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Research Article

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Survival and Disease-Free Survival by Breast Density and Phenotype in Interval Breast Cancers

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Abstract

Background: We aimed to evaluate survival and disease-free survival in different subtypes of interval cancers by breast density, taking into account clinical and biological characteristics.

Methods: We included 374 invasive breast tumors (195 screen-detected cancers; 179 interval cancers, classified into true interval, false-negatives, occult tumors and minimal-sign cancers) diagnosed in women ages 50–69 years undergoing biennial screening from 2000–2009, followed up to 2014. Breast density was categorized into non-dense (<25% dense tissue) and mixed dense breasts (≥25%). Survival curves were generated by the Kaplan–Meier method and compared by the log-rank test. Cox proportional hazard regression models were computed to estimate the adjusted hazard ratios (aHRs) and 95% confidence intervals (95% CIs) for death and recurrences by comparing women with interval and true interval cancers versus women with screen-detected cancers, controlling for tumor and patient characteristics. All analyses were stratified by breast density.

Results: Interval cancers were detected in younger women, at more advanced stages, in denser breasts and showed a higher proportion of triple-negative cancers, especially among true interval cancers. Women with interval cancer and non-dense breasts had an aHR for death of 3.40 (95% CI, 0.92–12.62). Women with true interval cancers detected in non-dense breasts had the highest adjusted risk of death (aHR, 6.55; 95% CI, 1.37–31.39).

Conclusions: Women with true interval cancer in non-dense breasts had a higher risk of death than women with screen-detected cancers.

Impact: These results support the advisability of routinely collecting information on breast density, both for further tailoring of screening strategies and as a prognostic factor for diagnosed breast cancers. *Cancer Epidemiol Biomarkers Prev; 27(8); 908–16. ©2018 AACR.*

Introduction

Approximately one third of cancer cases in women participating in population-based breast cancer screening programs are interval cancers (1, 2). Interval cancers are those primary breast cancers diagnosed after a screening mammogram with a normal result and before the next scheduled screening invitation (3). Because they are diagnosed by symptoms, affected women may lose the benefit of early detection. About a half of these tumors are true interval cancers (4), in which the previous screening mammogram showed normal or benign features, and about a quarter are false negatives, in which an abnormality suspicious for malignancy is retrospectively seen on the previous mammogram. Occult tumors (tumors showing clinical signs of the disease despite a lack of mammographic abnormalities either at screening or at diagnosis) and minimal-sign cancers (cancers showing detectable but nonspecific signs at the latest screen) are less frequent.

Interval cancers have been reported to have worse prognosis and survival than screen-detected cancers (5). This is partly due to the diagnostic delay and worse clinical features, but differences remain even after taking into account known clinical and biologic characteristics (6). Interval cancers are detected at more advanced tumor stages, have larger tumor size and increased tumor cell proliferation, and—especially for true interval cancers—show a higher proportion of the triple-negative phenotype and are more common in dense breasts (7–9).

The triple-negative phenotype has been associated with worse prognosis and survival (10, 11). In turn, the presence of dense breast tissue in the mammogram is a known risk factor for breast cancer and also decreases mammographic sensitivity (12). For this reason, the prognostic advantage associated with mammography screening could be less evident in women with denser breasts. However, the role of breast density as a prognostic factor for breast cancer is less clear.

Some studies have found an association between high breast density and the risk of death (13–15), whereas others have

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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found no association (16, 17), and there have even been reports of worse overall and disease-free survival in non-dense breasts (18, 19).

So far, few studies have considered breast density in the analysis of survival in interval cancer, and none of them have explored different interval cancer subtypes considering the tumor phenotype. Holm and colleagues (5) reported that interval breast cancers in non-dense breasts showed the most aggressive phenotype, strengthening previous findings by our group, which showed a higher percentage of triple-negative tumors in non-dense breasts, especially for women with true interval cancers (4). In this line, Eriksson and colleagues (20) found that survival among patients with interval cancer was worse only in women with non-dense breasts, which could be related to the higher proportion of the triple-negative phenotype in this group, which could not benefit from suitable treatment.

The aim of this study was to evaluate survival and disease-free survival (considering relapses and second neoplasms) in different subtypes of interval cancers according to breast density groups, taking into account clinical and biological characteristics.

Materials and Methods

Setting and study population

This study was conducted among the CAMISS retrospective cohort, which includes information on 1,086 screened women ages 50–69 years, diagnosed with breast cancer between 2000 and 2009 in two regions of Spain (Canary Islands and Catalonia) and followed up to June 2014. The study included women with breast cancer detected at screening and cancer that arose as interval cancers, with information on interval cancer subtypes and breast density.

The study population was restricted to women with invasive breast cancer. Breast density was assessed for all interval cancers with available screening and diagnostic mammograms ($n = 179$) and for a random sample of screen-detected cancers of the CAMISS retrospective cohort, matched by screening program and year of cancer diagnosis between screen-detected cancers and interval cancers. We extracted a 10% oversample for screen-detected cancers because we expected that not all mammograms from screen-detected cancers could be available for breast density assessment. We ended up with available screening mammograms of 195 screen-detected cancers, and all of them were assessed and included in the study (Supplementary Fig. S1).

Following the recommendations of the European Guidelines for Quality Assurance in breast cancer screening and diagnosis (3), the Spanish Breast Cancer Screening Program provides free biennial screening to women ages 50 to 69 years. Two mammographic projections (mediolateral-oblique and craniocaudal views) were made both in the initial and in successive rounds. The BI-RADS (Breast Imaging Reporting and Data System) classification was used for mammogram reading (21). Since 2004, digital mammography has been gradually incorporated in one region. Mammography registers are maintained by all screening programs, with data from participants and the final screening outcome. After histological confirmation of a tumor, the woman is referred to a hospital for treatment and follow-up and she is not further invited to screening.

Study data were collected using a protocol approved by the ethics committee of Parc de Salut Mar (CEIC Parc de Salut Mar), Barcelona. Specific patient consent was not required.

Cancer identification

Breast cancer could be detected in the routine screening or could emerge as an interval cancer. Both types of cancer were included in the analyses.

Screen-detected cancers are routinely recorded in the screening programs' databases. Interval cancers were defined as proposed in the European guidelines: "primary breast cancer arising after a negative screening episode, with or without further assessment, and before the next invitation to screening, or within 24 months for women who reached the upper age limit" (3). These tumors were identified by merging data from the registers of screening programs with population-based cancer registries, the hospital Minimum Basic Data Set, and hospital-based cancer registries. To classify interval cancer, three panels consisting of three experienced radiologists conducted a semi-informed retrospective review of both diagnostic and screening mammograms through independent double reading with arbitration. Interval cancers were definitively classified into true interval cancers, false negatives, minimal-sign cancers, and occult tumors (3). Further details on the identification and classification of interval cancers are explained elsewhere (4).

Study variables

Age at diagnosis was obtained from the date of birth and date of cancer detection. The presence of comorbidities at diagnosis was identified at clinical records review. To calculate the burden of disease, we used the Charlson Comorbidity Index (CCI; ref. 22), a method of predicting mortality by classifying or weighting 19 comorbidities. The CCI was stratified into 4 categories: CCI=0; CCI=1, CCI=2, CCI$\geq$3.

Because information on breast density is not routinely collected by screening programs, it was specifically assessed within a random sample of screen-detected and interval cancers of the CAMISS retrospective cohort. For breast density classification, following Domingo and colleagues (4), one radiologist from each panel determined the breast density of the cancer-free breast. The radiologists involved in the density assessment were especially trained and followed a consensual protocol based on the methodology described by others, showing good agreement in our context and in the framework of mammography screening (23). Breast density was evaluated using Boyd's scale, a semi-quantitative score of six categories using percentages of density: A: 0%; B: 1% to 10%; C: 10% to 25%; D: 25% to 50%; E: 50% to 75%; F: 75% to 100% (24). For statistical purposes, breast density was dichotomized into two groups: <25% of dense tissue (non-dense breasts) and $\geq$25% of dense tissue (mixed dense breasts).

Tumor-related information (pathological tumor-node-metastasis [TNM] status, tumor size, and receptor expression) was obtained from the cancer registries, hospital-based registers, and clinical records. The positivity criteria for receptor expression followed international recommendations and their updates throughout the study period (25, 26). Tumors were classified into four phenotypes based on the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2): (i) luminal A: $ER^+/HER2^-$ or $PR^+/HER2^-$; (ii) luminal B: $ER^+/HER2^+$ or $PR^+/HER2^+$; (iii) HER2: $ER^-/PR^-/HER2^+$; and (iv) triple-negative: ER^-, PR^-, $HER2^-$ (27).

Information on the treatments received was obtained from the clinical records. Two types of surgery were considered: radical and conservative. Radical surgery included all the mastectomies performed, whether radical or simple. In addition to breast surgery,

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some women could undergo an axillary lymph node dissection (ALND). Information on breast surgery and ALND treatments were collapsed into a single explicative variable. Information about adjuvant treatment was also available and categorized as: chemotherapy, radiotherapy, and hormonal therapy; radiotherapy and hormonal therapy; other treatment; and no adjuvant treatment.

Follow-up of cancer cases

All women were followed up to June 2014 and information on local or regional recurrence, distant recurrence, second breast neoplasms and vital status (alive without disease, alive with disease, deceased at the end of follow-up) was obtained from clinical records and cancer registries. Locoregional recurrence was defined as disease recurrence within the ipsilateral breast or chest wall, in the ipsilateral axillary nodes, internal mammary nodes, or supraclavicular nodes. Distant recurrence was defined as disease recurrence in sites other than the breast or regional lymph nodes (bone, skin, or visceral metastasis). A second neoplasm was considered as a second primary carcinoma developing in the ipsilateral or contralateral breast. Disease-free survival was defined as the time from diagnosis of breast cancer to the first occurrence of one or more of the following: a local or regional recurrence, distant metastasis, and second neoplasm, whichever occurred first. Overall survival (OS) was computed from the date of diagnosis of breast cancer to death from any cause.

When disease-free survival was computed, women lost to follow-up or those who died were censored either at the last visit or at death. For OS, patients were censored at the date of their last hospital visit. The median follow-up period was 8.4 years (interquartile range [IQR]: 7.2–10.6), 8.8 years (IQR: 7.3–10.5) for women with screen-detected cancer, 7.7 years (IQR: 6.2–9.4) for those women with interval cancers and 7.2 years (IQR: 5.7–9) for women with true interval cancer.

Statistical analysis

Comparisons between detection method (screen-detected and interval cancers), and interval cancer subtypes (true interval cancers, false negatives, minimal-sign cancers, and occult tumors) were established to assess possible differences in patient characteristics (age groups, breast density), tumor characteristics (TNM stage, tumor size, tumor phenotype), treatment approaches, and the occurrence of a second breast neoplasm, recurrences, and vital status at the end of follow-up. Statistical significance was estimated using the χ^2 or Fisher exact two-sided test, because all study variables were categorical.

Stratified analyses by breast density categories (non-dense breasts [<25%] and mixed dense breasts [≥25%]) were carried out for vital status at the end of follow-up, recurrences and second breast neoplasms. The proportion of deaths by breast density categories within the different cancer phenotypes was also explored.

Because of the small number of interval cancers in some categories, the survival analyses only included the subset of women with screen-detected cancers, all interval cancers and true interval cancers. Survival curves were generated by using the Kaplan–Meier method and were compared by the log-rank test. Disease-free survival and OS among study groups were plotted with stratification by breast density categories.

Cox proportional hazard models were used to estimate the hazard ratios (HRs) for death and recurrences and second breast neoplasms according to detection method (comparing women with interval cancers vs. women with screen-detected cancer, and restricting tumors to true interval cancers vs. screen-detected cancers). We tested the effect of different adjustment variables (TNM stage instead of tumor size) and possible confounders (diagnostic period, mammography type, study site, and treatment) by fitting separated Cox regression models. The covariates included in the final model were selected according to their statistical significance and to their clinical and biological relevance. The final models were adjusted for phenotype, tumor size, tumor grade, patient age, and CCI. We also tested interactions between mammographic density categories and phenotype and between mammographic density and the detection method. However, because of the small numbers of the outcome variables in some combinations of the explanatory covariates the model was not informative for all study factors. Instead, we stratified the analyses by breast density categories (non-dense breasts [<25%] and mixed dense breasts [≥25%]) to analyze the impact of detection method in each strata adjusted for the other covariates. Unadjusted and adjusted HR (aHR) and their 95% confidence intervals (CIs) were computed. The proportional hazards assumption was ascertained by assessment of log-log survival plots.

Sensitivity analyses were performed considering different cutoffs to define non-dense breasts. When non-dense breasts were considered as a percentage of dense tissue <10%, the results were in the same direction but the sample size was inadequate to obtain robust estimators.

Statistical tests were two-sided and all P values <0.05 were considered statistically significant. Analyses were performed using the statistical software SPSS version 22.0 (SPSS Inc.) and R version 3.3.2. (Development Core Team, 2014).

Results

This analysis included a total of 374 cases of invasive breast cancer: 195 screen-detected cancers and 179 interval cancers. Most interval cancers were true interval cancers (n = 88, 48.9%), followed by false negatives (n = 43, 23.9%), occult tumors (n = 25, 13.9%), and minimal-sign cancers (n = 23, 12.8%).

Table 1 summarizes patient- and tumor-related characteristics as well as treatment and the vital status of women diagnosed with screen-detected cancer or interval cancer, and different interval cancer subtypes. At diagnosis, interval cancers were more frequently detected in younger women than screen-detected cancers (64.2% and 47.2%, respectively; P < 0.001) and at more advanced stages (25.6% and 11.4% stage III/IV, respectively; P < 0.001). True interval cancers and occult tumors showed the highest proportion of cancers at stage III/IV (30.7% and 32.0%, respectively). The percentage of women with non-dense breasts was higher among women with screen-detected cancers than among those with interval cancers (57.9% vs. 44.1%; P = 0.008). The most frequent phenotype in both screen-detected and interval cancers was luminal A followed by luminal B. However, the proportion of triple-negative phenotype was non-significantly higher in interval cancers than in screen-detected cancers (14.3% vs. 13.0%, respectively), especially among true interval cancers (17.6%). Mortality was higher among women with interval cancers than among those with screen-detected cancers (20.7% vs. 10.8%, respectively). No statistically significant differences were observed in the percentage of a second breast

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Table 1. Patient- and tumor-related characteristics and survival outcomes according to mode of detection[a]

	Screen-detected cancers ($n = 195$) n (%)	Interval cancers ($n = 179$) n (%)	P^b	True interval cancers ($n = 88$) n (%)	False negatives ($n = 43$) n (%)	Occult tumors ($n = 25$) n (%)	Minimal sign cancers ($n = 23$) n (%)	P^c
Age group (y)								
50–59 years	92 (47.2)	115 (64.2)		59 (67.0)	27 (62.8)	15 (60.0)	14 (60.9)	
60–69 years	103 (52.8)	64 (35.8)	0.001	29 (33.0)	16 (37.2)	10 (40.0)	9 (39.1)	0.021
Breast density (%)								
Non-dense breasts (< 25%)	113 (57.9)	79 (44.1)		37 (42.0)	25 (58.1)	6 (24.0)	11 (47.8)	
Mixed dense breasts (≥ 25%)	82 (42.1)	100 (55.9)	0.008	51 (58.0)	18 (41.9)	19 (76.0)	12 (52.2)	0.005
TNM stage								
I	108 (56.0)	53 (30.1)		25 (28.4)	15 (34.8)	8 (32.0)	5 (23.8)	
II	63 (32.6)	78 (44.3)		36 (40.9)	20 (46.5)	9 (36.0)	13 (61.9)	
III	19 (9.8)	41 (23.3)		25 (28.4)	7 (16.3)	6 (24.0)	3 (14.3)	
IV	3 (1.6)	4 (2.3)	<0.001	2 (2.3)	0 (0)	2 (8.0)	0 (0)	<0.001
Phenotype								
Luminal type A	73 (47.4)	82 (48.8)		36 (42.4)	19 (48.7)	15 (65.2)	12 (57.1)	
Luminal type B	49 (31.8)	43 (25.6)		21 (24.7)	11 (28.2)	5 (21.7)	6 (28.6)	
HER2	12 (7.8)	19 (11.3)		13 (15.3)	4 (10.3)	1 (4.3)	1 (4.8)	
Triple-negative	20 (13.0)	24 (14.3)	0.52	15 (17.6)	5 (12.8)	2 (8.7)	2 (9.5)	0.611
Vital status								
Alive without disease	156 (80.0)	132 (73.7)		65 (73.9)	32 (74.4)	17 (68.0)	18 (78.3)	
Alive with disease	18 (9.2)	10 (5.6)		3 (3.4)	2 (4.7)	2 (8.0)	3 (13.0)	
Exitus	21 (10.8)	37 (20.7)	0.018	20 (22.7)	9 (20.9)	6 (24.0)	2 (8.7)	0.103

[a]Tumors with missing information were excluded for the calculation of percentage.
[b]Screen-detected and interval cancers are compared with χ^2 two-sided test or Fisher's exact two-sided test.
[c]Screen-detected, true interval, and false negative cancers are compared with χ^2 two-sided test or Fisher's exact two-sided test.

neoplasm or in the number of recurrences, although the latter were more frequent in interval cancers (Supplementary Table S1).

The percentages of vital status, second breast neoplasm and recurrences according to mode of detection and breast density categories are shown in Table 2. The proportion of deaths was higher among women with mixed dense breasts in both screen-detected (18.3% and 5.3% of deaths in mixed and non-dense breasts, respectively; $P < 0.05$) and interval cancers (23.0% and 17.7%, respectively; $P > 0.05$). However, among true interval cancers, the proportion of deaths and recurrences was non-significantly higher in women with non-dense breasts (24.3% and 21.6%, respectively).

When the tumor phenotype was taken into account (Table 3), mortality was highest among women with screen-detected cancers with HER2 phenotype and mixed dense breasts (62.5%), followed by women with true interval cancers with the triple-negative phenotype and non-dense breasts (37.5%). However, the differences were not statistically significant.

OS and disease-free survival in women with screen-detected, interval cancers and true interval cancers by breast density categories are shown in Fig. 1. Among screen-detected cancers, women with non-dense breasts had better OS than women with mixed dense breasts (log-rank test = 0.004). This effect decreased among interval cancers and true interval cancers. For the latter, no differences in OS were observed according to breast density, but disease-free survival during the first 5 years was worse among women with non-dense breasts.

The unadjusted and adjusted HR for death for women with interval cancer vs. women with screen-detected cancer, stratified by breast density categories are shown in Table 4. A separate model was restricted to women with true interval cancers versus screen-detected cancers. The adjusted model showed that only women with interval cancer and non-dense breasts had a borderline significant HR for death (aHR, 3.40; 95% CI, 0.92–12.62). The HR became higher and reached statistical significance when restricted to true interval cancers among women with non-dense breasts (aHR, 6.55; 95% CI, 1.37–31.39). No increased HR for death was observed for women with interval or true interval cancers among those with mixed dense breasts. In this group, a higher unadjusted risk for death was observed in women with

Table 2. Vital status, second breast neoplasms, and recurrences according to mode of detection and breast density

	Screen-detected cancers		Interval cancers		True interval cancers		False negatives		Occult tumors		Minimal-sign cancers	
	Breast density		Breast density		Breast density		Breast density		Breast density		Breast density	
	<25% ($n = 113$)	≥25% ($n = 82$)	<25% ($n = 79$)	≥25% ($n = 100$)	<25% ($n = 37$)	≥25% ($n = 51$)	<25% ($n = 25$)	≥25% ($n = 18$)	<25% ($n = 6$)	≥25% ($n = 19$)	<25% ($n = 11$)	≥25% ($n = 12$)
Vital status												
Alive	107 (94.7)	67 (81.7)	65 (82.3)	77 (77.0)	28 (75.7)	40 (78.4)	22 (88.0)	12 (66.7)	5 (83.3)	14 (73.7)	10 (90.9)	11 (91.7)
Exitus	6 (5.3)	15 (18.3)[a]	14 (17.7)	23 (23.0)	9 (24.3)	11 (21.6)	3 (12.0)	6 (33.3)	1 (16.7)	5 (26.6)	1 (9.1)	1 (8.3)
Second breast neoplasm	5 (4.4)	1 (1.21)	1 (1.3)	5 (5.0)	1 (2.7)	–	–	2 (11.1)	–	2 (10.5)	–	1 (8.3)
Recurrence	8 (7.1)	13 (15.9)	11 (13.9)	19 (19.0)	8 (21.6)	9 (17.6)	2 (8.0)	3 (16.7)	–	4 (20.1)	1 (9.1)	3 (25.0)

[a]$P < 0.05$. P values were obtained using the two-sided χ^2 or Fisher's exact two-sided test when appropriate, to assess the distribution of each variable among breast density categories within study groups.

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Table 3. Proportion of deaths according to detection mode, breast density, and tumor phenotype

		Luminal A		Luminal B		HER2		Triple-negative	
	Breast density	*N* Total	*N* Death (%)	*N* Total	*N* Death (%)	*N* Total	*N* Death (%)	*N* Total	*N* Death (%)
Screen-detected cancers	<25%	43	3 (7)	30	0 (0)[a]	4	0 (0)	14	1 (7.1)
	>25%	30	4 (13.3)	19	4 (21.1)[a]	8	5 (62.5)	6	0 (0)
Interval cancers	<25%	36	5 (13.9)	16	4 (25)	7	1 (14.3)	15	3 (20)
	>25%	46	9 (19.6)	27	7 (25.9)	12	3 (25)	9	2 (22.2)
True interval cancers	<25%	16	3 (18.8)	8	1 (12.5)	3	1 (33.3)	8	3 (37.5)
	>25%	20	3 (15)	13	3 (23.1)	10	3 (30)	7	2 (28.6)

[a]$P < 0.05$. *P* values were obtained using the two-sided χ^2 or Fisher's exact two-sided test when appropriate, to assess the distribution of deaths among breast density categories and phenotypes within study groups.

HER2 which was not statistically significant in the adjusted model. Women with interval cancer or true interval cancers and non-dense or mixed dense breasts did not have an increased HR for recurrences or a second breast neoplasm (Table 5). Sensitivity analyses revealed equivalent results to those of our main model when variables such as TNM stage, diagnostic period, mammography type, study site, and treatment, were included in the Cox regression models.

Discussion

This study suggests that women with interval cancer arising in non-dense breasts, especially those with true interval cancers, had an increased risk of death compared with women with screen-detected cancers after adjustment for age, tumor size, and tumor phenotype. The current results add to the scarce knowledge about the role of breast density as a prognostic factor in breast cancer.

Our findings are in line with those of previous studies conducted in Scandinavian countries. In a Finish population, Masarwah and colleagues (19) analyzed a series of 270 invasive breast cancers and found that very low breast density (<10%) at diagnosis was associated with worse disease-free and OS. Among Swedish women ages 50 to 74, Erikson and colleagues (20) observed worse survival in interval cancers detected in non-dense breasts. Their results were based on 1,115 women with screen-detected cancers and 285 women with interval cancers, and the authors concluded that women with interval cancers in non-dense breasts seemed to have a particularly aggressive phenotype. However, none of these works considered true interval cancers in the analyses or took the effect of tumor phenotype into consideration. In our study, we included interval cancer subtypes (including true interval cancers) and tumor phenotype and, indeed, among interval cancers, one of the highest proportions of deaths was observed among women with true interval cancers detected in non-dense breasts and with the triple-negative phenotype. Two previous studies based on larger series of cases reported that the worse phenotype (triple-negative) was more common in non-dense breasts (4, 5).

Breast density is a well-known risk factor for breast cancer, which has been associated with age, genetic factors, parity, menopausal status, use of estrogen, chemoprevention, and body mass index (BMI; ref. 28). It has been hypothesized that higher breast density could yield a worse prognosis. Although some studies have supported this hypothesis (13), others have found no relationship between high breast density and risk of death (15, 29), and some have reported an increased risk of death among women with non-dense breasts (18, 19), especially among obese women (30). However, most of these works included asymptomatic and symptomatic women, wider age ranges, and were not able to control for tumor characteristics such as biomarker expression, which is known to be related to cancer prognosis.

The high percentage of triple-negative tumors observed among non-dense breasts in women with true interval cancers is an important feature that may help to explain why interval cancers arising in non-dense breasts are more aggressive than those arising in mixed dense breasts. Previous studies by our group have revealed such a relationship (4, 31), which has further been confirmed by other groups (5). It could be hypothesized that the worse outcomes could be explained by the overrepresentation of triple-negative tumors among true interval cancers in non-dense breasts. Nevertheless, after adjustment for this variable, the relationship became small but remained statistically significant, suggesting underlying biological mechanisms associated with the worse prognosis. As far as we know, this has never been explored previously.

Our results support the idea of a differential effect of breast density, with high breast density being a risk factor for breast cancer but not necessarily conferring a worse prognosis in diagnosed cancers. Some authors have reported that non-dense breasts, which are associated with a higher percentage of fatty tissue, may be linked to lipogenic regulation pathways of prognostic significance (32). This idea is supported by the results of Gierach and colleagues, (30) who found an increased risk of death among obese women with non-dense breasts in a large cohort of U.S. women. Unfortunately, data on BMI was not available in our study. Moreover, some studies have described a positive association with stromal composition and the estrogenic microenvironment, with women exposed to higher levels of endogenous and/or exogenous hormones being more likely to have dense breasts (28). In support of this idea, some studies have found that women with greater breast densities were at higher risk of developing ER^+ and/or ER^+/PR^+ tumors (33, 34), making them more likely to benefit from adjuvant and neoadjuvant endocrine therapies (35). Mammographic density has strong genetic component that could also explain in part some of the observed findings. Several studies investigated whether polymorphisms in candidate genes related to hormone metabolism, nuclear hormone receptors and growth factors are associated with mammographic density (36, 37) but the effect on response to treatment and survival is not clear.

We found a nonsignificant trend to worse disease free-survival among women with interval cancers and true interval cancers and non-dense breasts. However, larger sample sizes will be needed to confirm or refute these findings. In a recent case–control study based on 242 patients with invasive breast cancer who underwent modified radical mastectomy, Huang and colleagues (16), found an increased risk of locorregional recurrence, distant metastasis

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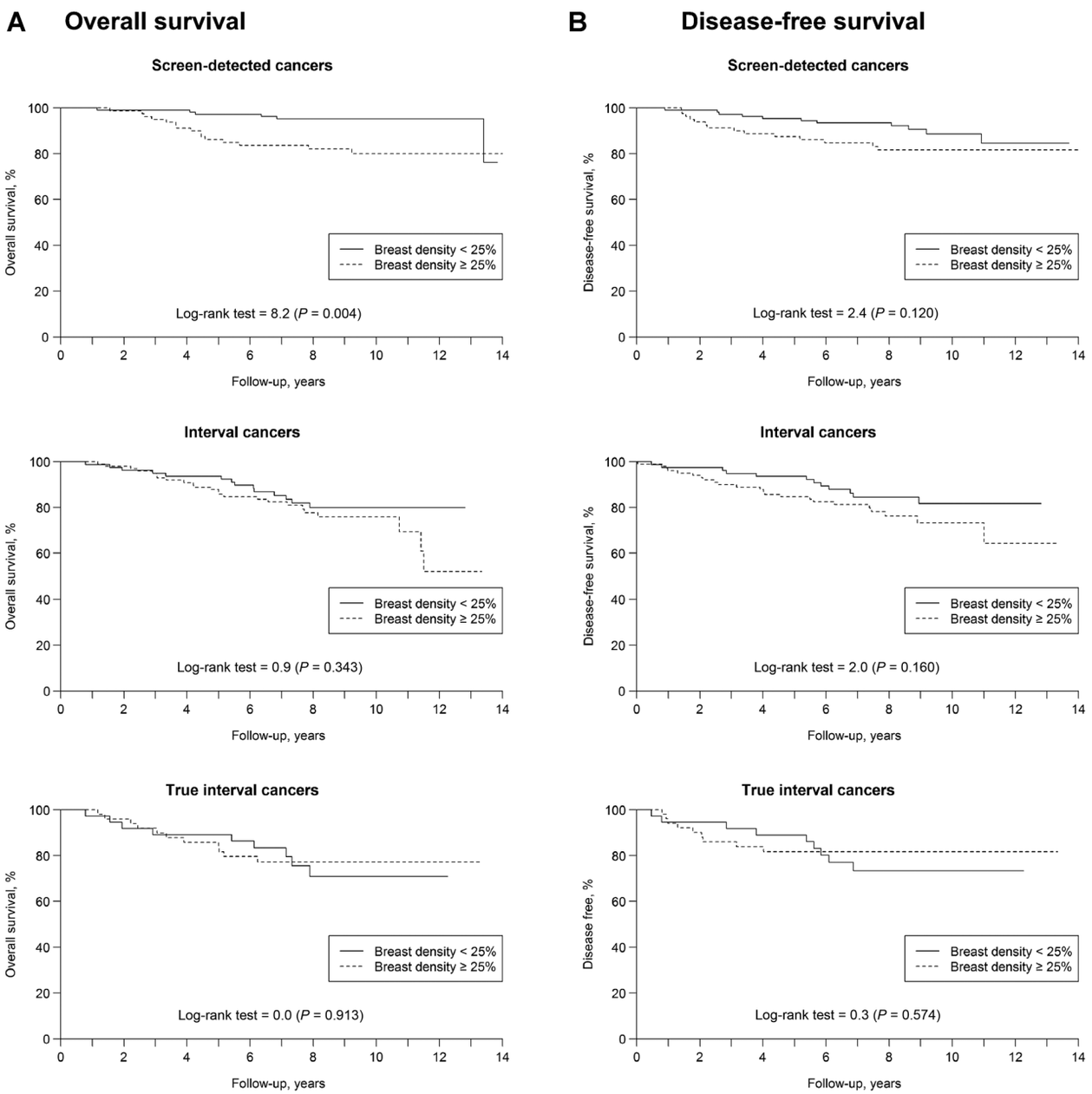

Figure 1.

OS and disease-free survival according to breast density. The figure shows OS (**A**) and disease-free survival (**B**) of women with screen-detected cancers, interval cancer and true interval cancers according to breast density.

and death in women with breast density >50% compared with <50% but only the risk for local recurrence was statistically significant. In a longitudinal study of 607 women with breast cancer, Maskarinec and colleagues (38) found that women with high breast density had a significantly elevated risk of dying only if they had not received radiation. In our study, 85% of patients received radiotherapy, and the results did not change after adjustment for radiotherapy.

Some of the differences between the findings of this study and those of others could be explained by the distinct methodologies used. Previous studies used different breast density classification systems and cutoffs, included distinct age groups and study periods, and most of them did not consider detection mode or tumor phenotype. We used a qualitative classification system for mammographic density assessment, with the possible limitation of subjectivity in the interpretation. However, the reading process was centralized and performed with a panel of trained and experienced radiologists, minimizing variability in the reading process. In addition, our study population consisted of women participating in population-based screening programs, ages

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Table 4. Unadjusted and adjusted HRs for death stratifying by breast density

	Non-dense breasts (<25%)					Mixed dense breasts (>25%)				
	n **(events)**	**HR (95% CI)**	**HR (95% CI)**	**aHR (95% CI)**	**aHR (95% CI)**	***n*** **(events)**	**HR (95% CI)**	**HR (95% CI)**	**aHR (95% CI)**	**aHR (95% CI)**
Detection method										
Screen-detected cancer	113 (6)	Ref.	Ref.	Ref.	Ref.	82 (15)	Ref.	Ref.	Ref.	Ref.
Interval cancer	79 (14)	4.44 (1.6–12.33)		3.40 (0.92–12.62)		100 (23)	1.45 (0.76–2.77)		1.07 (0.50–2.27)	
True interval cancer	37 (9)		6.46 (2.16–19.33)		6.55 (1.37–31.39)	51 (11)		1.32 (0.6–2.87)		0.97 (0.40–2.39)
Tumor phenotype										
Luminal A	79 (8)	Ref.		Ref.	Ref.	76 (13)	Ref.		Ref.	Ref.
Luminal B	46 (4)	0.82 (0.25–2.73)		1.20 (0.34–4.29)	1.38 (0.37–5.09)	46 (11)	1.36 (0.62–2.98)		1.34 (0.60–3.04)	1.31 (0.57–3.03)
HER2	11 (1)	0.95 (0.12–7.57)		0.70 (0.08–6.31)	0.78 (0.08–7.29)	20 (8)	3.51 (1.44–8.55)		2.44 (0.81–7.40)	2.37 (0.75–7.49)
Triple-negative	29 (4)	1.32 (0.40–4.39)		1.07 (0.22–5.09)	1.07 (0.21–5.47)	15 (2)	0.91 (0.21–4.06)		0.83 (0.17–4.14)	0.72 (0.14–3.65)

NOTE: Adjusted for age, comorbidities (Charlson index), tumor grade, and tumor size.

Table 5. Unadjusted and adjusted HRs for recurrences and second neoplasms stratifying by breast density

	Non-dense breasts (<25%)					Mixed dense breasts (>25%)				
	n **(events)**	**HR (95% CI)**	**HR (95% CI)**	**aHR (95% CI)**	**aHR (95% CI)**	***n*** **(events)**	**HR (95% CI)**	**HR (95% CI)**	**aHR (95% CI)**	**aHR (95% CI)**
Detection method										
Screen-detected cancer	113 (11)	Ref.	Ref.	Ref.	Ref.	82 (14)	Ref.	Ref.	Ref.	Ref.
Interval cancer	79 (12)	1.73 (0.76–3.94)		0.93 (0.3–2.92)		100 (22)	1.47 (0.75–2.86)		1.17 (0.52–2.65)	
True interval cancer	37 (9)		2.99 (1.23–7.28)		1.87 (0.52–6.77)	51 (9)		1.15 (0.50–2.67)		0.89 (0.33–2.43)
Tumor phenotype										
Luminal A	79 (8)	Ref.		Ref.	Ref.	76 (10)	Ref.		Ref.	Ref.
Luminal B	46 (4)	0.83 (0.25–2.75)		0.69 (0.19–2.51)	0.85 (0.22–3.25)	46 (10)	1.71 (0.72–4.02)		1.44 (0.58–3.59)	1.39 (0.54–3.55)
HER2	11 (1)	0.88 (0.11–7.05)		0.40 (0.05–3.51)	0.64 (0.07–6.08)	20 (8)	4.32 (1.69–11.02)		2.14 (0.73–6.26)	2.47 (0.77–7.85)
Triple negative	29 (5)	1.72 (0.56–5.26)		0.60 (0.15–2.35)	0.52 (0.12–2.20)	15 (3)	1.71 (0.47–6.24)		1.06 (0.25–4.42)	0.99 (0.23–4.22)

NOTE: Adjusted for age, comorbidities (Charlson index), tumor grade, and tumor size.

between 50 and 69 years. Therefore, they represent a relatively homogenous study group, making our conclusions especially relevant in the context of cancer screening but not directly extrapolable to other study groups. Despite using a quantitative method to assess breast density, the study by Erikson and colleagues (20) also focused on the context of screening. Their results were in the same direction as our findings, reinforcing the results currently presented. Nevertheless, the authors did not classify interval cancers into subtypes, but postulated that interval cancers arising in non-dense breasts were more likely to be true interval cancers. This assumption contrasts with our data and the results of other studies (4) that found a higher proportion of true interval cancers than false negatives among dense breasts.

Our study has some limitations, the most important being the relatively small size of events in the different categories of analysis. To our knowledge, this is the first survival analysis providing information on breast density, phenotype and interval cancer subtypes. However, we lacked complete information for the whole study cohort and other relevant information, such as BMI and breast-specific cancer mortality. Given that our findings are based in a limited number of events, it would be interesting to further explore such relationship in larger series of cases and in other contexts. Besides, cases came from women participating in a population-based screening program between 2000 and 2009 who were followed up until 2014. Because treatment for HER2 tumors was introduced in 2006, the effect of this treatment could not be fully determined even after taking the diagnostic period into account.

The main strength of this study is its classification of interval cancers into subtypes. Restricting the analyses to true interval cancers allowed us to minimize the possible confounding due to false negative cancers, since their characteristics in terms of tumor features and women's characteristics are equivalent to those of screen-detected cancers. At the same time, the availability of information on phenotype allowed us to control for this important prognostic factor for breast cancer, highlighting the need for future works to elucidate the underlying biological mechanisms that heighten tumor aggressiveness. Finally, we took into account women's burden of disease by considering the CCI in the adjusted models. This variable is an important predictor for mortality and therefore its inclusion in the models reinforces the robustness of our results. We also considered tumor size as an adjustment variable in an attempt to control for the lead time bias related to screening.

In conclusion, our results suggest that breast density plays a differential role in the prognosis of interval cancers, revealing that women with interval cancers arising in non-dense breasts, especially those with true interval cancers, had a higher risk of death than women with screen-detected cancers, even when we controlled for tumor-related characteristics. These findings reinforce the need to routinely assess and record information on breast density during the screening process, both for its information as a risk factor for breast cancer as for its role in cancer prognostic and its potential influence in prevention and therapy strategies. Further work is needed to explore the mechanisms associated with non-dense breasts and worse survival in order to improve treatment strategies and patient monitoring.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L. Domingo, M. Baré, J. Ferrer, M.C. Carmona-Garcia, T. Barata, F. Macià

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