Puerperal mastitis: a reproductive event of importance affecting anti-mucin antibody levels and ovarian cancer risk

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Puerperal Mastitis: a Reproductive Event of Importance Affecting Anti-Mucin Antibody Levels and Ovarian Cancer Risk

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Abstract

Purpose—Test the hypothesis that puerperal mastitis may alter immunity related to the mucin (MUC) family of glycoproteins and lower risk for ovarian cancer.

Methods—In two case-control studies conducted in New England between 1998–2008, we examined the association between self-reported mastitis and ovarian cancer in 1,483 women with epithelial ovarian cancer and 1,578 controls. IgG1 antibodies against (MUC1) CA15.3 and (MUC16) CA125 were measured using electrochemiluminescence assays in a subset of controls (n=200). Preoperative CA125 was recorded in 649 cases. The association between ovarian cancer and mastitis was assessed using unconditional logistic regression to calculate adjusted odds ratios, OR, and 95% confidence intervals (CI). Associations between mastitis and anti-CA15.3 and anti-CA125 antibodies and preoperative CA125 levels were evaluated using adjusted linear regression models.

Results—Prior mastitis was associated with a significantly lower risk for ovarian cancer: OR (and 95% CI) of 0.67 (0.48, 0.94) adjusted for parity, breastfeeding, and other potential confounders. The association was strongest with 2 or more episodes of mastitis; and risk declined progressively with increasing number of children and episodes of mastitis. Among controls, prior mastitis was associated with significantly higher anti-CA15.3 and anti-CA125 antibody levels and, among cases, with significantly lower preoperative CA125 levels.

Conclusion—Puerperal that mastitis may produce long-lasting anti-mucin antibodies that may lower the risk for ovarian cancer, plausibly through enhanced immune surveillance. Studying

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

immune reactions related to MUC1 and MUC16 in the 10–20% of breastfeeding women who develop mastitis may suggest ways to duplicate its effects through vaccines based on both antigens.

Keywords

CA125; CA15.3; Ovarian Cancer; Puerperal Mastitis

INTRODUCTION

An estimated 22,280 women in the U.S. were diagnosed with and 15,500 died of ovarian cancer in 2012 [1]. Prevention will require a comprehensive understanding of risk factors for this deadly disease and underlying mechanisms. Ages at menarche and menopause, births, and lengths of breastfeeding and oral contraceptive use (OC) are important reproductive events—often aggregated to estimate number of ovulatory cycles which are directly correlated with ovarian cancer risk [2]. Less easily explained by ovulation, events like hysterectomy (without oophorectomy) and tubal ligation lower ovarian cancer risk and endometriosis and genital talc use increase it [3,4,5]. To explain these associations, we hypothesized that they alter ovarian cancer risk through effects related to the mucin (MUC) family of cell surface glycoproteins, especially MUC1, or CA15.3, which is over-expressed in many cancers including ovarian. Acute events, like hysterectomy or tubal ligation, release a tumor-like form of MUC1 and elicit anti-MUC1 antibodies which signal enhanced immune surveillance, thereby reducing ovarian cancer risk [6]. Conversely chronic events, such as repeated ovulations, lead to more continuous exposure to MUC1, dampen mucin-specific immunity, and produce immune tolerance of an emerging MUC1+ cancer. Key elements of this model were confirmed in prospective data from the Nurses' Health Study [7].

Two protective events we considered were puerperal mastitis and mumps parotitis. Regarding mastitis, we presented limited case-control data showing mastitis may lower ovarian cancer risk [6]. Regarding mumps, we reviewed epidemiologic evidence that mumps reduced risk for ovarian cancer and showed that individuals going through a mumps infection do, in fact, have elevated levels of anti-MUC1 antibodies as well as elevated levels of CA125, or MUC16 [8]. In this report, we present new case-control data on the association between mastitis and ovarian cancer and examine plasma anti-MUC1 and anti-MUC16 antibody levels as possible biomarkers of an immune surveillance mechanism for ovarian cancer risk reduction.

METHODS

Study Design and Population

Data for this study arose from the last two enrollment periods of a case-control study of ovarian cancer in New England (period 4 (1998–2002) and period 5 (2003–2008)) described elsewhere [9]. Briefly, 2,877 women residing in eastern Massachusetts and New Hampshire with a diagnosis of ovarian cancer were identified through hospital tumor boards and statewide cancer registries. Of these 2,206 (77%) were eligible and 1,588 (72%) agreed to participate (1,483 epithelial and 105 non-epithelial ovarian cancers). 4,366 controls were identified through a combination of drivers' license and town resident lists, 2,940 (67%) were eligible, 1,362 (46%) declined to participate and 1,578 (54%) were enrolled. Controls were frequency matched to cases on age and state of residence.

After written informed consent, demographic information, reproductive and medical history, and habits were assessed by in-person interviews. Approximately 95% of cases and controls

provided a blood sample at the time of the interview. Pathology reports were reviewed for histologic type, grade, and stage of the ovarian cancer. From hospitals with searchable electronic records, CA125 levels prior to therapy were recorded [10]. In period 4, occurrence of mastitis was captured in responses to the question: "Did you have any problems with breastfeeding?" In period 5, subjects were specifically asked if they ever had a breast infection, how many episodes, whether they were associated with breastfeeding, and age at first episode. This study was approved by Brigham and Women's Hospital and Dartmouth Medical Center's institutional review boards.

Anti-CA125 and Anti-CA15.3 Antibody Detection

A sample of period 5 controls was selected for antibody assays. Forty controls were age-matched from each of the following categories: 1) nulliparous, 2) parous and never breastfed, 3) parous, breastfed, no mastitis, 4) parous, breastfed, 1 mastitis, and 5) parous, breastfed, >1 mastitis. Plasma antibodies to CA125 and CA15.3 were measured using the Meso Scale Discovery (MSD) electro-chemiluminescence (ECL) multiplex platform, which compares favorably with standard ELISA and immunobead assays [11]. Antigen-grade CA15.3 and CA125 purified from breast and ovarian cancer cell lines (Meridian Life Sciences Inc, Memphis, TN) were coated on multi-spot plates by MSD and inspected for coating quality control. The plates were provided to the Fichorova Laboratory for assay optimization by standardized operational procedures. The final assay design included the following steps: blocking with 200 μl/well MSD Blocker A for 1h followed by wash; adding 25 μl/well of MSD Assay Diluent and 2h incubation with samples (25 μl/ml) at multiple dilutions (10, 50, 250, 1250) followed by PBS/0.05% Tween-20 wash; detection of human IgG1 bound to the specific protein spots with MSD sulfoTag-labeled antibodies (1μg/ml) for 2h; washing and adding read buffer followed by detection of ECL using MSD Imager 2400. A plasma pool prepared from 5 ovarian cancer patients with high anti-MUC1 antibodies levels ELISA-determined previously [8] was used as a positive control. Split aliquots of this pool were tested at the same dilutions as the test samples on each assay plate and served to assess ECL inter-assay variation. The raw reading inter-plate coefficients of variation (CV) for all plasma dilutions were 17–19% for anti-CA15.3 and 9–11% for anti-CA125. Based on the most consistent inter-plate (CV<19%) and intra-plate (CV<5%) reproducibility, and the lowest frequency of non-zero readings, we chose the 10-fold dilution data to describe general relationships with mucin immunity.

Statistical Analysis

Associations between epidemiologic factors and mastitis among cases and controls were identified by Chi-square and Fisher's exact tests. Odds ratios and 95 percent confidence intervals (CI) were calculated using unconditional logistic regression with adjustment for age (continuous), study period, and study center. In addition, we included the following ovarian cancer and/or mastitis risk factors to assess the association between mastitis and ovarian cancer risk independent of these variables: menopausal status (pre, post), OC use (never, <1 year, 1–5 years, >5 years), endometriosis, bladder infection, colitis, parity (0, 1, 2, 3, >3), smoking (never, <9 pack years, ≥ 9 pack years) and duration of breastfeeding (never, <4 months, 4–9 months, 10–16 months, >16 months). Because puerperal mastitis occurs predominantly among breastfeeding women, analyses were repeated in women who had breastfed. The overall association between mastitis and ovarian cancer was quantified in all cases and then separately for serous borderline, serous invasive, mucinous, endometrioid, clear cell and other or undifferentiated histologic types of ovarian cancer.

Anti-CA15.3 antibodies, anti-CA125 antibodies, and preoperative CA125 levels were log-normalized. Means and confidence intervals (CI) were calculated on the log scale and exponentiated back to their original units (relative luminescence units for the antibodies and

units/ml for CA125). To examine the specific effect of mastitis on anti-CA15.3 antibodies, anti-CA125 antibodies, and preoperative CA125 levels, we used linear regression models and adjusted for age, parity, breastfeeding, and additional variables possibly affecting levels of these biomarkers (see footnote to Table 4). In a sensitivity analysis we repeated the linear regression models using antibody levels that had been corrected for possible batch effects [12].

RESULTS

Mastitis and Ovarian Cancer Risk Association

The likelihood of mastitis by various potential confounding factors is shown in Table 1. Self-reported history of mastitis was lower in period 4 of the study than in period 5. In controls, history of mastitis was more likely for premenopausal women, women with BMI <25, non-smokers, women with a later age at first livebirth, non-hysterectomized women, OC users, and women who breastfed more children or for a longer period. The latter three variables were also significantly related to the likelihood of mastitis in cases. Other variables predicting mastitis in cases were history of endometriosis or colitis and blood types O and A. After restricting these analyses to women who had breastfeed, variables that continued to predict a greater likelihood of mastitis were study period 5, premenopausal status (controls), number of infants breastfed and total months of breastfeeding (cases and controls), OC use (cases), endometriosis (cases), and colitis (cases). Variables examined but not included in Table 1 because they were not associated with mastitis were age, Jewish ethnicity, menopausal hormone use, history of infertility, genital talc use, fibroids, personal history of breast cancer, and a family history of ovarian or (premenopausal) breast cancer (data not shown).

History of puerperal mastitis was associated with significantly decreased risk for ovarian cancer adjusted OR (and 95% CI) of 0.67 (0.48, 0.94) (Table 2). No significant associations with age at first mastitis or years since first mastitis were observed. The association was more apparent in those who had experienced 2 or more episodes of mastitis with an adjusted OR (and 95% CI) of 0.34 (0.16, 0.72). When the analyses were restricted to women who had breastfed, the comparable ORs (and 95% CIs) were: 0.65(0.46, 0.93) and 0.34(0.16, 0.73). Examining the association within strata based upon number of livebirths, the association was least apparent for those with 1 pregnancy and most apparent for those with 2 pregnancies—the category with the largest number of subjects. Within each of these birth strata, history of 2 or more episodes of mastitis was associated with the lowest risk including women who had only 1 pregnancy but 2 episodes of mastitis. The final rows in Table 2 illustrate the additive effect of number of births and episodes of mastitis relative to women with one child and no mastitis. There is a progressive decline in risk for ovarian cancer with number of births and episodes of mastitis. Women who had 3 or more births had an OR (and 95% CI) of 0.59 (0.46, 0.77) if they had no mastitis but 0.20 (0.07, 0.61) if they had 2 or more episodes of mastitis. Similar associations were observed if the data were restricted to those who had breastfed, the comparable ORs (and 95% CIs) were: 0.57 (0.40, 0.82) and 0.20 (0.06,0.63). The association between mastitis and reduced risk for ovarian cancer applied least to mucinous histologic types of ovarian cancer (Table 3).

Anti-Mucin Antibodies Association with Mastitis and Ovarian Cancer Risk Factors

Table 4 shows how history of mastitis (and Table 1 variables) affected mean levels of anti-CA15.3 and anti-CA125 antibodies in controls and preoperative CA125 levels in cases. Compared to nulliparous women, women who had more than one episode of mastitis had a 37% increase in anti-CA15.3 and a 54% increase in anti-CA125 antibody levels. Both anti-CA15.3 and anti-CA125 antibody levels were about 50% lower for controls who had 9 or

more pack years of smoking compared to non-smokers, 24 to 43% lower for those with a history of endometriosis, and about 50% lower for those with history of colitis (borderline significance). Women with a history of bladder infections had about 70% higher levels of both anti-CA15.3 and anti-CA125 antibodies. Adjusting for these and other variables, compared to women without a history of mastitis, anti-CA15.3 antibody levels were 23 to 32% higher for controls: with any history of mastitis (p=0.02), 2 or more episodes of mastitis (p=0.01), and mastitis among women with more than one child (p=0.02). Anti-CA125 antibody levels were significantly higher for women who had more than 1 pregnancy and mastitis (p=0.04). Mastitis was associated with a 51% lower level of (preoperative) CA125 in cases (p=0.03). There was no clear trend in antibody levels with time since last episode of mastitis. Results were similar when antibody levels were adjusted for potential batch effects.

DISCUSSION

Puerperal mastitis affects about 10% of breastfeeding women in US surveys [13] but more than 20% in some populations [14]. In this study, we confirmed our prior observation that puerperal mastitis is associated with significantly decreased risk for ovarian cancer. The OR (and CL) for “any mastitis” from the earlier study which relied upon responses to an open-ended question [6] was 0.35 (0.16, 0.77) where the referent category was parous women who never breastfed. In study 5 the comparable OR for “any mastitis” was slightly higher, 0.48 (0.32,0.72). Subjects who remembered to mention mastitis were likely those who experienced multiple or more severe episodes. Indeed in Study 5 it appears that the association may be largely confined to women who had experienced 2 or more episodes of mastitis. Importantly, controls with history of mastitis had significantly higher anti-CA15.3 antibody levels. This is compatible with our original hypothesis that mastitis, similar to other acute events like mumps and tubal ligation, releases a tumor-like form of MUC1 and leads to anti-MUC1 antibodies that may reduce the risk of ovarian cancer, a MUC1-expressing tumor. We also observed higher anti-CA125 levels in controls with past mastitis and lower preoperative CA125 levels in cases with past mastitis.

Obvious confounders for the association between puerperal mastitis and ovarian cancer are parity and breastfeeding. Thus we examined the association in all women adjusted for parity and breastfeeding and then restricted to parous women who breastfed, with comparable and significant effects of mastitis in both analyses. The effect was best seen for the largest birth category (women with two children), but within all birth strata women with ≥2 episodes of mastitis had the greatest degree of protection. Other potential confounders include those related to mastitis occurrence including menopausal status, smoking history, OC use, and (in cases) colitis and endometriosis. Controlling for these factors did not negate the association between mastitis and reduced risk for ovarian cancer. Since the association is inverse, preferential recall by cases cannot account for the results. More controls than cases said they had mastitis in response to the open-ended question in period 4 and the closed-ended question in period 5. Chance must always be considered as an explanation for a novel finding, but seems less likely since the association was seen in two separate studies. Since not all controls approached agreed to participate, selection bias is possible if controls who were parous and breastfed were over-represented. However, our participation rate is not substantially lower than other case-control studies of ovarian cancer [15] and the frequency of livebirth and breastfeeding we observed among controls are not higher than the rates recently reported in another US based study [16].

Little has been written on the topic of mastitis and cancer risk. In the pre-antibiotic era, puerperal mastitis was sometimes treated with X-ray therapy, which appeared to be associated with subsequent increased risk for breast cancer [17]. There is also a cohort study

of women hospitalized for mastitis (not treated by radiotherapy) in Sweden which reported a statistically non-significant relative risk for breast cancer of 1.23[18]. Studies based upon women hospitalized for mastitis are not particularly relevant because the rate is only about 1 per 1000 deliveries [19]. Clearly, more human or animal studies specifically designed to examine the mastitis-ovarian cancer association are needed.

MUC1 is expressed in normal breast tissue at low levels compared with its expression in other organs [20]. Mammary expression of MUC1 increases during pregnancy and lactation [21], and MUC1 is an abundant component of breast milk. MUC1 is readily identified in the serum of pregnant and lactating women. Croce et al. reported that circulating anti-MUC1 antibodies can also be found in lactating women, proving that a humoral immune response to MUC1 occurs [21]. In Croce's study, the highest levels of anti-MUC1 antibodies were in lactating women who were multiparous, consistent with our findings of higher antibody levels in multiparous compared to uniparous women. Other than a case report of a woman with advanced breast cancer who developed puerperal mastitis and generated a high level of anti-MUC1 antibodies [22], there are no human data on MUC1 and anti-MUC1 antibodies during mastitis. In-vitro studies using bovine mammary cells found that MUC1 expression is up-regulated with exposure to bacterial endotoxin or to *E. coli*, a common pathogen in bovine mastitis [23]. Thus, it is reasonable to postulate that increased expression of MUC1 during mastitis releases MUC1 exposing it to the host immune system and generating anti-MUC1 antibodies.

MUC16 (CA125) is also expressed in human mammary tissue [24]. Like CA15.3, serum CA125 is elevated during pregnancy above the normal cutoff levels in about 35% of women, especially during the first trimester. CA125 declines in the second trimester but may increase again at delivery [25]. CA125 is expressed in decidua and found in amniotic fluid, suggesting that the source of serum CA125 during pregnancy is primarily from the uterus rather than the breasts, as it likely is for CA15.3 in pregnancy [26]. Although CA125 is present in colostrum [24], we found no published data to indicate that serum CA125 is elevated in lactating women or those with mastitis. Nevertheless, we observed that a history of mastitis was associated with elevated anti-CA125 antibodies in controls.

After restricting the analysis to subjects who breastfed, variables that predicted greater likelihood of mastitis in both cases and controls, (other than study period) included number of infants breastfed and duration of breastfeeding. These two variables may simply reflect greater opportunity to have developed mastitis, although veterinary literature suggests greater parity may increase the likelihood of bovine mastitis [27]. Fewer postmenopausal controls reported mastitis, which could reflect fading memory of the event or that fewer older women had breastfed compatible with secular trends in breastfeeding rates [28]. Variables that predicted higher rates of mastitis in cases included history of colitis and endometriosis. Although we could find no supporting literature for these associations and, while they may be due to chance, the fact that these events also affected anti-CA15.3 and anti-CA125 antibody levels suggest they may be biologically important in mucin immunity, as may be urinary tract infections (UTI) which were significantly associated with elevated antibodies to both CA125 and CA15.3 in this study. The potential significance of these associations may not be fully apparent until we have pre-diagnostic levels of anti-CA15.3 and anti CA125 antibodies in cases. We were not able to study bacterial type involved in mastitis or UTI but this is likely to be an important determinant of immune response.

Of course CA15.3 and CA125 are best known as tumor markers. Both are expressed in breast and ovarian cancer and other epithelial malignancies [29,30]. There is evidence that anti-CA15.3 antibodies also occur during malignancy, and these may be associated with better survival [31]. It has also been observed, both in cancer cases and healthy controls, that

anti-CA15.3 antibodies may bind with CA15.3 to form immune complexes which can interfere with detection of the antigen [32]. The inverse association between CA15.3 antigen and anti-CA15.3 antibody levels should be examined also for CA125. Our observation that mastitis may increase anti-CA125 antibodies which remain in circulation years after the acute event, as shown in controls, permit the speculation that mastitis-induced antibodies contributed to the lower pre-operative CA125 levels observed in cases who reported mastitis. A limitation of our study is that the association between history of mastitis and anti-mucin antibody levels could not be examined in ovarian cancer cases due to lack of preoperative blood samples available for antibody analysis; and, thus, we could only examine mastitis association with existing data on pre-operative CA125 levels.

In conclusion, puerperal mastitis is a common reproductive event that may lower ovarian cancer risk—an effect that may be mediated through immune reactions to mucins and signaled by elevated anti-CA15.3 and anti-CA125 antibodies. These observations are important because they lend support to our model explaining a broad range of risk factors for ovarian cancer through MUC1 immunity and because they suggest MUC16 should now be incorporated into this model. Puerperal mastitis affects between 10–20% of breastfeeding women, making “real time” mechanistic studies of the effect of mastitis feasible. Such studies may advance ovarian cancer biology and suggest innovative approaches to ovarian cancer prevention, including vaccines involving MUC1 and MUC16. To facilitate future epidemiologic studies of ovarian and other cancers that express MUC1 and MUC16, puerperal mastitis should become a routine part of a comprehensive past medical history and questionnaires designed to assess cancer risk.

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Table 1

Characteristics of cases and controls with and without a history of puerperal mastitis.

	All cases and controls						Restricted to those who breast fed					
	Cases			Controls			Cases			Controls		
	No mastitis N=1423 N (%)	Mastitis N=60 N (%)	p value*	No mastitis N=1440 N (%)	Mastitis N=138 N (%)	p value*	No mastitis N=461 N (%)	Mastitis N=58 N (%)	p value*	No mastitis N=692 N (%)	Mastitis N=137 N (%)	p value*
Study period												
Period 4	649 (98.6)	9 (1.4)	<0.0001	692 (96.0)	29 (4.0)	<0.0001	207 (96.3)	8 (3.7)	<0.0001	322 (92.0)	28 (8.0)	<0.0001
Period 5	774 (93.8)	51 (6.2)		748 (87.3)	109 (12.7)		254 (83.6)	50 (16.4)		370 (77.2)	109 (22.8)	
Study Center												
Massachusetts	1135 (95.8)	50 (4.2)	0.50	1184 (91.1)	115 (8.9)	0.74	354 (88.1)	48 (11.9)	0.30	578 (83.5)	114 (16.5)	0.93
New Hampshire	288 (96.6)	10 (3.4)		256 (91.8)	23 (8.2)		107 (91.5)	10 (8.5)		114 (83.2)	23 (16.8)	
Menopausal status												
Pre	591 (95.8)	26 (4.2)	0.78	570 (88.1)	77 (11.9)	0.0002	199 (88.8)	25 (11.2)	0.99	300 (79.8)	76 (20.2)	0.009
Post	832 (96.1)	34 (3.9)		870 (93.4)	61 (6.6)		262 (88.8)	33 (11.2)		392 (86.5)	61 (13.5)	
Race												
White	1353 (95.9)	58 (4.1)	0.99	1417 (91.1)	138 (8.9)	0.26	426 (88.4)	56 (11.6)	0.41	677 (83.2)	137 (16.8)	0.15
Non-white	68 (97.1)	2 (2.9)		23 (100.0)	0 (0)		34 (94.4)	2 (5.6)		15 (100.0)	0 (0)	
BMI quintile												
<25	680 (95.8)	30 (4.2)	0.74	684 (89.8)	78 (10.2)	0.05	235 (88.7)	30 (11.3)	0.93	346 (81.8)	77 (18.2)	0.20
≥25	741 (96.1)	30 (3.9)		747 (92.6)	60 (7.4)		225 (88.9)	28 (11.1)		343 (85.1)	60 (14.9)	
Pack years												
Never	692 (95.6)	32 (4.4)	0.63	693 (90.0)	77 (10.0)	0.02	247 (88.5)	32 (11.5)	0.96	362 (82.5)	77 (17.5)	0.34
<9	292 (95.7)	13 (4.3)		340 (90.2)	37 (9.8)		110 (89.4)	13 (10.6)		177 (82.7)	37 (17.3)	
≥9	438 (96.7)	15 (3.3)		401 (94.6)	23 (5.4)		103 (88.8)	13 (11.2)		150 (87.2)	22 (12.8)	
OC use												
No or <1 year	768 (97.3)	21 (2.7)	0.004	603 (93.5)	42 (6.5)	0.009	237 (91.9)	21 (8.1)	0.03	252 (85.7)	42 (14.3)	0.20
≥1 year	655 (94.4)	39 (5.6)		837 (89.7)	96 (10.3)		224 (85.8)	37 (14.2)		440 (82.2)	95 (17.8)	
Parity												
1	207 (95.4)	10 (4.6)	0.70	191 (93.6)	13 (6.4)	0.05	102 (91.1)	10 (8.9)	0.78	121 (90.3)	13 (9.7)	0.08
2	379 (93.6)	26 (6.4)		459 (88.1)	62 (11.9)		195 (89.0)	24 (11.0)		275 (81.8)	61 (18.2)	

	All cases and controls						Restricted to those who breast fed					
	Cases			Controls			Cases			Controls		
	No mastitis N=1423 N (%)	Mastitis N=60 N (%)	p value*	No mastitis N=1440 N (%)	Mastitis N=138 N (%)	p value*	No mastitis N=461 N (%)	Mastitis N=58 N (%)	p value*	No mastitis N=692 N (%)	Mastitis N=137 N (%)	p value*
3	219 (93.2)	16 (6.8)		271 (87.1)	40 (12.9)		106 (86.9)	16 (13.1)		165 (80.5)	40 (19.5)	
>3	151 (95.0)	8 (5.0)		246 (91.4)	23 (8.6)		58 (87.9)	8 (12.1)		131 (85.1)	23 (14.9)	
Age at first livebirth												
<25	457 (95.8)	20 (4.2)	0.06	482 (92.2)	41 (7.8)	0.02	157 (89.7)	18 (10.3)	0.54	223 (84.8)	40 (15.2)	0.51
25–28	262 (93.9)	17 (6.1)		300 (89.3)	36 (10.7)		152 (89.9)	17 (10.1)		171 (82.6)	36 (17.4)	
29–31	118 (90.1)	13 (9.9)		175 (85.0)	31 (15.0)		69 (84.1)	13 (15.9)		124 (80.0)	31 (20.0)	
>31	114 (91.9)	10 (8.1)		210 (87.5)	30 (12.5)		83 (89.2)	10 (10.8)		174 (85.3)	30 (14.7)	
Breastfed (among parous)												
No	495 (99.6)	2 (0.4)	<0.0001	475 (99.8)	1 (0.2)	<0.0001	0 (0.0)	0 (0.0)	--	0 (0.0)	0 (0.0)	--
Yes	461 (88.8)	58 (11.2)		692 (83.5)	137 (16.5)		461 (88.8)	58 (11.2)		692 (83.5)	137 (16.5)	
Number of infants breast fed												
1	206 (93.2)	15 (6.8)	0.03	262 (89.7)	30 (10.3)	0.003	206 (93.2)	15 (6.8)	0.03	262 (89.7)	30 (10.3)	0.003
2	170 (87.2)	25 (12.8)		268 (81.5)	61 (18.5)		170 (87.2)	25 (12.8)		268 (81.5)	61 (18.5)	
3	59 (81.9)	13 (18.1)		115 (77.2)	34 (22.8)		59 (81.9)	13 (18.1)		115 (77.2)	34 (22.8)	
>3	26 (83.9)	5 (16.1)		47 (79.7)	12 (20.3)		26 (83.9)	5 (16.1)		47 (79.7)	12 (20.3)	
Total months breast fed												
<4	133 (95.0)	7 (5.0)	0.02	174 (87.9)	24 (12.1)	0.008	133 (95.0)	7 (5.0)	0.02	174 (87.9)	24 (12.1)	0.008
4–9	119 (88.8)	15 (11.2)		176 (87.1)	26 (12.9)		119 (88.8)	15 (11.2)		176 (87.1)	26 (12.9)	
10–16	101 (87.8)	14 (12.2)		141 (83.4)	28 (16.6)		101 (87.8)	14 (12.2)		141 (83.4)	28 (16.6)	
>16	108 (83.1)	22 (16.9)		201 (77.3)	59 (22.7)		108 (83.1)	22 (16.9)		201 (77.3)	59 (22.7)	
Endometriosis/painful periods												
No	1279 (96.4)	48 (3.6)	0.01	1321 (91.2)	127 (8.8)	0.90	417 (90.1)	46 (9.9)	0.01	630 (83.3)	126 (16.7)	0.72
Yes	144 (92.3)	12 (7.7)		119 (91.5)	11 (8.5)		44 (78.6)	12 (21.4)		62 (84.9)	11 (15.1)	
Hysterectomy												
No	1287 (95.9)	55 (4.1)	0.75	1306 (90.8)	132 (9.2)	0.05	418 (88.7)	53 (11.3)	0.86	638 (83.0)	131 (17.0)	0.16
Yes	136 (96.5)	5 (3.5)		134 (95.7)	6 (4.3)		43 (89.6)	5 (10.4)		54 (90.0)	6 (10.0)	
Bladder infections												
No	1362 (96.0)	56 (4.0)	0.33	1382 (91.4)	130 (8.6)	0.32	446 (89.2)	54 (10.8)	0.25	659 (83.6)	129 (16.4)	0.60

	All cases and controls						Restricted to those who breast fed					
	Cases			Controls			Cases			Controls		
	No mastitis N=1423 N (%)	Mastitis N=60 N (%)	p value*	No mastitis N=1440 N (%)	Mastitis N=138 N (%)	p value*	No mastitis N=461 N (%)	Mastitis N=58 N (%)	p value*	No mastitis N=692 N (%)	Mastitis N=137 N (%)	p value*
Yes	61 (93.8)	4 (6.2)		58 (87.9)	8 (12.1)		15 (79.0)	4 (21.0)		33 (80.5)	8 (19.5)	
Colitis												
No	1385 (96.2)	55 (3.8)	0.03	1390 (91.1)	136 (8.9)	0.31	450 (89.5)	53 (10.5)	0.02	667 (83.2)	135 (16.8)	0.29
Yes	38 (88.4)	5 (11.6)		50 (96.2)	2 (3.8)		11 (68.8)	5 (31.3)		25 (92.6)	2 (7.4)	
Blood type												
O	519 (94.5)	30 (5.5)	0.06	573 (90.7)	59 (9.3)	0.28	170 (85.4)	29 (14.6)	0.15	253 (81.4)	58 (18.6)	0.26
A	499 (96.5)	18 (3.5)		478 (90.7)	49 (9.3)		453 (90.0)	17 (10.0)		238 (82.9)	49 (17.1)	
AB	46 (100.0)	0 (0)		40 (83.3)	8 (16.7)		14 (100.0)	0 (0.0)		19 (70.4)	8 (29.6)	
B	136 (98.6)	2 (1.4)		130 (92.9)	10 (7.1)		39 (95.1)	2 (4.9)		67 (87.0)	10 (13.0)	

*p-value from chi-square or Fisher's exact test.

Table 2

Association between puerperal mastitis and ovarian cancer.

	All cases and controls				Restricted to those who breast fed			
	Cases N=1483 N (%)	Controls N=1578 N (%)	Adjusted* OR (95% CI)	p-value	Cases N=519 N (%)	Controls N=829 N (%)	Adjusted* OR (95% CI)	p-value
Puerperal mastitis†								
Never	1423 (96.0)	1440 (91.3)	1.00		461 (88.8)	692 (83.5)	1.00	
Ever	60 (4.0)	138 (8.7)	0.67 (0.48, 0.94)	0.02	58 (11.2)	137 (16.5)	0.65 (0.46, 0.93)	0.02
Age at first mastitis‡								
<28	18 (36.7)	37 (33.9)	1.00		17 (35.4)	37 (33.9)	1.00	
28–30	14 (28.6)	30 (27.5)	0.70 (0.25, 1.94)	0.49	14 (29.2)	30 (27.5)	0.74 (0.27, 2.07)	0.57
>30	17 (34.7)	42 (38.5)	0.84 (0.31, 2.26)	0.73	17 (35.4)	42 (38.5)	0.90 (0.33, 2.44)	0.83
Years since first mastitis‡								
≤15 years	14 (28.6)	40 (36.7)	1.00		14 (29.1)	40 (36.7)	1.00	
16–29 years	21 (42.9)	35 (32.1)	1.44 (0.42, 4.94)	0.56	21 (43.8)	35 (32.1)	1.36 (0.40, 4.65)	0.62
≥30 years	14 (28.6)	34 (31.2)	1.15 (0.15, 8.85)	0.90	13 (27.1)	34 (31.2)	0.91 (0.11, 7.31)	0.93
Number of mastitis events								
0	1423 (96.0)	1440 (91.3)	1.00		461 (88.8)	692 (83.5)	1.00	
1	51 (3.4)	97 (6.1)	0.80 (0.56, 1.16)	0.24	49 (9.4)	96 (11.6)	0.78 (0.53, 1.14)	0.20
≥2	9 (0.6)	41 (2.6)	0.34 (0.16, 0.72)	0.005	9 (1.7)	41 (5.0)	0.34 (0.16, 0.73)	0.006
Mastitis among parity=1								
Never	207 (95.4)	191 (93.6)	1.00		102 (91.1)	121 (90.3)	1.00	
Ever	10 (4.6)	13 (6.4)	1.03 (0.41, 2.56)	0.96	10 (8.9)	13 (9.7)	1.06 (0.41, 2.73)	0.91
1	8 (3.7)	5 (2.4)	2.06 (0.62, 6.81)	0.23	8 (7.1)	5 (3.7)	1.91 (0.56, 6.48)	0.30
≥2	2 (0.9)	8 (3.9)	0.33 (0.06, 1.71)	0.18	2 (1.8)	8 (6.0)	0.38 (0.07, 2.10)	0.27
Mastitis among parity=2								
Never	379 (93.6)	459 (88.1)	1.00		195 (89.0)	275 (81.8)	1.00	
Ever	26 (6.4)	62 (11.9)	0.53 (0.32, 0.88)	0.02	24 (11.0)	61 (18.2)	0.49 (0.29, 0.84)	0.01
1	23 (5.7)	49 (9.4)	0.59 (0.34, 1.01)	0.05	21 (9.6)	48 (14.3)	0.54 (0.31, 0.96)	0.03
≥2	3 (0.7)	13 (2.5)	0.30 (0.08, 1.11)	0.07	3 (1.4)	13 (3.9)	0.30 (0.08, 1.12)	0.07
Mastitis among parity=3								

Cancer Causes Control. Author manuscript; available in PMC 2014 November 01.

	All cases and controls				Restricted to those who breast fed			
	Cases N=1483 N (%)	**Controls N=1578 N (%)**	**Adjusted* OR (95% CI)**	**p-value**	**Cases N=519 N (%)**	**Controls N=829 N (%)**	**Adjusted* OR (95% CI)**	**p-value**
Never	219 (93.2)	271 (87.1)	1.00		106 (86.9)	165 (80.5)	1.00	
Ever	16 (6.8)	40 (12.9)	0.79 (0.40, 1.55)	0.49	16 (13.1)	40 (19.5)	0.75 (0.37, 1.51)	0.42
1	14 (6.0)	27 (8.7)	0.93 (0.45, 1.93)	0.85	14 (11.5)	27 (13.2)	0.89 (0.42, 1.87)	0.76
≥2	2 (0.8)	13 (4.2)	0.37 (0.08, 1.81)	0.22	2 (1.6)	13 (6.3)	0.34 (0.07, 1.70)	0.19
Mastitis among parity>3								
Never	151 (95.0)	246 (91.4)	1.00		58 (87.9)	131 (85.1)	1.00	
Ever	8 (5.0)	23 (8.6)	0.84 (0.33, 2.15)	0.72	8 (12.1)	23 (14.9)	0.98 (0.37, 2.57)	0.96
1	6 (3.8)	16 (6.0)	1.00 (0.34, 2.90)	0.99	6 (9.1)	16 (10.4)	1.07 (0.36, 3.17)	0.90
≥2	2 (1.3)	7 (2.6)	0.55 (0.10, 2.98)	0.49	2 (3.0)	7 (4.6)	0.74 (0.13, 4.29)	0.74
Number of live births and mastitis events								
0 live births	467 (31.5)	273 (17.3)	1.28 (0.97, 1.69)	0.08				
1 live birth, no mastitis	207 (14.0)	191 (12.1)	1.00		102 (19.6)	121 (14.6)	1.00	
1 live birth, 1 mastitis	8 (0.5)	5 (0.3)	1.89 (0.59, 5.91)	0.29	8 (1.5)	5 (0.6)	2.01 (0.62, 6.52)	0.24
1 live birth, >1 mastitis	2 (0.1)	8 (0.5)	0.32 (0.06, 1.53)	0.15	2 (0.4)	8 (1.0)	0.33 (0.07, 1.64)	0.18
2 live births, no mastitis	379 (25.6)	459 (29.1)	0.79 (0.62, 1.01)	0.06	195 (37.6)	275 (33.2)	0.87 (0.62, 1.22)	0.43
2 live births, 1 mastitis	23 (1.6)	49 (3.1)	0.50 (0.29, 0.87)	0.01	21 (4.0)	48 (5.8)	0.48 (0.26, 0.87)	0.02
2 live births, >1 mastitis	3 (0.2)	13 (0.8)	0.27 (0.08, 0.99)	0.05	3 (0.6)	13 (1.6)	0.28 (0.08, 1.05)	0.06
≥3 live births, no mastitis	370 (25.0)	517 (32.8)	0.59 (0.46, 0.77)	<0.0001	164 (31.6)	296 (35.7)	0.57 (0.40, 0.82)	0.003
≥3 live births, 1 mastitis	20 (1.4)	43 (2.7)	0.52 (0.29, 0.94)	0.03	20 (3.8)	43 (5.2)	0.54 (0.29, 1.01)	0.05
≥3 live births, >1 mastitis	4 (0.3)	20 (1.3)	0.20 (0.07, 0.61)	0.005	4 (0.8)	20 (2.4)	0.20 (0.06, 0.63)	0.006

*Adjusted for study, center and period, reference age, menopausal status, oral contraceptive use (never, <1 year, 1–5 years, >5 years), endometriosis, bladder infection, colitis, parity (0, 1, 2, 3, >3), duration of breastfeeding (never, <4 months, 4–9 months, 10–16 months, >16 months), and pack years (never smoked, <9, ≥9).

†In the period 5 data, thirty subjects (18 cases, 12 controls) who reported mastitis not associated with pregnancy or breastfeeding were classified as never having had a puerperal mastitis. Estimates of risk were unchanged if these subjects were excluded.

‡Period 5 only.

Table 3

Association between puerperal mastitis and histologic type of ovarian cancer.

	All cases and controls				Restricted to those who breast fed			
	No mastitis N (%)	Mastitis N (%)	Adjusted* OR (95% CI)	p-value	No mastitis N (%)	Mastitis N (%)	Adjusted* OR (95% CI)	p-value
Controls	1440 (91.2)	138 (8.8)	1.00		692 (83.5)	137 (16.5)	1.00	
Serous borderline	158 (96.3)	6 (3.7)	0.60 (0.25, 1.45)	0.25	57 (90.5)	6 (9.5)	0.64 (0.26, 1.58)	0.34
Serous invasive	660 (95.8)	29 (4.2)	0.64 (0.42, 1.00)	0.05	235 (89.7)	27 (10.3)	0.61 (0.38, 0.97)	0.04
Mucinous	147 (93.0)	11 (7.0)	1.05 (0.52, 2.16)	0.88	48 (81.4)	11 (18.6)	1.10 (0.52, 2.32)	0.81
Endometrioid	251 (96.5)	9 (3.5)	0.65 (0.31, 1.36)	0.25	68 (88.3)	9 (11.7)	0.59 (0.27, 1.28)	0.18
Clear cell	86 (97.7)	2 (2.3)	0.54 (0.12, 2.45)	0.42	17 (89.5)	2 (10.5)	0.48 (0.10, 2.27)	0.36
Other/undifferentiated	121 (97.6)	3 (2.4)	0.44 (0.13, 1.48)	0.18	36 (92.3)	3 (7.7)	0.39 (0.11, 1.36)	0.14

*Adjusted for study, center and period, reference age, menopausal status, oral contraceptive use (never, <1 year, 1–5 years, >5 years), endometriosis, bladder infection, colitis, parity (0, 1, 2, 3, >3), duration of breastfeeding (never, <4 months, 4–9 months, 10–16 months, >16 months), and pack years (never smoked, <9, ≥9).

Table 4

Mean levels of anti-CA15.3 and anti-CA125 antibodies in 200 controls and preoperative CA125 in 649 cases by mastitis and other ovarian cancer risk factors.

	Controls					Cases		
	N=200 N (%)	**Anti-CA15.3 Antibody Geom. mean (95% CI)**	**Adjusted p-value**	**Anti-CA125 Antibody Geom. mean (95% CI)**	**Adjusted p-value**	**N=649 N (%)**	**Preoperative CA125 Geom. mean (95% CI)**	**Adjusted p-value**
Age adjusted models								
Study group								
Nulliparous	40 (20.0%)	4309 (3177, 5843)	Ref.	2986 (2292, 3889)	Ref.	--	--	--
Parous, never breastfed	40 (20.0%)	4911 (3622, 6660)	0.53	3953 (3034, 5149)	0.14	--	--	--
Breastfed, no mastitis	40 (20.0%)	4028 (2970, 5462)	0.77	3875 (2975, 5047)	0.17	--	--	--
1 mastitis	40 (20.0%)	5088 (3752, 6899)	0.42	4321 (3317, 5629)	0.05	--	--	--
>1 mastitis	40 (20.0%)	5897 (4349, 7996)	0.14	4592 (3525, 5981)	0.02	--	--	--
Menopausal status								
Pre	127 (63.5%)	5162 (4353, 6121)	0.47	4190 (3611, 4861)	0.32	248 (38.2%)	171 (135, 217)	0.43
Post	73 (36.5%)	4237 (3384, 5305)		3453 (2839, 4201)		401 (61.8%)	294 (244, 354)	
Race								
White	196 (98.0%)	4803 (4185, 5513)	0.97	3907 (3464, 4406)	0.91	605 (93.5%)	248 (212, 289)	0.20
Non-white	4 (2.0%)	4825 (1839, 12660)		3795 (1635, 8811)		42 (6.5%)	147 (82, 263)	
BMI								
<25	112 (56.0%)	5139 (4285, 6163)	0.31	3916 (3340, 4592)	0.98	311 (48.0%)	263 (212, 325)	0.08
≥25	88 (44.0%)	4408 (3591, 5411)		3890 (3250, 4655)		337 (52.0%)	218 (178, 268)	
Smoking								
Never smoked	104 (52.3%)	5425 (4532, 6494)	Ref.	4589 (3918, 5374)	Ref.	318 (49.1%)	233 (189, 289)	Ref.
<9 pack years	52 (26.1%)	6151 (4769, 7932)	0.43	4222 (3377, 5278)	0.55	130 (20.1%)	232 (167, 324)	0.93
≥9 pack years	43 (21.6%)	2638 (1995, 3490)	<0.0001	2375 (1858, 3036)	<0.0001	200 (30.9%)	254 (194, 331)	0.97
Endometriosis								
No	187 (93.5%)	4889 (4247, 5628)	0.41	4049 (3585, 4572)	0.03	589 (90.8%)	248 (212, 290)	0.23
Yes	13 (6.5%)	3725 (2184, 6354)		2317 (1461, 3675)		60 (9.2%)	168 (103, 273)	
Colitis								
No	194 (97.0%)	4900 (4270, 5623)	0.12	3983 (3533, 4490)	0.08	635 (97.8%)	242 (209, 282)	0.17
Yes	6 (3.0%)	2517 (1151, 5504)		2055 (1040, 4064)		14 (2.2%)	128 (47, 351)	

	Controls					Cases		
	N=200 N (%)	Anti-CA15.3 Antibody Geom. mean (95% CI)	Adjusted p-value	Anti-CA125 Antibody Geom. mean (95% CI)	Adjusted p-value	N=649 N (%)	Preoperative CA125 Geom. mean (95% CI)	Adjusted p-value
Hysterectomy								
No	187 (93.5%)	4641 (4036, 5338)	0.03	3846 (3401, 4349)	0.24	590 (90.9%)	243 (208, 284)	0.11
Yes	13 (6.5%)	7870 (4631, 13376)		4858 (3048, 7742)		59 (9.1%)	202 (124, 330)	
Bladder infections								
No	186 (93.0%)	4623 (4019, 5317)	0.05	3760 (3328, 4247)	0.02	618 (95.2%)	238 (204, 277)	0.78
Yes	14 (7.0%)	7995 (4799, 13319)		6444 (4132, 10050)		31 (4.8%)	275 (139, 541)	
Parity								
Nulliparous	40 (20.0%)	4309 (3181, 5837)	Ref.	2986 (2306, 3865)	Ref.	204 (31.4%)	180 (139, 234)	Ref
1	27 (13.5%)	5809 (4014, 8405)	0.27	5341 (3901, 7313)	0.008	90 (13.9%)	171 (115, 253)	0.58
2	59 (29.5%)	5588 (4352, 7175)	0.20	4896 (3958, 6056)	0.004	170 (26.2%)	259 (195, 345)	0.25
3	51 (25.5%)	3941 (3012, 5156)	0.70	3147 (2504, 3955)	0.74	113 (17.4%)	329 (231, 468)	0.10
>3	23 (11.5%)	4885 (3273, 7289)	0.46	3891 (2768, 5470)	0.17	72 (11.1%)	406 (261, 631)	0.07
Total months breast fed								
Nulliparous	80 (40.0%)	4600 (3712, 5700)	Ref.	3435 (2851, 4140)	Ref.	411 (63.3%)	237 (197, 286)	Ref.
<4	19 (9.5%)	6815 (4390, 10581)	0.09	5084 (3468, 7454)	0.06	58 (8.9%)	174 (106, 285)	0.16
4–9	22 (11.0%)	5897 (3919, 8875)	0.26	5119 (3587, 7304)	0.04	64 (9.9%)	251 (157, 402)	0.81
10–16	25 (12.5%)	5187 (3535, 7612)	0.54	4488 (3215, 6265)	0.15	55 (8.5%)	368 (221, 613)	0.15
>16	54 (27.0%)	4019 (3096, 5217)	0.39	3611 (2878, 4531)	0.80	61 (9.4%)	220 (136, 357)	0.83
Blood type								
O	86 (47.2%)	5097 (4144, 6269)	Ref.	4456 (3716, 5344)	Ref.	226 (41.8%)	239 (186, 307)	Ref.
A	68 (37.4%)	4344 (3442, 5482)	0.35	3411 (2781, 4184)	0.07	240 (44.4%)	239 (188, 306)	0.89
AB	10 (5.5%)	4804 (2618, 8813)	0.85	3951 (2319, 6731)	0.67	16 (3.0%)	408 (159, 1049)	0.21
B	18 (9.9%)	4342 (2762, 6826)	0.54	3390 (2279, 5044)	0.23	59 (10.9%)	273 (167, 447)	0.46
Multivariable adjusted models *								
Puerperal mastitis								
Never	122 (61.0%)	4425 (3719, 5265)	0.02	3576 (3074, 4160)	0.19	624 (96.1%)	244 (210, 284)	0.01
Ever	78 (39.0%)	5461 (4394, 6786)		4480 (3708, 5413)		25 (3.9%)	148 (69, 314)	
Number of mastitis events								
0	122 (61.0%)	4425 (3718, 5266)	Ref.	3576 (3073, 4161)	Ref.	624 (96.1%)	244 (210, 284)	Ref.

	Controls					Cases		
	N=200 N (%)	Anti-CA15.3 Antibody Geom. mean (95% CI)	Adjusted p-value	Anti-CA125 Antibody Geom. mean (95% CI)	Adjusted p-value	N=649 N (%)	Preoperative CA125 Geom. mean (95% CI)	Adjusted p-value
1	39 (19.5%)	5125 (3768, 6973)	0.16	4371 (3343, 5716)	0.51	20 (3.1%)	184 (79, 427)	0.03
≥2	39 (19.5%)	5818 (4277, 7915)	0.01	4591 (3511, 6003)	0.12	5 (0.8%)	62 (11, 335)	0.16
Years since first mastitis								
≤15 years	37 (47.4%)	6346 (4659, 8648)	Ref.	5221 (3912, 6967)	Ref.	4 (19.0%)	197 (23, 1685)	Ref.
16–29 years	28 (35.9%)	4081 (2861, 5823)	0.12	3449 (2475, 4805)	0.11	10 (47.6%)	129 (33, 501)	0.82
≥30 years	13 (16.7%)	6667 (3958, 11232)	0.49	5090 (3128, 8282)	0.87	7 (33.3%)	247 (49, 1251)	0.61
Mastitis among parity=1								
No	19 (70.4%)	5881 (3478, 9943)	0.34	5709 (3476, 9376)	0.36	87 (96.7%)	175 (117, 260)	0.86
Yes	8 (29.6%)	5640 (2511, 12670)		4560 (2123, 9796)		3 (3.3%)	93 (11, 799)	
Mastitis among parity>1								
No	63 (47.4%)	4131 (3236, 5273)	0.02	3482 (2830, 4285)	0.04	333 (93.8%)	320 (260, 394)	0.03
Yes	70 (52.6%)	5441 (4316, 6859)		4471 (3672, 5444)		22 (6.2%)	157 (70, 353)	

*All models adjusted for age, parity (0, 1, 2, 3, >3), and breastfeeding (never, <4 months, 4–9 months, 10–16 months, >16 months). CA15.3 IgG1 additionally adjusted for pack years, bladder infection, and hysterectomy. CA125 IgG1 additionally adjusted for smoking (never, <9 pack years, ≥9 pack years), endometriosis, colitis, bladder infection. Preoperative CA125 additionally adjusted for BMI, hysterectomy, histologic type (serous borderline, serous invasive, mucinous, endometrioid, clear cell, other/undifferentiated), and stage (1/2 vs. 3/4).