, the positive significant association between BI-RADS categories and BC risk emerged, ranging in multivariate analyses from a 79% increase in category 2 to a more than twofold increase in categories 3 and 4 in comparison to the lowest category (Table 2). The adjusted OR for a single category increase in the BI-RADS scale was 1.41 (95% CI 1.09–1.81) with a significant trend ($p = 0.008$).

In the multivariate models, we also observed a significant positive association of breast biopsy history with BC risk (OR 1.90; 95% CI 1.08–3.35).

The results did not change in analyses carried out separately by menopausal status (data not shown). No effect modification of the association between MBD and BC risk by BMI, previously reported breast biopsies, first-degree BC family history or HRT use emerged.

Discussion

In this case–control study nested in the EPIC Florence cohort, we confirmed the positive association between high mammographic breast density, classified according to BI-RADS (4th edition) and breast cancer risk.

In comparison with women in the lowest category of BI-RADS (1: <25% fibroglandular tissue), the risk to develop

Table 1 Distribution of 136 breast cancer cases and 635 matched controls, according to selected characteristics at enrolment and by BI-RADS classification as assessed by the study radiologist. Nested case–control study in the EPIC Florence cohort

	N	(%)	BC cases	Controls	*p*
Age (years)					
<50	176	22.8	34 (25.0%)	142 (22.4%)	
50–59	428	55.5	72 (52.9%)	356 (56.0%)	
60+	167	21.7	30 (22.1%)	137 (21.6%)	0.75
Education level					
Primary school	222	28.8	31 (22.8%)	191 (30.1%)	
Secondary school	143	18.5	31 (22.8%)	112 (17.6%)	
High school	273	35.4	56 (41.2%)	217 (34.2%)	
University	133	17.3	18 (13.2%)	115 (18.1%)	0.08
Smoking history					
Current smoker	216	28.0	37 (27.2%)	179 (28.2%)	
Former smoker	203	26.3	39 (28.7%)	164 (25.8%)	
Never smoker	352	45.7	60 (44.1%)	292 (46.0%)	0.79
BMI categories					
Underweight/normal (<25)	400	51.9	70 (51.5%)	330 (52.0%)	
Overweight (25–29.99)	273	35.4	53 (39.0%)	220 (34.6%)	
Obesity (≥30)	98	12.7	13 (9.5%)	85 (13.4%)	0.41
Waist circumference					
≤77.0 cm	393	51.0	65 (47.8%)	328 (51.6%)	
>77.0 cm	378	49.0	71 (52.2%)	307 (48.4%)	0.45
Menopausal status					
Yes	495	64.2	86 (63.2%)	409 (64.4%)	
No	276	35.8	50 (36.8%)	226 (35.6%)	0.80
Age at menarche (years)					
<12	168	21.8	32 (23.5%)	136 (21.4%)	
12	216	28.0	34 (25.0%)	182 (28.7%)	
13–14	327	42.4	61 (44.9%)	266 (41.9%)	
>14	60	7.8	9 (6.6%)	51 (8.0%)	0.75
Parity					
Nulliparous	106	13.7	19 (14.0%)	87 (13.7%)	
Parous	665	86.3	117 (86.0%)	548 (86.3%)	0.47
Number of children					
−1	224	33.7	42 (35.9%)	182 (33.2%)	
−2	338	50.8	60 (51.3%)	278 (50.7%)	
−3+	103	15.5	15 (12.8%)	88 (16.1%)	0.78
Breast feeding					
Never	327	42.4	57 (41.9%)	270 (42.5%)	
Ever	444	57.6	79 (58.1%)	365 (57.5%)	0.92
Duration of breast feeding*					
≤6 months	241	54.3	37 (46.8%)	204 (55.9%)	
>6 months	203	45.7	42 (53.2%)	161 (44.1%)	0.17
Current hormone replacement therapy					
Yes	90	11.7	14 (15.6%)	122 (17.91%)	
No	681	88.3	76 (84.44%)	559 (82.09%)	0.58
First-degree BC family history					
Yes	72	9.3	20 (14.7%)	52 (8.2%)	
No	699	90.7	116 (85.3%)	583 (91.8%)	0.02
Previous breast biopsy					
Yes	82	10.6	24 (17.6%)	58 (9.1%)	
No	689	89.4	112 (82.4%)	577 (90.9%)	0.02

Table 1 continued

	N	(%)	BC cases	Controls	*p*
Breast density (BI-RADS categories)					
1 (<25% fibroglandular tissue)	312	40.5	39 (28.7%)	273 (43.0%)	
2 (25–50%)	218	28.3	44 (32.4%)	174 (27.4%)	
3 (51–75%)	188	24.4	41 (30.0%)	147 (23.1%)	
4 (>5%)	53	6.9	12 (8.8%)	41 (6.5%)	0.02

* For women reporting breast feeding ($N = 444$)

Table 2 Association of BI-RADS classification with breast cancer risk Nested case–control study in the EPIC Florence cohort (each matched set with 1 BC and 1–6 matched controls)

BI-RADS categories (% fibroglandular tissue)	BC cases (*n* 136)	Controls (*n* 635)	Crude OR	(95% CI)	Adjusted OR[a]	(95% CI)
1 (<25%)	39	273	1	1	1	
2 (25–50%)	44	174	1.83	(1.12–3.00)	1.79	(1.06–3.01)
3 (51–75%)	41	147	2.29	(1.35–3.89)	2.09	(1.17–3.74)
4 (>75%)	12	41	2.68	(1.14–6.30)	2.67	(1.08–6.62)
p for trend				0.001		0.008

[a] Odds ratios (OR) and 95% confidence intervals (CI) estimated by multivariate model including terms for education (primary/secondary school, high school/university) BMI (continuous), number of children (0, 1–2, ≥3); breast feeding (≤6 months, >6 months); first-degree BC family history (yes/no); previous breast biopsy(yes/no); current HRT use (yes/no)

BC increased across categories with a significant trend up to a 2.67-fold increase in women in the most dense breast BI-RADS category (4: >75% of fibroglandular tissue). These results were obtained taking into account a series of variables known to affect both MBD and BC risk including BMI. No differences emerged in the evaluation of the association between high MBD BI-RADS categories and BC risk according to menopausal status. No interactions emerged by breast biopsy history, first-degree family history for BC or HRT use.

A series of studies have evaluated the association between BI-RADS and BC risk showing consistently an increase in risk over categories of increasing breast density although different designs that have been adopted could at least partially explain some differences in estimates. In a nested case–control study carried out in the cohort set up in the population-based screening programme in Denmark, an approximately two- and fourfold age-adjusted increase in BC risk emerged among women classified in the 3 and 4 BI-RADS categories, respectively [12]. The BI-RADS classification in this study was based on a negative mammogram performed on average 26 months before diagnosis [12]. In a population-based case–control study including both Afro-American and Caucasian women in which MBD assessment using reported BI-RADS was performed on mammograms performed between 5 years before and 1 year after diagnosis, an increase in BC risk emerged for all women with extremely dense breast having a threefold increase in BC risk in comparison with women in the lowest BI-RADS category [13]. The association was more evident in Caucasian women, and a significant effect modification emerged by HRT use [13]. The proportion of current HRT users in our Mediterranean population was low as expected and no effect modification emerged in our analyses.

The association between BI-RADS categories of MBD and BC risk has been also evaluated in a case–control study in which MBD assessment was based on full-field digital mammograms and performed with different tools and also using BI-RADS classification extracted by mammography reports. Women in the highest BI-RADS category had a twofold increase in BC risk in comparison with women in the lowest category in models adjusted for BMI, parity, and menopausal status and the BI-RADS was as accurate as computer-assisted methods in discriminating cases and controls [14]. Some studies have evaluated the association of BI-RADS categories with BC risk by BC subtypes and no specific differences emerged [15, 16].

We have also reported the independent contribution of MBD and previous self-reported breast biopsies to BC risk. Having a history of breast biopsies per se increases the BC risk and this information has been incorporated in

predictive models for the assessment of BC risk [17–19]. We can postulate a higher occurrence of breast biopsies in women with higher MBD. Moreover, some studies showed the independent effect of previous histologically confirmed benign breast disease and high MBD in increasing BC risk [20]. In our study, we confirm that high MBD and a previous history of breast biopsies are both associated with increased BC risk thus supporting the independent role of these two characteristic in identifying high-risk women.

In spite of its relatively small size, this study has a number of strengths, especially based on the case–control design nested in a cohort in which standardized and well-established procedures for the identification of cases have been applied. BMI measures were obtained through a standardized protocol, and series of other BC risk factors to adjust for were collected. The evaluation of MBD was performed by a single experienced radiologist and based on mammograms obtained in the same setting. Moreover, all mammographic examination included in the study were performed with the same imaging technology. Limitations of the study are mainly related to the method used to assess MBD that allow only a broad categorization of MBD thus possibly leading to a misclassification, and consequently to an attenuation of the risk estimates.

In conclusion, we confirm, in a Mediterranean population, the positive association between higher MBD assessed by BI-RADS, a method of qualitative assessment of MBD widely used in clinical settings, and increased BC risk also taking into account the aspects related to personal characteristics, reproductive variable and anthropometry well known to influence both MBD and BC risk. These results support the possibility to use this classification as a tool to identify women with differential BC risk to be targeted by specific interventions of risk reduction or by different diagnostic/screening protocols.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study has been approved by the local Florence Ethical Committee (2001/96) and have been performed in accordance with the ethical standard as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

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