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Short term effects of night shift work on risk of overall breast cancer and breast cancer classified by oestrogen and HER2 receptor status

PhD dissertation

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PREFACE

The studies presented in this PhD thesis were carried out at the Department of Occupational Medicine, Danish Ramazzini Centre, Aarhus University Hospital, Denmark between 2011 and 2015.

I wish to thank my supervisors, Henrik Kolstad, Anne Helene Garde, and Peer Christiansen, who have been very enthusiastic about and engaged in all the studies. I also wish to thank my other co-authors, Morten Frydenberg, Jens Peter Bonde, Johnni Hansen, and Åse Marie Hansen who have all been very engaged in the studies and providing many different points of view. A special thank to Morten Frydenberg for your engagement, help, and critique – it has been priceless.

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Helene Tilma Vistisen

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THIS THESIS IS BASED ON THE FOLLOWING PAPERS:

I. Short term risk of breast cancer following night shift work: a cohort study of pay roll data (Manuscript)

II. Short term effects of night shift work on risk of breast cancer classified by oestrogen and HER2 receptor status (Manuscript)

III. Short term risk of breast cancer following consecutive night shifts: a cohort study of pay roll data (Manuscript)

ABBREVATIONS

CI: Confidence interval

RR: Risk ratio

ER: Oestrogen receptor

ER-: Tumours that lack oestrogen receptors

ER+: Oestrogen dependent tumours

PR: Progesterone

PR-: Tumours that lack progesterone receptors

PR+: Progesterone dependent tumours

HER2: Human epidermal growth factor 2

HER2-: Tumours that lack the expression of human epidermal growth factor 2

HER2+: Tumours over expressing the human epidermal growth factor 2

CONTENTS

INTRODUCTION AND BACKGROUND

Breast cancer is the most frequent diagnosed cancer among women worldwide and the most common cause of deaths among women.(1) Several risk factors for breast cancer have been recognized, such as familial history of breast cancer, prolonged exposure to endogenous estrogens, exogenous hormone use, alcohol use, overweight, and physical inactivity.(2, 3) However, the majority of breast cancer cases cannot be accounted for by these risk factors.

In 2007 the International Agency for Research on Cancer (IARC) classified night shift work that involves circadian disruption as a risk factor for breast cancer as they concluded that "*Shift work that involves circadian disruption is probably carcinogenic to humans*".(4)

CIRCADIAN DISRUPTION

The endogenous circadian rhythm is generated by the master pacemaker in suprachiasmatic nuclei.(5, 6) Several biologic markers follow an approximately 24-hour rhythm and especially melatonin is a strong predictor of the circadian rhythm.(5, 6) Melatonin is a hormone produced by the pineal gland during the night. Light suppresses melatonin production and daytime melatonin production is near to nil. As a consequence of the endogenous circadian rhythm, the nocturnal melatonin release persists even during night time light exposure though attenuated.(7, 8) This nocturnal melatonin rhythm may be shifted as an attendant to repeated exposure to light at night causing disequilibrium of the circadian rhythm with the peripheral oscillators in the tissues throughout the body during the following days. The new rhythm can be synchronized within few days. Disruption of the circadian rhythm is predominantly characterized by the relationship between melatonin attenuation and phase shifting.(9, 10)

The degree of nocturnal melatonin suppression is dependent on the intensity of the light level and the wavelength where the suppression is more pronounced at high light intensities and short wavelengths (blue light).(5, 6) Furthermore, the amplitude

and phase of nocturnal melatonin is also dependent of the daytime exposure to light and the timing of the exposure.(11, 12)

Anticancer properties of melatonin

Experimental animal studies have shown that melatonin possess anti cancer properties.(5, 7, 10, 13-15) These studies have shown a strong evidence of nocturnal melatonin in uninterrupted darkness to inhibit the growth of established tumours.(7, 16, 17) This may serve as a protective mechanism against the growth of breast cancer and may result in non-clinically detectable neoplasm.(7, 18-20) Furthermore, the malignant progression seems to additionally increase as malignant tumours become more advanced.(7) Hence, nocturnal melatonin suppression may act as a promoter of carcinogenesis and is thus, expected to exert its effect on significant tumour growth in the years preceding clinical detection and diagnosis.

MELATONIN DURING NIGHT SHIFT WORK

Shift work and night shift work in particular has been used as a surrogate for circadian disruption and suppression of nocturnal melatonin in epidemiological studies of breast cancer. A non-day shift may induce some degree of circadian disruption, but, a night shift is assumed to be the most disruptive shift.(9) Several studies have examined melatonin levels in relation to night shift work.(21-35) Schernhammer et al, Peplonska et al, and Mirick et al observed a lower level of melatonin among night shift workers.(22, 31, 32) A decreased melatonin level during night shift work were observed in most studies (26, 29, 34, 35), but not all.(27, 28, 30) Only few studies have examined the phase of melatonin; these observed a phase shift following consecutive night shifts.(21, 33-35). Hence, melatonin suppression may occur as a result of a single night shift (24) whereas several consecutive night shifts are required for a phase shift to occur. Night shift workers may experience a circadian rhythm that is out of synchrony during the first days of night shift work as well as the first days subsequent to night shift work.(21, 33, 36, 37)

NIGHT SHIFT WORK AND BREAST CANCER

Several epidemiologic studies have examined the association between night shift work and breast cancer.(38-59) These included 9 case-control studies (39, 40, 42, 46, 51, 52, 54, 58, 59), 6 nested case-control studies (43, 48-50, 57), and 8 cohort studies.(38, 41, 44, 45, 47, 53, 55, 56) Various definitions of night shift work have been used across the studies:

- Graveyard shifts and overnight shifts (39, 42, 46-55, 57, 58)
- Rotating night shifts (41, 44, 48, 57, 58)
- Night shifts without precise definitions.(38, 40, 41, 43-45, 56, 59)
- In addition, permanent night shift work has been studied in a few studies(48, 58)

Various exposure metrics have also been used in analyzing the risk:

- Standard incidence rate (38, 45)
- Duration including both short term exposure (<10 years) (39, 42, 44, 46, 48-51, 54, 56, 58) and long term exposure (≥20 years) (41, 43, 44, 46-49, 51, 52, 56)
- Cumulated number of night shifts (46-48, 57, 58)
- The timing of exposure, i.e. different exposure windows (39, 46, 51)
- Consecutive night shifts (49) or other models assumed to cause phase shift (51)
- Frequency of night shifts (47, 50, 54)
- A few studies only included analysis of never versus ever night shift work (53, 59)

This heterogeneity across studies has been considered an Achilles heel for the interpretation and comparison of results across the studies in five out of six previous reviews.(60-65) In addition, Kolstad, Jia et al, Ijaz et al, and Kamdar et al also considered the self-reported information on night shift work a limitation in the studies as well as the high amount of case-control studies.(60-63) Megdal et al included 13, of which 7 were studies on fly attendants, in a meta analysis and observed a significant increase of breast cancer among night shift workers.(64) Kolstad concluded, based on 8 studies, that the evidence to support a causal association between night shift work and breast cancer was insufficient.(63) This was also the conclusion of the meta-analysis of both Ijaz et al (16 studies) (60) and

Kamdar et al (15 studies including fly attendants).(62) However, the meta-analysis of Jia et al (13 studies) (61) and Wang et al (10 studies) (65) concluded that there was an indication of an association between night shift work and breast caner. The reviews of Jia et al, Ijaz et al, Kamdar et al, and Wang et al were all published in 2013. The most recent studies of Fritschi et al (51), Grundy et al (52), Koppes et al (55), Åkerstedt et al (56), Li et al (57), Papantoniou et al (58), and Wang et al (59) were not included in these reviews. Grundy et al observed a statistically significant increased risk of breast cancer at ≥30 years of night shift work (odds ratio (OR) 2.21 with a 95% confidence interval (CI) of 1.14 to 4.31).(52) A borderline significant increased risk for ever night shift work was observed by Wang et al (OR 1.34, 95% CI 1.05 to 1.72) (59) and Fritschi et al (phase shift OR 1.22, 95% CI 1.01 to 1.47).(51) An increased by not significantly increased risk was observed by Papantoniou et al for ever night shift work (OR 1.18, 95% CI 0.97 to 1.43) (58) and by Åkerstedt et al for ≥20 years of night shift work (Hazard ratio (HR) 1.68, 95% CI 0.98 to 2.88).(56) Li et al and Koppes et al did not observe any association.(55, 57) These seven recent studies relied on self-reported information on night shift work, and as described earlier in this section, they also used different night shift definitions and exposure risk assessments, and the results are inconsistent. Thus, they are subject to the same limitations as pointed out by the recent reviews (60-63) and the need for cohorts with objective and individual information on night shift work is still existing.

Recent night shift work

As mentioned earlier, there is evidence that nocturnal melatonin suppression may act as a promoter of carcinogenesis. This promoter of tumour growth is expected to be exerted in the years preceding clinical detection and diagnosis thus the most recent years prior to diagnosis.

The effect of recent night shift work has been studied by Davis et al, Pesch et al, and Fritschi et al.(39, 46, 51) Davis et al examined the risk of breast cancer during the ten years prior to diagnosis and observed an increased OR of 1.6 (95% CI 1.0 to 2.5) among women who worked graveyard shifts during the recent ten years.(39) Pesch et al examined the risk by years since last night shift and observed an OR of 1.10 (95% CI 0.51 to 2.38) for current night shift workers, and an OR of 1.04 (95% CI 0.31

to 3.53) among women with their last night shift between less than a year and nine years.(46) However, these confidence intervals were too broad to contribute to the association. Fritschi et al examined different exposure windows prior to diagnosis and observed an OR of 1.02 (95% CI 0.73 to 1.43) among women working graveyard shifts during the recent ten years, and an OR of 1.23 (95% CI 0.83 to 1.83) among women exposed to phase shifts during the recent ten years.(51) Overall these findings may indicate an association between recent night shift work and the risk of breast cancer.

Consecutive night shifts

As described earlier, nocturnal melatonin suppression may occur as a result of a single night shift whereas a phase shift of melatonin require more consecutive nights to occur. As this phase shift is suggested to cause the circadian to become out of synchrony, consecutive night shifts are believed to be an important shift domain to capture in epidemiologic studies of breast cancer.(9) This is supported by the only epidemiological study that has examined the effect of consecutive night shifts on the risk of overall breast cancer (49) as well as subtypes of breast cancer.(66) In the study of overall breast cancer (49), a statistically increased risk of breast cancer among women who worked five or more years in schedules including 6 six or more consecutive night shifts (OR 1.8, 95% CI 1.1 to 2.8) was observed. In addition, the observations indicated an increased risk by increased number of consecutive night shifts. In the same study they did not find a significant association between the duration of any night shifts (the highest OR being 1.1 for ≥30 years of night shifts), or by the cumulative number of night shifts (the OR was 1.2 for both <1007 night shifts and ≥1007 night shifts). Thus, this study indicates, that the risk of breast cancer may be related to the number of consecutive nights.

BREAST CANCER

Breast cancer is a heterogeneous disease with different biology, pathology, and prognosis according to the different tumour subtypes.(67) Breast cancer tumours can be classified into intrinsic subtypes with a variety of clinical and pathological

features: luminal A, luminal B, epidermal growth factor 2 (HER2) over expression, and basal like which is also denoted triple negative tumours.(68-71) In clinical settings, status of oestrogen receptors (ER), and HER2.(72, 73) Progesterone receptor (PR) has been used in the classification of breast cancer subtypes, however, PR status has shown to be strongly associated with ER status and has limited predictive value compared to ER status.(70, 73, 74) Luminal A (ER+/HER2-) tumours are slow growing, have high survival rates, and account for the majority of breast cancer cases (~ 40%).(68) Tumours over expressing HER2 (HER2+) (~ 10%) as well as triple negative tumours (ER-/HER2-) (10-20%) grow and spread more aggressively and the patients are younger at the time of diagnosis.(68) Luminal B represents a subgroup of HER2+ tumours that are ER+.(73) These tumours (10-20%) have high proliferation rates and a worse prognosis than luminal A tumours.(68)

Risk factors

Older age, high social economic status, family history of breast cancer or ovarian cancer, late age at first pregnancy, and prolonged exposure to estrogens are well established risk factors for overall breast cancer.(2, 3) Oestrogen is released during the menstrual cycle and thus, early menarche, late menopause, and no or few pregnancies increases the exposure to oestrogen as do use of sex hormones like oral contraception and hormone replacement treatment.

Prolonged exposure to oestrogen has different effects on breast cancer subtypes and is most consistent for the hormone dependent tumours.(68, 75, 76) The most distinct effects on breast cancer risk by breast cancer subtypes is shown for age, age at first pregnancy, the number of pregnancies, family history of breast cancer and ovarian cancer, and hormone replacement treatment.(77-79) Thus, breast cancer subtypes may have distinct etiologic pathways and point to the importance of accounting for these subtypes when examining new risk factors.

One of the mechanisms suggested to link nocturnal melatonin suppression and increased breast cancer risk, is through an increase of oestrogen production which especially should increase the risk of oestrogen dependent (ER+) tumours.(80) In experimental studies, nocturnal melatonin suppression has been shown to increase

tumour growth in HER2+ tumours (81-83), but there is no established mechanisms linking melatonin and HER2. This is also the case for triple negative breast cancer. However, due to the aggressive tumour doubling times of HER2+ and triple negative tumours (77-79), a more pronounced effect of recent night shift work on these subtypes could be expected - if an association between night shift work and breast cancer exists.

The association between night shift work and breast cancer subtypes has been studied in several previous studies.(41, 42, 52, 54, 58, 59, 66, 84) Schernhammer et al and Wang et al supported the hypothesis that night shift work (as a surrogate for nocturnal melatonin suppression) increases the risk of ER+ tumours.(41, 59) Grundy et al observed an increased risk of ER+/PR+ tumours among women who worked ≥30 years of night shift work.(52) Papantoniou et al observed an increased risk of ER+ tumours among night shift workers, but this was not statistically significant.(58) Lie et al, Menegaux et al, O'Leary et al, and Rabstein et al did not observe an association between night shift work and ER+ breast cancer.(42, 54, 66, 84) In contrast, Rabstein et al observed an increased risk of ER- breast cancer.(84) Only Wang et al and Papantoniou et al included information on HER2 status.(58, 59) Wang et al observed increased risks of both HER2- and HER2+ breast cancer, but only HER2+ was statistically significant.(59) Papantoniou et al also observed increased risks of both HER2- and HER2+ breast cancer, but these were not statistically significant.(58)
The studies are few, the results were inconsistent, and only two studies included HER2 status and none of the studies examined when the exposure occurred relative to diagnosis.

The melatonin hypothesis has been suggested to be associated with oestrogen dependent tumours through an increase in oestrogen production. Experimental studies have observed increased growth of HER2+ tumours following melatonin suppression. There is no established hypothesis linking triple negative tumours and night shift work and there no experimental studies that have examined this.

However, if there is an association between night shift work and HER2+ and triple negative tumours, then the effect of recent night shift work may be more pronounced for these fast growing subtypes.

THE DANISH WORKING HOUR DATABASE

With the newly established database of the Danish Working Hour Database, a new opportunity to scrutinize the association between night shift work and breast cancer has emerged. The data are unique as they are based on pay-roll data from all employees in the Danish Regions and, thus encompass a majority of the public healthcare professionals in Denmark. Besides healthcare professionals, the Regions also employ administrative and service personnel among others. The pay-roll data include individual information on personal identification number, occupation, seniority, the date, hour, and minute for the beginning and end of every work duty. Hence, night shift exposure can be scrutinized in every possible way within this cohort. The only limitation so far is the available data period which includes data as of January 1, 2007.

SYNTHESIS AND HYPOTHESES

There is strong evidence from experimental studies that inhibition of nocturnal melatonin is related to increased tumour growth and proliferation in existing tumours. Thus, night shift work as a surrogate of nocturnal melatonin suppression may act as a promoter of carcinogenesis and lead to clinical detectable tumours and diagnosis. Previous studies of the effect of recent night shift work are few, but they may indicate an association between recent night shift work and breast cancer.

Breast cancer is a heterogeneous disease and distinct etiologic pathways are suggested for the individual breast cancer subtypes. Clinically, breast cancer is most frequently divided into ER+/HER2- (luminal A), HER2+, and ER-/HER2- (triple negative breast cancer). Night shift work has been suggested to increase the risk of oestrogen dependent tumours though an increase in the oestrogen production. Experimental studies have shown to increase growth of existing HER2+ tumours.

There are no established mechanisms linking melatonin and HER2+ tumours as well as melatonin and triple negative tumours. However, as both HER2+ and triple negative tumours are aggressive in terms of growth, the effect of recent night shift work is expected to be more pronounced among these breast cancer subtypes.
A single night shift may suppress melatonin and cause some circadian disruption whereas consecutive night shifts are expected to cause a phase shift and a circadian rhythm that are out of synchrony with the peripheral oscillators in the tissues during the following days. Hence, consecutive night shifts are believed to be an important domain to capture when studying night shift work and breast cancer.

Based on the evidence presented in this section as well as on the available data from the Danish Working Hour Database, the aim of this thesis was to test the following hypotheses:

1. Does recent night shift work increases the risk of overall breast cancer?
2. Does the effect of recent night shift work differ by breast cancer subtypes?
3. Does consecutive night shifts increase any risk of overall breast cancer and breast cancer subtypes additionally?

AIMS OF THE THESIS

STUDY I:

The aim of this study was to examine the effect of recent short term night shift work on the risk of breast cancer.

STUDY II:

The aim of this study was to examine if the association between recent night shift work and breast cancer differs by tumour subtypes defined by ER and HER2 status.

STUDY III:

The aim of this study was to assess the association between number of consecutive night shifts and HER2+ breast cancer as well as overall breast cancer.

METHODS

DATA SOURCES

Denmark has an early tradition for registration of residents such as family connections, birth, death, residence, immigration, and emigration. As of 1968 the civil registration number has been given to all individuals living in Denmark.(85) The individual registration has been expanded over time and now provides a unique and excellent opportunity for epidemiological research with the civil registration number as the key element in all national registers.(86)

The studies in this thesis are based on information from seven Danish registries linked on individual level by the civil registration number:

THE DANISH WORKING HOUR DATABASE:

- Encompasses all employees of the five Danish Regions. A majority of the employees are health professionals. Data are based on individual pay-roll information with information on job title classified by the Danish version of the International Standard Classification of Occupations (DISCO), As well as day, hour, and minute of the start and end of every work shift.
- *Available data period:* Data are available as of January 1, 2007. Updated annually.
- *Retrieved data period:* January 1, 2007 to December 31, 2012.

THE CIVIL REGISTRATION SYSTEM:

- Encompasses all individuals living in Denmark since 1968 with information on date of birth, sex, parents, siblings, children, partner, and vital status.(87)
- *Available data period:* Data are available as of 1968. Updated continuously.
- *Retrieved data period:* 1968 to December 31, 2012.

THE CLINICAL DATABASE OF THE DANISH BREAST CANCER COOPERATIVE GROUP:

- Encompasses all women diagnosed and treated with breast cancer since 1977. Includes date of biopsy and operation as well as pathological and clinical information on the tumours.(88)
- *Available data period:* Data are available as of 1977. Updated continuously.
- *Retrieved data period:* 1977 to December 31, 2012.

THE NATIONAL CANCER REGISTRY:

- Encompasses date of diagnosis and type of cancer for all cancers diagnosed since 1943. The cancers are classified according to ICD-7 and ICD-10 (the International Classification of Diseases).(89)
- *Available data period:* Data are available as of 1943. Updated annually.
- *Retrieved data period:* 1943 to December 31, 2011. Data were not available for 2012 at the time of retrieval.

THE NATIONAL REGISTER OF MEDICINAL PRODUCT STATISTICS:

- Encompasses date of purchase and ATC kodes (the Anatomical Therapeutic Chemical Classification System) for all purchases of prescription drugs at private pharmacies.(90)
- *Available data period:* Data are available as of 1995. Updated monthly.
- *Retrieved data period:* 1995 to December 31, 2012.

THE FAMILY INCOME REGISTRY:

- Includes information on the highest educational level in a family living at the same address.(91)
- *Available data period:* Data on the educational level in families are available as of 2000. Updated annually.
- *Retrieved data period:* 2007 to 2012

THE CLINICAL DATABASE OF MAMMOGRAPHY SCREENING:

- Encompasses all women invited to participate in the national mammography screening programme. Includes date of invitation and date of examination since the start of the programme by the end of 2007. All women between age 50 and 69 are invited to participate.(92)
- *Available data period:* Data are available since the start of the programme in ultimo 2007. Updated continuously.
- *Retrieved data period:* 2007 to December 31, 2012.

The Danish Data Protection Agency approved the study (j.no. 2011-41-6850). In Denmark, register studies do not need to be approved by the Danish Health Research Ethics Committee System.

POULATIONS

In the Danish Working Hour Database, women with at least one registration of work between January 1, 2007 and December 31, 2011 and who were 18 years or older on the date of their first registration of work were identified (n=156,927). One woman was excluded due to missing date of breast cancer diagnosis, and 1357 women were excluded due to breast cancer diagnosis prior to the date of the first registration of work. Hence, a total of 155,569 women were identified.

No information on working time prior to 2007 was available. The first date of employment in the Region at January 1, 2007 or later was used to obtain a population which was more likely to have a more complete night shift history (the inception population). It is possible that a woman had been employed in another Region before 2007, but unfortunately this information was not available. Among the 155,569 women a total of 71,479 women had their first date of employment in the Region at January 1, 2007 or later.

BREAST CANCER

From the Danish Breast Cancer Cooperative Group breast cancer cases and date of diagnosis were identified for all available years (1977 to 2012) and supplemented

with breast cancer cases and date of diagnosis from the National Cancer Registry (ICD10=DC50 or ICD7=170) for al available years (1943 to 2011).

NIGHT SHIFT WORK

A night shift was defined as at least three hours of work between midnight and 05:00 AM as recommended by the 2009 IARC Working Group.(9) Thus, a woman working at least three hours between midnight and 05:00 AM on a specific date was classified as exposed to a night shift on this particular date and otherwise classified as not exposed to a night shift on this date.

COVARIATES

The registries provided individual information on the following potential confounders: calendar year (2008 to 2012), age (<40, 40-44, 45-49, and every second year from age 50), age at birth of the first child (<20, 20-29, ≥30, no children), number of births (0, 1, 2, 3, ≥4), female 1st degree relatives with breast cancer before age 50 or ovarian cancer at any time (0, ≥1 , no information), oral contraception, ATC: G03A (no, yes), hormone replacement therapy, ATC: G03c, G03D, G03F (no, yes), other sex hormones, ATC: G03B, G03G, G03H (no, yes), medication related to alcoholism, ATC: N03AA, N05AB, N07BB (no, yes), mammography screening attendance (invited but not screened, invited and screened, not invited), and highest family educational level at the first registration of work (unspecified, primary and secondary school, advanced level education, vocational education, undergraduate and bachelor degree, higher education, and no information on education). These potential confounders were defined a priori based on a review of the literature (2, 3).

STATISTICAL ANALYSIS

In all the analysis conducted in the three studies, the basic concept of the analysis was the same.

We had date on every exposure, covariates, and breast cancer diagnosis. This made it possible to individually evaluate changes in exposure group, covariates, and breast

cancer diagnosis for every single day during follow-up. End of follow-up was determined by the date of first primary breast cancer diagnosis, death, disappearance, emigration, or end of follow-up at December 31, 2012. The association between night shift work and the incidence of breast cancer was estimated by rate ratios (RR). Both crude and adjusted estimates were reported, where the adjusted models included the potential confounders described in previous section. All data management and data analysis were done using Stata 13.1.

Data were complete for all variables except for female first degree relatives (5% missing) and highest family educational level (<0.5% missing). The missing values were evenly distributed across night shift groups.

STUDY I

Night shift work

Five different exposure windows of recent night shift work were examined: from the previous one year to the previous five years. The cumulated amounts of night shifts were grouped with respect to the distribution of person days at risk.

Statistical analysis

As a consequence of the exposure windows examined (from the last years to the last five years) follow-up started one to five years after the first registration of work, respectively (Figure 1). Thus, the earliest start of follow-up was not before January 1, 2008. Data were analysed as incidence rate, i.e. as the number of incident breast cancer cases per time units at risk using Poisson regression. Interactions were not included in the models because of no a priori hypotheses of such effects. Estimates were reported with a 95% confidence interval. Trend analyses were done across the unexposed and exposed groups, and a comparison was made between the low exposure (1 to 29 night shifts) and high exposure (≥30 night shifts).

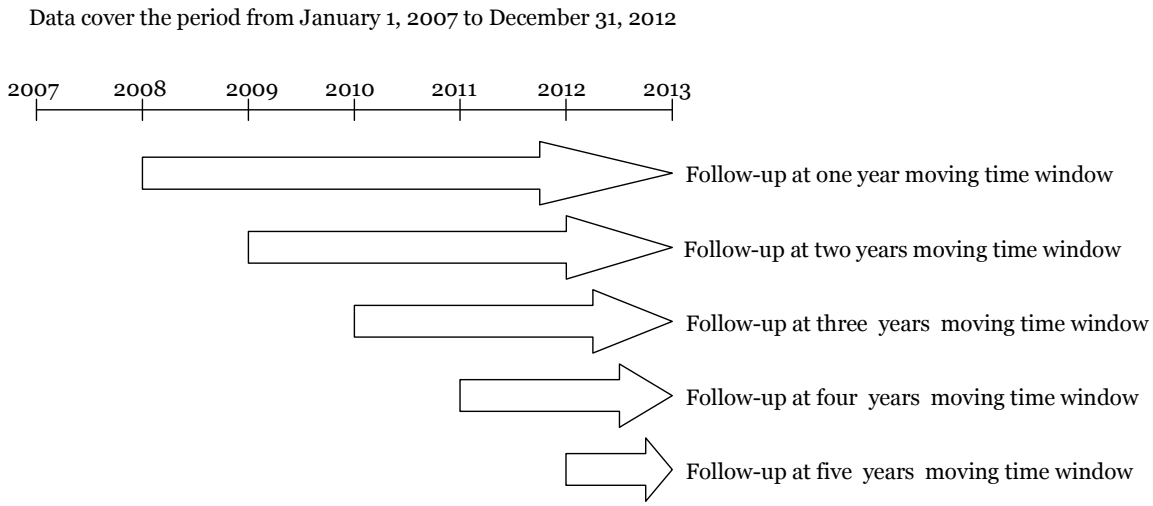

Figure 1 The follow-up periods with respect to the moving time windows.

Supplemental analysis (Study I)

The aim of the inception population was to eliminate the possibility of night shift work prior to follow-up as some studies have observed significant increased risk of breast cancer following long term night shift work. We used the first employment date or the first date of registration of work (whichever came first) in the present Region to identify women who were most likely to have their first employment during follow-up. However, there is a possibility that some women in the inception population might have been employed elsewhere prior to 2007. Therefore, to further scrutinize the effect of recent night shift work on the risk of breast cancer and to further eliminate the possibility of night shift work prior to follow-up affecting the results, I have included a supplementary analysis based on a subpopulation of women employed in occupations which are unlikely to include night shift work, and women employed in occupations with a high prevalence of night shift work. This analysis is not presented in Paper I.

Women who at study entry (the first registration of work) were employed as either office workers (DISCO: 25, 16, 33, 34, 41, and 43), nurses (DISCO: 2221), or midwifes (DISCO: 2222) were identified and included a total of 69,864 women.

The exposure status in this population was defined as: 1) Office workers, 2) nurses/midwifes with no night shifts, and 3) nurses/midwifes with ≥1 night shift during follow-up. Thus, the second category contributed person time in this group until her first night shift after which she contributed person time in the last category until the end of follow-up.

Follow-up started at the first registration of work the earliest being January 1, 2007. Data were analysed as the number of incident overall breast cancer cases per time units at risk using Poisson regression.

STUDY II

Night shift work

From study entry the amounts of night shifts were summed day by day and categorized in respect to a reasonable number of person time, but also to maintain the same categories in all the analyses in the total population as well as in the inception population.

Breast cancer

Information on ER and HER2 status was obtained for breast cancer cases in the period January 1, 2007 to December 31, 2012 from the clinical database of the Danish Breast Cancer Cooperative Group. The cases were stratified into subtypes according to the Danish clinical guidelines (93): 1) Tumours that were ER+ and HER2- (ER+/HER2-), 2) tumours that were HER2+ regardless of ER status, and 3) tumours that were ER- and HER2- (ER-/HER2-). ER tumours were defined using a cut off at 10% positive oestrogen cells. HER2 status was based on immunohistological markers from 0 to 3+, where 2+ is regarded 'equivocal', and 3+ as positive. For HER2 2+ cases the immunohistological test was supplied with fluorescence (FISH test), or chomogenic in situ hybridization (CISH test), and tumours were regarded as positive if oncogenic amplification was found.(94)

PR status is strongly related to ER status and not as strongly a predictor as ER. Therefore, PR has not been routinely analyzed in Denmark since 2007 and were only available for a subset of the cases and therefore not used in the analyses.

Statistical analysis

Follow-up started at the first registration of work the earliest being January 1, 2007. A combined analysis was conducted to test if the effects of night shift work differed by breast cancer subtypes. This analysis was made by stacked Poisson regression based on a table combining person years at risk and number of events for: ER+/HER2-, HER2+, ER-/HER2-, and unclassified tumours with no receptor status available.

As age, age at first pregnancy, the number of pregnancies, family history of breast cancer and ovarian cancer, and hormone replacement treatment has different effect in the breast cancer subtypes.(77-79) The potential confounders were divided into two sets:

A) Age (<40, 40-44, 45-49, and every second year from age 50), age at birth of the first child (<20, 20-29, ≥30, no children), number of births (0, 1, 2, 3, ≥4), female 1st degree relatives with breast cancer before age 50 or ovarian cancer at any time (0, ≥1, no information), hormone replacement therapy, ATC: G03c, and G03D, G03F (no, yes)

B) Calendar year (2008 to 2012), oral contraception, G03A (no, yes), other sex hormones, G03B, G03G, G03H (no, yes), medication related to alcoholism, ATC: N03AA, N05AB, N07BB (no, yes), mammography screening attendance (invited but not screened, invited and screened, not invited), and highest family educational level at the first registration of work (unspecified, primary and secondary school, advanced level education, vocational education, undergraduate and bachelor degree, higher education, and no information on education).

In the adjusted models the effects of the covariates in set A were allowed to differ between breast cancer subtypes while the covariates in set B were assumed to have the same effect on the rate independently of the subtype.

STUDY III

Night shift work

Every event of two to seven consecutive night shifts were identified. Three comparison groups were used:

1) Never night shift work
2) Night shift work without ≥2, ≥3, ≥4, ≥5, ≥6, or ≥7 consecutive nights, respectively
3) Night shift work with ≥2, ≥3, ≥4, ≥5, ≥6, or ≥7 consecutive nights, respectively.

A woman contributed person time to the first category until the date of her first night shift and in this group until the date of her first event ≥2, ≥3, ≥4, ≥5, ≥6, or ≥7 consecutive nights. She contributed to the highest attained of the latter categories until the end of follow-up.

In addition to the consecutive night shift groups, the amount of night shifts were calculated and categorized into three groups: 1-29, 30-99, and ≥100 night shifts.

Breast cancer

Overall breast cancer cases as well as HER2+ breast cancer cases were obtained and categorized in accordance with Study II.

Statistical analysis

Follow-up started at the first registration of work the earliest being January 1, 2007. Data was analysed as the number of incident overall breast cancer cases and incident HER2+ breast cancer cases per time units at risk using Poisson regression. Separate analyses were conducted for each consecutive night shift category: ≥2, ≥3, ≥4, ≥5, ≥6, and ≥7 consecutive night shifts. These separate analyses were also conducted for each of the three night shift groups: 1-29, 30-99, and ≥100 night shifts for HER2+ breast cancer.

SUMMARY OF RESULTS

Of the 155,569 women in the total study population, a substantial part of these were health professionals like nurses, midwifes, doctors, and physiotherapists (40%) and personal care workers like health care assistants (23%), Table 1.

Table 1 Occupations among the study population of 155,569 women employed in the Danish Regions, 2007 to 2012.

Occupation	DISCO	Persons	%
Managers	1	195	0.1
Professionals	20	295	0.2
Science and engineering professionals	21	262	0.2
Health professionals	22	61,392	39.5
Teaching professionals	23	6,852	4.4
Information and communications technology professionals	25	98	0.1
Legal, social, and cultural professionals	26	4,307	2.8
Technicians and associate professionals	3	22,683	14.6
Clerical support workers	4	7,472	4.8
Service and sales workers	50	74	0.0
Personal service workers	51	194	0.1
Personal care workers	53	35,910	23.1
Skilled agricultural, forestry, and fishery workers	6	7	0.0
Craft and related trades workers	7	182	0.1
Elementary occupations	9	14,702	9.5
Missing		944	0.6
Total		155,569	100.0

During the period from January 1, 2007 to December 31, 2012, the 155,569 women contributed a total of 771,417 person years and 1245 overall breast cancer cases. Of the 1245 breast cancer cases, ER status was available for 1177 (95%) cases, HER2 status for 1123 cases (90%), and both ER and HER2 status was available for 1118 (90%) cases. This resulted in a total of 797 ER+/HER2- tumours, 187 HER2+ tumours, 136 ER-/HER2- tumours, and 123 tumours that were not classifiable because of missing receptor status.

The inception population included a total of 71,479 women in the period from January 1, 2007 to December 31, 2012. These contributed a total of 286,050 person

years and 321 overall breast cancer cases and a total of 204 ER+/HER2- tumours, 43 HER2+ tumours, 44 ER-/HER2- tumours, and 30 not classifiable tumours.
The occupation population, i.e. the population of office workers, nurses, and midwifes, included 69,864 women in the period from January 1, 2007 to December 31, 2012. These contributed a total of 361,060 person years and 644 breast cancer cases.

Among women who ever worked night shifts, there was a not insignificant higher fraction of person years among women below age 40 as well as a higher fraction of age-standardized person years among women who had a higher educational level in the family (undergraduate and bachelor degree, and higher education) compared to women who never worked night shifts. In addition, among women who ever worked night shifts, there was a slightly higher fraction of age-standardized person years among women who had three or more children. Comparable distributions were observed for the occupation population, however, in this population of nurses/midwifes there was a lower fraction of age-standardized person years among women who were ≥30 years at birth of first child, among women who were nulliparous, and among women who used oral contraception compared to the office workers.

Among women who worked night shifts, or who were employed in occupations with a high prevalence of night shift work (i.e. nurses and midwifes), the distributions of covariates in the low and high exposure groups did not vary significantly. Except for age and educational level in family: among women who worked consecutive night shifts, and women employed as nurses/midwifes with night shift work there was a higher fraction of person years among women below age 40 and among nurses/midwifes without night shifts there was a higher fraction of person years among women with a high educational level in the family, Table 2 and Table S. 1.

Age, age at birth of first child, family history of breast cancer or ovarian cancer, mammography screening attendance, and family educational level were associated

with increased breast cancer risk, all as expected. Though, the association between the family educational level and breast cancer was not significant in the population of office workers, nurses, and midwifes, Table S. 2.

Table 2 The distributions of age-standardized percentages covariates in the low and high exposure groups in the three studies and the supplementary study to Study I. Only covariates with ≥4% difference between low and high exposure groups are presented in the table.

	STUDY I		**Suppl.**		**STUDY II**		**STUDY III**	
Covariate	1-29 night shifts the previous one year	≥30 night shifts the previous one year	Nurses/midwifes without night shift work	Nurses/midwifes with night shift work	1-29 night shifts since study entry	≥30 night shifts since study entry	Night shift work, but not ≥2 consecutive night shifts	Night shift work with ≥2 consecutive night shifts
<40 years of age*	50%	45%	39%	51%	50%	48%	39%	51%
Nulliparous	22%	25%	12%	18%	23%	22%	24%	22%
≥30 years at first child	18%	14%	21%	19%	16%	17%	17%	16%
High educational level in family**	73%	69%	99%	99%	68%	72%	68%	71%

* Not age-standardized

** Undergraduate and bachelor degree, and higher education

THE EFFECT OF RECENT NIGHT SHIFT WORK ON THE RISK OF OVERALL BREAST CANCER (STUDY I)

Among women who worked night shifts the previous year, a decreased rate ratio (RR) of 0.82 (95% CI 0.71 to 0.96) was observed compared to women who did not work night shifts the previous year. No increased risk was observed when the previous two to five years were considered and in addition, no increased risk was observed in the inception population. Including additional exposure groups did not change the results. These results are in line with the supplementary analysis (not

presented in Paper I) of office workers and nurses/midwifes, Table 3. In this analysis nurses/midwifes had a decreased risk of breast cancer compared to office workers (RR 0.74, 95% CI 0.57 to 0.96 among nurses/midwifes with no night shifts, and RR 0.75, 95% CI 0.58 to 0.96 among nurses/midwifes with ≥1 night shift).

Table 3 The rate ratio (RR) of breast cancer by night shift status among 69,864 women in the Danish public healthcare sector 2007 to 2012.

	Person years	**Cases**	**Crude RR (95% CI)**	**Adjusted* RR (95% CI)**
Total	361,060	644		
Office workers	119,825	259	1	1
Nurses/midwifes with no night shifts	84,812	169	0.92 (0.76 to 1.12)	0.74 (0.57 to 0.96)
Nurses/midwifes with ≥1 night shift	156,423	216	0.64 (0.53 to 0.77)	0.75 (0.58 to 0.96)
Trend			>0.001	p=0.07
Total	241,235	385		
Nurses/midwifes with no night shifts	84,812	169	1	1
Nurses/midwifes with ≥1 night shift	156,423	216	0.69 (0.57 to 0.85)	1.01 (0.82 to 1.24)

CI: confidence interval

* Poisson regression model adjusted for changes in calendar year, age (<40, 40 to 44, 45 to 49, and every second year from age 50), age at birth of first child (<20, 20 to 29, ≥30, no children), number of births (0, 1, 2, 3, ≥4), female 1st degree relatives with breast cancer before age 50 or ovarian cancer at any time (0, ≥1 female, no information), oral contraception, ATC: G03A (no, yes), hormone replacement therapy, ATC: G03c, G03D, G03F (no, yes), other sex hormones, ATC: G03B, G03G, G03H (no, yes), medication related to alcoholism, ATC: N03AA, N05AB, N07BB (no, yes), mammography screening attendance (invited but not screened, invited and screened, not invited), and highest family educational level at first registration of work (unspecified, primary and secondary school, advanced level education, vocational education, undergraduate and bachelor degree, higher education, and no information on education).

THE EFFECT OF RECENT NIGHT SHIFT WORK ON THE RISK OF BREAST CANCER SUBTYPES DEFINED BY ER STATUS AND HER2 STATUS (STUDY II)

In the total population, an increased risk of HER2+ breast cancer was found among women who worked ≥1 night shifts (RR 1.35, 95% CI 1.01 to 1.81) and among women who worked ≥30 night shifts (RR 1.49, 95% CI 1.04 to 2.13). In the inception population, no increased risk of HER2+ breast cancer was observed.

In both the total population and in the inception population, the point estimates of ER+/HER2- breast cancer as well as ER-/HER2- breast cancer were below one, but these estimates were only significant for ER+/HER2- breast cancer among women who worked ≥1 night shifts (RR 0.83, 95% CI 0.71 to 0.96).

THE EFFECT OF RECENT EXPOSURE TO CONSECUTIVE NIGHT SHIFTS ON THE RISK OF OVERALL BREAST CANCER AND HER2+ BREAST CANCER (STUDY III)

Overall breast cancer was not associated with ever working consecutive night shifts compared to never working night shifts. The RR among women working ≥7 consecutive night shifts was 0.95 (95% CI 0.76 to 1.19).

A significant increased risk of HER2+ was observed among women ever working consecutive night shifts and indicated an increased risk by increasing number of consecutive night shifts. The RR among women who worked ≥6 consecutive night shifts was 1.45 (95% CI 1.06 to 1.98) and 1.94 (95% CI 1.23 to 3.08) for women who worked ≥7 consecutive night shifts. When restricting the analysis to women who worked night shifts and stratifying to the amount of night shifts worked since study entry, the risk of HER2+ breast cancer was increased among women who had worked consecutive night shifts compared to women who had not worked consecutive night shifts in the group of women who had worked 1-29 night shifts since study entry. Furthermore, in the group of women who had worked 30-99 night shifts since study entry, an increased risk of HER2+ breast cancer was observed

among women who had worked ≥6 consecutive night shifts. However, none of these point estimates were statistically significant and the confidence intervals were wide. There were too few data to examine the risk of HER2+ breast cancer following consecutive night shifts among women who had worked ≥100 night shifts since study entry.

DISCUSSION

KEY FINDINGS

In the studies the association between overall breast cancer and recent exposure to night shift work was examined by:

- Exposure to night shift work in five moving exposure windows: from the previous year to the previous five years. Within these exposure windows, the amount of night shift work was grouped to account for the intensity of night shift work. This was examined in the inception population, a population less likely to include women with previous night shift work. (Study I)
- Office workers, who are assumed to never have had night shift work, were compared to nurses and midwifes, who most likely have had night shift work early in their career. Nurses and midwifes were divided in those without night shift work during follow-up and those with ≥1 night shift during follow-up. (Supplementary analysis)
- Exposure to≥2, ≥3, ≥4, ≥5, ≥6, or ≥7 consecutive night shifts during follow-up. (Study III)

No increased risk of overall breast cancer was observed in any of these analyses. On the contrary, a significant decreased risk was observed among women who worked night shift the previous year, as well as among nurses/midwifes compared to office workers.

The association between breast cancer subtypes and recent exposure to night shift work was examined by:

- The number of night shifts since study entry. (Study II)
- Exposure to≥2, ≥3, ≥4, ≥5, ≥6, or ≥7 consecutive night shifts during follow-up. (Study III)

A significant increased risk of HER2+ breast cancer was observed among women who worked ≥30 night shift during follow up. In addition, there were indications of an increased risk of HER2+ breast cancer by increasing number of consecutive night shifts. No increased risk of ER+/HER2- and ER-/HER2- breast cancer was observed.

On the other hand a significant decreased risk of ER+/HER2- breast cancer was observed among women who worked ≥1 night shift during follow-up.

METHODOLOGICAL CONSIDERATIONS

Breast cancer

The analyses were based on information obtained from national registries which do not require permission from the study subjects. Hence, selection bias is not expected to be an issue in the analyses.

Information on breast cancer was obtained from both the clinical database of the Danish Breast Cancer Cooperative Group (1977-2012) and the National Cancer Registry (1943-2011). The validity of these registries are high.(89, 95) ER and HER2 status was available for 90% and 95% of the breast cancer cases, respectively. It is unlikely, that the missing receptor status is associated with night shift work.

Exposure assessment

Information on night shift work was obtained from pay-roll registers which besides being objective and detailed are also assumed to be valid.
A night shift was defined according to the recommendations from the 2011 IARC working group as at least three hours of work between midnight and 05 AM.(9) Several exposure models of recent night shift work were analysed: The number of night shifts the previous one to five years, the number of night shifts since entry, and ever exposed to ≥2, ≥3, ≥4, ≥5, ≥6, or ≥7 consecutive night shifts during follow-up. Capturing number of consecutive night shifts is suggested to be important in epidemiological studies of night shift work and breast cancer, as consecutive night shifts are suggested to impact the circadian rhythm significantly.(9)
The comparison group in most of the analyses were non-night shift workers. Permanent day workers are assumed to be less exposed to circadian disruption than non-night shift workers. However, in the occupational population, office workers, who are assumed to be permanent day workers, were used as reference group to

nurses/midwifes with and without recent night shift work and this did not change the results.

Several epidemiological studies have indicated that long term exposure (≥20 years) to night shift work may bee associated with breast cancer.(41, 43, 44, 46, 48, 52, 56) In addition, observations by Fritschi et al indicated that night shift work >30 years ago (prior to diagnosis) increased the risk of breast cancer. If long term night shift work is associated with breast cancer, this could have biased the findings. However, the findings were approximately identical when analyses were restricted to the inception population as well as to women employed as office workers, nurses or midwifes. The inception was generated by the first available date of employment in the Danish Regions. We were not able to take employment outside the Regions prior to this date into account. This may be of minor importance among the younger women because approximately every doctor and the majority of nurses and midwifes are employed in the Regions after graduation. In the population restricted to office workers, nurses, and midwifes, office workers may fairly be assumed not be or at least less exposed to night shift work than nurses and midwifes. Nurses and midwifes have a high prevalence of night shift work and the prevalence of night shift work is observed to be highest among women at 20 to 29 years of age.(96) It is therefore reasonable to assume that the majority of nurses and midwifes have had night shift work if not early then at some point in their career. The findings from the inception population and the population of office workers, nurses, and midwifes are thus, less likely to be biased from long term exposure. The affects of left truncation bias are also assumed to be less likely in the inception population.

Some women experience more discomfort in relation to night shift work than others. This can lead to a selection away from night shift work. If the individual's ability to cope with night shift work is related to the degree of circadian disruption, it may influence the association between night shift work and breast cancer. Still, in the population of office workers, nurses, and midwifes, the nurses and midwifes have been divided into two groups: those with no night shift work during follow-up and

those with night shift work during follow-up. Both groups had a significantly lower risk of breast cancer than the office workers, and in addition, there were no difference in risk between the two groups. Hence, the healthy worker effect is less likely to have affected the findings in this population.

Confounding

Covariates of possible confounders were obtained from national registries. Changes in these covariates during follow-up were accounted for in the analysis.
Reproductive factors, hormone replacement treatment, and family history of breast cancer or ovarian cancer are well established risk factors for breast cancer and were all included in the adjusted models. The reproductive factors included number of children (twins are considered as one child) and age at birth of first child. Hormone replacement treatment were only available as of 1995 thus, women who bought hormone replacement treatment before and not after 1995 were classified as not exposed to hormone replacement treatment.
To my knowledge, no previous studies have accounted for mammography screening attendance. Even though the majority of the invited women were screened for breast cancer, the risk of diagnosed breast cancer among the screened women was significantly increased. This was accounted for in the adjusted models.
Adjustment for alcohol consumption was based on prescribed medication related to alcoholism. This will to some extend account for severe alcohol consumption, but will not account for moderate alcohol consumption.
The highest educational level in the family was used as a surrogate measure for socio economic status. This made it possible to differentiate socio economic status within a single occupation with the same income and educational background. Increased educational level in the family was associated with breast cancer, as expected.

BMI, menarche, menopause, physical activity, and smoking were not accounted for in the adjusted models. These variables have been suggested as risk factors for breast cancer, but previous studies of night shift work and breast cancer have only demonstrated minor if any confounding from these.(63)

INTERPRETATION OF FINDINGS

The studies in this thesis are the first studies of night shift work and breast cancer based on objective and day-by-day information on night shift work. Opposite previous studies, this study population was large and had a high prevalence of night shift work and included a variety of occupations with different educational levels. Though, health professional accounted for approximately 40% of the population.

We found no increased risk of overall breast cancer by the number of night shifts worked the previous one, two, three, four, or five years (Study I) or by ever working ≥2, ≥3, ≥4, ≥5, ≥6, or ≥7 consecutive night shifts since study entry (Study III). In addition, no increased risk was observed among nurses/midwifes who worked night shifts during follow-up compared to office workers and compared to nurses/midwifes who did not work night shifts during follow-up (Supplementary study). The effect of recent night shift work has been studied by Davis et al, Pesch et al, and Fritschi et al.(39, 46, 51) They all examined the effect of ever working night shift the previous ten years prior to diagnosis and Pesch et al (46) also examined current night shift work. However, the study by Pesch et al (46) had too broad confidence intervals to contribute to the association. Fritschi et al observed no association between recent graveyard shifts and breast cancer, but did however observe an insignificant increased risk by recent phase shift.(51) Davis et al also observed indications on an increased risk of breast cancer by recent night shift work.(39) The definition of phase shift in the study of Fritschi et al is not quite clear and thus, these observations may not be applicable with the consecutive night shifts examined in Study III. To sum up, contrary to the observations of this thesis, previous epidemiological studies indicate that recent night shift work may increase the risk of breast cancer. However, these observations were not statistically significant and in addition, based on self-reported information on night shift work.

This is the first study to examine the effect of recent exposure to consecutive night shifts and risk of breast cancer. Only one other study included information on number of consecutive night shifts.(49) This study observed a statistically increased

risk of breast cancer among women who worked five or more years in schedules including six or more consecutive night shifts and the observations also indicated an increased risk by increased number of consecutive night shifts. Still, this study did not account for the timing of the exposure to consecutive night shifts.

Previous studies have examined the risk of breast cancer among occupations with high prevalence of night shift work compared to occupations without night shift work. Schwartzbaum et al, O'Leary et al, and Hansen et al compared women employed in occupations with a high prevalence of night shift work with non-night shift occupations.(40, 42, 45) Both Schwartzbaum et al and O'Leary et al observed no increased risk of breast cancer among women employed in occupations with high prevalence of night shift work, the point estimates were below one and statistically significant in the study of O'Leary.(42, 45) Hansen et al observed a statistically significant increased risk among women employed in occupations with a high prevalence of night shift work.(40) To my knowledge, no previous studies have examined the risk of breast cancer among nurses or midwifes compared with occupations without night shift work. However, some studies have examined the risk of breast cancer among nurses and midwifes compared to the general population.(97-103) These studies, observed increased standard incidence ratios which were significant in a few (98, 99, 102, 103) of the studies, but moderately raised. Hence, the finding in this thesis of no increased risk among nurses and midwifes is consistent with other studies comparing night shift occupations with non-night shift occupations, but is not in line with studies comparing nurses to the general population.

We observed no increased risk of ER+/HER2- breast cancer or ER-/HER2- breast cancer following recent night shift work, but did on the other hand, observe an increased risk of HER2+ breast cancer and there were indications of an increased risk by increasing number of consecutive night shifts. ER-/HER2- tumours can be regarded as triple negative tumours, as the PR status has shown to be strongly associated with ER status and has limited predictive value compared to ER

status.(70, 73, 74) Triple negative tumours as well as HER2+ tumours have high tumour doubling time by which it takes approximate 8.5 years ± 3.5 years for triple negative and 13 years ± 5 years for HER2+ to reach a detectable tumour of one cm^3.(77-79) Hence, a follow-up of six years should cover the majority of the relevant exposure time for these breast cancer subtypes. Nocturnal melatonin suppression may increase oestrogen production (80), but because there is a strong evidence of an association between prolonged exposure to oestrogen and breast cancer (68, 75, 76), ER+ tumours may be associated with long term night shift work rather than recent night shift work. No previous studies have examined the effect of recent night shift work on breast cancer subtypes. Among studies that examined breast cancer subtypes in relation to night shift work, an increased risk of ER+ tumours has been observed in some studies (41, 52, 58, 59), but not all.(42, 54, 66, 84) Only two studies included information on HER2 status.(58, 59) Papantoniou et al observed an insignificantly increased risk of HER2+ breast cancer among women who worked night shifts.(58) Wang et al used a broader definition of HER2+, as they included all cases of HER2 that were 2+ (equivocal), but in spite of this they observed a significant increased risk of HER2+ breast cancer among women who worked night shifts. Equivocal HER2 cases are usually classified as HER2+ on the basis of fluorescence (FISH) or chomogenic in situ hybridization (CISH) tests.(94) No previous studies have examined the effect of consecutive night shifts on the risk of HER2+ breast cancer.

CONCLUSION AND PERSPECTIVES

No increased risk of overall breast cancer was observed among women who worked night shift the previous one to five years, regardless of the amount of night shift in these time windows, among women who worked night shifts during follow-up, regardless of the number of consecutive nights, or among nurses and midwifes who worked night shift during follow-up when compared to nurses and midwifes without night shift work during follow-up or when compared to office workers. Furthermore, no increased risk of breast cancer was observed among all nurses or midwifes compared to office workers.

A statistically significant increased risk of HER2+ breast cancer was observed among women who worked night shift during follow up and the observations also indicated an increased risk by increasing number of consecutive night shifts. No increased risk of ER+/HER2- and ER-/HER2- breast cancer following recent night shift work was observed.

Night shift work is inevitable in some industries. If night shift work is associated with breast cancer or other diseases, it is important to organize work schedules to reduce this risk. Our findings of an increased risk of HER2+ breast cancer which was indicated to increase with increasing number of consecutive night shifts only applies for 10-20% of all breast cancer cases and additionally, the treatment and thereby also the prognosis has improved. Hence, the gain of organizing schedules differently on behalf of these finding is small and may in addition, cause unnecessary concerns among night shift workers. Any recommendations of how to organize shift work schedules should thus, be based on strong evidence and associations. Strong evidence should be gained from additional cohort studies with a longer follow-up, and with objective and detailed information on night shift work so that a variety of exposure metrics can be explored. In that respect, the Danish Working Hour Database is a strong candidate especially in the future, when more years are added.

Until the Danish Working Hour Database has grown older, combining existing datasets is also a possibility. This has been among studies of night shift work overall breast cancer, but not among studies that have examined the effect of night shift work on breast cancer subtypes. Several studies have included receptor status and thus, there is a lot of data to merge and explore.

ENGLISH SUMMARY

AIM: The main aim was to examine, if recent night shift work increase the risk of breast cancer. Specifically, the aim was to examine if: 1) night shift work the previous one to five years increase the risk of overall breast cancer, 2) the effect of night shift work during the previous six years differs by breast cancer subtypes classified by oestrogen and HER2 receptor status, and 3) the number of consecutive night shifts increase the risk of HER2+ and overall breast cancer.

METHOD: The studies were based on a cohort of 155,569 women employed in the public Danish healthcare sector between 2007 and 2012 and without a previous breast cancer diagnosis. For each woman, individual information on objective, detailed, and daily working hours were available from pay roll registers. Breast cancer cases and information on potential confounders were based on national register linkage.

RESULTS: No increased risk of overall breast cancer was observed for night shift work the last one to five years, for consecutive night shift work during the previous six years, or among nurses or midwifes with night shift work during the previous six years compared to office workers without night shift work as well as nurses and midwifes without night shift work during the previous six years. An increased risk of HER2+ breast cancer was observed for night shift work during the previous six years (risk ratio (RR) 1.35, 95% confidence interval (CI) 1.01 to 1.81) and this risk was additionally increased among women who worked consecutive night shifts during the previous six years (RR 1.45, 95% CI 1.06 to 1.98 for ≥2 consecutive night shifts and RR 1.96, 95% CI 1.23 to 3.08 for ≥7 consecutive night shifts). This risk seemed to increase by increasing number of consecutive night shifts. No increased risk was observed for ER+/HER2- or ER-/HER2- breast cancer for night shift work during the previous six years.

CONCLUSION: Recent night shift work did not increase the risk of overall breast cancer, ER+/HER2-, or ER-/HER2- breast cancer in these studies. On the other hand, an increased risk of HER2+ breast cancer was observed among women who worked night shifts during the previous six years and even more so among women who

worked consecutive night shifts during the previous six years. The risk seemed to increase by increasing number of consecutive night shifts.

DANISH SUMMARY

FORMÅL: Det overordnede formål var at undersøge, om der er en øget risiko for brystkræft forbundet med en kvindes seneste års natarbejde, dvs. om natarbejde er en promoter for udviklingen af brystkræft. Konkret var formålet at undersøge om: 1) natarbejde det seneste år til de seneste fem år er forbundet med en øget risiko for brystkræft, 2) effekten af natarbejde inden for de seneste seks år har forskellige effekter på brystkræftsubtyper defineret ud fra østrogen og HER2 receptor status og 3) antallet af konsekutive nattevagter er forbundet med en forøget risiko for HER2+ brystkræft og for brystkræft generelt.

METODE: Studierne var baseret på en kohorte af 155.569 kvinder ansat i de Danske Regioner mellem 2007 og 2012 uden forudgående brystkræft diagnoser. Fra løndata var der for hver kvinde individuelle, objektive, detaljerede og daglige oplysninger om natarbejde. Oplysninger om brystkræftdiagnoser og potentielle risikofaktorer forbundet med brystkræft var baseret på nationale registerudtræk.

RESULTATER: Der blev ikke fundet en forøget risiko for brystkræft generelt, hverken for natarbejde det seneste år til de seneste fem år, for konsekutive nattevagter indenfor de seneste seks år, eller blandt sygeplejersker og jordemødre med natarbejde indenfor de seneste seks år i forhold til både kontorarbejdere og sygeplejersker og jordemødre uden natarbejde indenfor de seneste seks år. Der blev fundet en forøget risiko for HER2+ brystkræft blandt kvinder som havde natarbejde indenfor de seneste seks år (relativ risiko (RR) 1,35, 95% konfidensinterval (KI) 1,01 til 1,81), dette var yderligere forøget blandt kvinder som havde arbejdet konsekutive nattevagter indenfor de seneste seks år (RR 1,45, 95% KI 1,06 til 1,98 for ≥2 konsekutive nattevagter og RR 1,96, 95% KI 1,23 til 3,08 for ≥7 konsekutive nattevagter). Denne risiko så ud til at stige med stigende antal konsekutive nattevagter. Der blev ikke fundet en forøget risiko for ER+/HER2- eller ER-/HER2- brystkræft blandt kvinder som have natarbejde indenfor de seneste seks år.

KONKLUSION: De seneste år natarbejde var ikke forbundet med en forøget risiko for brystkræft generelt, for ER+/HER2- eller for ER-/HER2- brystkræft i disse studier. Derimod var natarbejde indenfor de seneste seks år forbundet med en

forøget risiko for HER2+ brystkræft og denne risiko var yderligere forøget blandt kvinder som arbejde konsekutive nattevagter indenfor de seneste seks år. Risikoen for HER2+ brystkræft så ud til at sige med antallet af konsekutive nattevagter.

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SUPPLEMENTAL ANALYSIS – TABLES

Table S. 1 Distribution of person years 2007 to 2012 by office works and nurses/midwifes and nights shift work status among 69,864 women in the Danish public healthcare sector.

	Office workers		Nurses/midwifes with no night shifts		Nurses/midwifes with ≥1 night shift	
	Person years	Age-standardized %*	Person years	Age-standardized %*	Person years	Age-standardized %*
Total	119825	100	84812	100	156423	100
Age (years)**						
<40	45891	40	21459	39	76682	51
40-49	32755	25	26798	28	42622	25
50-59	25805	24	26160	25	29350	19
≥60	15375	11	10295	8	7769	5
Calendar year						
2007	12261	10	13848	17	15622	10
2008	17644	15	15304	19	23570	15
2009	20217	17	14608	17	26966	17
2010	22056	18	14095	16	29029	19
2011	23531	20	13741	16	30260	20
2012	24116	20	13215	14	30976	21
Age at birth of first child (years)						
<20	4463	3	1419	1	2526	2
20-29	68518	57	56811	66	92148	60
≥30	21574	18	17672	21	30090	19
No children	25271	22	8909	12	31659	18
No. of children						
0	25271	22	8910	12	31659	18
1	23195	19	12832	16	26641	16
2	53544	44	41578	48	62599	41
3	15305	13	18251	21	29725	20
≥4	2510	2	3241	3	5799	4
Female 1st degree relatives with breast cancer before the age of 50 or ovarian cancer at any time						
No	107977	91	76287	91	145409	92
Yes	2985	3	2249	3	4177	3
No information on female 1st degree relatives	8864	6	6276	6	6837	6
Oral contraception						
No	45276	36	44072	42	53506	41
Yes	74549	64	40739	58	102917	59
Hormone replacement therapy						
No	86167	74	57791	73	122397	75
Yes	33658	26	27021	27	34027	25

Other sex hormones						
No	104126	87	75717	87	133917	87
Yes	15700	13	9095	13	22507	13
Medication related to alcoholism						
No	117869	98	83794	99	154755	99
Yes	1957	2	1018	1	1668	1
Mammography screening attendance						
No	4829	4	3781	3	4663	4
Yes	21896	16	19002	16	20657	18
Not invited	93100	80	62029	80	131103	78
Highest educational level in family						
Unspecified	327	0	212	0	767	0
Primary and secondary school	5682	5	23	0	136	0
Advanced level education	63180	53	423	1	1583	1
Vocational education	9117	8	65	0	186	0
Undergraduate and bachelor degree	18746	16	67711	80	133957	86
Higher education	22728	19	16330	19	19677	13
Missing	44	0	47	0	117	0

* Age-standardized (<40, 40-44, 45-49, and every second year from age 50)
** Age is not age-standardized

Table S. 2 The rate ratio (RR) of breast cancer by participant characteristics among 69,864 women employed as office workers, nurses, or midwifes in the Danish public healthcare sector 2007 to 2012.

Covariates	Adjusted RR (95% CI)*
Age (years)	
<40	1
40 to 44	2.61 (1.83 to 3.71)
45 to 59	4.23 (3.04 to 5.91)
50 to 51	4.65 (3.06 to 7.08)
52 to 53	5.00 (3.24 to 7.71)
54 to 55	4.93 (3.15 to 7.70)
56 to 57	5.94 (3.80 to 9.27)
58 to 59	5.57 (3.49 to 8.89)
60 to 61	6.44 (4.01 to 10.34)
62 to 63	5.42 (3.23 to 9.10)
64 to 65	8.34 (4.96 to 14.54)
66 to 67	7.78 (4.17 to 14.54)
68 to 69	14.83 (7.63 to 28.85)
≥70	10.54 (4.06 to 27.38)
Age at birth of first child (years)	
<20	1
20 to 29	1.52 (0.89 to 2.60)
≥30	2.25 (1.28 to 3.93)
No. of children	
0	1
1	0.55 (0.30 to 1.01)
2	0.56 (0.31 to 1.01)
3	0.45 (0.25 to 0.81)
≥4	0.66 (0.34 to 1.28)
Female 1st degree relatives with breast cancer before the age of 50 or ovarian cancer at any time	
No	1
Yes	2.18 (1.52 to 3.12)
No information on female 1st degree relatives	1.13 (0.87 to 1.46)
Oral contraception	
No	1
Yes	0.96 (0.79 to 1.17)
Hormone replacement therapy	
No	1
Yes	1.00 (0.84 to 1.19)
Other sex hormones	
No	1
Yes	1.19 (0.90 to 1.57)
Medication related to alcoholism	
No	1
Yes	1.71 (1.10 to 2.64)
Mammography screening attendance	
No	1
Yes	1.49 (1.06 to 2.08)

Not invited	0.85 (0.58 to 1.25)
Highest educational level in family	
Primary and secondary school	1
Advanced level education	1.46 (0.81 to 2.65)
Vocational education	1.46 (0.71 to 3.00)
Undergraduate and bachelor degree	1.59 (0.86 to 2.93)
Higher education	1.69 (0.91 to 3.11)
Unspecified	1.73 (0.38 to 7.83)
Missing	6.66 (0.85 to 51.86)

CI: confidence interval

*Poisson regression model adjusted for changes in calendar year, age (<40, 40 to 44, 45 to 49, and every second year from age 50), age at birth of first child (<20, 20 to 29, ≥30, no children), number of births (0, 1, 2, 3, ≥4), female 1st degree relatives with breast cancer before age 50 or ovarian cancer at any time (0, ≥1 female, no information), oral contraception, ATC: G03A (no, yes), hormone replacement therapy, ATC: G03c, G03D, G03F (no, yes), other sex hormones, ATC: G03B, G03G, G03H (no, yes), medication related to alcoholism, ATC: N03AA, N05AB, N07BB (no, yes), mammography screening attendance (invited but not screened, invited and screened, not invited), and highest family educational level at first registration of work (unspecified, primary and secondary school, advanced level education, vocational education, undergraduate and bachelor degree, higher education, and no information on education).

ORIGINAL MANUSCRIPTS

I. Vistisen HT, Garde AH, Frydenberg M, Chistiansen P, Hansen ÅM, Hansen J, Bonde JPE, Kolstad H. Short term risk of breast cancer following night shift work: a cohort study of pay roll data (Submittet to the Scandinavian Journal of Work, Environment, and Health)

II. Vistisen HT, Garde AH, Frydenberg M, Chistiansen P, Hansen J, Bonde JPE, Hansen ÅM, Kolstad H. Short term effects of night shift work on risk of breast cancer classified by oestrogen and HER2 receptor status (Submittet to the Scandinavian Journal of Work, Environment, and Health)

III. Vistisen HT, Garde AH, Frydenberg M, Chistiansen P, Hansen J, Bonde JPE, Hansen ÅM, Kolstad H. Short term risk of breast cancer following consecutive night shifts: a cohort study of pay roll data (In prep to Cancer Causes and Control)

MANUSCRIPT I

Short term risk of breast cancer following night shift work: a cohort study of pay roll data

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ABSTRACT

Suppression of nocturnal melatonin may act as a promoter of oncogenesis and the aim was to examine if recent night shift work is a short term risk factor for breast cancer. The authors conducted a cohort study of 154,751 female employees of the public healthcare sector in Denmark 2007 to 2012. Day-by-day night shift information was available from pay roll registers and 1,050 incident cases of breast cancer were identified in national cancer registries. A rate ratio of 0.82 (95% confidence interval (CI): 0.71, 0.96) was observed for women who worked night shifts during the last year compared with those who did not after adjustment for age, age at first child, parity, family history of breast or ovarian cancer, sex hormones, medications related to alcoholism, family educational level, mammography screening, and other potential confounders. No increased risk was observed when the last two to the last five years of exposure were considered. Night shift work during the last five years showed an adjusted RR of 1.00 (95% CI: 0.74, 1.37). These results are reassuring, but only in the short run as it are still unclear if there is a long term risk of night shift work.

In 2007, a working group convened by the International Agency for Research on Cancer (IARC), classified night shift work that involves circadian disruption as probably carcinogenic to humans based on sufficient evidence in animals, and limited evidence in humans (1). The same year, Denmark became the first country to regard breast cancer as an occupational disease, which attracted international attention (2, 3) Since then several new epidemiologic studies have been published (4-16). In 2013 no less than four systematic reviews and meta-analyses were published on this issue, but despite these efforts the epidemiological evidence is still limited (17-20).

The pineal hormone melatonin is considered a key biomarker of circadian rhythms, including the biological day and night, and attenuation of nocturnal melatonin secretion is assumed to be a pivotal element of the causal mechanisms linking night shift work and breast cancer (21-29). From animal studies it is known that melatonin reduces the growth of chemically induced mammary tumours (30, 31). It has been shown that melatonin at physiological levels suppresses the proliferation of human breast cancer xenografts (25, 32, 33). Furthermore, melatonin may reduce the invasiveness of human breast cancer, and the suppression of melatonin during the biological night may act as a promoter of oncogenesis (30, 32). This experimental evidence suggests that night shift work may exert its response downstream the complex casual pathways that lead to breast cancer. Hence, recent night shift work may be associated with short term risk of breast cancer and progression in humans.

Only few epidemiologic studies have examined the short term risk of night shift work (7, 11, 34). Davis et al's observations were in line with the experimental findings and indicated that recent night shift work may increase the risk of breast cancer (34). Fritschi et al observed an association with recent work which included phase shifts, but no association with recent graveyard night shift work and the observations by Pesch et al did not contribute to the association (7, 11). These studies relied on self reported information on working time, as most other studies of night shift work and cancer, and such measures might have influenced findings by recall bias. Furthermore, the prevalence of night shift work was low. In this study, we circumvented these limitations by utilising a large national cohort of women with pay roll register data on day-by-day information on exact working time, and a high prevalence of night shift work that we linked to national cancer registries. The aim was to examine if recent night shift work is a short term risk factor for breast cancer.

METHODS

Data sources

This study linked information from seven Danish registries on the individual level by use of the civil registration number given to all individuals living in Denmark since 1968:

The Danish Working Hour Database is a newly established database encompassing all employees of each of the five administrative Regions, who operate the public healthcare sector. The majority of the employees are healthcare professionals. Data covers individual pay-roll data with information on day, hour, and minute of the beginning and end of every work shift, and job title classified by DISCO (the Danish version of ISCO, the International Standard Classification of Occupations). Data have been available since January 1, 2007.

The Civil Registration System encompasses all individuals living in Denmark with information on sex, vital status, date of birth and links to relatives since 1968 (35).

The clinical database of the Danish Breast Cancer Corporative Group includes pathological and clinical information on all breast cancers diagnosed and treated since 1977 (36).

The National Cancer Registry keeps records on all cancers diagnosed and classified according to ICD-7 and ICD-10 codes (the International Classification of Diseases), and date of diagnosis since 1943 (37).

The National Register of Medicinal Product Statistics encompasses all purchases of prescription drugs at private pharmacies with information on the medication by ATC codes (the Anatomical Therapeutic Chemical Classification System), date of purchase, and purchaser (38). Data have been available since 1995.

The Family Income Registry from Statistics Denmark encompasses all individuals born or living in Denmark with information on the highest educational level in a family living at the same address (39). We included information as of January 1, 2007.

The Clinical Database of Mammography Screening encompasses women invited to participate in the national mammography screening programme for all women between age 50 and 69 (40). The database includes information on date of invitation and date of examination since the start of the programme by the end of 2007.

Data were retrieved up to and including December 31, 2012 for all registers, though data from the National Cancer Registry were only available up to December 31, 2011.

The Danish Data Protection Agency approved the study (j.no. 2011-41-6850). In Denmark, register studies do not need to be approved by the Danish Health Research Ethics Committee System.

Study population

The study population was women aged 18 years or older with at least one registration of work in the Danish Working Hour Database between January 1, 2007 and December 31, 2011 (n=156,927). We excluded 1,552 women diagnosed with breast cancer prior to follow-up, one woman with missing date of breast cancer diagnosis, and 623 women who died or emigrated prior to follow-up as follow-up began one year after the first registration of work (i.e. not before January 1, 2008). The final study population included 154,751 women free of breast cancer at start of follow-up.

We had no information on the study participants' working time prior to 2007. Therefore, to reduce possible bias from night shift work prior to 2007 we established a sub population of recently employed workers. This was possible for four of the five Regions and included workers first employed in that Region by January 1, 2007 or later (inception population). In total 70,985 fulfilled the criterion.

Breast cancer

Breast cancer cases and date of diagnosis were identified in the clinical database of the Danish Breast Cancer Corporative Group for all available years, and supplemented with breast cancer cases and date of diagnosis from the National Cancer Registry (IDC10=DC50 or ICD7=170).

Night shift work

A night shift was defined according to a 2009 IARC Working Group as at least three hours of work between midnight and 05:00 AM (41). If a registration met this criterion the woman was classified as exposed to a night shift on that specific date. For each day during follow up the total number of night shifts were recorded for the previous one, two, three, four, and five years, respectively. The number of night shifts was then categorized into three groups in respect to a reasonable number of person time within each category for the five exposure windows in the total population as well as the inception population: 0, 1-29, and ≥30 night shifts.

Statistical analysis

Five separate analyses were conducted for both the total population and the inception population.

Each woman was followed on a daily basis from start of follow-up, that was subsequent to the end of an exposure window and the earliest one year after the first registration of work (i.e. not before January 1, 2008) for the one-year exposure window and continued until the date of first primary breast cancer diagnosis, death, disappearance, emigration, or end of follow-up at December 31, 2012.

Data were analysed as incidence rate, i.e. as the number of incident breast cancer cases per time units at risk using Poisson regression. All variables were time-dependent, i.e. varied for each date from start until the end of follow-up. The association between night shift work and the incidence of breast cancer was estimated by rate ratios (RR). Both crude and adjusted estimates were reported, where the adjusted models included the following potential confounders: calendar year (2008 to 2012), age (<40, 40-44, 45-49, and every second year from age 50), age at birth of the first child (<20, 20-29, ≥30, no children), number of births (0, 1, 2, 3, ≥4), female 1st degree relatives with breast cancer before age 50 or ovarian cancer at any time (0, ≥1 , no information), oral contraception, ATC: G03A (no, yes), hormone replacement therapy, ATC: G03c, G03D, G03F (no, yes), other sex hormones, ATC: G03B, G03G, G03H (no, yes), medication related to alcoholism, ATC: N03AA, N05AB, N07BB (no, yes), mammography screening attendance (invited but not screened, invited and screened, not invited), and highest family

educational level at the first registration of work (unspecified, primary and secondary school, advanced level education, vocational education, undergraduate and bachelor degree, higher education, and no information on education). These potential confounders were defined a priori based on a review of the literature (42, 43).

Interactions were not included in the models because of no a priori hypotheses of such effects. Estimates were reported with a 95% confidence interval. Trend analyses were done across the unexposed and exposed groups, and a comparison was made between the low exposure (1 to 29 night shifts) and high exposure (≥30 night shifts). All data management and data analysis were done using Stata 13.1.

Data were complete for all variables except for female first degree relatives (5%) and highest family educational level (<0.5%). The missing values were evenly distributed across night shift groups.

RESULTS

The 154,751 women contributed a total of 615,925 person years and 1,050 breast cancer cases during follow-up. The 70,985 women in the inception population correspondingly contributed a total of 214,673 person years and 258 breast cancer cases. Table 1 presents the distribution and the age standardized percentages of person years by participant covariates and number of night shifts during the last year for the total population. Among women who worked night shifts compared

to women who did not work night shifts, there was a higher fraction of person years among women who had a higher level of education in the family. Among women who worked 30 night shifts or more compared to women who worked 1 to 29 night shifts, there was a higher fraction of person years among women who were nulliparous, and a lower fraction of women who were above age 30 at the birth of their first child. Except for these, age standardized person years were evenly distributed by participant characteristics and number of night shifts. The sub populations defined by increasing exposure periods within the total as well as the inception population showed comparable person year distributions.

Table 2 presents rate ratios (RR) of breast cancer by cumulated night shifts during the last one to five year exposure windows. By increasing exposure window, the fraction of person years following ever working night shifts and more than 30 night shifts increased and the absolute numbers decreased, as expected. For night shifts during the last year, a decreased adjusted overall breast cancer RR of 0.82 (95% CI: 0.71, 0.96) was observed. No difference in overall breast cancer risk between night shift workers and non night shift workers was observed when the last two to five years of exposure was considered. Working night shifts during the last five years showed an adjusted RR of 1.00 (95% CI: 0.74, 1.37). Working more than 30 night shifts during the last one and last four years showed decreased adjusted RRs of 0.74 (95% CI: 0.57, 0.95) and 0.73 (95% CI: 0.54, 0.99), respectively. For these two exposure periods we observed decreasing trends across all workers (P=0.01, P=0.04), but no differences between 1 to 29 and ≥30 night shifts (P=0.90,

P=0.69). There were no indications of trends by number of night shifts in any of the other sub analyses.

In the inception population there were no associations between night shift work and breast cancer in the crude or the adjusted analyses (Table 3). There was no indication of positive trends, and the overall findings were in line with those obtained for the total study population.

Using 1 to 29, 30 to 99, and 100 or more night shifts as exposure categories did not change our results (Supplementary, Table S.1).

We found age, age at birth of first child, family history of breast cancer or ovarian cancer, mammography screening attendance, and family educational level to be associated with increased breast cancer risk, all as expected (Supplementary, Table S.2).

DISCUSSION

In this large population of women with a high prevalence of night shift work, we observed no elevated overall risk of breast cancer following the most recent one to five years of night shift work and there were no positive trends by number of night shifts within these five periods. Thus, we could not corroborate a short term association between night shift work and breast cancer occurence as suggested by

experimental data (30, 32). This finding is partly consistent with that of Fritschi et al who observed no elevated risk among women working graveyard shifts the recent 10 years (odds ratio (OR) 1.02, 95% CI: 0.73, 1.43) however, they observed a non-significant increased risk among women who were exposed to phase shifts during the recent 10 years (OR 1.23, 95% CI: 0.83, 1.83) (11). Our findings are not consistent with the findings of Davis et al who observed a slightly elevated risk during the recent 10 years of night shift work (OR 1.6, 95% CI: 1.0, 2.5) (34). As opposed to our study, these studies relied on self-reported information on night shift work.

Several epidemiological studies have observed an increased risk of breast cancer following long term night shift work that we were not able to assess due to lack of work schedule data prior to 2007 (5-7, 10, 44-46). Long term night shift work beginning prior to 2007 could, however, have biased our findings if causally related with breast cancer. But this should only be a problem if recent night shift work is inversely associated with long term night shift work. In our opinion this is an unlikely explanation. Additionally, findings were approximately identical when analyses were restricted to the inception population less likely to include women with previous night shift work.

Strengths and limitations

This study had a number of strengths, namely a large study population with a high prevalence of night shift work generated from an objective pay-roll register that is presumed to be complete. Information on night shift work was based on daily records of paid working hours. Since the salary varies by working hours during the day these recordings should be precise and valid given that employers and employees have a common interest in correct recordings. Cases of breast cancer were identified in national databases encompassing all breast cancers diagnosed in Denmark since 1943 (36, 37) Thus, information bias with respect to exposure and outcome as well as selection bias can hardly explain our findings. A further strength was that we accounted for changes in reproductive factors, hormonal treatment, and family history of breast cancer during follow-up, which are all well established risk factors for breast cancer. However, hormone treatment was based on information as of 1995. Thus, for women above age 60 the information on hormone replacement therapy was not comprehensive as was the information on oral contraception. Our extensive data allowed detailed adjustment for age and year of follow-up. This should account for ageing as well as secular changes in breast cancer occurrence.

During recent years the possible risk of breast cancer following night shift work has attracted public interest in Denmark (3). For that reason night shift workers may have been more willing to participate in breast cancer screening programs and more likely to be diagnosed with breast cancer than non-night shift workers. We had access to complete national screening data and could therefore also adjust for this possible confounder. We used prescription of medications related to

alcoholism as a surrogate measure for alcohol consumption (involving about 2% of the population). This will to some extent account for severe, but not moderate alcohol consumption. Income and education was not expected to vary substantially in this rather homogenous study population and therefore we used the highest education in the family as a surrogate measure for socioeconomic status.

There were also limitations. Although the study population is large, the number of exposed person time was small in several of the subanalyses and the power thus limited. About half of the participants were hired prior to January 1, 2007 and they may represent a subset less susceptible to the effects of night shift work (47). Such left truncation bias is expected to provide underestimates of risk and could explain our negative results, but results from the inception population should not to the same extent be affected by this bias and were in line with those from the total population. We used a cutpoint of 30 night shifts in all the analyses which correspond to a low exposure to night shift work especially in the five year time window. However, using 1 to 29, 30 to 99, and 100 or more night shifts as exposure categories did not change our results.

We were not able to account for alcohol habits in the lower and average end, age at menarche and menopause, BMI, and physical activity, all well documented risk factors for breast cancer and potential confounders. However, most studies on night shift work and breast cancer have only demonstrated minor confounding from these exposures, if any (48).

Conclusion

We observed no increased risk of breast cancer among women working night shifts during the recent five years compared with women not working night shifts during the same period. Thus, our data did not support a short term association between night shift work and breast cancer occurence. These results are reassuring for the many women working night shifts, but only in the short run. It is still unclear if night shift work has long term effects on breast cancer risk.

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All authors have completed the Conflicts of Interest and declare: HK has received financial support from LO, the Danish Confederation of Trade Unions, and prepares specialists' statements for the National Board of Occupational Injuries that processes notifications of breast cancer as occupational disease; no relationships with any organizations that might have an interest in the submitted work in the previous 3 years; their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and no non-financial interests that may be relevant to the submitted work.

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Table 1. Distribution of Person Years 2008 to 2012 by Participant Characteristics and Number of Night Shift the Last Year Among 154,752 Women In the Danish Public Healthcare Sector.

	Number of night shifts the previous year							
	0		1-29		≥30		Ever (≥1)	
Characteristics	1,000 person years	Age-standardized %*	1,000 person years	Age-standardized %*	1,000 person years	Age-standardized %*	1,000 person years	Age-standardized %*
Total	436	100	121	100	59	100	180	100
Age (years)**								
<40	168	39	61	50	26	45	87	48
40-49	109	25	30	25	16	27	47	26
50-59	105	24	25	20	13	23	38	21
≥60	54	12	5	4	3	5	9	5
Calendar year								
2008	52	12	20	16	10	16	29	16
2009	78	18	25	21	12	20	37	20
2010	92	21	26	21	13	21	38	21
2011	103	24	25	21	13	22	38	21
2012	111	25	25	21	12	21	38	21
Age at birth of first child (years)								
<20	22	5	4	4	2	5	6	4
20-29	249	56	66	57	33	56	99	56
≥30	72	17	22	18	8	14	30	17
No children	93	22	29	22	16	25	45	23
No. of children								
0	93	22	29	22	16	25	45	23
1	77	18	22	17	9	15	30	16
2	182	41	46	40	22	38	68	39
3	70	16	20	18	10	18	30	18
≥4	14	3	4	4	2	4	6	4
Female 1st degree relatives with breast cancer before the age of 50 or ovarian cancer at any time								

No	392	91	112	90	54	91	166	90
Yes	11	3	3	3	1	2	5	2
No information on female 1st degree relatives	33	7	6	7	4	7	10	7
Oral contraception								
No	176	38	40	39	21	40	61	40
Yes	259	62	82	61	38	60	119	60
Hormone replacement therapy								
No	314	73	94	74	46	76	140	75
Yes	122	27	27	26	13	24	40	25
Other sex hormones								
No	383	87	103	87	52	88	155	87
Yes	53	13	18	13	7	12	25	13
Medication related to alcoholism								
No	428	98	120	99	58	99	178	99
Yes	7	2	1	1	1	1	2	1
Mammography screening attendance								
No	21	4	4	4	2	5	6	5
Yes	101	21	17	20	9	19	27	19
Not invited	314	75	100	76	47	76	147	76
Highest educational level in family								
Unspecified	28	1	2	1	1	1	3	1
Primary and secondary school	149	6	27	2	15	2	41	2
Advanced level education	23	34	3	23	1	26	4	24
Vocational education	165	5	66	2	35	2	101	2
Undergraduate and bachelor degree	69	38	23	54	6	58	30	55
Higher education	2	16	1	19	1	11	1	16
Missing	0	0	0	0	0	0	0	0

* All covariates except for age are age-standardized (<40, 40-44, 45-49, and every second year from age 50)

** Age is not age-standardized

Table 2. The Rate Ratio (RR) of Breast Cancer by Number of Night Shifts During the Last One to the Last Five Years Among 154,752 Women in the Danish Public Healthcare Sector 2008 to 2012.

Number of night shifts	1,000 person years	Cases	Crude RR	95% CI	Adjusted RR*	95% CI
Last year						
Total	616	1050				
0	436	825	1		1	
1 to 29	121	157	0.68	0.58-0.81	0.87	0.73-1.04
≥30	59	68	0.61	0.48-0.78	0.74	0.57-0.95
Ever night shift	180	225	0.66	0.57-0.76	0.82	0.71-0.96
1 to 29 vs. ≥30			*P*=0.20		*P*=0.90	
Trend			*P*<0.001		*P*=0.01	
Last two years						
Total	465	826				
0	306	606	1		1	
1 to 29	83	116	0.70	0.58-0.86	0.89	0.73-1.10
≥30	76	104	0.69	0.56-0.85	0.87	0.71-1.08
Ever night shift	159	220	0.70	0.60-0.81	0.88	0.75-1.04
1 to 29 vs. ≥30			*P*=0.12		*P*=0.68	
Trend			*P*<0.001		*P*=0.15	
Last three years						
Total	324	586				
0	202	415	1		1	
1 to 29	56	84	0.72	0.57-0.92	0.88	0.69-1.12
≥30	66	87	0.64	0.51-0.81	0.82	0.65-1.04
Ever night shift	122	171	0.68	0.57-0.81	0.85	0.71-1.02
1 to 29 vs. ≥30			*P*=0.42		*P*=0.81	
Trend			*P*<0.001		*P*=0.08	
Last four years						
Total	194	359				
0	114	248	1		1	
1 to 29	34	56	0.76	0.57-1.02	0.91	0.68-1.22
≥30	45	55	0.56	0.42-0.75	0.73	0.54-0.99
Ever night shift	79	111	0.64	0.52-0.81	0.81	0.65-1.03
1 to 29 vs. ≥30			*P*=0.91		*P* =0.69	
Trend			*P* <0.001		*P* =0.04	
Last five years						
Total	80	182				
0	44	113	1		1	
1 to 29	14	32	0.87	0.59-1.29	1.06	0.71-1.58

≥30	21	37	0.70	0.48-1.01	0.96	0.65-1.40
Ever night shift	35	69	0.77	0.57-1.04	1.00	0.74-1.37
1 to 29 vs. ≥30			*P* =0.82		*P* =0.68	
Trend			*P* =0.05		*P* =0.88	

CI: confidence interval

*Poisson regression model adjusted for changes in calendar year, age (<40, 40-44, 45-49, and every second year from age 50), age at birth of first child (<20, 20-29, ≥30, no children), number of births (0, 1, 2, 3, ≥4), female 1st degree relatives with breast cancer before age 50 or ovarian cancer at any time (0, ≥1, no information), oral contraception, ATC: G03A (no, yes), hormone replacement therapy, ATC: G03c, G03D, G03F (no, yes), other sex hormones, ATC: G03B, G03G, G03H (no, yes), medication related to alcoholism, ATC: N03AA, N05AB, N07BB (no, yes), mammography screening attendance (invited but not screened, invited and screened, not invited), and highest family educational level at first registration of work (unspecified, primary and secondary school, advanced level education, vocational education, undergraduate and bachelor degree, higher education, and no information on education).

Table 3. The Rate Ratio (RR) of Breast Cancer by Number of Night Shifts During the Last One to the Last Five Years Among 73,990 Women in the Danish Public Healthcare Sector 2008 to 2012, Results From the Inception Population.

	1,000		Crude		Adjusted	
Number of night shifts	person years	Cases	RR	95% CI	RR*	95% CI
Last year						
Total	215	258				
0	157	206	1		1	
1 to 29	40	36	0.69	0.48-0.98	0.85	0.59-1.22
≥30	18	16	0.67	0.41-1.12	0.78	0.47-1.31
Ever night shift	58	52	0.68	0.50-0.93	0.83	0.61-1.13
1 to 29 vs. ≥30			*P* =0.40		*P* =0.86	
Trend			*P* =0.02		*P* =0.23	
Last two years						
Total	148	178				
0	100	128	1		1	
1 to 29	25	30	0.92	0.62-1.37	1.16	0.78-1.75
≥30	22	20	0.70	0.44-1.13	0.90	0.56-1.45
Ever night shift	48	50	0.82	0.59-1.14	1.04	0.74-1.46
1 to 29 vs. ≥30			*P* =0.67		*P* =0.35	
Trend			*P* =0.15		*P* =0.90	
Last three years						
Total	89	119				
0	57	79	1		1	
1 to 29	15	20	0.99	0.61-1.62	1.12	0.68-1.84
≥30	17	20	0.84	0.52-1.38	1.05	0.63-1.73
Ever night shift	32	40	0.91	0.62-1.33	1.08	0.73-1.60
1 to 29 vs. ≥30			*P* =0.77		*P* =0.73	
Trend			*P* =0.53		*P* =0.78	
Last four years						
Total	41	49				
0	25	33	1		1	
1 to 29	7	11	1.26	0.64-2.49	1.29	0.65-2.59
≥30	9	5	0.42	0.16-1.06	0.48	0.18-1.24
Ever night shift	16	16	0.77	0.42-1.40	0.85	0.46-1.57
1 to 29 vs. ≥30			*P* =0.08		*P* =0.10	
Trend			*P* =0.13		*P* =0.24	
Last five years						
Total	8	<18				
0	5	10	1		1	
1 to 29	1	<4**	0.36	0.05-2.83	0.38	0.05-3.09

≥30	2	<4**	0.24	0.03-1.84	0.32	0.04-2.64
Ever night shift	3	<8**	0.29	0.06-1.30	0.35	0.07-1.67
1 to 29 vs. ≥30			*P* =0.80		*P* =0.74	
Trend			*P* =0.12		*P* =0.21	

CI: confidence interval

*Poisson regression model adjusted for changes in calendar year, age (<40, 40-44, 45-49, and every second year from age 50), age at birth of first child (<20, 20-29, ≥30, no children), number of births (0, 1, 2, 3, ≥4), female 1st degree relatives with breast cancer before age 50 or ovarian cancer at any time (0, ≥1, no information), oral contraception, ATC: G03A (no, yes), hormone replacement therapy, ATC: G03c, G03D, G03F (no, yes), other sex hormones, ATC: G03B, G03G, G03H (no, yes), medication related to alcoholism, ATC: N03AA, N05AB, N07BB (no, yes), mammography screening attendance (invited but not screened, invited and screened, not invited), and highest family educational level at first registration of work (unspecified, primary and secondary school, advanced level education, vocational education, undergraduate and bachelor degree, higher education, and no information on education).

SUPPLEMENTARY

Table S.1 The Rate Ratio (RR) of Breast Cancer by Number of Night Shifts During the Last One to the Last Five Years Among 154,752 Women in the Danish Public Healthcare Sector 2008 to 2012.

Number of night shifts	1,000 person years	Cases	Crude RR	95% CI	Adjusted RR*	95% CI
Last year						
Total	616	1050				
0	436	825	1		1	
1 to 29	121	157	0.68	0.58-0.81	0.87	0.73-1.04
30-99	50	57	0.60	0.46-0.79	0.78	0.59-1.02
≥100	9	11	0.65	0.36-1.18	0.58	0.32-1.05
Ever night-shift	180	225	0.66	0.57-0.76	0.82	0.71-0.96
Trend among night-shift workers			*P*=0.08		*P*=0.88	
Trend all levels			*P*<0.001		*P*=0.01	
Last two years						
Total	465	826				
0	306	606	1		1	
1 to 29	83	116	0.70	0.58-0.86	0.90	0.73-1.10
30-99	57	80	0.71	0.57-0.90	0.95	0.75-1.21
≥100	20	24	0.62	0.41-0.93	0.69	0.46-1.04
Ever night-shift	159	220	0.70	0.60-0.81	0.88	0.75-1.04
Trend among night-shift workers			*P*=0.08		*P*=0.97	
Trend all levels			*P*<0.001		*P*=0.10	
Last three years						
Total	324	586				
0	202	416	1		1	
1 to 29	56	83	0.72	0.57-0.92	0.88	0.69-1.12
30-99	41	57	0.67	0.51-0.88	0.91	0.69-1.21
≥100	25	30	0.59	0.41-0.85	0.69	0.48-1.00
Ever night-shift	122	170	0.68	0.57-0.81	0.84	0.71-1.02
Trend among night-shift workers			*P*=0.26		*P*=0.98	
Trend all levels			*P*<0.001		*P*=0.05	
Last four years						
Total	194	359				
0	114	248	1		1	
1 to 29	34	56	0.76	0.57-1.02	0.91	0.68-1.22
30-99	24	24	0.46	0.30-0.70	0.65	0.42-0.99

≥100	21	31	0.67	0.46-0.97	0.81	0.56-1.19
Ever night-shift	79	111	0.64	0.52-0.81	0.81	0.65-1.03
Trend among night-shift workers			*P*=0.28		*P* =0.77	
Trend all levels			*P* <0.001		*P* =0.04	
Last five years						
Total	80	182				
0	44	113	1		1	
1 to 29	14	32	0.87	0.59-1.29	1.06	0.71-1.58
30-99	10	18	0.72	0.44-1.18	1.05	0.63-1.75
≥100	11	19	0.68	0.42-1.10	0.88	0.54-1.44
Ever night-shift	35	69	0.77	0.57-1.04	1.00	0.74-1.37
Trend among night-shift workers			*P* =0.95		*P* =0.58	
Trend all levels			*P* =0.06		*P* =0.76	

CI: confidence interval

*Poisson regression model adjusted for changes in calendar year, age (<40, 40-44, 45-49, and every second year from age 50), age at birth of first child (<20, 20-29, ≥30, no children), number of births (0, 1, 2, 3, ≥4), female 1st degree relatives with breast cancer before age 50 or ovarian cancer at any time (0, ≥1, no information), oral contraception, ATC: G03A (no, yes), hormone replacement therapy, ATC: G03c, G03D, G03F (no, yes), other sex hormones, ATC: G03B, G03G, G03H (no, yes), medication related to alcoholism, ATC: N03AA, N05AB, N07BB (no, yes), mammography screening attendance (invited but not screened, invited and screened, not invited), and highest family educational level at first registration of work (unspecified, primary and secondary school, advanced level education, vocational education, undergraduate and bachelor degree, higher education, and no information on education).

Table S.2 The Rate Ratio (RR) of Breast Cancer by Participant Characteristics Among 154,752 Women in the Danish Public Healthcare Sector 2008 to 2012.

Covariates	Adjusted RR*	95% CI
Age (years)		
<40	1	
40 to 44	2.77	2.05-3.75
45 to 59	5.25	4.02-6.87
50 to 51	5.93	4.25-8.29
52 to 53	4.55	3.16-6.54
54 to 55	4.40	3.03-6.38
56 to 57	4.65	3.19-6.80
58 to 59	5.42	3.70-7.95
60 to 61	7.24	4.96-10.58
62 to 63	7.13	4.79-10.61
64 to 65	8.00	5.24-12.22
66 to 67	8.33	5.10-13.59
68 to 69	14.90	8.78-25.29
≥70	11.01	5.35-22.65
Age at birth of first child (years)		
<20	1	
20 to 29	1.03	0.79-1.34
≥30	1.45	1.08-1.96
No. of children		
0	1	
1	0.81	0.58-1.14
2	0.82	0.60-1.13
3	0.75	0.54-1.04
≥4	0.70	0.46-1.08
Female 1st degree relatives with breast cancer before the age of 50 or ovarian cancer at any time		
No	1	
Yes	2.08	1.55-2.79
No information on female 1st degree relatives	1.12	0.92-1.36
Oral contraception		
No	1	
Yes	1.13	0.97-1.31
Hormone replacement therapy		
No	1	

Yes	1.00	0.88-1.15
Other sex hormones		
No	1	
Yes	1.14	0.91-1.44
Medication related to alcoholism		
No	1	
Yes	1.35	0.94-1.93
Mammography screening attendance		
No	1	
Yes	1.84	1.42-2.39
Not invited	0.79	0.58-1.08
Highest educational level in family		
Primary and secondary school	1	
Advanced level education	1.28	0.94-1.75
Vocational education	1.13	0.75-1.72
Undergraduate and bachelor degree	1.32	0.97-1.79
Higher education	1.46	1.05-2.02
Unspecified	1.51	0.60-3.80
Missing	-	

CI: confidence interval

*Poisson regression model adjusted for changes in calendar year, age (<40, 40 to 44, 45 to 49, and every second year from age 50), age at birth of first child (<20, 20 to 29, ≥30, no children), number of births (0, 1, 2, 3, ≥4), female 1st degree relatives with breast cancer before age 50 or ovarian cancer at any time (0, ≥1 female, no information), oral contraception, ATC: G03A (no, yes), hormone replacement therapy, ATC: G03c, G03D, G03F (no, yes), other sex hormones, ATC: G03B, G03G, G03H (no, yes), medication related to alcoholism, ATC: N03AA, N05AB, N07BB (no, yes), mammography screening attendance (invited but not screened, invited and screened, not invited), and highest family educational level at first registration of work (unspecified, primary and secondary school, advanced level education, vocational education, undergraduate and bachelor degree, higher education, and no information on education).

MANUSCRIPT II

Short term effects of night shift work on risk of breast cancer classified by oestrogen and HER2 receptor status

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ABSTRACT

Objective The objective of this study was to examine if the association between recent night shift work and breast cancer differed by tumour subtypes defined by oestrogen (ER) and human epidermal growth factor 2 (HER2) status.

Methods A cohort study of 155,569 female employees in the public healthcare sector in Denmark 2007 to 2012. Day-by-day information on working time was available from pay roll registers and 1245 incident cases of breast cancer were identified in national cancer registries: 136 ER-/HER2-, 797 ER+/HER2-, 187 HER2+, and 125 not classifiable due to missing receptor status.

Results A significantly increased rate ratio (RR) was observed for HER2+ breast cancer among women who worked ≥30 night shifts (RR 1.49, 95% confidence interval (CI) 1.04 to 2.13) and ever night shifts (RR 1.35, 95% CI 1.01 to 1.81) compared with those who did not work night shifts after adjustment for age, age at first child, parity, family history of breast or ovarian cancer, sex hormones, medications related to alcoholism, family educational level, mammography screening, and other potential confounders. However, no association was observed among women likely to be recently hired. No increased risk was observed for ER+/HER2- and ER-/HER2- receptor subtypes.

Conclusion We observed an increased risk of HER2+ breast cancer following recent night shift work. Other breast cancer subtypes defined by ER and HER2 status were not associated with recent night shift work.

A number of epidemiologic studies have during the last approximately 20 years suggested an association between night shift work and increased risk of breast cancer (1). However, heterogeneous assessment of night shift work and heterogeneity in results complicate the casual interpretation. In addition, breast cancer has been classified as one disease entity in most studies, even though breast cancer is known to be a diverse group of tumours with different biology, pathology, and prognosis (2). Based on molecular profiles breast cancer can be classified into intrinsic subtypes which show a variety of clinical and pathological features: luminal A, luminal B, epidermal growth factor receptor 2 (HER2) overexpression, and basal like, which is also denoted triple negative tumours (3-6). In clinical settings, status of oestrogen receptor (ER) status, progesterone receptor (PR), and HER2, and antigen KI 67 (Ki67 proliferation index) are used to differentiate between these subtypes (7-9). PR status is strongly associated with ER status and the predictive value of PR status is limited when ER is known (3, 8, 10). Luminal A tumours (ER+/HER2-) account for about 40% of all breast cancer cases, are slow growing, and survival rates are high (4). HER2 overexpressing (HER2+) and triple negative tumours (ER-/PR-/HER2-) which account for approximately 10% and 10 to 20% of all breast cancers, respectively, on the other hand grow and spread more aggressively, and the patients are younger at diagnosis (4). HER2+ tumours can also be separated in ER- and ER+ subgroups, and the ER+ subgroups included in the luminal B subtype (8). Luminal B tumours (ER+/HER2+) account for 10 to 20% of all breast cancer cases, have high proliferation rates, and prognosis is worse than for luminal A (4).

The well established effects of prolonged exposure of breast tissue to oestrogen vary according to breast cancer subtypes and are most consistent for the hormone dependent tumours (4, 11, 12). Especially age, age at birth of first child, number of children, a family history of either breast cancer or ovarian cancer, and hormone replacement therapy has shown to have distinct effects on the breast cancer subtypes (13-15). This suggests distinct etiologic pathways for breast cancer subtypes and new risk factors may be overseen if this is not accounted for (16).

Inhibition of nocturnal melatonin is assumed to be a pivotal element in the causal mechanism linking night shift work and breast cancer and night shift work may exert long term as well as short term effects on breast cancer risk (17-25). Inhibition of nocturnal melatonin is proposed to influence the risk of ER dependent tumours through an increase in oestrogen production (23). Experimental studies have also shown that melatonin plays an important role in HER2+ carcinogenesis (26-28). There is no established mechanism linking triple negative breast cancer and night shift work, but we hypothesize that if there is an association between night shift work and the risk of breast cancer, we should expected a more pronounced effect in triple negative and HER2+ overexpressing tumours would be seen because of their more aggressive tumour doubling times (13-15).

Only few studies have considered receptor subtypes of breast cancer in relation to night shift work (29-36). Rabstein et al found a significant positive association

between night shift work and ER- tumours, on the other hand, Shernhammer et al and Wang et al found positive significant associations between night shift work and ER+ tumours (29, 30, 36). Wang et al and Papantoniou et al included as the only studies information on HER2 status (35, 36). Wang et al reported comparable associations between night shift work and HER2- and HER2+ tumours, but only the first observations was of statistical significance (36). Papantoniou et al reported positive non-significant associations with a tendency of higher risk in premenopausal than postmenopausal women (35). None of these studies accounted for whether night shift work occurred distantly or recently in time and thus, did not assess short term versus long term effects. Furthermore, a majority of these studies relied on self reported information on working time and may therefore be influenced by recall bias. In this study we used objective day-by-day information on exact working time during a period of up to six years prior to diagnosis.

In a previous study on the same dataset, we did not find an increased risk of breast cancer following recent night shift work (Vistisen et al). The objective in this study was to examine if the association between recent night shift work and breast cancer differed by tumour subtypes defined by ER and HER2 status.

MATERIALS AND METHODS

Data sources

This study is based on register linkage between seven Danish registries that is described in detail in a previous study (Vistisen HT et al). These registers provide a unique opportunity to examine the association between recent night shift work and breast cancer objectively by linkage on the individual level by use of the civil registration number given to all individuals living in Denmark since 1968 (37). The seven Danish registries are: The Danish Working Hour Database (January 1, 2007 to December 31, 2012), the Civil Registration System (1968 to December 31, 2012), the clinical database of the Danish Breast cancer Cooperative Group (1977 to December 31, 2012), the National Cancer Registry (1943 to December 31, 2011), the National Register of Medicinal Product Statistics (1995 to December 31, 2012), the Family Income Register from Statistics Denmark (January 1, 2007 to December 31, 2012), and the Clinical Database of Mammography Screening (2007 to December 31, 2012) (38-43).

The Danish Data Protection Agency approved the study (j.no. 2011-41-6850). In Denmark, register studies do not need to be approved by the Danish Health Research Ethics Committee System.

Populations

The study population was women aged 18 or older with at least one registration of work in the DWHD between January 1, 2007 and December 31, 2011 (n=156,927).

We excluded 1357 women who had been diagnosed with breast cancer prior to start of follow-up (date of first registration of work), and one woman with missing date of breast cancer diagnosis. The final study population included 155,569 women.

We had no information on the study participants working time prior to 2007. Therefore, to obtain a population with more complete night shift history we established a sub population of recently employed women from four out of five Regions contributing data to the Danish Working Hour Database with information on first date of employment in the current Region at January 1, 2007 or later (inception population). In total 71,479 women fulfilled the criteria.

Breast cancer

Breast cancer cases with information on ER or HER2 status together with date of diagnosis were identified in the clinical database of the Danish Breast Cancer Corporative Group for all available years. The cases were stratified into subtypes on the basis of their ER and HER2 status according to the Danish clinical guidelines (Figure 1) (44). Hence, we stratified breast cancer tumours into three subtypes: 1) tumours that were ER+ and HER2- (ER+/HER2-), 2) tumours that were HER2+ regardless of ER status, and 3) tumours that were ER- and HER2- (ER-/HER2-). Because PR status is strongly associated with ER status, PR status have not been routinely analysed in Denmark since 2007 and were therefore only available for a subset of cases and hence, not used in our classification of breast

cancer subtypes. ER status was defined using a cut off at 10% positive oestrogen cells. HER2 status was established using immunohistological markers from 0 to 3+, where 2+ is regarded as 'equivocal', and 3+ as positive. In HER2+ 2+ cases the immunohistological test was supplied with fluorescence, or chromogenic in situ hybridization (FISH and CISH test, respectively), and the tumour was classified as positive (HER2+) if oncogenic amplification was found (45).

Night shift work

We used a definition of night shift defined by the 2009 IARC Working Group as at least three hours of work between midnight and 05:00 AM (46). If a woman had a registration that met this criterion the woman was classified as exposed to a night shift on that specific date. Otherwise, she was classified as not exposed to night shift work (reference). From study entry a woman's night shifts were summed day by day. The number of night shifts was then categorized into three groups in respect to a reasonable number of person time for each of the three breast cancer subtype in the total population as well as the inception population: 0, 1-29, and ≥30 night shifts.

Covariates

From the registries, information was retrieved on age, age at birth of first child, number of children, a family history of either breast cancer before the age of 50 or ovarian cancer at any age among female first degree relatives, use of oral contraception, hormone replacement therapy or other hormone medications in the

G03 ATC group, use of medications related to alcohol over-consumption and addiction (ATC group: N03AA, N05AB and N07BB), highest educational level in the family, and attending mammography screening. These potential confounders were defined a priori based on a review of the literature (47, 48).

Statistical analysis

Each woman was followed on a daily basis from start of follow-up, which was the first registration of work until the date of first primary breast cancer diagnosis, death, disappearance, emigration, or end of follow-up at December 31, 2012. The association between night shift work and the incidence of breast cancer subtypes was estimated by rate ratios (RR). The analysis was made by a stacked Poisson regression based on a table combining person years at risk and number of events for: ER+/HER2-, HER2+, ER-/HER2-, and unclassified tumours (no receptor status available). This combined analysis allowed us to test whether the association between night shift work and the incidence of breast cancer differed between subtypes. Both crude and adjusted estimates were reported. The potential confounders were divided into two sets: A) age (<40, 40-44, 45-49, and every second year from age 50), age at birth of the first child (<20, 20-29, ≥30, no children), number of births (0, 1, 2, 3, ≥4), female 1st degree relatives with breast cancer before age 50 or ovarian cancer at any time (0, ≥1, no information), hormone replacement therapy, ATC: G03c, and G03D, G03F (no, yes), and B) calendar year (2008 to 2012), oral contraception, G03A (no, yes), other sex hormones, G03B, G03G, G03H (no, yes), medication related to alcoholism, ATC: N03AA, N05AB,

N07BB (no, yes), mammography screening attendance (invited but not screened, invited and screened, not invited), and highest family educational level at the first registration of work (unspecified, primary and secondary school, advanced level education, vocational education, undergraduate and bachelor degree, higher education, and no information on education). Age, age at birth of first child, number of children, a family history of either breast cancer or ovarian cancer, and hormone replacement therapy have distinct effects on the breast cancer subtypes (13-15). In the adjusted models the effects of the covariates in set A were allowed to differ between breast cancer subtypes while the covariates in set B were assumed to have the same effect on the rate independently of the subtype. Estimates were reported with a 95% confidence interval. All data management and analysis were done using Stata 13.1.

Data were almost complete for all variables except for female first degree relatives (5% missing) and highest family educational level (<0.5% missing). The missing values were evenly distributed across night shift groups.

RESULTS

A total of 155,569 women were followed between January 1, 2007 and December 31, 2012 and contributed a total of 771,417 person years, and 1245 overall breast cancer cases. Of these ER status was available for 1177 (95%) cases, HER2 status for 1123 (90%) cases, and both ER and HER2 status for 1118 (90%) cases. In total 136 ER-/HER2-, 797 ER+/HER2-, 187 HER2+, and 125 not classifiable (because of

missing receptor status) breast cancer cases were included. The inception population included 71,479 women and contributed a total of 286,050 person years and 321 overall breast cancer cases, 44 ER-/HER2-, 204 ER+/HER2-, 43 HER2+, and 30 not classifiable breast cancer cases. In the total population about 40% of the person years were cumulated below the age of 40, while this percentage was about 56 in the inception population.

Table 1 presents the crude distributions and age standardized percentages of person years by participant characteristics and number of night shifts for the total population. Family educational level and the number of children increased while nulliparity decreased by increasing number of night shifts. A smaller fraction of the night shift workers was 50 years or older compared to the non night shift workers (26% vs. 35%). Except for these, age standardized person years were evenly distributed by participant characteristics and number of night shifts. The inception population showed comparable person year distributions. The median age of the total population was 39 years and 35 years for the inception population.

Table 2 presents rate ratios (RR) of ER-/HER2-, ER+/HER2-, and HER2+ breast cancer subtypes by number of night shifts since study entry. There were no indications of increased risk of ER-/HER2-, and ER+/HER2- breast cancer among women who worked night shifts compared to women who did not work night shifts in the crude or adjusted analyses. In contrast, a significantly decreased RR was observed for ER+/HER2- among womenwho worked night shifts A

significant increased adjusted RR was observed for HER2+ breast cancer among women who worked ≥30 night shifts (1.49, 95% CI 1.04 to 2.13) and ever night shifts (1.35, 95% CI 1.01 to 1.81). Finally, there seems to be a tendency of negative confounding when comaring crude and adjusted RR.

In the inception population, we observed no association between night shift work and ER-/HER2-, ER+/HER2-, or HER2+ breast cancer subtypes in the crude or the adjusted analyses (Table 3).

DISCUSSION

The present study demonstrated a significant increased risk of HER2+ breast cancer among women working night shifts during follow up. No increased risk was found for ER-/HER2- or ER+/HER2- breast cancer.

The association between night shift work and HER2+ breast cancer has been examined in only two previous studies (35, 36). In spite of using a broader definition of HER2+ tumours (including equivocal cases) than the present study, Wang et al also observed an increased risk of HER2+ tumours among night shift workers though not significant (odds ratio(OR) 1.35 (95% CI 0.94 to 1.94)) (36). Papantoniou et al found a non-significant increased relative risk ratio of 1.31 (95% CI 0.93 to 1.85) for HER2+ breast cancer among women who worked night shifts compared to women who never worked night shifts (35). In the subgroup of premenopausal women this estimate was 1.56 (95% CI 0.94 to 2.59). Taken

together, the present study and the studies by Wang et al and Papantoniou et al indicate an association between HER2+ breast cancer and night shift work, even though results were only of statistically significant in the present study. In addition, these findings are supported by the results from experimental studies, which have linked melatonin suppression (as a surrogate for light at night or night shift work) to HER2+ carcinogenesis (26-28).

The inconsistency in findings for the HER2+ breast cancer subtype between the total and the inception population is puzzling. One explanation could be due to the inclusion of more long term night shift workers in the first but not in the latter population. The overall evidence of an association with breast cancer is strongest for long term night shift work and our findings, although based on information on recent night shift work, could represent an effect of long term night shift work (29, 34, 51, 53-55) because it is assumed that current night shift workers more often have been long term shift workers than workers currently not working night shifts. Unfortunately, we did not have access to working time data prior to 2007 that allowed us to elucidate this. Further studies are needed to further explore the effect of different exposure metrics as well as the effect of age.

ER-/HER2- tumours grow and spread aggressively and are more prevalent among young women (4). Our short term follow up is expected to cover a significant part of the relevant exposure time in relation to ER-/HER2- breast cancer because of its high tumour doubling times (13-15) and hence, if night shift work is associated

with ER-/HER2- breast cancer in all ages or solely young age these data would most likely have shown some effect. Our finding of no increased risk of ER+ tumours following recent night shift work are consistent with a majority of the studies which examined night shift work in general and who did not find a significant association (30-35). Only the studies by Shernhammer et al and Wang et al have described a positive association between night shift work and ER+ tumours (29, 36). However, none of these studies accounted for the timing of night shift work or HER2 status. Prolonged exposure of to oestrogen is a well established risk factor for breast cancer and in particular hormone dependent tumours (4, 11, 12). Night shift work, as a surrogate for nocturnal melatonin suppression, is suggested to increase in oestrogen production and thus, influence the risk of ER dependent tumours (23), but may be associated with long term exposure rather than recent expsoure. We were not able to assess long term night shift work due to lack of work schedule data prior to 2007.

In a previous study we had additional exposure groups of 30-99 and ≥100 night shifts, however, these exposure groups did not change the overall results and thus, it is not likely they would have changed the results in this study (Vistisen et al). The adjusted risk estimates were generally higher than the crude estimates and could be explained by lower age, lower fraction of nulliparity, and more children among the night shift workers.

Rate ratio estimates for ER-/HER- and ER+/HER2- breast cancer below unity were observed in the total population as well as the inception population and statistically significant for ER+/HER2- breast cancer.

The strengths of this study were mainly the objective and detailed information on working time including night shift work generated from a pay-roll register that is presumed to be complete for the period 2007 to 2012. Information on night shift work was based on daily records of paid working hours. Since the salary varies by working hours during the day these recordings are expected to be precise and valid given that employers and employees have a common interest in correct recordings. Cases of breast cancer before follow up were identified in national databases encompassing all breast cancers diagnosed in Denmark since 1943, and cases of breast cancer during follow up were identified in the clinical database of the Danish Breast Cancer Corporative Group includes pathological and clinical information on all breast cancers diagnosed and treated since 1977 (39, 40). A total of 90 to 95% of the breast cancer cases had information on HER2 and ER status. Thus, information bias with respect to exposure and outcome as well as selection bias can hardly explain our findings. This is expected to apply for confounding as well because we had comprehensive data on other risk factors. We have recently found these covariates to be associated with night shift work in manners that are consistent with previous studies (Vistisen HT).

Conclusion

We observed an increased risk of HER2+ breast cancer among women who have had recent night shift work. No increased risk was observed for other breast cancer subtypes defined by ER and HER2 status following recent night shift work. Further research will be needed to corroborate or refute these findings.

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HTV had full access to all data and takes responsibility for the integrity and accuracy of the data analysis. HK, AHG, MF, PC, and JPB were involved in the study concept and design. HK, AHG, JH, ÅMH, and HTV acquired pay-roll data. HTV acquired additional register data. MF was involved in the statistical analyses.

HK, AHG, MF, PC, JH, JPB, and HTV were involved in interpretation of data. HTV wrote the paper. HK, AHG, MF, PC, JH, JPB, and ÅMH critically revised the paper and approved the final version.

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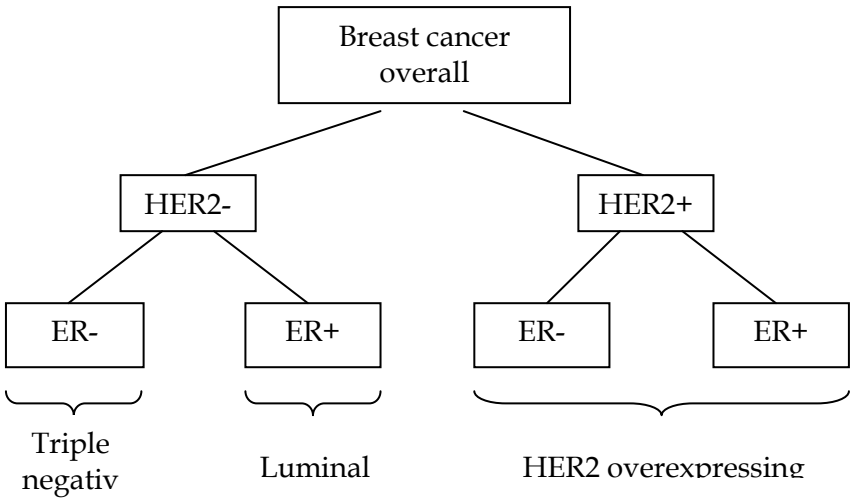

Figur 2 Clinical classification of breast cancer subtypes according to ER and HER2 status.

Table 1 Distribution of person years 2007 to 2012 by participant characteristics and number of night shift the last year among 155,569 women in the Danish public healthcare sector.

	Number of night shifts since study entry							
	0		1-29		≥30		Ever (≥1)	
Characteristics	100 person years	Age-standardized %*	100 person years	Age-standardized %*	100 person years	Age-standardized %*	100 person years	Age-standardized %*
Total	4558	100	1707	100	1449	100	3156	100
Age (years)**								
<40	1811	40	852	50	691	48	1544	49
40-49	1137	25	423	25	370	26	793	25
50-59	1104	24	335	20	301	21	636	20
≥60	506	11	97	6	87	6	184	6
Calendar year								
2007	544	12	231	13	44	3	275	9
2008	714	16	287	17	157	11	443	14
2009	779	17	294	17	239	16	533	17
2010	819	18	301	18	297	20	597	19
2011	851	19	300	18	340	24	640	20
2012	851	19	295	18	373	26	668	21
Age at birth of first child (years)								
<20	227	5	67	5	55	4	123	4
20-29	2513	54	938	56	812	57	1750	57
≥30	710	16	275	16	246	17	521	16
No children	1107	26	426	23	336	22	763	23
No. of children								
0	1107	25	426	23	336	22	763	23
1	775	17	297	17	250	17	547	17
2	1858	40	640	39	558	39	1198	39
3	679	15	285	17	251	18	536	18
≥4	138	3	59	4	54	4	113	4
Female 1st degree relatives with breast cancer before the age of 50 or ovarian cancer at any time								
No	4089	91	1567	91	1331	91	2898	91
Yes	109	2	45	3	36	2	82	3

No information on female 1st degree relatives	360	7	95	7	81	7	176	7
Oral contraception								
No	1875	38	578	39	493	38	1071	38
Yes	2683	62	1129	61	956	62	2085	62
Hormone replacement therapy								
No	3339	75	1320	74	1119	75	2438	75
Yes	1219	25	388	26	331	25	718	25
Other sex hormones								
No	4039	88	1474	87	1243	87	2717	87
Yes	519	12	233	13	206	13	439	13
Medication related to alcoholism		0		0		0		
No	4484	98	1683	98	1432	99	3115	99
Yes	74	2	24	2	17	1	41	1
Mammography screening attendance								
No	181	4	52	4	54	4	106	4
Yes	848	16	220	16	235	19	455	18
Not invited	3528	80	1435	80	1160	76	2595	78
Highest educational level in family								
Unspecified	28	1	11	1	10	1	21	1
Primary and secondary school	327	7	35	2	22	2	57	2
Advanced level education	1651	36	449	27	319	23	768	25
Vocational education	260	6	44	3	31	2	74	2
Undergraduate and bachelor degree	1580	35	878	51	837	57	1715	54
Higher education	710	16	290	17	229	15	519	16
Missing	2	0	1	0	1	0	2	0

* All covariates except age are age-standardized (<40, 40-44, 45-49, and every second year from age 50)
** Age is not age-standardized

Table 2 The rate ratio (RR) in the total population of ER-/HER2-, ER+/HER2-, and HER2+ breast cancer by number of night shifts since entry in the Danish public healthcare sector 2007 to 2012.

Number of night shifts since entry	Person years	Cases	Crude RR	95% CI	Adjusted RR*	95% CI
ER-/HER2- breast cancer						
Total	771,417	136				
0	455,783	87	1		1	
1-29	170,727	27	0.83	0.54-.28	0.94	0.61-1.45
≥30	144,907	22	0.80	0.50-1.27	0.90	0.56-1.45
Ever night-shift	315,633	49	0.81	0.57-1.15	0.92	0.65-1.31
ER+/HER2- breast cancer						
Total	771,417	797				
0	455,783	548	1		1	
1-29	170,727	133	0.65	0.54-0.78	0.83	0.69-1.01
≥30	144,907	116	0.67	0.54-0.81	0.82	0.67-1.01
Ever night-shift	315,633	249	0.66	0.56-0.76	0.83	0.71-0.96
HER2+ breast cancer						
Total	771,417	187				
0	455,783	102	1		1	
1-29	170,727	42	1.10	0.77-1.57	1.24	0.86-1.78
≥30	144,907	43	1.33	0.93-1.89	1.49	1.04-2.13
Ever night-shift	315,633	85	1.20	0.90-1.60	1.35	1.01-1.81

RR: rate ratio; CI: confidence interval

*Stacked Poisson regression model adjusted for: A) age (<40, 40-44, 45-49, and every second year from age 50), age at birth of first child (<20, 20-29, ≥30, no children), number of births (0, 1, 2, 3, ≥4), family history of breast cancer or ovarian cancer (no female 1. degree relatives with breast cancer before age 50 or ovarian cancer at any time, ≥1 female 1. degree relatives with breast cancer before age 50 or ovarian cancer at any time, no information of 1. degree relatives), and hormone replacement therapy, ATC: G03c, G03D, G03F (no, yes), and B) calendar year, oral contraception use, ATC: G03A (no, yes), use of other sex hormones, ATC: G03B, G03G, G03H (no, yes), use of medication related to alcoholism, ATC: N03AA, N05AB, N07BB (no, yes), attending national mammography screening (invited but not screened, invited and screened, not invited), and highest education in the family/household (unspecified, primary and secondary school, advanced level education, vocational education, undergraduate and bachelor degree, higher education, and no information on education). Where the effects of the covariates in A were allowed to differ between subtypes the covariates in B were assumed to have the same effect on the rate independently of the subtype.

Table 3 The rate ratio (RR) in the inception population of ER-/HER2-, ER+/HER2-, and HER2+ breast cancer by number of night shifts since entry in the Danish public healthcare sector 2007 to 2012.

Number of night shifts since entry	Person years	Cases	Crude RR	95% CI	Adjusted RR*	95% CI
ER-/HER2- breast cancer						
Total	286,050	44				
0	182,624	32	1		1	
1-29	61,000	8	0.75	0.34-1.62	0.82	0.38-1.78
≥30	42,425	4	0.54	0.19-1.52	0.59	0.21-1.67
Ever night-shift	103,426	12	0.66	0.34-1.29	0.72	0.37-1.41
ER+/HER2- breast cancer						
Total	286,050	204				
0	182,624	27	1		1	
1-29	61,000	34	0.71	0.49-1.03	0.87	0.59-1.26
≥30	42,425	143	0.81	0.54-1.23	0.98	0.65-1.49
Ever night-shift	103,426	177	0.75	0.56-1.02	0.91	0.67-1.24
HER2+ breast cancer						
Total	286,050	43				
0	182,624	4	1		1	
1-29	61,000	10	1.03	0.50-2.12	1.09	0.53-2.25
≥30	42,425	29	0.59	0.21-1.69	0.64	0.22-1.82
Ever night-shift	103,426	39	0.85	0.45-1.61	0.91	0.48-1.73

RR: rate ratio; CI: confidence interval

*Stacked Poisson regression model adjusted for: A) age (<40, 40-44, 45-49, and every second year from age 50), age at birth of first child (<20, 20-29, ≥30, no children), number of births (0, 1, 2, 3, ≥4), family history of breast cancer or ovarian cancer (no female 1. degree relatives with breast cancer before age 50 or ovarian cancer at any time, ≥1 female 1. degree relatives with breast cancer before age 50 or ovarian cancer at any time, no information of 1. degree relatives), and hormone replacement therapy, ATC: G03c, G03D, G03F (no, yes), and B) calendar year, oral contraception use, ATC: G03A (no, yes), use of other sex hormones, ATC: G03B, G03G, G03H (no, yes), use of medication related to alcoholism, ATC: N03AA, N05AB, N07BB (no, yes), attending national mammography screening (invited but not screened, invited and screened, not invited), and highest education in the family/household (unspecified, primary and secondary school, advanced level education, vocational education, undergraduate and bachelor degree, higher education, and no information on education). Where the effects of the covariates in A were allowed to differ between subtypes the covariates in B were assumed to have the same effect on the rate independently of the subtype.

MANUSCRIPT III

Short term risk of breast cancer following consecutive night shifts: a cohort study of pay roll data

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ABSTRACT

Purpose

The objective of this study was to assess the association between number of consecutive night shifts and HER2+ breast cancer as well as overall breast cancer.

Methods

A cohort of 155,569 women employed in the public healthcare sector in Denmark. Detailed and individual information on date, hour, and minute on working time was available from the Danish Working Hour Database from January 1, 2007 to December 31, 2012. A total of 1245 breast cancer cases were identified in national cancer registries, of which 187 were HER2+ tumours and 125 were missing on receptor status.

Results

An increased risk of HER2+ was observed among women who ever worked consecutive night shifts (RR 1.45, 1.06-1.98) when compared with women never working night shifts. This risk appeared to increase by number of consecutive night shifts (RR 1.94, 1.23-3.08 among women who worked ≥7 consecutive night shifts). Among women who worked 1 to 29 night shifts there was increased risk of HER2+ following consecutive night shifts (RR 1.56, 0.77-3.14) and among women who worked 30 to 99 night shifts there was an increased risk following ≥6 night shifts (RR 1.25, 0.52-3.01). No increased risk of overall breast cancer was observed for women working consecutive night shifts (RR 0.95, 0.83-1.08) compared to women not working night shifts.

Conclusions

We observed associations between consecutive night shifts during recent years and HER2+ when compared with no night shift work. Among night shift workers, however, analyses displayed inconsistent results that were not in agreement with casual effects.

INTRODUCTION

Night shift work that involves circadian disruption has been suggested to be carcinogenic to humans.(1) Disruption of the circadian rhythm by reduced melatonin secretion or phase shifting and as a consequence of this, disequilibrium of the master circadian pacemaker with peripheral oscillators may be linked to breast cancer though different mechanisms which might work together.(2-5) Reduced melatonin secretion may occur during a single night shift whereas a phase shift and circadian disequilibrium require consecutive night shifts to occur.(2, 6-9)

Night shift work is inevitable in several industries and sectors and the organization of work schedules to reduce any risk of breast cancer is therefore important.(10) Restriction of the number of consecutive night shifts has been recommended in order to reduce breast cancer risk. Although, this recommendation is supported by epidemiologic observations which indicate an increased risk by increased number of consecutive night shifts, evidence is scarce.(11)

We recently observed an increased risk of breast cancer overexpressing the human epidermal growth factor 2 (HER2+), but no increased overall risk of breast cancer or other subtypes defined by HER2 or oestrogen receptor status following night shift work during recent years.(Vistisen et al) The objective of this study was to assess the association between number of consecutive night shifts and HER2+ breast cancer as well as overall breast cancer.

MATRIALS AND METHOD

Data sources

This study is based on objective and individual register linkage between seven Danish registries described in a previous study.(Vistisen et al) The seven Danish registries are: The Danish Working Hour Database (January 1, 2007 to December 31, 2012) encompasses all employees in the Danish Regions, the Civil Registration System (1968 to December 31, 2012) encompasses all individuals born or lived in Denmark since 1968, the clinical database of the Danish Breast cancer Cooperative Group (DBCG) (1977 to December 31,

2012) encompasses all women diagnosed and treated for breast cancer, the National Cancer Registry (1943 to December 31, 2011) encompasses all cancers diagnosed, the National Register of Medicinal Product Statistics (1995 to December 31, 2012) encompasses all purchases of prescription drugs, the Family Income Register from Statistics Denmark (January 1, 2007 to December 31, 2012) encompasses information on socio economic variables, and the Clinical Database of Mammography Screening (2007 to December 31, 2012) encompasses all women invited and screened in the national screening programme (15-20).

The Danish Data Protection Agency approved the study (j.no. 2011-41-6850). In Denmark, register studies do not need to be approved by the Danish Health Research Ethics Committee System.

Populations

From the Danish Working Hour Database women aged 18 or older with at least on registration of work between January 1, 2007 and December 31, 2012 were identified (n=156,927). We excluded 1357 women who had been diagnosed with breast cancer prior to start of follow up (date of first registration of work), and 1 women with missing date of breast cancer diagnosis. The final study population included 155,569 women.

Breast cancer

Breast cancer cases with information on HER2 status together with date of diagnosis were identified in the DBCG database from January 1, 2007 to December 31, 2012. HER2+ status was established using immunohistological markers from 0 to 3+, where 2+ is regarded as 'equivocal', and 3+ as positive. In Her2 2+ cases the immunohistological test was supplied with fluorescence, or chromogenic in situ hybridization (FISH and CISH test, respectively), and the tumour was classified as positive if oncogenic amplification was found.(21)

The DBCG database has registrations as of 1977. To exclude women diagnosed with breast cancer before study entry information on breast cancer cases prior to 1977 was retrieved from the National Cancer Registry (IDC10=DC50 or ICD7=170).

Night shift work

Information on night shifts obtained from the Danish Working Hour Database which encompasses daily recordings of working time on an individual level. A night shift was defined according to the 2009 IARC Working Group as at least three hours of work between midnight and 05:00 AM.(2) Exposure to night shifts was classified as: 1) never night shift work, 2) night shift work, but no consecutive night shifts, and 3) consecutive night shifts: ≥2, ≥3, ≥4, ≥5, ≥6, or ≥7. Each woman contributed person time to the first category until the date of her first night shift, and to the second category until the date of her first ≥2, ≥3, ≥4, ≥5, ≥6, or ≥7 consecutive night shifts, and contributed to the highest attained of the latter categories until the end of follow up.

Covariates

Potential confounders were defined a priori based on a review of the literature.(22, 23) Information on age, age at birth of first child, number of children, a family history of either breast cancer before the age of 50 or ovarian cancer at any age among female first degree relatives, use of oral contraception, hormone replacement therapy or other hormone medications in the G03 ATC group, use of medications related to alcohol over-consumption and addiction (ATC group: N03AA, N05AB and N07BB), highest educational level in the family, and attending mammography screening was retrieved from registry linkage between the seven national registers. Additionally, the number of night shifts worked was calculated for each day during follow up.

Statistical analysis

Each woman was followed on a daily basis from start of follow-up, that was the date of the first registration of work, and until the date of first primary breast cancer diagnosis, death, disappearance, emigration, or end of follow-up at December 31, 2012. Data were analysed as incidence rate, i.e. as the number of incident overall breast cancer cases and incident HER2+ breast cancer cases per time units at risk using Poisson regression. All variables were time-dependent, i.e. varied for each date from start until the end of follow-up. The association between consecutive night shift work and the incidence of overall breast cancer and HER2+ breast cancer was estimated by rate ratios (RR). Separate analyses were done for each consecutive night shift category: ≥2, ≥3, ≥4, ≥5, ≥6, and ≥7 consecutive night shifts. The reference group were women who did not work night shifts. The analyses were also done for different groups of night shift work: 1-29, 30-99, and ≥100 night shifts. The reference group in these analyses was women who worked night shifts

which did not include ≥2, ≥3, ≥4, ≥5, ≥6, or ≥7 consecutive night shifts, respectively. Both crude and adjusted estimates were reported, where the adjusted models included the following potential confounders: age (<40, 40-44, 45-49, and every second year from age 50), age at birth of the first child (<20, 20-29, ≥30, no children), number of births (0, 1, 2, 3, ≥4), female 1st degree relatives with breast cancer before age 50 or ovarian cancer at any time (0, ≥1 , no information), hormone replacement therapy, ATC: G03c, G03D, G03F (no, yes), calendar year (2008 to 2012), oral contraception, ATC: G03A (no, yes), other sex hormones, ATC: G03B, G03G, G03H (no, yes), medication related to alcoholism, ATC: N03AA, N05AB, N07BB (no, yes), mammography screening attendance (invited but not screened, invited and screened, not invited), and highest family educational level at the first registration of work (unspecified, primary and secondary school, advanced level education, vocational education, undergraduate and bachelor degree, higher education, and no information on education). Estimates were reported with a 95% confidence interval. All data management and data analysis were done using Stata 13.1.

Data were complete for all variables except for female first degree relatives (5% missing) and highest family educational level (<0.5% missing). The missing values were evenly distributed across night shift groups.

RESULTS

The 155.569 women followed from January 1, 2007 to December 31, 2012 contributed a total of 771,417 person years and 1245 breast cancer cases. A total of 1123 cases had information on HER2 status and 187 cases of HER2+ breast cancer cases were observed during follow up. Table 1 presents the distribution of person years by age and consecutive night shift status and the age standardized distributions by other participant characteristics and consecutive night shift status. Among women who ever worked consecutive night shifts, there was a higher fraction of person years below age 40, a higher fraction of age standardized person years among women who had a higher level of education in the family, and a lower fraction among nulliparous women. Number of consecutive night shifts was strongly associated with the number of night shifts. Comparable distributions were observed across covariates for ≥3, ≥4, ≥5, ≥6, and ≥7 consecutive night shifts (data not shown).

Table 2 presents rate ratios (RR) of overall breast cancer by number of consecutive night shifts during follow up using a dichotome categorization (ever/never number of consecutive night shifts). Overall breast cancer was not associated with ever working consecutive night shifts compared with never working night shifts. This was irrespective of the number of consecutive night shifts. Internal analyses restricted to night shift workers showed comparable estimates for the high and low categories of consecutive night shifts.

A significant increased adjusted HER2+ breast cancer RR was observed for consecutive night shifts compared with never working night shifts and it was furthermore indicated that the risk increased by increasing number of consecutive night shifts (Table 3). For women working seven or more consecutive night shifts the RR for HER2+ breast cancer was 1.94 (95% CI 1.23 to 3.08).

Table 4 presents the association between consecutive night shifts and overall breast cancer for different numbers of night shifts. Thus, the reference group was women who worked night shifts which did not include ≥2, ≥3, ≥4, ≥5, ≥6, or ≥7 consecutive night shifts, respectively. Among women who worked 1 to 29 night shifts, an increased risk of HER2+ breast cancer following consecutive night shifts was observed. Among women who worked 30 to 99 night shifts, an increased risk of HER2+ breast cancer was observed among those who worked six or more consecutive night shifts. However, this was not significant and the confidence intervals were wide. There was not enough data to examine the risk of HER2+ breast cancer following consecutive night shifts among women who worked more than 100 night shifts. As in the case for overall breast cancer, the risk of HER2+ breast cancer also seemed to increase by increasing number of night shifts, Table S.1.

DISCUSSION

An increased risk of HER2+ breast cancer was observed among women who ever worked consecutive night shifts when compared with women never working night shifts. This risk appeared to increase by number of consecutive night shifts. Among women who worked 1 to 29 night shifts there was increased risk of HER2+ breast cancer following consecutive night shifts and among women who worked 30 to 99 night shifts there was an increased risk following six or more night shifts. However, the data was limited. No increased risk

of overall breast cancer was observed for women who worked consecutive night shifts compared to women who did not work night shifts.

To our knowledge, only Lie et al have examined number of consecutive night shifts and risk of breast cancer.(11, 24) In this study, a 70% increased odds ratio (OR 1.7, 95% CI 1.1 to 2.8) of overall breast cancer following five or more years with seven or more consecutive night shifts was observed.(11) In additions, the observations indicated an exposure response effect.(11) Analyses of breast cancer subtypes classified by oestrogen and progesterone receptor status showed high odds ratios for progesterone dependent breast cancer (2.4, 95% CI 1.3 to 4.3), but these analysis did not consider timing of exposure or HER2 receptor status.(24) However, women who never worked night shifts were used as reference group and thus, did not examine the effect of consecutive night among night shift workers. The observed effect could be attributed the number of night shifts rather than the consecutive night shifts.

In a previous study of the same dataset we found an increased risk of HER2+ breast cancer among women who worked 30 or more night shifts (RR 1.49, 95% confidence interval (CI) 1.04 to 2.13) and ever working night shifts (RR 1.35, 95% CI 1.01 to1.81) compared to women who did not work night shifts. Because consecutive night shifts were highly correlated with any night shifts, the suggested association between consecutive night shifts and HER2+ breast cancer could be confounded by any night shifts. For that reason we stratified by the number of night shifts. These analyses suggested effects of consecutive night shifts for overall as well as HER2+ breast cancer, but only among those working less than 30 night shifts. This could be because the more susceptible workers avoid or leave night shift work earlier. Chonotype is suggested to mediate such effects.(2) In our opinion, this can, however, not explain that no effect was seen in those working ≥30 night shifts, because the reference group was women with the same exposure to night shifts. Even if other selection processes and chance may be at play we would have expected consistent associations across the night shift groups – if the underlying data represents causual associations.

Several consecutive night shifts may cause a phase shift and circadian disequilibrium that has been linked to breast cancer carcinogenesis in experimental studies.(2, 6-9) WE were, however, not able to support this by epidemiological data for recent night shift work.

Major strengths were a large study population with a high prevalence of night shift work and objective and detailed information on work hours generated from pay roll registers. This allowed us to for the first time to assess the number of consecutive night shifts in detail over several years. Furthermore we expect our information on night shift to be unaffected by recall bias compared with many other studies. Cases of breast cancer and HER2 receptor status were identified in national databases encompassing all breast cancers diagnosed in Denmark since 1943 and should neither be affected by recall bias.(16, 17) This is expected to apply for confounding as well because we had comprehensive data on other risk factors from national registers.

A major limitation was the lack of information on night shift work prior to 2007 that hindered us to assess the effect of early and long term night shift work.

Conclusion

We observed associations between consecutive night shifts during recent years and HER2+ when compared with no night shift work. Among night shift workers, however, analyses displayed inconsistent results that were not in agreement with casual effects.

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Table 1 Distribution of person years 2007 to 2012 by participant characteristics and consecutive nights shift work status among 155,569 women in the Danish public healthcare sector.

	Never night shift work		Night shift work but never ≥2 consecutive night shifts		Ever (≥1) ≥2 consecutive night shifts	
	100 person years	Age standardized %*	100 person years	Age standardized %*	100 person years	Age standardized %*
Total	4575	100	552	100	2588	100
Age (years)**						
<40	1821	40	215	39	1318	51
40-49	1141	25	154	28	635	25
50-59	1106	24	137	25	496	19
≥60	507	11	46	8	138	5
Calendar year						
2007	548	12	65	12	206	8
2008	716	16	86	16	354	13
2009	782	17	94	17	436	17
2010	822	18	100	18	494	19
2011	854	19	103	19	534	21
2012	854	18	103	19	563	22
Age at birth of first child (years)						
<20	228	5	23	4	99	4
20-29	2522	54	309	55	1432	57
≥30	712	16	94	17	424	16
No children	1113	26	125	24	632	22
No. of children						
0	1113	26	125	24	632	22
1	778	17	88	16	456	17
2	1863	40	220	39	972	39
3	682	15	98	17	436	18
≥4	139	3	21	4	91	4
Female 1st degree relatives with breast cancer before the age of 50 or ovarian cancer at any time						
No	4105	91	500	91	2382	91
Yes	110	2	15	3	66	3
No information on female 1st degree relatives	360	7	36	7	139	7
Oral contraception						
No	1880	38	224	39	842	38
Yes	2695	62	327	61	1746	62
Hormone replacement therapy						
No	3353	75	406	74	2018	75
Yes	1222	25	146	26	569	25
Other sex hormones						
No	4053	88	487	88	2215	87
Yes	522	12	64	12	372	13
Medication related to alcoholism						
No	4501	98	543	98	2556	99
Yes	74	2	9	2	32	1
Mammography screening						

attendance						
No	182	4	23	4	83	4
Yes	850	16	99	17	355	18
Not invited	3544	80	430	79	2150	78
Highest educational level in family						
Unspecified	28	1	3	1	18	0
Primary and secondary school	327	7	14	3	43	2
Advanced level education	1659	37	141	26	618	25
Vocational education	260	6	15	3	59	2
Undergraduate and bachelor degree	1587	35	277	50	1431	55
Higher education	712	16	101	18	417	16
Missing	2	0	0	0	1	0
Number of night shifts						
0	4575	100	-	-	-	-
1 to 29	-	-	543	98	1147	44
30 to 99	-	-	8	2	900	35
≥100	-	-	0	0	540	22

* Age-standardized (<40, 40-44, 45-49, and every second year from age 50)

** Age is not age-standardized

Table 2 The rate ratio (RR) of overall breast cancer by number of consecutive nights shifts among 155,569 women in the public Danish healthcare sector (2007 to 2012).

Number of consecutive nights since entry	100 PYR	Cases	Crude RR (95% CI)	Adjusted RR (95% CI)*
≥2 consecutive nights				
Total	7615	1245		
Never night shift	4575	825	1	1
Night shift but never ≥2 consecutive nights	552	73	0.73 (0.58-0.93)	0.74 (0.59-0.95)
Ever ≥2 consecutive nights	2588	347	0.74 (0.66-0.84)	0.95 (0.83-1.08)
≥3 consecutive nights				
Total	7615	1245		
Never night shift	4575	825	1	1
Night shift but never ≥3 consecutive nights	552	158	0.79 (0.67-0.94)	0.86 (0.73-1.02)
Ever ≥3 consecutive nights	2588	262	0.72 (0.62-0.82)	0.93 (0.81-1.07)
≥4 consecutive nights				
Total	7615	1245		
Never night shift	4575	825	1	1
Night shift but never ≥4 consecutive nights	552	239	0.76 (0.66-0.88)	0.88 (0.76-1.02)
Ever ≥4 consecutive nights	2588	181	0.72 (0.61-0.84)	0.93 (0.79-1.10)
≥5 consecutive nights				
Total	7615	1245		
Never night shift	4575	825	1	1
Night shift but never ≥5 consecutive nights	552	291	0.75 (0.65-0.85)	0.89 (0.78-1.03)
Ever ≥5 consecutive nights	2588	129	0.73 (0.61-0.88)	0.92 (0.76-1.11)
≥6 consecutive nights				
Total	7615	1245		
Never night shift	4575	825	1	1
Night shift but never ≥6 consecutive nights	552	314	0.73 (0.64-0.83)	0.88 (0.77-1.01)
Ever ≥6 consecutive nights	2588	106	0.79 (0.65-0.97)	0.97 (0.79-1.19)
≥7 consecutive nights				
Total	7615	1245		
Never night shift	4575	825	1	1
Night shift but never ≥7 consecutive nights	552	333	0.73 (0.64-0.82)	0.89 (0.78-1.01)
Ever ≥7 consecutive nights	2588	87	0.81 (0.65-1.01)	0.95 (0.76-1.19)

RR: rate ratio; CI: confidence interval

*Poisson regression model adjusted for changes in calendar year, age (<40, 40-44, 45-49, and every second year from age 50), age at birth of first child (<20, 20-29, ≥30, no children), number of births (0, 1, 2, 3, ≥4), family history of breast cancer or ovarian cancer (no female 1. degree relatives with breast cancer before age 50 or ovarian cancer at any time, ≥1 female 1. degree relatives with breast cancer before age 50 or ovarian cancer at any time, no information of 1. degree relatives), oral contraception use, ATC: G03A (no, yes), hormone replacement therapy, ATC: G03c, G03D, G03F (no, yes), use of other sex hormones, ATC: G03B, G03G, G03H (no, yes), use of medication related to alcoholism, ATC: N03AA, N05AB, N07BB (no, yes), attending national mammography screening (invited but not screened, invited and screened, not invited), and highest education in the family/household (unspecified, primary and secondary school, advanced level education, vocational education, undergraduate and bachelor degree, higher education, and no information on education).

Table 3 The rate ratio (RR) of HER2+ breast cancer by number of consecutive night shifts among 155,569 women in the public Danish healthcare sector (2007 to 2012).

Number of consecutive nights since entry	1,000 PYR	Cases	Crude RR (95% CI)	Adjusted RR (95% CI)*
≥2 consecutive nights				
Total	771	187		
Never night shift	458	103	1	1
Night shift but never ≥2 consecutive nights	55	11	0.89 (0.48-1.65)	0.87 (0.47-1.62)
Ever ≥2 consecutive nights	259	73	1.25 (0.93-1.69)	1.45 (1.06-1.98)
≥3 consecutive nights				
Total	771	187		
Never night shift	458	103	1	1
Night shift but never ≥3 consecutive nights	111	24	0.96 (0.62-1.50)	0.99 (0.63-1.55)
Ever ≥3 consecutive nights	203	60	1.31 (0.95-1.80)	1.55 (1.11-2.16)
≥4 consecutive nights				
Total	771	187		
Never night shift	458	103	1	1
Night shift but never ≥4 consecutive nights	174	43	1.10 (0.77-1.56)	1.18 (0.82-1.69)
Ever ≥4 consecutive nights	140	41	1.30 (0.91-1.87)	1.54 (1.06-2.24)
≥5 consecutive nights				
Total	771	187		
Never night shift	458	103	1	1
Night shift but never ≥5 consecutive nights	216	54	1.11 (0.80-1.54)	1.22 (0.87-1.71)
Ever ≥5 consecutive nights	98	30	1.36 (0.91-2.04)	1.59 (1.05-2.41)
≥6 consecutive nights				
Total	771	187		
Never night shift	458	103	1	1
Night shift but never ≥6 consecutive nights	240	58	1.07 (0.78-1.48)	1.19 (0.85-1.66)
Ever ≥6 consecutive nights	74	26	1.56 (1.01-2.39)	1.80 (1.16-2.78)
≥7 consecutive nights				
Total	771	187		
Never night shift	458	103	1	1
Night shift but never ≥7 consecutive nights	255	61	1.06 (0.78-1.46)	1.18 (0.85-1.64)
Ever ≥7 consecutive nights	59	23	1.72 (1.10-2.71)	1.94 (1.23-3.08)

RR: rate ratio; CI: confidence interval

*Poisson regression model adjusted for changes in calendar year, age (<40, 40-44, 45-49, and every second year from age 50), age at birth of first child (<20, 20-29, ≥30, no children), number of births (0, 1, 2, 3, ≥4), family history of breast cancer or ovarian cancer (no female 1. degree relatives with breast cancer before age 50 or ovarian cancer at any time, ≥1 female 1. degree relatives with breast cancer before age 50 or ovarian cancer at any time, no information of 1. degree relatives), oral contraception use, ATC: G03A (no, yes), hormone replacement therapy, ATC: G03c, G03D, G03F (no, yes), use of other sex hormones, ATC: G03B, G03G, G03H (no, yes), use of medication related to alcoholism, ATC: N03AA, N05AB, N07BB (no, yes), attending national mammography screening (invited but not screened, invited and screened, not invited), and highest education in the family/household (unspecified, primary and secondary school, advanced level education, vocational education, undergraduate and bachelor degree, higher education, and no information on education).

Table 4 The rate ratio (RR) of HER2+ breast cancer by number of consecutive night shifts among who worked 1-29, 30-99, or ≥100 night shifts. A total of 155,569 women in the public Danish healthcare sector were followed from 2007 to 2012.

	Women 1-29 night shifts			Women 30-99 night shifts			Women ≥100 night shifts		
Number of consecutive nights since entry	100 PYR	Cases	Adjusted RR* (95% CI)	100 PYR	Cases	Adjusted RR* (95% CI)	1,000 PYR	Cases	Adjusted RR* (95% CI)
≥2 consecutive nights									
Total	1680			909			540		
Never night shift									
Night shift but never ≥2 consecutive nights	543	11	1	8	0		0	0	
Ever ≥2 consecutive nights	1147	30	1.56 (0.77-3.14)	900	27	Few data	540	16	Few data
≥3 consecutive nights									
Total	1680			909			540		
Never night shift									
Night shift but never ≥3 consecutive nights	996	23	1	105	1		6	0	
Ever ≥3 consecutive nights	694	18	1.37 (0.73-2.56)	803	26	Few data	534	16	Few data
≥4 consecutive nights									
Total	1680			909			540		
Never night shift									
Night shift but never ≥4 consecutive nights	1387	32	1	319	11	1	37	0	
Ever ≥4 consecutive nights	303	9	1.58 (0.75-3.35)	590	16	1.00 (0.45-2.22)	503	16	Few data
≥5 consecutive nights									
Total	1680			909			540		
Never night shift									
Night shift but never ≥5 consecutive nights	1547	36	1	524	17	1	88	1	
Ever ≥5 consecutive nights	143	5	1.88 (0.73-4.84)	385	10	0.98 (0.43-2.23)	452	15	Few data
≥6 consecutive nights									
Total	1680			909			540		
Never night shift									
Night shift but never ≥6 consecutive nights	1605	37		647	19	1	145	2	
Ever ≥6 consecutive nights	85	4	Few data	261	8	1.25 (0.52-3.01)	395	14	Few data
≥7 consecutive nights									
Total	1680			909			540		
Never night shift									
Night shift but never ≥7 consecutive nights	1632	38		718	21	1	196	2	
Ever ≥7 consecutive nights	58	3	Few data	191	6	1.23 (0.47-3.25)	344	14	Few data

RR: rate ratio; CI: confidence interval

*Poisson regression model adjusted for changes in calendar year, age (<40, 40-44, 45-49, and every second year from age 50), age at birth of first child (<20, 20-29, ≥30, no children), number of births (0, 1, 2, 3, ≥4), family history of breast cancer or ovarian cancer (no female 1. degree relatives with breast cancer before age 50 or ovarian cancer at any time, ≥1 female 1. degree relatives with breast cancer before age 50 or ovarian cancer at any time, no information of 1. degree relatives), oral contraception use, ATC: G03A (no, yes), hormone replacement therapy, ATC: G03c, G03D, G03F (no, yes), use of other sex hormones, ATC: G03B, G03G, G03H (no, yes), use of medication related to alcoholism, ATC: N03AA, N05AB, N07BB (no, yes), national mammography screening (invited but not screened,

invited and screened, not invited), and highest education in the family/household (unspecified, primary and secondary school, advanced level education, vocational education, undergraduate and bachelor degree, higher education, and no information on education).

SUPPLEMENTARY

Table S.1 The rate ratio (RR) of overall breast cancer by number of consecutive nights shifts among who worked 1-29, 30-99, or ≥100 nights shifts. A total of 155,569 women in the public Danish healthcare sector were followed from 2007 to 2012.

	Women 1-29 night shifts			Women 30-99 night shifts			Women ≥100 night shifts		
Number of consecutive nights since entry	100 PYR	Cases	Adjusted RR* (95% CI)	100 PYR	Cases	Adjusted RR* (95% CI)	1,000 PYR	Cases	Adjusted RR* (95% CI)
≥2 consecutive nights									
Total	1680	225		909	112		540	83	
Never night shift									
Night shift but never ≥2 consecutive nights	543	72	1	8	1		0	0	
Ever ≥2 consecutive nights	1147	153	1.32 (0.99-1.75)	900	111	Few data	540	83	Few data
≥3 consecutive nights									
Total	1680	225		909	112		540	83	
Never night shift									
Night shift but never ≥3 consecutive nights	996	138	1	105	20	1	6	0	
Ever ≥3 consecutive nights	694	87	1.16 (0.88-1.52)	803	92	0.84 (0.51-1.38)	534	83	Few data
≥4 consecutive nights									
Total	1680	225		909	112		540	83	
Never night shift									
Night shift but never ≥4 consecutive nights	1387	182	1	319	52	1	37	5	1
Ever ≥4 consecutive nights	303	43	1.40 (1.00-1.96)	590	60	0.77 (0.52-1.14)	503	78	1.44 (0.57-3.62)
≥5 consecutive nights									
Total	1680	225		909	112		540	83	
Never night shift									
Night shift but never ≥5 consecutive nights	1547	201	1	524	76	1	88	14	1
Ever ≥5 consecutive nights	143	24	1.66 (1.08-2.54)	385	36	0.75 (0.49-1.13)	452	69	1.16 (0.64-2.12)
≥6 consecutive nights									
Total	1680	225		909	112		540	83	
Never night shift									
Night shift but never ≥6 consecutive nights	1605	208	1	647	86	1	145	20	1
Ever ≥6 consecutive nights	85	17	1.93 (1.17-3.18)	261	26	0.84 (0.53-1.33)	395	63	1.34 (0.79-2.27)
≥7 consecutive nights									
Total	1680	225		909	112		540	83	
Never night shift									
Night shift but never ≥7 consecutive nights	1632	213	1	718	91	1	196	29	1
Ever ≥7 consecutive nights	58	12	1.91 (1.07-3.44)	191	21	0.92 (0.56-1.52)	344	54	1.13 (0.70-1.84)

RR: rate ratio; CI: confidence interval

*Poisson regression model adjusted for changes in calendar year, age (<40, 40-44, 45-49, and every second year from age 50), age at birth of first child (<20, 20-29, ≥30, no children), number of births (0, 1, 2, 3, ≥4), family history of breast cancer or ovarian cancer (no female 1. degree relatives with breast cancer before age 50 or ovarian cancer at any time, ≥1 female 1. degree relatives with breast cancer before age 50 or ovarian cancer at any time, no information of 1. degree relatives), oral contraception use, ATC: G03A (no, yes), hormone replacement therapy, ATC: G03c, G03D, G03F (no, yes), use of other sex hormones, ATC: G03B, G03G, G03H (no, yes), use of medication related to alcoholism, ATC:

N03AA, N05AB, N07BB (no, yes), attending national mammography screening (invited but not screened, invited and screened, not invited), and highest education in the family/household (unspecified, primary and secondary school, advanced level education, vocational education, undergraduate and bachelor degree, higher education, and no information on education).