The Relation of Reproductive Factors to Mortality from Breast Cancer[1]

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Abstract

Young women with breast cancer have been reported to have an increased risk of dying from their disease if they have given birth in <2 years before diagnosis. The prognostic factors associated with the tumors of these women have not been thoroughly studied. We examined the tumors of the women who had a recent birth and compared the tumor characteristics with those of women who were nulliparous or had given birth ≥5 years before diagnosis. A follow-up study was conducted of 1174 women <45 years old whose invasive ductal breast cancer was diagnosed from January 1983 to December 1992 in three counties of western Washington. These women had participated previously in a population-based, case-control study. Mean follow-up time was 105.4 months. Histological slides were collected for 79.1% of the tumors and reviewed by the study pathologist. Using immunoperoxidase assays, tumor tissue was tested for prognostic markers for 70.4% of the tumors from the women. Cox proportional hazards models were used to estimate the relative risk of dying from breast cancer associated with reproductive events. Logistic regression was used to obtain estimates of the association between various reproductive factors and tumor characteristics. At the end of follow-up, 48.2% of the women ($n = 83$) whose last birth occurred in <2 years of diagnosis had died, compared with 23.3% of nulliparous women ($n = 189$) and 24.4% of the women ($n = 661$) whose last birth was ≥5 years before diagnosis. The tumors of the women with a recent birth (<2 years before diagnosis) were more likely to be progesterone receptor negative, odds ratio (OR) = 2.2, 95% confidence interval (CI) = 1.2–3.9, to be p53 positive, OR = 2.6, 95% CI = 1.5–4.7, to be of high histological grade, OR = 5.9, 95% CI = 1.7–20.1, to have high mitotic count, OR = 2.2, 95% CI = 1.4–4.4, to be node positive, OR = 2.1, 95% CI = 1.3–3.5, to have a high S phase fraction, OR = 2.3, 95% CI = 1.1–4.8, and to have a high American Joint Committee on Cancer stage (III+), OR = 2.8, 95% CI 1.3–5.8, compared with the tumors of nulliparous women. After adjusting for tumor characteristics and treatment, the risk of mortality associated with a birth in <2 years of diagnosis of breast cancer remained an independent predictor of mortality, hazard radio (HR) = 2.7, 95% CI = 1.6–4.3. Our study provides evidence that reproductive factors influence the biological behavior of breast cancer in young women and prognosis. Clinicians need to be aware that women who have delivered a child in <2 years before diagnosis are at increased risk of having tumors with especially adverse prognostic profiles and have a poorer survival rate than women who are nulliparous or whose last birth was some years in the past.

Introduction

Two recent reports relating to women diagnosed with breast cancer <45 years old indicate that having ever had a live birth and having had a birth in the 2 years before breast cancer diagnosis are associated with an increased risk of death compared with nulliparous women (1, 2). Although these reports included some pathological and tumor characteristic information (*i.e.*, tumor size, lymph node status, histology, ER[3] status, and histological grade), a comprehensive assessment of tumor characteristics known to be related to prognosis was not possible. This limited the authors' abilities to fully evaluate which prognostic molecular markers may account, in part, for the poor survival and explain the mechanisms involved.

To evaluate the effect of pregnancy-related factors to prognosis among women diagnosed with breast cancer at age <45, we conducted a population-based cohort study of mortality among women diagnosed with breast cancer from 1983 through 1992 in the Puget Sound area of western Washington. We assessed specifically how reproductive history relates to the risk of dying, as well as to markers of prognosis in tumor tissue.

Materials and Methods

Case Ascertainment and Interviews. The breast cancer patients studied were women who had been interviewed previously in two population-based, case-control studies. The methods for both studies have been described previously (3, 4). The CSS, a population-based cancer registry, which is part of the

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[3] The abbreviations used are: ER, estrogen receptor; CSS, Cancer Surveillance System; PR, progesterone receptor; CI, confidence interval; OR, odds ratio; AJCC, American Joint Committee on Cancer; HR, hazard ratio.

Surveillance, Epidemiology, and End Results Program of the National Cancer Institute, was used to identify women with breast cancer who resided in King, Pierce, and Snohomish Counties in western Washington state. The first study ascertained all incident cases of first primary breast cancer among women who were diagnosed from January 1, 1983 through April 30, 1990 at age <45 and who were born after 1944. Interviews were completed on 845 cases (83.3% of eligible cases). The second study ascertained all incident first primary breast cancer cases that were diagnosed from May 1, 1990 through December 31, 1992 at age <45. Interviews were completed on 643 (83.9%) of all eligible cases. The women were interviewed in their homes by a trained interviewer. Respondents were asked about the timing and outcome of all pregnancies.

Tissue Collection, Pathology Review, and Testing for Prognostic Markers. Women who were pregnant at diagnosis (n = 15) were excluded from all analyses. Women with *in situ* disease (n = 197) or whose tumors were of medullary (n = 54), lobular (n = 43), and other histologies (n = 5) were also excluded. The remaining 1174 women with invasive ductal breast cancer form the basis of this analysis.

Tumor specimens were requested from hospital and commercial pathology laboratories. Histological slides have been collected for 929 of the initial cohort of 1174 women (79.1%) diagnosed with invasive breast cancer of the ductal histologies of interest. The study pathologist (P. L. P.) conducted a complete histopathological review for all tumors collected. If slides were not available for a case, original pathology reports or cancer registry abstracted pathology summaries were used to obtain stage, histology, tumor size, and nodal status.

Tumor tissue sufficient for immunoperoxidase assays was available on 826 (70.4%) of the tumors. Expression of ER, PR, p53 tumor suppression gene protein, Ki-67 proliferation-related antigen, and c-erbB-2 oncogene protein were evaluated on sections from a single tumor block from each tumor.

Antibodies were scored using a subjective interpretation of staining intensity and/or the percentage of tumor cells positive and incorporated known expression patterns and cellular location of the proteins. Categories of intensity and/or percentage of cells positive were collapsed into positive/high or negative/low categories as follows: for ER and PR, any staining above negative was considered positive; percentage of Ki-67-positive tumor cells averaged over four high power fields (HPF), and >25% was considered high proliferation; nuclear staining of >10% tumor cells for p53 was considered positive; and a distinct membranous staining pattern was considered positive for c-erbB-2.

Bivariate flow cytometric analysis of DNA content and S phase fraction was completed for 688 (58.6%) of the tumors from the women (5).

Follow-Up for Mortality. The primary source of information on deaths in the cohort was the cancer registry, which follows the patients for vital status. The registry annually contacts the hospital tumor registrar for information on the disease and vital status of each patient. The tumor registrar then contacts the physician following each patient for an updated determination of vital status. If there is no physician who has had sufficiently recent contact with the patient, then the registrar sends a letter to the patient. This follow-up for both disease and vital status is performed for cases, regardless of whether or not they currently reside in the CSS catchment area. In addition, passive surveillance through routine computer linkage of patients with Washington State death certificates, National Death Index, and the Health Care Finance Administration tapes is conducted. Because this cohort is part of a continuing follow-up study assessing exposures after diagnosis of breast cancer, we also receive updates on vital status from relatives who are aware of the woman's participation in the study or at the time we attempt to recontact the women.

Subjects underwent follow-up until the earliest of the date of death, the date last known to be alive, or the end date of our designated follow-up period (June, 2000). Of the 862 women not reported to be dead as of June, 2000, >92% had been verified to be alive in ≤2 years preceding June, 2000, and 96% had been located in ≤3 years of June, 2000. The mean and median lengths of follow-up for the 1174 women were 105.4 and 106 months, respectively.

Medical Record Review. Medical records were reviewed by trained medical record abstractors for 87% of the women. Abstracted from the medical records were all courses of treatment, including surgery, radiation therapy, chemotherapy, and/or hormonal therapy.

Statistical Analysis. Estimates of the relative risk of dying and 95% CIs were calculated using Cox proportional hazards models. This hazard rate was used to adjust for the time lag between diagnosis and interview (median time = 7 months). To account for the left truncation of subjects' survival times by the dates they were interviewed, we considered the women to be at risk of dying only for the time period after they had completed the original interview. We did not calculate the risk of dying as a function of time since the interview date; rather, we calculated the risk of dying associated with the reproductive factors among women who had similar periods of survival after diagnosis, conditional on their having completed their interview in a similar period after diagnosis. However, in the "Results" and "Discussion" sections, for ease, we will refer to survival as if it was from diagnosis. Observations were censored at either the date of last known follow-up or the end date of the follow-up period, if death had not occurred. All analyses were adjusted by age at diagnosis and reference year. We assessed the following additional variables as potential confounders: family history (first degree, second degree, and none), race (white and other), smoking (ever and never), alcohol (<1, 1 to <3, and 3+ drinks/week in the 5 years before diagnosis), oral contraceptive use (never or <1 year, 1 to <5 years, and 5+ years), exercise (low, medium, and high), education (<high school, high school/some college, and college graduate), income (<$15,000/year and $15,000+/year), lactation (<2 months and 2+ months), and body mass index (quartiles). None of these variables altered the estimates of risk of mortality associated with the reproductive events studied. Trends were evaluated among parous women for selected variables using the likelihood ratio test statistic.

To assess the associations between various reproductive factors and tumor characteristics, we used logistic regression to obtain estimates of the relative risk (OR) and their 95% CIs, adjusted for age and diagnosis year. Not all tumors included sufficient tissue to test for all of the markers.

The multivariate model that included time since last birth was developed by first including the tumor characteristics that were significantly associated on a univariate basis with time since last birth. We didn't consider ploidy and S phase fraction, because those data were only available for a subset of the tested tumors. The significantly associated variables were PR status, p53, histological grade, tumor size, and nodal status. Alternately, the AJCC stage (grouped as I, IIA, IIB, and III+) was substituted for nodal status and tumor size. We then looked to

Table 1 Relationship of pregnancy variables to mortality[a]

	Overall mortality			5-year Mortality		
	Alive	Dead (%)	HR1[b]	Alive	Dead (%)	HR1[b]
Ever pregnant						
No	139	50 (26.5)		161	28 (14.8)	
Yes	723	262 (26.6)	1.1 (0.8–1.5)	838	147 (14.9)	1.1 (0.7–1.6)
Ever had a live/still birth						
No	237	75 (23.3)		271	38 (12.3)	
Yes	625	240 (27.8)	1.4 (1.1–1.8)	728	137 (15.8)	1.4 (1.0–2.1)
Number of live/still births						
Nulliparous	237	72 (23.2)		271	38 (12.3)	
1	148	52 (26.0)	1.3 (0.9–1.8)	172	28 (14.0)	1.3 (0.8–2.0)
2–3	426	166 (28.0)	1.4 (1.1–1.9)	496	96 (16.2)	1.5 (1.0–2.2)
4+	51	22 (30.1)	1.5 (1.0–2.5)	60	13 (17.8)	1.7 (0.9–3.2)
Age at first live birth						
Nulliparous	237	72 (23.3)		271	38 (12.3)	
<20	129	38 (22.8)	1.1 (0.8–1.7)	144	23 (13.8)	1.3 (0.8–2.2)
20–29	393	158 (28.7)	1.4 (1.0–1.9)	465	86 (15.6)	1.4 (0.9–2.0)
30+	102	44 (30.1)	1.7 (1.2–2.5)	118	28 (19.2)	1.9 (1.2–3.2)
Age at last live/still birth						
Nulliparous	237	72 (23.3)		271	38 (12.3)	
<20	27	7 (20.6)	1.0 (0.5–2.2)	31	3 (8.8)	0.8 (0.2–2.6)
20–29	344	132 (27.7)	1.4 (1.0–1.8)	403	73 (15.3)	1.4 (0.9–2.0)
30+	253	101 (28.5)	1.5 (1.1–2.1)	293	61 (17.2)	1.6 (1.1–2.5)
Time since last live/still birth						
Nulliparous	237	72 (23.3)		271	38 (12.3)	
5+ yrs	500	161 (24.4)	1.2 (0.9–1.6)	573	88 (13.3)	1.2 (0.8–1.8)
2–<5 yrs	81	39 (32.5)	1.5 (1.0–2.1)	95	25 (20.8)	1.7 (1.0–2.8)
<2 yrs	43	40 (48.2)	2.3 (1.5–3.4)	59	24 (28.9)	2.3 (1.4–3.9)
Ever had induced abortion						
No	487	184 (27.4)		564	107 (16.0)	
Yes	235	78 (24.9)	0.9 (0.7–1.1)	273	40 (12.8)	0.7 (0.5–1.0)
Age at first induced abortion						
Never	487	184 (27.4)		564	107 (16.0)	
<18	19	7 (26.9)	0.8 (0.4–1.8)	20	6 (23.1)	1.1 (0.5–2.6)
18+	216	71 (24.7)	0.9 (0.7–1.2)	253	34 (11.9)	0.7 (0.5–1.0)
Ever had a spontaneous abortion						
No	765	274 (26.4)		880	159 (15.3)	
Yes	96	38 (28.4)	0.9 (0.6–1.2)	118	16 (11.9)	0.7 (0.4–1.3)

[a] Not all tests were completed on the tumors of all women.
[b] HR1, adjusted for age and diagnosis year.

see if any of the other tumor characteristics added significantly to these models. C-erbB-2 was the only characteristic that was also predictive of mortality once the other variables (above) were included in the model. To arrive at our final model, we eliminated all tumor characteristics that did not independently predict mortality in the context of the multivariate model.

Results

Women who had ever had a birth were at a 40% increase in risk of dying of their disease during the first 5 years after diagnosis, HR = 1.4 (95% CI = 1.0–2.1), compared with women who had never given birth (Table 1). The increased risk of mortality remained at the end of follow-up, HR = 1.4 (95% CI = 1.1–1.8). There was increased risk of mortality in the first 5 years after diagnosis in women whose first birth or last birth was at 30+ years, HR = 1.9 (95% CI = 1.2–3.2) and 1.6 (95% CI = 1.1–2.5) respectively, compared with women who had never given birth. Although the data were suggestive of patterns of increasing risk, trends were not significant after accounting for ever having had a live/still birth.

Women whose last birth occurred in <2 years of diagnosis had the highest risk of dying in the first 5 years after diagnosis, HR = 2.3 (95% CI = 1.4–3.9, *P* for trend = 0.02; Table 1), relative to women who had never given birth. The high mortality associated with a recent birth persisted at the end of follow-up, at which time 48.2% of the women with a recent birth had died compared with 23.3% of nulliparous women and 24.4% of women whose last birth occurred ≥5 years before diagnosis. There was little excess risk among women whose last birth occurred ≥5 years before diagnosis, HR = 1.2 (95% CI = 0.8–1.8). The increased risk of dying associated with recency of giving birth did not change substantially according to whether the woman was diagnosed <35 years or ≥35 years (data not shown).

There was no increase in risk of mortality associated with having a prior spontaneous or induced abortion or with having an induced abortion at a young age (*i.e.*, <18 years of age; Table 1).

The characteristics of the women from whom we were able to obtain sufficient tissue for tumor marker assays differed on a number of factors from those of women from whom we were unable to obtain tissue (Table 2). The women whose tumors were not tested were younger, more likely to have four or more live births, and less likely to have had their first birth at age ≥30. The mortality was somewhat higher among the women from whom we obtained tissue for testing, *i.e.*, 27.7%

Table 2 Characteristics of breast cancer cases and their tumors according to whether tumor tissues were available for immunohistochemistry testing

	Cases without available tissue (n = 348)	Cases with available tissue (n = 826)	P
Vital Status			
Alive	265 (76.2)	597 (72.3)	
Dead	83 (23.9)	229 (27.7)	0.170
Age at diagnosis			
20–29	24 (6.9)	28 (3.4)	
30–34	61 (17.5)	126 (15.3)	
35–39	142 (40.8)	310 (37.5)	
40+	121 (34.8)	362 (43.8)	0.004
AJCC stage			
I	129 (39.2)	314 (38.2)	
IIA	103 (31.3)	247 (30.1)	
IIB	59 (17.9)	166 (20.2)	
IIIA+	38 (11.6)	95 (11.6)	0.850
Tumor size			
<2 cm	174 (53.7)	434 (52.9)	
>2–5 cm	120 (37.0)	325 (39.6)	
>5 cm	30 (9.3)	62 (7.6)	0.532
Ever pregnant			
No	61 (17.5)	128 (15.5)	
Yes	287 (82.5)	698 (84.5)	0.387
Ever had a live/still birth			
No	95 (27.3)	214 (25.9)	
Yes	253 (72.7)	612 (74.1)	0.621
Number of live/still births			
Nulliparous	95 (27.3)	214 (25.9)	
1	54 (15.5)	146 (17.7)	
2–3	167 (48.0)	425 (51.5)	
4+	32 (9.2)	41 (5.0)	0.037
Age at first live/still birth			
Nulliparous	95 (27.3)	214 (25.9)	
<20	62 (17.8)	105 (12.7)	
20–29	163 (46.8)	388 (47.0)	
30+	28 (8.1)	118 (14.3)	0.006
Age at last live/still birth			
Nulliparous	95 (27.3)	214 (25.9)	
<20	10 (2.9)	24 (2.9)	
20–29	157 (45.1)	319 (38.7)	
30+	86 (24.7)	268 (32.5)	0.056
Time since last live/still birth			
Nulliparous	95 (27.3)	214 (25.9)	
5+ years	194 (55.8)	467 (56.6)	
2–< 5 years	36 (10.3)	84 (10.2)	
<2 years	23 (6.6)	60 (7.3)	0.948
Ever had induced abortion			
No	264 (75.9)	596 (72.2)	
Yes	84 (24.1)	229 (27.8)	0.200
Age at first induced abortion			
Never	264 (75.9)	596 (72.2)	
<18	8 (2.3)	18 (2.2)	
18+	76 (21.8)	211 (25.6)	0.397

compared with 23.9%, but was within the limits of chance. There was no association between time since last birth and the availability of tissue. Among the women for whom we were able to obtain tumor tissue and test for prognostic markers, the tumors of women who had ever given birth were more likely to be p53 positive, OR = 1.7 (95% CI = 1.2–2.4), and have an elevated Ki-67 ratio, OR = 1.5 (95% CI = 1.1–2.1; Table 3). Women who had given birth were also more likely to have positive nodes at diagnosis, OR = 1.6 (95% CI = 1.2–2.1). The risk of having a p53-positive tumor was elevated among women whose first or last birth occurred at age ≥20; ORs range from 1.5 to 1.8, compared with women who had never given birth (data not shown). The elevated risk of having positive nodes at diagnosis among women who had given birth compared with nulliparous women did not vary substantially with age at first or last birth and was elevated throughout the age range; ORs range from 1.5 to 1.8. There was a ~2-fold increase in the risk of an AJCC stage III or higher tumor among women whose first or last birth occurred at age ≥30 (data not shown).

Neither spontaneous nor induced abortion had any association with the distribution of tumor characteristics (data not shown).

Women who had given birth in <2 years of diagnosis were more likely to have tumors of high histological grade (OR = 5.9, 95% CI = 1.7–20.1), a high level of mitoses (OR = 2.2, 95% CI = 1.1–4.5), a high S phase fraction (OR = 2.3, 95% CI = 1.1–4.8), and positive nodes (OR = 2.1, 95% CI = 1.3–3.5). In addition, their tumors were more likely to be PR negative (OR = 2.2, 95% CI = 1.2–3.9) and p53 positive (OR = 2.6, 95% CI = 1.5–4.7), compared with women who had never given birth (Table 3). Women who had given birth in <5 years of diagnosis were more likely to have large tumors (>5 cm) relative to women who had never given birth or whose last birth was ≥5 years before diagnosis (OR = 2.1, 95% CI = 1.3–3.4; data not shown). Similarly, the tumors of women who had given birth in <5 years of diagnosis were more than two times more likely to be AJCC stage III or higher compared with nulliparous women.

We incorporated the tumor characteristics with the time since last birth variable in a multivariate model (see "Materials and Methods"). The risk of dying associated with having given birth in <2 years of diagnosis remained elevated, HR = 2.7, 95% CI = 1.7–4.2. The association did not change substantially whether the model included tumor size and node positivity or alternatively AJCC stage (Table 4).

Information on the treatment that the women received was available from the medical records for 656 women for whom we had tumor characteristics and AJCC stage. After adjusting for treatment in the multivariate model, the risk of mortality associated with birth in <2 years of diagnosis was 2.7 (95% CI = 1.6–4.3; Table 4).

Finally, in an attempt to disentangle the relationship between age at last birth and time since last birth, as well as the risk of mortality associated with ever having a birth, we calculated the risk of mortality associated with age at last birth and time since last birth separately and then with both variables in the model among parous women (Table 5). Having given birth in <2 years of diagnosis remained the more predictive reproductive variable associated with mortality among parous women.

Discussion

There are some limitations to our study that should be considered in the interpretation of the study results. In our original case-control studies, we were unable to interview ~15% of the women who were eligible for the study. The mortality of the noninterviewed women (43.5%) at 5 years was substantially different from those we were able to interview (15.4%). If the survival in relation to reproductive history of these women differed from that of women we did interview, our results may not reflect the true relationship of reproductive events to mortality.

A second limitation to be considered is that we were only able to do immunoperoxidase assays on the tumors for 70.7% of the 1174 women in our study. If the distribution of tumor

Table 3 Relationship of time since last live/still birth to tumor characteristics[a]

Tumor characteristic	Time since last live/still birth				Age-adjusted OR[b]			
	Never had	5+ yrs	2–<5 yrs	<2 yrs	Ever	5+ yrs	2–<5 yrs	<2 yrs
AJCC stage								
I	125 (41.3)	259 (39.9)	33 (28.5)	25 (30.5)				
IIA	91 (30.0)	201 (31.0)	36 (31.0)	22 (26.8)	1.2 (0.9–1.6)	1.2 (0.8–1.6)	1.5 (0.9–2.6)	1.2 (0.6–2.2)
IIB	56 (18.5)	123 (19.0)	28 (24.1)	18 (22.0)	1.4 (0.9–2.0)	1.3 (0.8–1.9)	1.9 (1.0–3.4)	1.5 (0.7–2.9)
III+	31 (10.2)	66 (10.2)	19 (16.4)	17 (22.0)	1.3 (0.8–2.1)	1.0 (0.6–1.6)	2.3 (1.2–4.6)	2.8 (1.3–5.8)
Nodal status								
Negative	199 (65.9)	392 (59.7)	59 (50.4)	38 (46.9)				
Positive	103 (34.1)	265 (40.3)	58 (49.6)	43 (53.1)	1.6 (1.2–2.1)	1.4 (1.1–1.9)	1.9 (1.2–2.9)	2.1 (1.3–3.5)
Ploidy								
Diploid	69 (37.7)	156 (39.7)	23 (33.8)	14 (32.6)				
Aneuploid	114 (62.3)	237 (60.3)	45 (66.2)	29 (67.4)	1.0 (0.7–1.4)	0.9 (0.6–1.3)	1.2 (0.7–2.1)	1.3 (0.6–2.6)
Tumor size								
≤2 cm	164 (54.5)	354 (54.9)	51 (44.0)	38 (46.3)				
>2–5 cm	113 (37.5)	250 (38.8)	49 (42.2)	33 (40.2)	1.2 (0.9–1.6)	1.2 (0.9–1.6)	1.4 (0.9–2.2)	1.2 (0.7–2.0)
>5 cm	24 (8.0)	41 (6.4)	16 (13.8)	11 (13.4)	1.1 (0.7–1.9)	0.8 (0.5–1.4)	2.1 (1.1–4.3)	2.0 (0.9–4.4)
Histologic grade								
Low	45 (19.1)	127 (24.1)	12 (12.4)	3 (4.6)				
Intermed	95 (40.3)	193 (36.6)	45 (46.4)	21 (31.8)	1.0 (0.6–1.5)	0.8 (0.5–1.3)	1.7 (0.8–3.6)	3.1 (0.9–10.9)
High	96 (40.7)	208 (39.4)	40 (41.2)	42 (63.6)	1.2 (0.8–1.8)	1.0 (0.6–1.5)	1.5 (0.7–3.2)	5.9 (1.7–20.1)
Mitoses								
Low	91 (38.6)	227 (43.0)	42 (43.3)	15 (22.7)				
Intermed	77 (32.6)	161 (30.5)	27 (27.8)	25 (37.9)	0.9 (0.6–1.3)	0.9 (0.6–1.3)	0.8 (0.4–1.3)	1.9 (0.9–3.9)
High	68 (28.8)	140 (26.5)	28 (28.9)	26 (39.4)	1.0 (0.7–1.5)	0.9 (0.6–1.4)	0.9 (0.5–1.6)	2.2 (1.1–4.5)
S phase fraction								
Low	90 (53.3)	175 (50.1)	28 (45.2)	13 (32.5)				
High	79 (46.8)	174 (49.9)	34 (54.8)	27 (67.5)	1.3 (0.9–1.9)	1.2 (0.8–1.8)	1.4 (0.8–2.5)	2.3 (1.1–4.8)
Ki-67 ratio								
0–24%	139 (65.9)	278 (60.6)	48 (57.1)	33 (55.0)				
25–100%	72 (34.1)	181 (39.4)	36 (42.9)	27 (45.0)	1.5 (1.1–2.1)	1.5 (1.1–2.2)	1.5 (0.9–2.5)	1.5 (0.8–2.7)
ER								
Positive	130 (61.0)	290 (62.6)	50 (59.5)	30 (50.0)				
Negative	83 (39.0)	173 (37.4)	34 (40.5)	30 (50.0)	1.1 (0.8–1.6)	1.1 (0.8–1.5)	1.1 (0.6–1.8)	1.5 (0.8–2.7)
PR								
Positive	138 (64.8)	289 (62.6)	48 (57.8)	27 (45.0)				
Negative	75 (35.2)	173 (37.5)	35 (42.2)	33 (55.0)	1.4 (1.0–1.9)	1.3 (0.9–1.8)	1.4 (0.8–2.3)	2.2 (1.2–3.9)
c-erbB-2								
Negative	116 (54.5)	253 (54.5)	40 (48.2)	30 (50.0)				
Positive	97 (45.5)	211 (45.5)	43 (51.8)	30 (50.0)	1.1 (0.8–1.5)	1.0 (0.7–1.4)	1.3 (0.8–2.1)	1.2 (0.7–2.1)
P53								
Negative	144 (67.3)	286 (61.8)	36 (42.9)	26 (43.3)				
Positive	70 (32.7)	177 (38.2)	48 (57.1)	34 (56.7)	1.7 (1.2–2.4)	1.4 (1.0–2.0)	2.8 (1.6–4.7)	2.6 (1.5–4.7)

[a] Not all tests were completed on the tumors of all women.
[b] Adjusted for age and diagnosis year.

characteristics differs with regard to reproductive history for the tumors we could obtain and test compared with those that were not available, our results may be biased. We did find that the women whose tumors we were unable to obtain were younger, had more live births, and were less likely to have had a first birth at age ≥30. However, stage at diagnosis and tumor size, as well as time since last birth, did not vary by availability of tumor tissue. The mortality was somewhat higher, although not significantly ($P = 0.17$), among the women from whom we were able to obtain tissue. This could have led to some overestimation of the relationship of the reproductive factors to the tumor markers and mortality.

Finally, our results only relate to ductal tumors in young women and cannot be extrapolated to all breast cancers. This, however, is also a strength of our study, in that restricting our analysis to the most common and homogenous subset of tumors resulted in a more accurate estimation of the main effects.

Strengths of our study include its large size, the population-based nature of the study, the centralized and comprehensive pathological review by a single pathologist, and the testing of tumor markers in a single centralized laboratory.

Among the studies that primarily included premenopausal women or a separate analysis focusing on premenopausal women, our findings of an increased risk of dying associated with ever having a birth are in accord with those of Olson *et al.* (2), Mohle-Boetani *et al.* (6), and Black *et al.* (7) but in contrast to Lethaby *et al.* (8), who focused on node-negative women where, although parous women had a lower survival at 5 years (*i.e.*, 83 *versus* 92%), the difference in survival was not statistically significant. Consistent with Black *et al.* (7) and Guinee *et al.* (9), we found that parous women were more likely to present with positive nodes; however, we did not find parous women to have a significantly higher stage at diagnosis. We found that parous women (particularly those who have given birth in <5 years of diagnosis) were more likely to have tumors that were p53 positive by immunohistochemistry. The tumor

Table 4 Multivariate model of mortality according to time since last pregnancy and tumor characteristics

	HR1[a]	HR2[b]	HR3[c]
Time since last birth			
5+ yrs	1.3 (0.9–1.8)	1.4 (0.9–1.9)	1.5 (1.0–2.3)
2–<5 yrs	1.5 (0.9–2.4)	1.5 (0.9–2.4)	2.2 (1.3–3.6)
<2 yrs	2.7 (1.7–4.2)	2.4 (1.5–3.8)	2.7 (1.6–4.3)
Tumor size			
>2–5 cm	1.5 (1.1–2.0)	[d]	
>5 cm	2.0 (1.3–3.2)		
Node positive	2.9 (2.2–4.0)		
AJCC stage			
IIA		1.7 (1.2–2.6)	2.0 (1.2–3.2)
IIB		3.5 (2.4–5.3)	4.1 (2.5–6.7)
IIIA+		6.0 (3.9–9.1)	7.2 (4.3–12.2)
PR negative	1.9 (1.4–2.5)	1.8 (1.4–2.4)	1.8 (1.3–2.4)
Positive c-erbB-2	1.6 (1.2–2.1)	1.6 (1.2–2.1)	1.5 (1.2–2.1)
Positive p53	1.2 (1.0–1.6)	1.3 (1.0–1.7)	1.3 (1.0–1.8)
Any radiation			0.9 (0.7–1.2)
Any chemo			0.8 (0.5–1.2)
Any hormonal therapy			0.9 (0.6–1.3)

[a] HR1: $n = 805$, adjusted for age, diagnosis year, and all other variables in the model.
[b] HR2: $n = 812$, adjusted for age, diagnosis year, and all other variables in the model.
[c] HR3: $n = 656$, adjusted for age, diagnosis year, and all other variables in the model.
[d] Information not entered indicates not in model.

Table 5 Association of reproductive events with mortality among parous women

	Overall mortality	Overall mortality	Overall mortality
	HR1[a]	HR2[b]	HR3[c]
Age at last live/still birth			
<20	1.0 (ref)	[d]	1.0 (ref)
20–29	1.3 (0.6–2.9)		1.3 (0.6–2.8)
30+	1.5 (0.7–3.3)		1.2 (0.5–2.6)
Time since last live/still birth			
5+ yrs		1.0 (ref)	1.0 (ref)
2–<5 yrs		1.2 (0.8–1.7)	1.2 (0.8–1.9)
<2 yrs		1.9 (1.3–2.7)	2.0 (1.2–3.1)

[a] HR1: includes age at last live/still birth, age, and diagnosis year, $n = 864$.
[b] HR2: includes time since last live/still birth, age, and diagnosis year, $n = 864$.
[c] HR3: includes age at last live/still birth, time since last live/still birth, age, and diagnosis year, $n = 864$.
[d] Information not entered indicates not in model.

suppressor gene, *p53*, plays an important role in cell cycle control. The relationship between p53 overexpression and p53 mutation is not one to one, and it was not possible to assess the mutational status of the tumor cells in this study. Because our study suggests that a recent full-term pregnancy is more strongly related to p53-positive tumors, it is possible that early tumors or preneoplastic cells that contain a p53 mutation may be selected for enhanced growth via exposure to pregnancy-related hormone changes. Few studies have evaluated the relationship of p53 in the tumors to parity. Van der Kooy *et al.* (10) showed that parous women were equally likely to present with a p53-positive tumor as with a p53-negative tumor. Gammon *et al.* (11) found a similar proportion of tumors of parous and nulliparous women to be p53 positive, 44.8 and 43.2 respectively. One explanation for the different results could be the age of the women studied. The women (41%) in van der Kooy's study (10) and 46.4% of the women in Gammon's study (11) were <40 years, whereas in our study, 69% of the women were <40 years. In contrast to Olsson *et al.* (12) and Olsson *et al.* (13), we did not find that ever having a spontaneous abortion or an induced abortion at a young age were related to tumor characteristics indicative of poor survival. They reported that the tumors of women with a prior abortion (induced or spontaneous) were more likely to have a higher S phase fraction and amplification of the *INT2* gene than women without a prior abortion, whereas c-erbB-2 was not amplified.

The most striking results of our study reflect those of Kroman *et al.* (1) and Olson *et al.* (2), in that giving birth in <2 years before the diagnosis of breast cancer is associated with a poor prognosis, compared with never having given birth or giving birth ≥5 years before diagnosis. Consistent with Kroman *et al.* (1), we found that the tumors of women with a birth in <2 years of diagnosis were more likely to have positive nodes and be of higher histological grade than those of nulliparous women or women who had given birth >5 or >6 years in the past. Our study extends knowledge of the possible reasons for the poor prognosis by showing that the tumors of women who had given birth in the 2 years before diagnosis were also more likely to be PR negative, P53 positive, to have high mitoses, and to have a high S phase fraction. When time since last pregnancy, the tumor characteristics, and treatment were included in a multivariate model, having given birth in <2 years of diagnosis remained related independently to mortality. This suggests that other unmeasured factors related to a recent pregnancy have an impact on the course of disease.

We found that the increased risk of mortality associated with ever having a birth is likely attributable to the elevation in risk among women who have had a recent birth. We base this assumption on the lack of any significant increase in risk of mortality associated with having given birth ≥5 years before diagnosis relative to nulliparous women and the elevated risk among parous women with a recent birth relative to women who had given birth ≥5 years before diagnosis. The increased risk associated with high age at first birth (30+ years) and high age at last birth (30+ years) likely reflect the recency of birth. This is supported by the analysis among parous women in which the risk of mortality associated with a recent birth remained elevated, whereas the risk associated with last birth after age 30 did not once both variables were accounted for in the analysis.

Hormones play a major role in the etiology of breast cancer, and it is likely that the high levels of hormones during pregnancy may in part be related to the poor prognosis we observed among women diagnosed with breast cancer <2 years after giving birth. The fact that the tumors of women who had recently given birth were of high histological grade, had a high S phase fraction, and had high mitoses would support the notion that they might be more rapidly growing than the tumors of women who were nulliparous or had given birth ≥5 years before diagnosis. Hormonal changes associated with pregnancy have been shown to stimulate the growth of established mammary cancers in humans (14), and *in vitro* studies using animal models show that pregnancy stimulates the growth of tumor cells (15).

Alternately, it is possible that detection bias may also account for our finding that women who have experienced a recent birth before diagnosis of breast cancer may be at increased risk for having a tumor with poor prognosis. Women shortly after delivery may fail to detect tumors quickly, allowing the tumors to grow large in size and develop an aggressive phenotype (16, 17). Although we were unable to determine

whether delayed diagnosis is an important factor in these women, finding that the association between recent pregnancy and increased mortality was present among women with stage I (HR = 3.1), stage IIA (HR = 2.6), and stage IIB (HR = 2.2) tumors (data not shown) would argue that delay in diagnosis does not account for the aggressive behavior of these tumors.

The data presented here provide additional evidence that reproductive factors influence the biological behavior of breast cancer in young women. Clinicians need to be particularly aware that women who have delivered a child within the last 5 years are at higher risk of having positive nodes and P53-positive tumors and poorer survival than women who are nulliparous or whose last birth was some years in the past.

References

1. Kroman, N., Wohlfahrt, J., Andersen, K. W., Mouridsen, H. T., Westergaard, T., and Melbye, M. Parity, age at first childbirth and the prognosis of primary breast cancer. Br. J. Cancer, *78:* 1529–1533, 1998.

2. Olson, S. H., Zauber, A. G., Tang, J., and Harlap, S. Relation of time since last birth and parity to survival of young women with breast cancer. Epidemiology, *9:* 669–671, 1998.

3. Daling, J. R., Malone, K. E., Voigt, L. F., White, E., and Weiss, N. S. Risk of breast cancer among young women: relationship to induced abortion. J. Natl. Cancer Inst. (Bethesda), *86:* 1584–1592, 1994.

4. Brinton, L. A., Daling, J. R., Liff, J. M., Schoenberg, J. B., Malone, K. E., Stanford, J. L., Coates, R. J., Gammon, M. D., Hanson, R., and Hoover, R. N. Oral contraceptives and breast cancer risk among younger women. J. Natl. Cancer Inst. (Bethesda), *87:* 827–835, 1995.

5. Glogovac, J., Porter, P., Banker, D., and Rabinovich, P. Cytokeratin labeling of breast cancer cells extracted from paraffin embedded tissue for bivariate flow cytometric analysis. Cytometry, *24:* 260–267, 1996.

6. Mohle-Boetani, J. C., Grosser, S., Whittemore, A. S., Malec, M., Kampert, J. B., and Paffenbarger, R. S. Body size, reproductive factors, and breast cancer survival. Prev. Med., *17:* 634–642, 1988.

7. Black, M. M., Hankey, B. F., and Barclay, T. H. C. Parity as a prognostic factor in young breast cancer patients. J. Natl. Cancer Inst. (Bethesda), *70:* 27–30, 1983.

8. Lethaby, A. E., Mason, B. H., Harvey, V. J., and Holdaway, I. M. Survival of women with node negative breast cancer in the Auckland region. NZ Med. J., *109:* 330–333, 1996.

9. Guinee, V. F., Olsson, H., Moller, T., Hess, K. R., Taylor, S. H., Fahey, T., Gladikov, J. V., van den Blink, J. W., Bonichon, F., and Dische, S. Effect of pregnancy on prognosis for young women with breast cancer. Lancet, *343:* 1587–1589, 1994.

10. van der Kooy, K., Rookus, M. A., Peterse, H. L., and van Leeuwen, F. E. p53 protein overexpression in relation to risk factors for breast cancer. Am. J. Epidemiol., *144:* 924–933, 1996.

11. Gammon, M. D., Hibshoosh, H., Terry, M. B., Bose, S., Schoenberg, J. B., Brinton, L. A., Bernstein, J. L., and Thompson, W. D. Cigarette smoking and other risk factors in relation to p53 expression in breast cancer among young women. Cancer Epidemiol. Biomarkers Prev., *8:* 255–263, 1999.

12. Olsson, H., Borg, A., Ferno, M., Ranstam, J., and Sigurdsson, H. Her-2/*neu* and INT2 proto-oncogene amplification in malignant breast tumors in relation to reproductive factors and exposure to exogenous hormones. J. Natl. Cancer Inst. (Bethesda), *83:* 1483–1487, 1991.

13. Olsson, H., Ranstam, H., Baldetorp, B., Ewers, S-B., Ferno, M., Killander, D., and Sigurdsson, H. Proliferation and DNA ploidy in malignant breast tumors in relation to early oral contraceptive use and early abortions (Olsson 1991b). Cancer (Phila.), *67:* 1285–1290, 1991.

14. Muggia, F. M., Cassileth, P. A., Ochoa, M., Flatow, F. A., Gellhorn, A., and Hyman, G. A. Treatment of breast cancer with medroxyprogesterone acetate. Ann. Intern. Med., *68:* 328–337, 1968.

15. Grubbs, C. J., Hill, D. L., McDonough, K. C., and Peckham, J. C. *N*-nitroso-n-methylurea-induced mammary carcinogenesis: effect of pregnancy on preneoplastic cells. J. Natl. Cancer Inst. (Bethesda), *71:* 625–628, 1983.

16. Moore, H. C. F., and Foster, R. S., Jr. Breast cancer and pregnancy. Semin. Oncol., *27:* 646–653, 2000.

17. Gemignani, M. L., Petrek, J. A., and Borgen, P. I. Breast cancer and pregnancy. Surgical Clinics of North America, *79:* 1157–1169, 1999.

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