Research Article

Cancer Epidemiology, Biomarkers & Prevention

High Mammographic Density in Long-Term Night-Shift Workers: DDM-Spain/Var-DDM

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Abstract

Background: Night-shift work (NSW) has been suggested as a possible cause of breast cancer, and its association with mammographic density (MD), one of the strongest risk factors for breast cancer, has been scarcely addressed. This study examined NSW and MD in Spanish women.

Methods: The study covered 2,752 women aged 45–68 years recruited in 2007–2008 in 7 population-based public breast cancer screening centers, which included 243 women who had performed NSW for at least one year. Occupational data and information on potential confounders were collected by personal interview. Two trained radiologist estimated the percentage of MD assisted by a validated semiautomatic computer tool (DM-scan). Multivariable mixed linear regression models with random screening center–specific intercepts were fitted using log-transformed percentage of MD as the dependent variable and adjusting by known confounding variables.

Results: Having ever worked in NSW was not associated with MD [e^{β}:0.96; 95% confidence interval (CI), 0.86–1.06]. However, the adjusted geometric mean of the percentage of MD in women with NSW for more than 15 years was 25% higher than that of those without NSW history ($MD_{>15\ years}$:20.7% vs. MD_{never}:16.5%;e^{β}:1.25; 95% CI,1.01–1.54). This association was mainly observed in postmenopausal participants (e^{β}:1.28; 95% CI, 1.00–1.64). Among NSW-exposed women, those with $\leq$2 night-shifts per week had higher MD than those with 5 to 7 nightshifts per week (e^{β}:1.42; 95% CI, 1.10–1.84).

Conclusions: Performing NSW was associated with higher MD only in women with more than 15 years of cumulated exposure. These findings warrant replication in futures studies.

Impact: Our findings suggest that MD could play a role in the pathway between long-term NSW and breast cancer. *Cancer Epidemiol Biomarkers Prev; 26(6); 905–13. ©2017 AACR.*

Introduction

The study of circadian disruption in the context of cancer development has been receiving increasing attention in cancer epidemiology. Specifically, night-shift workers have been the population of interest in several studies for being one of the main groups exposed to substantial alterations in their circadian rhythm. According to a 2015 European survey, 19% of employees worked at night (1). Similarly, 22% of employees in Spain worked at night at least once a month (1) and 12.5% performed night-shift work (NSW) regularly (2).

In 2007, the International Agency for Research on Cancer (IARC) concluded that shift work involving circadian disruption is probably carcinogenic to humans (Group 2A), mainly due to its association with increased breast cancer risk (3). A recent meta-analysis showed a pooled relative risk of 1.21 for women with a history of NSW exposure (4), and other studies not included in that review have confirmed this positive association (5–8). It is noteworthy to point out that this association is mostly found in women with long term NSW exposure (i.e., 15–20 years; refs. 5, 9). Unfortunately, the biological mechanisms underlying this association are not yet well understood, although the most commonly alleged pathway focuses on the reduced production of melatonin caused by exposure to light at night (10).

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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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doi: 10.1158/1055-9965.EPI-16-0507

Mammographic density (MD) is currently the most interesting marker of risk for breast cancer due to its strong association with this tumor (11). Mammogram images reflect breast tissue composition; light (dense or non-radiolucent) areas on the mammogram represent the fibrous and glandular tissues in the breast, whereas the dark (radiolucent or less dense) areas are primarily fat (12). Breast cancer risk in women with highly dense breasts is four to five times greater compared with those with slightly or not dense breast controlling for age and BMI (11, 13); this association holds regardless of breast cancer subtype (14). Thus, MD has been proposed as an intermediate phenotype for breast cancer (13) and it has been used as outcome variable in prevention trials (15).

MD is highly hereditary (16), but this phenotype has also been independently associated with other breast cancer risk factors such as reproductive and anthropometric characteristics (17, 18); postmenopausal hormone therapy (19), and alcohol or tobacco consumption (20). However, the relationship between MD and NSW has only been explored in a previous study with inconclusive results (21). If confirmed, this association could have practical effects: Being MD the leading potentially modifiable intermediate marker for breast cancer, preventive strategies to reduce MD (22) might moderate the excess of breast cancer risk associated to NSW.

Here, we examine the association between NSW and MD in DDM-Spain/Var-DDM, a research project with over 3,000 women designed to evaluate determinants of MD in Spanish pre/peri- and postmenopausal women attending breast cancer–screening programs.

Materials and Methods

This is a cross-sectional multicenter study included in DDM-Spain (Determinants of Mammographic Density in Spain)/Var-DDM (Variability of the Mammographic Density in Spanish Women) research project. More detail regarding the design of the study is provided elsewhere (17, 22). Briefly, in Spain, the publicly funded and population-based breast cancer screening network invites all women between 50 and 69 years old (45–69 in some regions) to be screened every 2 years, with attendance rates ranging from 62.9% to 88.0% of its target population, depending on the region (23). For DDM-Spain/Var-DDM women were recruited consecutively at 7 breast cancer screening centers of several Regions (Aragon; Balearic Islands; Castile-Leon; Catalonia; Galicia; Navarre; and Valencia) in 2007 and 2008. Each center had to enroll at least 500 participants; thus, the total number of participants was 3,584 with an average participation rate of 74.5% (range: 64.7%–84.0%). The exclusion criteria were having been diagnosed of breast or ovarian cancer, having had breast surgery or implants and, in a second step, we also excluded 10 women who developed breast cancer within 6 months of the mammogram.

DDM-Spain/Var-DDM projects were formally approved by the Bioethics and Animal Welfare Committee at the Carlos III Institute of Health. All participants signed an informed consent form and, on the same day of the mammographic exam, they answered a detailed epidemiological questionnaire on obstetric and lifestyle factors administered by trained interviewers. This questionnaire included specific items to explore NSW exposure, namely, whether they had ever worked the night-shift, in which occupation, for how many years, usual number of nights worked per week (None, up to 2, 3–4, 5–7 nights/week), age at beginning of NSW and, if applicable, age when they stopped NSW. Combining this information with the age at mammogram we constructed two additional variables: timing of first childbirth (nulliparous; before onset of NSW; after onset of NSW) and years since last NSW (No NSW; Current or recent NSW ($\leq$1 year); 1–5 years, 5–15 years, >15 years). Postmenopausal status was defined as self-reported absence of menstruation in the last 12 months. Women's height and weight were also measured twice by the interviewer in accordance with pre-established protocols, with a third measurement if the first two were substantially different. Average values were used to compute body mass index (BMI).

We obtained the cranio-caudal mammogram of the left breast for 3309 women (ages 45–68 years; 99.2% Caucasian) in the following formats: (i) Analogical mammograms digitalized with a Totalook MammoAdvantage scanner (maximum optical density 4.2; $n = 1{,}781$, 53.8%); (ii) fully digital images using Senographe 2000D Full Field Digital Mammography System; Hologic-Lorad M-IV; or Siemmens MAMMOMAT Novation DR depending on recruitment site ($n = 1{,}376$, 41.6%), and (iii) Originally digital mammograms printed on film and digitalized with a Microtek Medi-700scanner (maximum optical density 4.0) in ($n = 152$, 4.6%).

Two experienced radiologists estimated the percentage of MD in the mammograms in a continuous scale, assisted by DM-Scan, a semiautomated computer tool specifically designed by the Polytechnic University of Valencia for this purpose. This free tool (http://dmscan.iti.upv.es) has shown a high reproducibility and validity, with a substantial discriminative power to predict subsequent breast cancer development (24, 25). One radiologist read the mammograms from Castile-Leon, Balearic Islands and Navarre and the other one read those from Aragon, Catalonia, Galicia and Valencia. To evaluate intra- and inter-rater intraclass correlation (ICC), each radiologist repeated MD estimation in 60 images, and 243 mammograms (~35 per region) were read by both of them. We obtained high ICCs in both cases (>0.9).

We excluded participants that had never held remunerated jobs (housewives; $n = 476$) to avoid possible bias due to a healthy worker effect as well as 26 participants with missing information for some of the main confounding variables. In addition, because we decided to set one year as the minimum time of cumulated NSW needed to be considered as ever exposed to NSW, 55 women working NSW for less than a year, which could not be clearly classified, were also excluded.

We adjusted multivariate mixed linear regression models with random screening center-specific intercepts using log-transformed percentage of MD as dependent variable. We also included in the model a number of potential confounder variables as fixed effects, which could be classified into three categories: (i) basic confounders that must be included when studying percent density: BMI (restricted quadratic spline), age at time of mammogram (continuous) and menopausal status (pre/perimenopausal and postmenopausal); (ii) lifestyle and personal variables associated to MD and breast cancer: parity (continuous), HRT(no use, previous use, current use), first-degree relative with breast cancer (yes/no), smoking (never smoker, ex-smoker $\geq$6 months and current smoker or ex-smoker <6 months), alcohol intake (non-drinker, <10 gram/day, $\geq$10 gram/day), average level of physical activity (low, moderate and vigorous), daily calorie intake (continuous); and (iii) other possible sources of variability derived of the study design: type of mammogram (analogic, full digital and scanned digital), and radiologist.

We used these log-lineal models to calculate geometric means of MD for nightshift exposure variables adjusted by all the mentioned confounding factors. In addition, the estimated regression coefficients and standard errors of these models were exponentiated to calculate the relative change of the adjusted geometric mean of MD comparing groups of participants across categories of the studied epidemiological variables. Models were also constructed separately for pre/peri- and postmenopausal women and we also explored the possible interactions between menopausal status and the four NSW exposure variables using likelihood ratio test. Interaction terms between cumulative NSW exposure and the demographic, obstetric, and lifestyle characteristics presented in Table 1 were tested as well. In addition, we evaluated the association between MD and NSW among women with current or recent exposure (stopped NSW <1 before mammogram; See Supplementary Table S1).

In a second step, we fitted models restricted only to the ever-exposed women (i.e., the 243 women with a history of NSW >1 year) to study the association of MD with all NSW characteristics simultaneously. These multivariable mixed linear regression models, with random screening center-specific intercepts, were adjusted by the previously mentioned confounding variables and the selected NSW characteristics (time since last NSW, total years of NSW, NSW usual frequency in nights per week, and parity at the beginning of NSW). Again, we calculated adjusted geometric means of MD for nightshift exposure variables.

We also performed additional sensitivity analyses by testing whether a number of factors (education, socioeconomic level, waist to hip ratio, and years since menopause in postmenopausal women) might cause residual confounding and by fitting the models classifying as exposed also those women that had NSW <1 year (*n*, 55) or including housewives (*n*, 476) among non-exposed participants as well as excluding (i) all women that had ever had HRT (*n*, 282) and (ii) those women that were using HRT at the moment of the mammograms (*n*, 69; See Supplementary Table S2).

Finally, we explored non-linear associations for total years of NSW exposure in all women, using restricted cubic splines with 3 knots located in 5, 10, and 15 years. Departure from linearity was evaluated with non-lineal spline terms. All analyses were carried out with STATA/MP 13.1 software (StataCorp. 2013).

Results

Overall, 8.8 % of the 2,752 women (*n*, 243) reported having performed NSW for at least one year in their lifetime. Detailed information of the main characteristics of the women included in this study, stratified by their NSW status, is shown in Table 1.

The prevalence of ever performing NSW was higher among younger women, nulliparous and among those with low or normal weight. Also, NSW was more common in women with a university degree, due to the high proportion of nurses and health care professionals within the NSW group (102 out of 243 women). Other frequent occupational sectors with NSW exposure were industry (35 women), geriatric care (30 women), cleaning services (30 women), and administrative work (25 women). Balearic Islands and Valencia had the highest prevalence of women reporting NSW (11.7% and 11.4%, respectively). Smokers and former smokers were more likely to be engaged in NSW than nonsmokers (Table 1) and, on average, night-shift workers reported slightly higher daily caloric intake, although the difference was not statistically significant (NSW_{mean}: 2112 vs. non-NSW_{mean}: 2061 cals/d; *P*, 0.112). The median of years in NSW was 8 (Interquartile range: 3–12) and the mean age at onset of NSW was 32 years (Standard Deviation: 11.4). Almost one third of the participants reporting NSW history (76 out of 243) were currently doing NSW at the time of interview.

Table 2 shows the association between MD and NSW overall and stratified by menopausal status. Having ever performed NSW was not associated with MD, neither for the entire sample (MD_{ever}:15.9% vs. MD_{never}:16.5%; e^{β}_{all}: 0.96; 95% confidence Intervals (CI), 0.86–1.06), nor by menopausal status ($MD_{ever\ pre}$:23.3% vs. $MD_{never\ pre}$:25.0%; e^{β}_{pre}: 0.93; 95% CI, 0.78–1.11;$MD_{ever\ post}$:14.3% vs. $MD_{never\ post}$:14.7%; e^{β}_{post}: 0.98; 95% CI, 0.86–1.11). However, the adjusted geometric mean of the percentage of MD in women with NSW for more than 15 years was 25% higher than that of those without NSW history ($MD_{>15\ years}$:20.7% vs. MD_{never}:16.5%; e^{β}:1.25; 95% CI, 1.01–1.54). The association did not change when we also took into account vitamin D consumption ($MD_{>15\ years}$:22.1% vs. MD_{never}:16.4%; e^{β}:1.25; 95% CI, 1.01–1.54). We repeated the analysis, including housewives and performed different sensitivity analyses with similar results (See Supplementary Table S1), whereas none of the interaction terms between cumulative NSW exposure and the demographic, obstetric and lifestyle characteristics presented in Table 1 were statistically significant. It is interesting to note that MD was also higher in women who were current/recent night workers or had stopped working night shifts less than 5 years before the mammogram, although these associations were not statistically significant.

Concerning NSW frequency, women working 5 to 7 nights per week had lower MD than those which had never had NSW, both globally ($MD_{5\text{-}7\ nights}$:13.6% vs. MD_{never}:16.5%; e^{β}: 0.82; 95% CI, 0.68–0.99), and among postmenopausal women ($MD_{5\text{-}7\ nights}$:10.9% vs. MD_{never}:16.5%; e^{β}: 0.75; 95% CI, 0.60–0.92). However, in premenopausal women this association was not observed ($P_{interaction}$:0.029). There were no differences in MD regarding timing of first child (Table 2) or regarding occupational sector of the NSW (Health Services e^{β}: 1.04; 95% CI, 1.04–0.89; Geriatric Care e^{β}: 0.94; 95% CI, 0.71–1.24; Industry e^{β}: 0.88; 95% CI, 0.68–1.14; Cleaning Services e^{β}: 0.89; 95% CI, 0.67–1.18; Administrative work e^{β}: 1.00; 95% CI, 0.74–1.36; Others e^{β}: 0.98; 95% CI, 0.64–1.51).

When we restricted the analysis to current workers (See Supplementary Table S2) the percentage of MD was again 20% higher in women who had performed NSW for more than 15 years (e^{β}:1.20; 95% CI, 0.92–1.59), although the smaller number of exposed women reduced the power to obtain statistical significance. As in the previous analyses, there was a significant interaction between NSW frequency and menopausal status (*P*, 0.036).

In further analysis, we focused only in the subgroup of 243 women exposed for over a year to be able to evaluate the effect of all the characteristics of NSW within the same model (Table 3). Again, the geometric mean of MD, adjusted by the other NSW exposure variables, was higher in women who had performed NSW for more than 15 years ($MD_{>15years}$: 23.6% vs. MD_{never}: 16.0%; e^{β}:1.39; 95% CI, 1.05–1.83). In this case, we observed

Table 1. Main characteristics of DDM-Spain/VarDDM-Spain participants (2007–2008) according to their nightshift work (NSW) status

	Total (*n*: 2,752)		Never NSW (*n*: 2,509)	Ever NSW[a] (*n*: 243)	
	N	Mamographic density %[c]	*n* (%)	*n* (%)	*P*[b]
Age, y					
<50	433	29.6	377 (87.1)	56 (12.9)	
50–54	788	18.8	705 (89.5)	83 (10.5)	
55–59	787	14.5	725 (92.1)	62 (7.9)	
>59	744	11.4	702 (94.4)	42 (5.6)	0.001
Current socioeconomic level[d]					
Low/middle-low	674	13.5	615 (91.2)	59 (8.8)	
Middle	1,929	17.2	1,762 (91.3)	167 (8.7)	
Middle-high/high	137	21.2	122 (89.1)	15 (10.9)	0.657
Educational level[e]					
Primary education or less	912	12.7	873 (95.7)	39 (4.3)	
Secondary education	1,527	18.0	1,394 (91.3)	133 (8.7)	
University graduate	308	22.1	237 (76.9)	71 (23.1)	0.001
Body mass index					
Low or normal (<25 Kg/m^2)	806	27.8	717 (89)	89 (11.0)	
Overweight (25–29 Kg/m^2)	943	16.9	864 (91.6)	79 (8.4)	
Obese (≥30 Kg/m^2)	1,003	10.4	928 (92.5)	75 (7.5)	0.024
Region					
Galicia	390	17.8	351 (90.0)	39 (10.0)	
Catalonia	438	8.9	412 (94.1)	26 (5.9)	
Castile-León	325	26.8	297 (91.4)	28 (8.6)	
Balearic Islands	384	14.0	339 (88.3)	45 (11.7)	
Navarre	404	29.5	375 (92.8)	29 (7.2)	
Aragon	434	14.2	401 (92.4)	33 (7.6)	
Valencia	377	14.7	334 (88.6)	43 (11.4)	0.024
Current menopausal status					
Pre/perimenopausal	644	25.4	569 (88.4)	75 (11.6)	
Postmenopausal	2,108	14.3	1,940 (92.0)	168 (8.0)	0.004
Parity (Number of live births)					
Nulliparous	20.1	22.9	231 (85.2)	40 (14.8)	
1	18.3	21.1	402 (89.9)	45 (10.1)	
2	16.2	16.0	1,209 (92.0)	105 (8.0)	
≥3	15.1	12.8	667 (92.6)	53 (7.4)	0.001
First-degree relative with breast cancer					
No	2,565	16.2	2,339 (91.2)	226 (8.8)	
Yes	187	19.1	170 (90.9)	17 (9.1)	0.896
Hormonal replacement therapy					
Never	2,470	16.4	2,255 (91.3)	215 (8.7)	
Past use	213	15.1	196 (92.0)	17 (8.0)	
Current use	69	20.4	58 (84.1)	11 (15.9)	0.102
Tobacco use					
Never	1,612	14.7	1,516 (94.0)	96 (6.0)	
Former >6 months ago	500	18.9	434 (86.8)	66 (13.2)	
Smoker or former <6 months	640	19.4	559 (87.3)	81 (12.7)	0.001
Current alcohol consumption					
Non-drinkers	1,094	15.5	1,002 (91.6)	92 (8.4)	
<10 grams/day	1,166	16.3	1,058 (90.7)	108 (9.3)	
≥10 grams/day	492	18.7	449 (91.3)	43 (8.7)	0.773
Physical activity					
Low	652	14.0	595 (91.3)	57 (8.7)	
Moderate	1,401	16.8	1,286 (91.8)	115 (8.2)	
Vigorous	699	18.1	628 (89.8)	71 (10.2)	0.331
Type of mammogram					
Analogic	1,441	21.5	1,320 (91.6)	121 (8.4)	
Digital	1,179	12.0	1,067 (90.5)	112 (9.5)	
Digital printed on film	132	13.1	122 (92.4)	10 (7.6)	0.535

[a]Ever NSW is consider when cumulative NSW ≥ 1 years.
[b]The χ^2 test comparing distribution in ever and never NSW.
[c]Geometric mean of the percentage of MD.
[d]Twelve participants did not report socioeconomic level.
[e]Five participants did not report Educational level.

also an inverse association between MD and years since last NSW ($e^{\beta}_{5\text{ years decrease}}$: 1.04; 95% CI, 1.00–1.09), which seems more clear in pre/peri-menopausal women ($e^{\beta}_{5\text{ years decrease}}$: 1.13; 95% CI, 1.03–1.23). The association between NSW frequency and MD varied by menopausal status ($P_{\text{interaction}}$, 0.006). Among post-menopausal women, MD was higher as the number of nightshifts

Table 2. Association of nightshift work (NSW) and mammographic density (MD) in DDM-Spain/Var-DDM study (2007–2008), overall and by menopausal status[a]

	Total (*n*: 2,752)				Pre/Perimenopausal (*n*: 644)				Postmenopausal (*n*: 2,108)			
	n	**%MD[a]**	**e^{β} (95% CI)**	***P***	***n***	**%MD[a]**	**e^{β} (95% CI)**	***P***	***n***	**%MD[a]**	**e^{β} (95% CI)**	***P***
NSW												
Never	2,509	16.5	1.00		569	25.0	1.00		1,940	14.7	1.00	
Ever	243	15.9	0.96 (0.86–1.06)	0.432	75	23.3	0.93 (0.78–1.11)	0.444	168	14.3	0.98 (0.86–1.11)	0.696
<5 years of cumulative NSW	88	14.9	0.90 (0.77–1.07)	0.234	24	24.3	0.97 (0.72–1.31)	0.842	64	13.0	0.89 (0.73–1.08)	0.231
5–15 years of cumulative NSW	102	14.5	0.88 (0.75–1.03)	0.105	38	21.0	0.84 (0.66–1.07)	0.149	64	13.3	0.91 (0.74–1.11)	0.333
>15 years of cumulative NSW	53	20.7	1.25 (1.01–1.54)	0.040	13	29.7	1.19 (0.80–1.77)	0.395	40	18.7	1.28 (1.00–1.64)	0.054
Trend (per 5-years)	2,752		1.01 (0.98–1.04)	0.664	644		0.98 (0.93–1.04)	0.539	2,108		1.02 (0.98–1.05)	0.397
Years since last NSW[b]												
No NSW	2,509	16.5	1.00		569	25.0	1.00		1,940	14.7	1.00	
>15 years	83	14.2	0.86 (0.73–1.02)	0.087	21	21.1	0.84 (0.61–1.16)	0.297	62	12.8	0.87 (0.71–1.07)	0.189
5–15 years	46	13.5	0.81 (0.65–1.02)	0.077	17	15.1	0.60 (0.43–0.85)	0.004	29	14.4	0.98 (0.73–1.31)	0.886
1–5 years	30	19.8	1.20 (0.90–1.58)	0.212	10	27.6	1.10 (0.70–1.73)	0.672	20	18.4	1.26 (0.89–1.78)	0.200
Current or recent NSW (≤1 year)	82	18.3	1.10 (0.93–1.31)	0.256	27	31.5	1.26 (0.95–1.67)	0.107	55	15.2	1.04 (0.84–1.28)	0.745
Trend (5-years decrease)	2,750		1.00 (0.99–1.01)	0.991	644		1.00 (0.98–1.02)	0.802	2106		1.00 (0.99–1.02)	0.831
NSW frequency[c,d]												
No NSW	2,509	16.5	1.00		569	25.0	1.00		1,940	14.7	1.00	
5–7 nights/week	69	13.6	0.82 (0.68–0.99)	0.038	15	29.9	1.19 (0.82–1.73)	0.357	54	10.9	0.75 (0.60–0.92)	0.008
3–4 nights/week	59	15.7	0.95 (0.77–1.16)	0.601	18	18.0	0.72 (0.51–1.01)	0.060	41	15.7	1.07 (0.84–1.37)	0.582
≤2 nights/week	112	17.6	1.07 (0.92–1.24)	0.389	42	23.9	0.95 (0.76–1.20)	0.687	70	16.8	1.14 (0.95–1.38)	0.168
Trend (per category)	2,749		1.00 (0.96–1.05)	0.897	644		0.97 (0.90–1.04)	0.367	2105		1.02 (0.97–1.08)	0.418
First child after or before first NSW[b]												
No NSW	2,509	16.5	1.00		569	25.0	1.00		1,940	14.7	1.00	
Nulliparous	40	15.9	0.96 (0.75–1.23)	0.759	12	22.1	0.88 (0.57–1.37)	0.578	28	14.7	1.00 (0.74–1.36)	0.990
1th childbirth after 1th NSW	82	15.6	0.94 (0.79–1.12)	0.503	30	22.0	0.88 (0.67–1.15)	0.347	52	14.2	0.97 (0.78–1.21)	0.796
1th childbirth before 1th NSW	119	16.3	0.98 (0.85–1.14)	0.823	33	25.1	1.00 (0.78–1.30)	0.979	86	14.5	0.99 (0.83–1.17)	0.890

NOTE: Log-lineal models are adjusted for: age, BMI, parity, HRT, family history of breast cancer, physical activity, tobacco and alcohol consumption, calorie intake, type of mammogram, radiologist, menopausal status (only for all women), and include screening center as a random term

[a]Adjusted geometric mean of percentage of MD derived from the multivariated log-lineal models.

[b]Two participants did not report the age at beginning and/or end of NSW.

[c]Three participants did not report the frequency of the shifts.

[d]$P_{heterogeneity}$ (interaction term between menopausal status and NSW frequency):0.029.

per week reported decreased. Compared with women reporting 5–7 night-shifts per week, risk of having high MD was significantly higher in those working 3-4 nights/week (e^{β}: 1.57; 95% CI, 1.11–2.21) or ≤ 2 nights/week (e^{β}: 1.83; 95% CI, 1.32–2.52). This trend was not observed in pre/perimenopausal women. There were no differences in MD by timing of first child birth.

Table 3. Association of mammographic density (MD) and exposure characteristics among night-shift workers (NSW) in DDM-Spain/Var-DDM study (2007–2008), overall and by menopausal status

	Total (*n*: 243)				Pre/Perimenopausal (*n*: 75)				Postmenopausal (*n*: 168)			
	n	**%MD[a]**	**e^{β} (95% CI)**	***P***	***n***	**%MD[a]**	**e^{β} (95% CI)**	***P***	***n***	**%MD[a]**	**e^{β} (95% CI)**	***P***
Years of NSW												
≤15 years	190	16.0	1.00		62	21.4	1.00		128	13.8	1.00	
>15 years	53	23.6	1.39 (1.05–1.83)	0.021	13	34.7	1.33 (0.82–2.16)	0.248	40	21.1	1.49 (1.07–2.07)	0.019
Trend (per 5-years)	243		1.02 (0.96–1.09)	0.485	75		0.95 (0.84–1.09)	0.481	168		1.05 (0.98–1.12)	0.164
Years since last NSW[b]												
>15 years	83	15.3	1.00		21	19.8	1.00		62	13.4	1.00	
5–15 years	46	15.1	0.98 (0.73–1.33)	0.920	17	15.0	0.77 (0.48–1.25)	0.295	29	16.2	1.24 (0.86–1.79)	0.246
1–5 years	30	21.7	1.42 (1.02–1.97)	0.037	10	26.5	1.40 (0.85–2.31)	0.191	20	19.6	1.47 (0.99–2.17)	0.053
Current or recent NSW (≤ 1 year)	82	20.2	1.25 (0.97–1.61)	0.083	27	34.0	1.79 (1.15–2.78)	0.010	55	16.2	1.07 (0.79–1.44)	0.662
trend (5-years decrease)	241		1.04 (1.00–1.09)	0.067	75		1.13 (1.03–1.23)	0.008	166		1.02 (0.97–1.07)	0.397
NSW frequency[c,d]												
5–7 nights/week	69	13.9	1.00		15	26.2	1.00		54	10.6	1.00	
3–4 nights/week	59	16.7	1.22 (0.91–1.64)	0.182	18	16.5	0.62 (0.37–1.01)	0.056	41	16.0	1.57 (1.11–2.21)	0.011
≤2 nights/week	112	20.6	1.42 (1.10–1.84)	0.008	42	26.4	1.02 (0.66–1.57)	0.922	70	19.8	1.83 (1.32–2.52)	0.001
Trend (per category)	240		1.19 (1.05–1.35)	0.008	75		1.06 (0.85–1.32)	0.609	165		1.34 (1.14–1.58)	0.001
First child after or before first NSW[b]												
Nulliparous	40	18.1	1.00		12	19.9	1.00		28	17.5	1.00	
1th child after 1th NSW	82	17.3	0.98 (0.66–1.47)	0.932	30	24.1	1.40 (0.72–2.72)	0.318	52	15.8	0.85 (0.53–1.37)	0.501
1th child before 1th NSW	119	17.5	0.97 (0.64–1.46)	0.870	33	24.0	1.06 (0.54–2.09)	0.862	86	14.6	0.84 (0.51–1.38)	0.487

NOTE: Log-lineal models are adjusted for: age, BMI, parity, HRT, family history of breast cancer, physical activity, tobacco and alcohol consumption, calorie intake, type of mammogram, radiologist, menopausal status (when appropriate), and for the other shiftwork variables (time since last NSW, usual frequency of the night-shifts, total years of NSW, parity status at the beginning of NSW), and include screening center as a random term.

[a]Adjusted geometrical mean of the percentage of MD derived from the multivariated log-lineal models.

[b]Two women with NSW≥ 1 years did not report the age at beginning and/or end of NSW.

[c]Three women with NSW≥ 1 years did not report the frequency of the shifts.

[d]$P_{heterogeneity}$ (interaction term between menopausal status and NSW frequency):0.006.

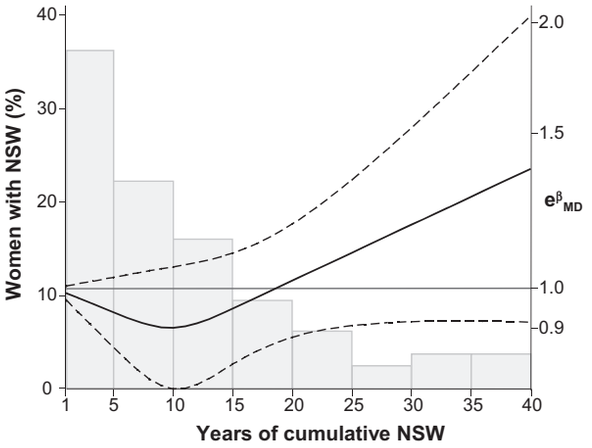

Figure 1.

Dose–response association between cumulative years of NSW and MD, in DDM-Spain/Var-DDM study (2007-2008)[a]. [a]e^{β} (95% CIs) obtained from a multivariate log-lineal–mixed model with ln-transformed percentage of MD as dependent variable, and based on restricted cubic splines for cumulative years of NSW with knots at 5, 10, and 15 years. e^{β} adjusted for age, BMI, parity, HRT, family history of breast cancer, physical activity, tobacco and alcohol consumption, caloric intake, type of mammogram, radiologist, menopausal status and including screening center as a random term. Lines represent the e^{β} (thick line) and 95% CIs (dotted lines). Bars represent distribution of cumulative years of night-shift work in the study participants

Finally, the analysis of the dose–response relationship for cumulative years of NSW is presented in Fig. 1. Starting from 15 to 20 years of cumulative NSW, the percentage of MD was higher than in women without NSW history, suggesting the existence of an exposure threshold, although departure from linearity was not statistically significant (*P*, 0.209).

Discussion

Up to date this is the only study examining NSW and MD carried out in general population. Our results show that having ever worked in NSW is not related to MD; however, performing NSW for more than 15 years is associated to higher MD, with no evidence of a lineal dose-response relationship. Our findings suggest that MD may play an intermediate role in the not fully understood association between long term NSW and breast cancer. In addition, our data indicate that the frequency of weekly night-shifts may affect MD, although the effect may be substantially different in pre/perimenopausal and postmenopausal women. Shorter total years of NSW or other characteristics of this exposure do not seem to be associated with MD.

As far as we are aware of, the relationship between MD and NSW has only been explored in a cross-sectional study with 640 middle aged nurses/midwives. In that study, there was no association with cumulated NSW, whereas women currently working in rotating night-shifts had non-significant higher MD and women with higher number of night-shifts per month had slightly lower MD than women with lower night-shift frequency. As 90% of the participants had performed NSW for over 5 years, the very low proportion of unexposed women could explain the lack of concordance with our findings (21).

Circadian disruptive NSW has been classified as probably carcinogenic to humans by the IARC (3). Even though some studies, particularly in Asian populations, have reported no association (26), most evidence shows an increased risk of breast cancer after NSW exposure (4–8, 27–31). Up to six meta-analyses published in recent years have reported risk ratios for breast cancer ranging from 1.06 to 1.21 (4, 28–32) after NSW exposure. Two of the most recent meta-analyses observed a dose-response effect, with an increase in breast cancer risk per 5-year rise in NSW exposure (28, 30). However, often the association was only observed when long exposure to NSW was considered (5, 8, 9, 33, 34). MD is a dynamic trait, but our results seem to suggest that prolonged exposures might induce effects in MD that could remain after the end of NSW, and supports the inclusion of MD in the biological pathways that connect long term NSW to breast cancer. In addition, we observed an inverse relationship between years since last NSW and MD among those that had ever had NSW, which could reflect a decreasing effect of the exposure with time, but this trend is only clear in premenopausal women.

There are several biological mechanisms that could contribute to the observed higher MD of long-term night-shift workers (see Supplementary Fig. S1). Recent research suggests that long-term exposure to NSW-related light at night (LAN) may induce epigenetic dysregulation of microRNA that are involved in apoptotic and proliferative gene networks in breast cancer cells (35). There is also the hypothesis that phase shift (alteration of the peripheral functions due to the desynchronization with sleep-wake cycle) may confuse the body's master clock and produce variations in the circadian clock genes expression, promoting cellular proliferation and breast cancer (36, 37). However, the association of MD with cellular proliferation markers such as Ki67 is not clear (38, 39).

Other possible mechanism is LAN-related melatonin suppression. Melatonin, which is regulated by the light/dark cycle, presents antioxidant, antimitotic, antiangiogenic and antiestrogenic activity (10). Night-shift workers have substantially reduced 6-sulfatoxy melatonin levels during night work and daytime sleep, remaining low even when sleeping at night (40, 41). However, recent studies have not found any association between this biomarker and breast cancer risk (42, 43), or with MD (44).

Growth hormone (GH) and insulin-like growth factor 1 (IGF -1) may also play a role in the NSW and MD association. GH, which stimulates IGF-1 production, is secreted in a pulsatile fashion, with sleep and exercise being their strongest physiologic stimuli (45). Sleep deprivation due to NSW can desynchronize GH release timing. Individuals adapted to NSW experience lower GH nocturnal release, more frequent daily pulses and sporadic random releases (46). GH and IGF-1 promote breast cell proliferation and both have been associated with higher MD among women with high levels of estrogen (47).

Vitamin D could also mediate the association between MD and NSW as shift workers may have lower vitamin D levels due to reduced sun exposure (36, 37). Vitamin D is known to inhibit cell proliferation and induce apoptosis and differentiation (48). Inconclusive evidence links high circulating vitamin D levels with lower breast cancer risk (49) and it has also been hypothesized

that it could reduce MD (50). It should be noted that our participants had low intake of vitamin D, as reported in a prior paper (51), but these levels did not differ by NSW history and the inclusion of vitamin D consumption in the model did not change our results

Lifestyle factors may also mediate the association between NSW and MD. There is some evidence that night-shift workers are more likely than others to smoke, to be less physically active, and to report poor diet choices (52), three known breast cancer risk factors (53–55). Indeed, in our sample night-shift workers were more likely to smoke and report a slightly, although non-significant, higher caloric intake than other workers. However, those in NSW had lower BMI, and alcohol consumption and physical activity did not differ between the two groups. In any case, our results are adjusted for these lifestyle factors, so they are not likely to explain the association observed.

Interestingly, number of night-shifts per week had different effects on pre/peri- and postmenopausal women. Postmenopausal women working 5 to 7 night-shifts per week had even lower MD than those unexposed to NSW. Among women exposed to NSW, two or less night-shifts per week were associated with higher MD. Five to seven shifts per week may correspond to regular night-shift workers who may not be at higher breast cancer risk according to a recent meta-analysis of cohort studies. This meta-analysis concluded that women in rotating NSW showed an increased risk of breast cancer whereas women with regular night work showed a marginally statistically significant decrease in breast cancer risk (28); a differential effect explained by variations in adaptation to NSW (56). In contrast, our results for pre/perimenopausal participants present a different pattern: MD was slightly higher in women working 5 to 7 night shifts per week compared with those unexposed to NSW.

This study has several strengths. The population-based nature of the study sample and the relatively high participation rate of women to breast cancer screening network in the Spanish regions analyzed (23) support the external validity of our results. In fact, according to the data supplied by the Spanish National Health Survey (57) our participants were very similar to the national population in the same age range in terms of lifestyle factors such as smoking habits, alcohol consumption, use of HRT and prevalence of night-shift workers (2). With regard to MD estimations, we have used a validated software to obtain a continuous measure of MD which allows to explore associations more accurately, as recommended by previous authors (58). We would like to emphasize that DDM-Spain logistic organization made it possible to use mammograms performed as part of regular clinical practice, that is, no additional mammograms were performed for this study.

Our study also has several limitations. First, we have a reduced number of NSW women in the subgroup of long-term exposure. Second, our sample reflects the Spanish target population of a breast cancer screening program (women between the ages of 45 and 68 years), so we had relatively small numbers of premenopausal women. This limited the power of our analyses to assess possible effect modification due to menopausal status. Third, as a cross-sectional study, changes in MD patterns across time and through changes in NSW could not be investigated. Fourth, NSW exposure information was self-reported and collected retrospectively and, thus, susceptible to recall bias. Nevertheless, since density assessment was blind and anonymous, any recall bias would not be differential. In turn, it may result in an underestimation of the effects studied. In addition, the possibility that the associations found could be due to other confounders not included in the analysis (e.g., sleep duration) can never be ruled out, although we made sensitivity analyses to evaluate the more plausible candidates. Fifth, we combined different images (digital and digitized digital or analogical images) as well as the estimations of two radiologists. We have adjusted for these possible sources of error by including these variables (type of mammogram and radiologist) into the model. Finally, we lack sufficient information to distinguish between rotating or permanent shifts, and information on chronotypes and sleep characteristics was not registered.

Our results show that long-term NSW is associated with MD, which could play an intermediate role in the still not fully understood relationship between NSW and breast cancer. Future studies with more detailed information on NSW and with larger sample size for night shift workers, especially pre/perimenopausal ones, are needed to confirm our results and to help identify the biological pathways involved in this association.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Acknowledgments

We would like to thank the participants in the DDM-Spain/Var-DDM-Spain study for their contribution to breast cancer research. Other members of DDM-Spain/Var-DDM: Gonzalo. López-Abente, Roberto Pastor-Barriuso, Pablo Fernández-Navarro, Anna Cabanes, Soledad Laso, Manuela Alcaraz, María Casals, Inmaculada Martínez, Juan Carlos Pérez Cortés, Joaquín Antón, Nieves Ascunce, Isabel González-Román, Ana Belén Fernández, Montserrat Corujo, Soledad Abad, and Jesús Vioque.

Grant Support

A.M. Pedraza-Flechas FI14CIII/00013 PFIS; B. Perez-Gomez FIS PS09/0790; M. Pollán FIS PI060386, EPY1306/06 collaboration agreement between AstraZeneca and ISCIII, and FECMA 485 EPY 1170–10; R. LLobet Gent per Gent Fund (EDEMAC Project); All authors: European Regional Development Fund (FEDER).

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Received June 20, 2016; revised July 8, 2016; accepted January 13, 2017; published OnlineFirst March 23, 2017.

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