Breast Cancer Risk in Women With Abnormal Cytology in Nipple Aspirates of Breast Fluid

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Background: **We previously showed that women with abnormal cytology in breast fluid obtained by nipple aspiration had an increased relative risk (RR) of breast cancer compared with women from whom fluid was not obtained and with women whose fluid had normal cytology. This study extends the follow-up in the original study group (n = 4046) and presents the first follow-up for a second group of women (n = 3627).** ***Methods:*** **We collected nipple aspirate fluid from women in the San Francisco Bay Area during the period from 1972 through 1991, classified the women according to the most severe epithelial cytology observed in fluid specimens, and determined breast cancer incidence through March 1999. We estimated RRs for breast cancer using Cox regressions, adjusting for age and year of study entry. All statistical tests were two-sided.** ***Results:*** **For women in the first and second study groups, the median years of follow-up were 21 years and 9 years, respectively, and breast cancer incidences were 7.8% (285 cases in the 3633 women for whom breast cancer status could be determined) and 3.5% (115 of 3271), respectively. Compared with women from whom no fluid was obtained, whose incidences of breast cancer were 4.7% (39 of 825) and 3.3% (65 of 1950) for those in group 1 and group 2, respectively, incidences and adjusted RRs were 8.1% (34 of 422), with RR = 1.4 (95% confidence interval [CI] = 0.9 to 2.3), and 0% (0 of 31), respectively, for those with unsatisfactory aspirate specimens and 8.2% (148 of 1816), with RR = 1.6 (95% CI = 1.1 to 2.3), and 3.1% (25 of 811), with RR = 1.2 (95% CI = 0.8 to 2.0), respectively, for those with normal cytology in aspirates. Compared with women from whom no fluid was obtained, incidences and adjusted RRs for women in group 1 with epithelial hyperplasia and atypical hyperplasia in aspirates were 10.8% (52 of 483), with RR = 2.4 (95% CI = 1.6 to 3.7), and 13.8% (12 of 87), with RR = 2.8 (95% CI = 1.5 to 5.5), respectively, while those for women in group 2 were 5.5% (25 of 457) and 0% (0 of 22), respectively, with a combined RR = 2.0 (95% CI = 1.3 to 3.3).** ***Conclusion:*** **The results obtained with the newly followed women independently confirmed previous findings that women with abnormal cytology in nipple aspirates of breast fluid have an increased risk of breast cancer. [J Natl Cancer Inst 2001;93:1791–8]**

For the past 30 years, we and other researchers *(1–30)* have investigated epidemiologic, biochemical, and cytologic features of breast fluid obtained by the simple, noninvasive technique of nipple aspiration and have studied the relationship between breast fluid characteristics and the development of benign and malignant breast diseases. Breast fluids have been obtained from a large proportion of women who are neither pregnant nor lactating, with reports of percentages of women from whom fluid could be obtained ranging from 25% *(26)* to more than 95% *(27)*. In a previous review *(18)*, we discussed factors consistently associated with the ability to obtain breast fluid and some of the reasons for variability in reported rates of obtaining breast fluids with nipple aspiration. Although all women produce some fluid in their breast ducts, the ability to obtain the fluid probably depends on the quantity of fluid present, duct and nipple characteristics, age and other characteristics of the woman, and the skill of the technician collecting the fluid.

We previously showed that women with epithelial hyperplasia and atypia diagnosed in nipple aspirates of breast fluids *(3,4,10)* were 2.5 and 4.9 times more likely, respectively, to develop breast cancer than women from whom fluid was not obtained *(1,2)*.

This report presents results from an additional median 8 years of follow-up of the original cohort and a first follow-up of an independent cohort of women who underwent nipple aspiration.

Subjects and Methods

Description of Cohorts

We followed a total of 8338 women who participated in breast fluid studies during the period from 1972 through 1991 to determine their breast cancer status. Our analyses were restricted to the 7673 women who both were free of breast cancer at study entry and had not developed the disease within 6 months of study entry and for whom we could obtain a cytologic diagnosis of their breast fluid obtained by nipple aspiration. Of the 665 (8%) women excluded from our analyses, 630 had no cytologic diagnosis of their breast fluid and 35 had developed breast cancer within 6 months of nipple aspiration.

We studied two groups of women who were recruited from different sources during different time periods. Previous reports *(1,2,5,7,10–20,31–33)* have characterized most of the participants. Briefly, women in group 1 (n = 4046) were volunteers recruited during the period from 1972 through 1980 from outpatient clinics at the University of California, San Francisco (UCSF) (35%), the American Cancer Society/National Cancer Institute's Breast Cancer Detection and Diagnosis Project at the Breast Screening Center of Northern California at Merritt Hospital (Oakland, CA) (59%), and other community sources, such as local health fairs and screening programs (6%). These women were self-referred or were referred by physicians *(8)*. Women in group 2 (n = 3627) were volunteers who either had been diagnosed with benign breast disease or non-breast-related conditions at UCSF or Children's Hospital (San Francisco, CA) during the period from 1981 through 1987 *(12)* or were UCSF employees or patients at UCSF mammography and breast surgery clinics *(20)* who participated in breast fluid studies during the period from 1988 through 1991. In our previous studies *(1,2)*, we followed the women in group 1 through 1991. This report is the first follow-up of the women in group 2. Over the nearly 20-year period of subject recruitment, we administered an evolving series of baseline questionnaires to the women in both groups that included questions about standard breast cancer risk factors, such as age, family history of breast cancer, parity, ethnicity, other

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See "Notes" following "References."

demographic factors, reproductive and menstrual histories, and histories of breast diseases and procedures (e.g., biopsy, fine-needle aspiration, mastectomy, and mammography).

Nipple Aspiration and Analysis of Breast Fluids

We used the method of Sartorius et al. *(30)* to obtain breast fluids by nipple aspiration from the women in our cohort. The nipple was first cleaned with a detergent, after which a small plastic cup attached to a 10-mL syringe by a short plastic tube was placed over the nipple. While the subject gently compressed the breast with both hands, the plunger on the syringe was retracted to the 5- to 6-mm^3 mark. If fluid did not appear at the nipple surface within 5 seconds, the plunger was withdrawn to the full 10-mm^3 mark and held for an additional 10–15 seconds. Up to three such attempts were made alternately on each breast. If no fluid appeared after these attempts, the subject was considered to be a nonyielder. The pressure on the nipple created by the aspiration device is similar to that created by a nursing infant. We avoided aspiration in women with permanently retracted nipples. If fluid appeared during one of the attempts, it was collected in capillary tubes and was processed for cytology with the use of previously described techniques *(10)*. Our project pathologist (E. B. King) noted the cytologic diagnosis of epithelial and other cell types contained within the breast fluid samples on standardized coding forms by using criteria described in detail elsewhere *(3,10)*. Each breast fluid specimen was classified according to the most severe epithelial change observed, i.e., normal, mild hyperplasia, moderate hyperplasia, or atypia. For this report, mild hyperplasia and moderate hyperplasia were combined into a single category of hyperplasia.

Written informed consent was obtained from each participant. The Committee on Human Research of the University of California, San Francisco, approved this study of human subjects.

Follow-up Methods

Initial follow-up methods for the women in group 1 through 1991 are presented in detail elsewhere *(1)*. Women in group 1 who were identified either as being deceased or as having had breast cancer during the first follow-up were not followed further. Beginning in May 1996, all remaining women in group 1 and all women in group 2 were mailed a short, structured questionnaire inquiring about their personal history of breast cancer and various breast procedures (such as biopsy, fine-needle aspiration, mastectomy, and mammography), family history of breast cancer, parity, menstrual status, and other characteristics relevant to breast cancer risk. We used several methods to trace the women who did not respond to this questionnaire. These methods included requests for information from contacts previously provided by the study participants, the California Department of Motor Vehicles, the Northern California Cancer Center [the San Francisco Bay Area cancer registry and member of the Surveillance, Epidemiology, and End Results (SEER)[1] Program since 1973 *(34)*], and the California Cancer Registry. We also searched California mortality data, the online National Death Index for subjects with Social Security numbers, and a variety of Internet search engines, such as Infoseek. Next of kin of deceased women from both groups were sent a questionnaire that was slightly modified from the original to reflect that questions were to be asked of proxy respondents. Follow-up through questionnaire and tracing sources ended in February 1999.

Ascertainment and Validation of Breast Cancer Incidence

We initially ascertained the breast cancer status of the women in both groups through self-reports or next-of-kin reports. For women whose history of breast cancer could not be determined in this way, we examined death certificates and reports of breast cancer to the Northern California Cancer Center and the California Cancer Registry. When possible, self-reports or proxy reports of breast cancer were confirmed by using one of these three sources or by checking medical records. Regardless of whether the questionnaire was returned, attempts were made to match women who were known to reside at certain time periods within California and/or the boundaries of the local cancer registry to persons listed within California mortality, California Cancer Registry, and Northern California Cancer Center databases to determine or to verify breast cancer status. This process of matching is called "linkage." We submitted a dataset consisting of the names of women in the two study groups along with their most recent address information (supplied either during original participation, during previous follow-up, during current follow-up, or through Department of Motor Vehicle records), Social Security number (when available), last name, first name, middle initial, date of birth, and race/ethnicity to the California Cancer Registry of the California Department of Health Services for linkage with all cancers diagnosed among female residents of California from 1988 through 1998 and death certificates for 1988 through 1997. At the time of our linkage, statewide reporting of invasive breast cancer cases was estimated to be 100% complete for 1988–1992, more than 99% complete for 1993–1996, 88.9% complete for 1997, and 15.5% complete for 1998. The death certificate information obtained from the California Department of Health Services Center for Health Statistics Death Certificate Master Files was complete through 1997.

The linkage was performed with the use of Integrity Data Re-engineering Environment AutoMatch software, version 3.3 (Vality Technology, Inc., Boston, MA), which is a probabilistic linkage program that uses selected variables to come up with one linkage score for each pair of records. Matching variables are weighted and combined into a single score. This weighting takes into account the reliability of the variable (the conditional probability that this variable will be a match, given that the pair of records is a matched pair) and the probability of a random agreement for this variable. AutoMatch software calculates the probability of a chance agreement for all variables with the use of a frequency analysis of all variables in both datasets. During file preparation for linkage, names were standardized for case subjects for whom nicknames had been used. For example, Meg is changed to Margaret. Names were then transformed with the use of New York State Identification and Intelligence System codes, which are a maximum of eight characters.

The California Cancer Registry then sent us a file containing exact and possible matches between women in our study and women in their databases, along with date of diagnosis, primary cancer site, vital status, date of death and cause of death (if deceased), or date of last contact if alive. We manually inspected each reported match to determine whether the woman was identified correctly.

After receipt and verification of the California Cancer Registry file, we sent the Northern California Cancer Center a list of the women whose last known addresses were within the catchment area of the Northern California Cancer Center for linkage to their records. Thus, a woman was considered to be within the study's linkage area if her last known address was within California as of January 1, 1988, or later or her last known address prior to 1988 was within Alameda, Contra Costa, Marin, San Mateo, or San Francisco counties. These criteria for linkage were used because the California Cancer Registry registered all cancers occurring in California on or after January 1, 1988, whereas continuous cancer registration has occurred since 1973 in the five counties served by the Northern California Cancer Center.

After receiving the linkages, we reviewed the available records to classify women into one of the following five categories: 1) lost to follow-up, 2) probably no breast cancer diagnosed (woman reported no breast cancer but lived out of the catchment area for cancer registration), 3) definitely no breast cancer diagnosis (woman reported no breast cancer and lived within the cancer registration area or woman lived within the cancer registration area according to Department of Motor Vehicles records), 4) breast cancer diagnosis probable (self-report of breast cancer without additional validation), or 5) breast cancer definitely diagnosed (breast cancer was indicated in cancer registry data, on a death certificate, or in medical records).

Statistical Analysis

We compared the breast cancer incidence among women in our study according to cytologic diagnoses made on nipple aspirates of breast fluid. We classified the women according to the following categories of cytologic diagnoses: nipple aspiration attempted and fluid not obtained, fluid specimen obtained but unsatisfactory for cytologic diagnosis, normal cytology, epithelial hyperplasia without atypia, and epithelial atypia. Women who did not yield breast fluid upon nipple aspiration were chosen as the referent group for most analyses in accordance with our previous reports *(1,2)*.

We used Cox regression analyses [life-table methods *(35)*] to compare the distributions of time to breast cancer development (controlling for age, age squared, and year at study entry) in women with different cytologic diagnoses compared with those in women from whom breast fluid could not be obtained (nonyielders). Potential interactions between breast fluid cytology and other breast cancer risk factors were assessed by a comparison of the –2 log likelihood statistics for models that included and excluded the interaction terms. Other breast cancer risk factors examined in this way for interaction were history of biopsy with benign findings either by surgery or by fine-needle aspiration, first-degree relative with breast cancer, age

at first pregnancy (categorized as ⩽19 years old, 20–24 years old, ⩾25 years old, and nulliparous), age at menarche (categorized as ⩽12 years old, 13 or 14 years old, and ⩾15 years old), and white versus nonwhite ethnicity. The final multivariate model considered time to breast cancer as a function of age, age squared, year of study entry, nipple aspirate cytology in conjunction with history of breast biopsy and the other breast cancer risk factors listed above, and stratification by study group. For women who had undergone more than one nipple aspiration, we used only the most severe cytologic findings obtained for these analyses. We used SAS statistical software, version 6 *(36–38),* for data management and PROC PHREG *(39)* for Cox regression analyses. Plots of the log–log survivor function versus follow-up time showed constant separation between the breast fluid and cytologic diagnosis categories, which indicates that the proportional hazards assumption was reasonable. All statistical tests were two-sided.

The SAS program LifeTest was used to compute unadjusted cumulative probabilities of developing breast cancer at specific times for women in the different breast fluid categories. The SAS program Plot was then used to plot these data. Although some women had multiple visits for additional nipple aspiration up to a year after their initial visit, the date of the first visit was used as the baseline time for regression analyses because the questionnaires with other pertinent risk factor data were completed at that time.

RESULTS

Description of the Cohort and Follow-up

Of the women in our study cohort, 10.0% (769 of 7673) did not return questionnaires or could not be positively identified through the Department of Motor Vehicles or other sources. Table 1 shows follow-up rates and baseline characteristics of the women who were followed. At study entry, the women in group 1 were, on average, 5–6 years older than the

Table 1. Baseline characteristics, breast fluid cytologic diagnoses, and follow-up percentages of women participating in nipple aspiration breast fluid studies in the San Francisco Bay Area, California, 1973–1999*

	Sources of subjects		
	Overall: groups 1 and 2	Group 1: Oakland Breast Screening Center, variety of San Francisco Bay Area clinics, and health fairs	Group 2: UCSF and Children's Hospital breast and other clinic patients and UCSF employees
Dates of nipple aspiration (median y of study entry)	1972–1991 (1979)	1972–1980 (1975)	1981–1991 (1987)
Person-years of follow-up	100 892	68 610	32 282
Total No. of women followed/total No. of women (% with complete follow-up)	6904/7673 (90)	3633/4046 (90)	3271/3627 (90)
Characteristics			
Cytologic diagnosis,† No. (%)			
No breast fluid	2775 (40.2)	825 (22.7)	1950 (59.6)
Unsatisfactory specimen	453 (6.6)	422 (11.6)	31 (0.9)
Normal	2627 (38.1)	1816 (50.0)	811 (24.8)
Hyperplasia	940 (13.6)	483 (13.3)	457 (14.0)
Atypia	109 (1.6)	87 (2.4)	22 (0.7)
Age groups, y, No. (%)			
18–24	298 (4.3)	163 (4.5)	135 (4.1)
25–34	1354 (19.6)	456 (12.6)	898 (27.5)
35–44	2109 (30.5)	1024 (28.2)	1085 (33.2)
45–54	1815 (26.3)	1101 (30.3)	714 (21.8)
55–64	954 (13.8)	623 (17.1)	331 (10.1)
⩾65	374 (5.4)	266 (7.3)	108 (3.3)
Median age, y	43	46	40
Parity, No. (%)			
Nulliparous	1693 (24.5)	679 (18.7)	1014 (31.0)
Parous	5062 (73.3)	2806 (77.2)	2256 (69.0)
Missing	149 (2.2)	148 (4.1)	1 (0.03)
Age at menarche, y, No. (%)			
⩽12	3019 (43.7)	1468 (40.4)	1551 (47.4)
13–14	2918 (42.3)	1548 (42.6)	1370 (41.9)
⩾15	787 (11.4)	451 (12.4)	336 (10.3)
Missing	180 (2.6)	166 (4.6)	14 (0.4)
Ethnicity, No. (%)			
White	4921 (71.3)	2477 (68.2)	2444 (74.7)
Nonwhite‡	1969 (28.5)	1143 (31.5)	826 (25.3)
Missing	14 (0.2)	13 (0.4)	1 (0.03)
First-degree relative with breast cancer, No. (%)			
No	5886 (85.3)	3082 (84.8)	2804 (85.7)
Yes, 1	769 (11.1)	406 (11.2)	363 (11.1)
Yes, ⩾2	53 (0.8)	29 (0.8)	24 (0.7)
Missing	196 (2.8)	116 (3.2)	80 (2.4)
History of breast biopsy, No. (%)			
No	4929 (71.4)	2842 (78.2)	2087 (63.8)
Yes, FNA only	405 (5.9)	0 (0.0)	405 (12.4)
Yes, biopsy	1362 (19.7)	600 (16.5)	762 (23.3)
Missing	208 (3.0)	191 (5.3)	17 (0.5)

*UCSF = University of California, San Francisco; FNA = fine-needle aspiration.

†Among the 4129 women from whom nipple aspirate fluid was obtained, cytologic diagnoses were based on the most severe finding at a single visit for 3593 (87%) women, at two visits for 269 (6.5%) women, and at three or more visits for 267 (6.5%) women.

‡Nonwhites include 38% black, 39% Asian, 16% Hispanic, and 7% other nonwhites.

women in group 2. At study entry in groups 1 and 2, respectively, fluid was obtained from 77.3% and 40.4% of the women, 2.4% and 0.7% of the women had atypical cytologic findings, 18.7% and 31.0% of the women were nulliparous, 68.2% and 74.7% of the women were white, and 16.5% and 23.3% of the women reported breast biopsy. About 12% of the women in each group reported having at least one first-degree relative with breast cancer. As of March 1999, 400 (5.8%) of 6904 women overall had developed breast cancer: 3.5% of the women in group 2, with a median of 9 years of follow-up, compared with 7.8% of the women in group 1, with a median of 21 years of follow-up (Table 2). Overall, 13 (3.3%) breast cancers were solely self-reported, and two (0.5%) were identified only by death certificate review; the remaining 96.3% of breast cancer cases were confirmed through either the tumor registry (n = 381) or medical records (n = 4).

Breast Cancer Incidence by Cytologic Diagnosis

Overall, women from whom breast fluid was obtained were somewhat more likely to develop breast cancer than women from whom fluid was not obtained (relative risk [RR] = 1.5; 95% confidence interval [CI] = 1.2 to 1.9). Women with normal breast fluid cytologic findings were 30% more likely to develop breast cancer than women from whom breast fluid was not obtained, whereas women who produced breast fluid with either hyperplasia or atypia were twice as likely to develop breast cancer as women from whom breast fluid was not obtained (Table 2). Age-adjusted RRs of breast cancer for women in group 1 with normal cytology, hyperplasia, or atypia were 1.6, 2.4, and 2.8, respectively, and for women in group 2 with normal or abnormal (hyperplasia or atypia) cytology were 1.2 and 2.0, respectively, compared with women from whom no breast fluid was obtained (Table 2). In group 2, there were very few women (n = 22) in the atypical hyperplasia category and none developed breast cancers. Since we are unaware of software that will calculate exact CIs for the RR with adjustment for other covariates, we combined the women with atypical hyperplasia with the women with hyperplasia to form a category of women with proliferative nipple aspirate findings.

As shown in Fig. 1 (top), overall, women with atypical hyperplasia were somewhat more likely to be diagnosed with breast cancer than were women with hyperplasia. The latter women were more likely to be diagnosed with breast cancer than women with normal cytology or women from whom fluid could not be obtained. Fig. 1 also shows an excess breast cancer risk among women with atypical hyperplasia or hyperplasia versus normal cytology in both group 1 (middle panel) and group 2 (bottom panel); age-adjusted RRs and 95% CIs for these comparisons are given in Table 2. As expected, based on usual risk factors for breast cancer, the RRs of breast cancer were higher in women who were nulliparous or whose first pregnancy occurred when they were 25 years old or older than in women whose first pregnancy occurred when they were 24 years old or younger, in women with earlier versus later age at menarche, in women with a first-degree relative with breast cancer than in those without a first-degree relative with breast cancer, and in women with history of breast fine-needle aspiration or biopsy than in women with no history of breast biopsy (Table 3). Breast cancer risk did not differ among whites and nonwhites in this cohort; however, there were insufficient numbers of women to determine risk for those with specific nonwhite ethnic backgrounds.

We did not find evidence for interactions between breast fluid cytologic diagnoses and age, history of a first-degree relative with breast cancer, age at first pregnancy, study group, ethnicity, or age at menarche with respect to breast cancer risk (*P* values of .78, .86, .87, .21, .89, and .77, respectively). In contrast, there was a suggestion of an interaction between cytologic diagnoses of breast fluid and history of biopsy prior to nipple aspiration ($P = .08$), but that interaction did not achieve statistical significance. Thus, the multivariate model included variables for the joint effects of biopsy history and cytologic diagnosis in addition to the other breast cancer risk factors (Table 3). Because positive family history is such an important risk factor for breast cancer, we note that, among women who reported such a history, those with hyperplasia or atypical hyperplasia in nipple aspirate fluids were 3.4 times (95% CI = 1.9 to 6.1 times) and 4.5 times (95% CI = 1.4 to 14.3 times) more likely, respectively, to develop breast cancer as women with no family history who did

Table 2. Age-adjusted and year-of-entry-adjusted relative risks (RRs) and 95% confidence intervals (CIs) of breast cancer by breast fluid cytologic diagnosis for women participating in nipple aspiration breast fluid studies, San Francisco Bay Area, CA, 1973–1999

	Overall			Group 1			Group 2		
Cytologic diagnosis	No. with breast cancer/total No. of women	% with breast cancer	RR* (95% CI)	No. with breast cancer/total No. of women	% with breast cancer	RR* (95% CI)	No. with breast cancer/total No. of women	% with breast cancer	RR* (95% CI)
No breast fluid	104/2775	3.7	1.0 (referent)	39/825	4.7	1.0 (referent)	65/1950	3.3	1.0 (referent)
Unsatisfactory specimen	34/453	7.5	1.2 (0.8 to 1.7)	34/422	8.1	1.4 (0.9 to 2.3)	0/31	0.0	—
Normal	173/2627	6.6	1.3 (1.0 to 1.7)	148/1816	8.2	1.6 (1.1 to 2.3)	25/811	3.1	1.2 (0.8 to 2.0)
Hyperplasia†	77/940	8.2	2.0 (1.5 to 2.8)	52/483	10.8	2.4 (1.6 to 3.7)	25/457	5.5	2.0 (1.3 to 3.3)‡
Atypia†	12/109	11.0	2.1 (1.1 to 3.9)	12/87	13.8	2.8 (1.5 to 5.5)	0/22	0.0	
Total	400/6904	5.8		285/3633	7.8		115/3271	3.5	

*RR adjusted by Cox regression for age, age squared, and year of specimen collection.

†RRs (95% CIs) for hyperplasia or atypia versus normal cytologic findings were 1.6 (1.2 to 2.1), 1.6 (1.2 to 2.1), and 1.7 (0.96 to 2.9) for both groups (overall), group 1, and group 2, respectively.

‡In group 2, there were very few women (n = 22) in the atypical hyperplasia category and none developed breast cancers. Since we are unaware of software that will calculate exact CIs for the RR with adjustment for other covariates, we combined the women with atypical hyperplasia with the women with hyperplasia to form a category of women with proliferative nipple aspirate findings. The RR for women with hyperplasia versus those with no breast fluid adjusted for age, age-squared, and year of specimen collection was 2.1 (95% CI = 1.3 to 3.5).

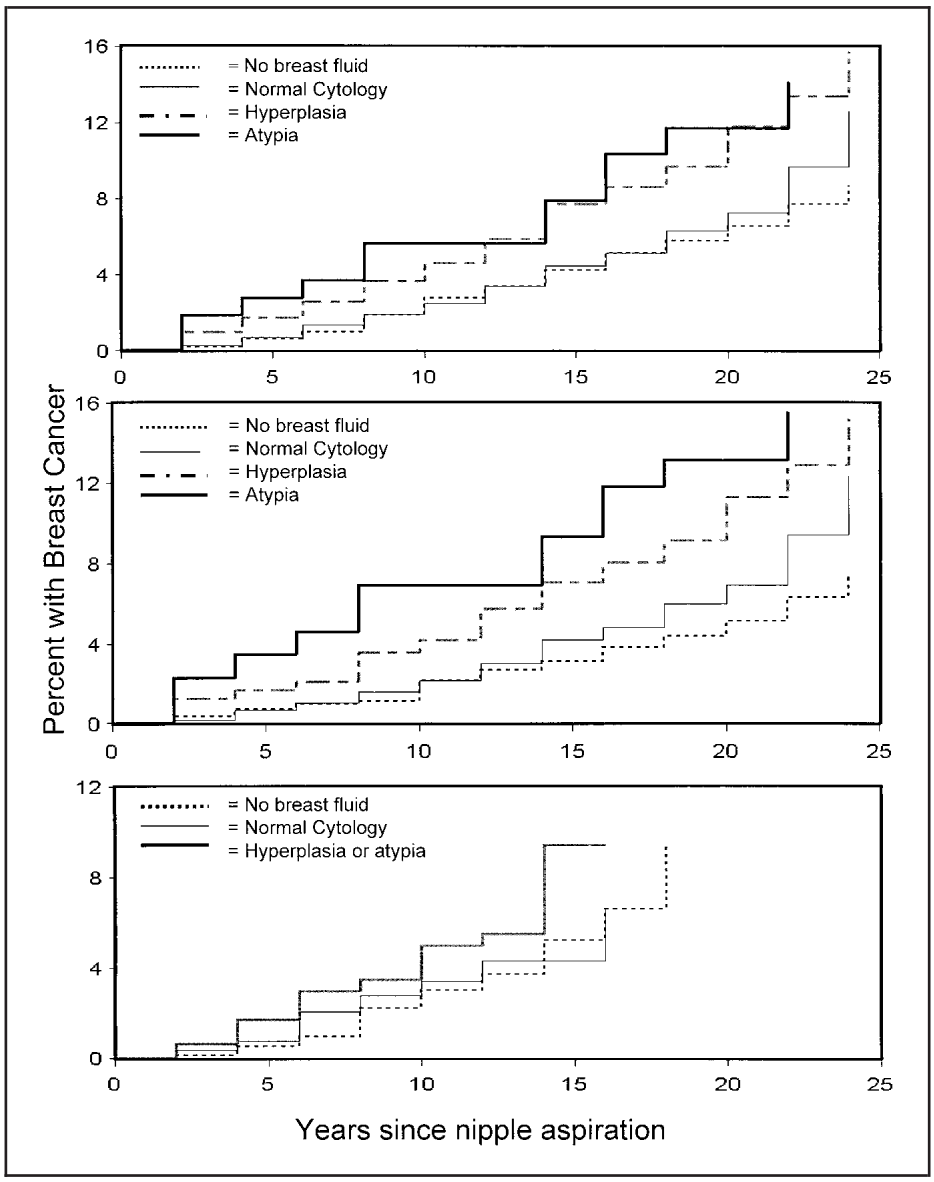

Fig. 1. Unadjusted cumulative percentages of volunteer women from the San Francisco Bay Area developing breast cancer by cytologic diagnosis in relation to time since nipple aspiration. **Top panel** shows all women followed from 1973 through 1999. For each category of cytologic diagnosis, the cumulative percentages (95% confidence intervals [CIs]) at 10 and 20 years, respectively, are as follows: no breast fluid, 2.7% (95% CI = 2.1% to 3.4%) and 6.5% (95% CI = 5.0% to 8.1%); normal cytology, 2.5% (95% CI = 1.9% to 3.1%) and 7.2% (95% CI = 6.0% to 8.4%); hyperplasia, 4.6% (95% CI = 3.2% to 6.0%) and 11.8% (95% CI = 9.0% to 14.6%); atypia, 5.6% (95% CI = 1.3% to 10.0%) and 11.7% (95% CI = 5.1% to 18.3%). **Middle panel** shows women from study group 1 only, who were followed from 1973 through 1999. For each category of cytologic diagnosis, the cumulative percentages (95% CIs) at 10 and 20 years, respectively, are as follows: no breast fluid, 2.1% (95% CI = 1.1% to 3.1%) and 5.1% (95% CI = 3.4% to 6.8%); normal cytology, 2.1% (95% CI = 1.5% to 2.8%) and 6.9% (95% CI = 5.7% to 8.2%); hyperplasia 4.2% (95% CI = 2.4% to 6.0%) and 11.3% (95% CI = 8.2% to 14.3%); atypia, 6.9% (95% CI = 1.6% to 12.2%) and 13.1% (95% CI = 5.9% to 20.4%). **Bottom panel** shows women from study group 2 only, who were followed from 1981 through 1999. For each category of cytologic diagnosis, the cumulative percentages (95% CIs) at 10 years are as follows: no breast fluid, 3.0% (95% CI = 2.2% to 3.9%); normal cytology, 3.4% (95% CI = 1.9% to 4.9%); hyperplasia or atypia, 5.0% (95% CI = 2.8% to 7.2%).

not yield nipple aspirate fluid (data not shown).

Discussion

This study both extends our original follow-up studies *(1,2)* of breast cancer risk associated with nipple aspirate fluid cytologic diagnoses in group 1 for a median of 8 years and enlarges the total number of women studied by adding those in group 2. Differences in breast cancer risk estimates in relation to breast fluid cytologic findings between this report and our original report *(1,2)* may be due to the previous report having been restricted to white women. However, the increasing gradient of breast cancer risk with increasing severity of the cytologic diagnosis is similar to that found previously, with the highest risk occurring among women with cytologic diagnosis of atypical hyperplasia. Most importantly, this study independently confirms, in a previously unreported group of 3271 women (group 2), that abnormal cytologic findings in breast fluids are associated with an increased risk of breast cancer. Because many of the women in group 2 were recruited from special breast diagnostic and treatment clinics, it is not surprising that their RR of breast cancer risk was statistically significantly higher than that of women in group 1, who were recruited from a breast-screening clinic or were volunteers at health fairs conducted throughout San Francisco. Nevertheless, the results for both groups were consistent, in that women with abnormal (proliferative) nipple aspirate fluid cytologic findings had an approximately twofold increased risk of breast cancer compared with women from whom fluid could not be obtained. Taken together, these results strongly support the hypothesis that abnormal cytology predicts an increased risk of breast cancer.

We have shown previously that a woman's age is related to her ability to produce breast fluid upon nipple aspiration and that breast fluid is most readily produced by women younger than 55 years *(18)*. We, therefore, believe that cytologic diagnosis of nipple aspirates is likely to be most useful in determining breast cancer risk among women younger than 55 years. Similar to the findings that we reported in our initial follow-up studies of the original cohort *(1,2)*, we found that women with either a family history of breast cancer or a personal history of a previous benign breast biopsy had statistically significantly increased breast cancer risks compared with women without such histories. Moreover, as in the first study, a history of biopsy and proliferative nipple aspirate fluid cytologic findings together increased breast cancer risk more than expected if the two characteristics were independent, although the interaction was of borderline statistical significance. This finding suggests that women with proliferative cytology in breast fluids who also had a history of lumps requiring biopsy may have had breast cells that had accumulated more of

Table 3. Age-adjusted and year-of-entry-adjusted and multivariate-adjusted relative risks (RRs) and 95% confidence intervals (CIs) stratified by study group for breast cancer by age at first pregnancy, age at menarche, family history of breast cancer, history of breast biopsy, white versus nonwhite ethnicity, and interaction of history of breast biopsy with nipple aspiration cytologic findings for all women participating in nipple aspiration breast fluid studies, San Francisco Bay Area, CA, 1973–1999*

Breast cancer risk factor	No. with breast cancer/total No. of women	% with breast cancer	Age-adjusted RR† (95% CI)	Multivariate-adjusted RR‡ (95% CI)
Age at first pregnancy, y				
≤24	160/2979	5.4	1.0 (referent)	1.0 (referent)
≥25	138/2003	6.9	1.3 (1.0 to 1.6)	1.3 (1.0 to 1.6)
Nulliparous	87/1693	5.1	1.6 (1.2 to 2.1)	1.5 (1.2 to 2.1)
Age at menarche, y				
≥15	37/787	4.7	1.0 (referent)	1.0 (referent)
13–14	175/2918	6.0	1.3 (0.9 to 1.9)	1.3 (0.9 to 1.8)
≤12	174/3019	5.8	1.4 (0.96 to 2.0)	1.3 (0.9 to 1.9)
First-degree relative with breast cancer				
No	311/5886	5.3	1.0 (referent)	1.0 (referent)
Yes, 1	66/769	8.6	1.6 (1.2 to 2.0)	1.5 (1.1 to 2.0)
Yes, ≥2	9/53	17.0	2.8 (1.5 to 5.5)	2.0 (0.95 to 4.3)
History of breast biopsy				
No	243/4929	4.9	1.0 (referent)	—
Yes, FNA only	16/405	4.0	1.7 (0.97 to 2.9)	
Yes, biopsy	128/1362	9.4	1.9 (1.5 to 2.3)	
Ethnicity				
Nonwhite	97/1969	4.9	1.0 (referent)	1.0 (referent)
White	303/4921	6.2	1.2 (0.94 to 1.5)	1.0 (0.8 to 1.3)
Breast biopsy and nipple aspirate fluid cytology				
No previous biopsy—cytologic diagnosis				
No breast fluid	58/1939	3.0	1.0 (referent)	1.0 (referent)
Unsatisfactory specimen	24/360	6.7	1.3 (0.8 to 2.1)	1.3 (0.7 to 2.1)
Normal	118/1904	6.2	1.6 (1.1 to 2.2)	1.6 (1.1 to 2.3)
Hyperplasia	38/652	5.8	1.9 (1.2 to 2.9)	1.7 (1.1 to 2.7)
Atypia	5/74	6.8	1.6 (0.6 to 3.9)	1.7 (0.7 to 4.3)
Previous biopsy—cytologic diagnosis				
No breast fluid	45/793	5.7	2.0 (1.4 to 3.0)	2.1 (1.4 to 3.1)
Unsatisfactory specimen	8/71	11.3	2.2 (1.0 to 4.6)	2.2 (1.0 to 4.8)
Normal	47/597	7.9	2.1 (1.4 to 3.1)	2.1 (1.4 to 3.2)
Hyperplasia	37/274	13.5	4.8 (3.2 to 7.3)	5.0 (3.2 to 7.7)
Atypia	7/32	21.9	6.3 (2.8 to 13.9)	6.1 (2.7 to 13.6)
Study group				
1	285/3633	7.8	1.0 (referent)	—
2	115/3271	3.5	2.0 (1.2 to 3.3)	

*FNA = fine-needle aspiration.

†All variables are adjusted for age, age squared, and year of breast fluid specimen collection. Study group is included as a stratification variable for all variables except study group.

‡Each variable presented is adjusted for age, age squared, year of breast fluid specimen collection, and the other risk factors in this column with stratification by study group.

the changes necessary for malignant transformation *(2)*.

There is enormous interest in early detection of breast cancer or breast cancer precursors and in the development of markers of high breast cancer risk because early diagnosis is believed to be a key for minimizing mortality from this disease *(40–45)*. It is the hope and expressed goal of many breast cancer advocacy groups that preventive strategies will be developed eventually so that even those women at very high risk of breast cancer need not face the fear of developing or dying of this disease. Timely assessment of the efficacy of preventive programs will require that such programs identify women at high risk and incorporate intermediate endpoints of breast cancer development. Other investigators *(46–50)* have discussed at length the strengths and limitations of various currently used breast cancer prediction models, including those that consider risks attributable to inherited susceptibility. Such discussions show that there is clearly a need for additional markers of breast cancer risk.

It is now widely accepted that histologically diagnosed breast epithelial hyperplasia and atypical hyperplasia in breast biopsy specimens are precursor, or "marker," lesions that are associated with an increased risk of breast cancer *(51–61)*, thus alerting the patient and physician to the need for increased surveillance. However, given that the cytologic or histologic condition of breast epithelia before breast cancer diagnosis is typically not known for most women who have no prior indication for breast biopsy *(25)* and epithelial proliferation in the breast is relatively common *(62,63)*, alternative methods, such as nipple aspiration or other techniques, are needed to identify women with high-risk breast cytology. Fabian et al. *(47)* used one such alternative method, random periareolar fine-needle aspiration, in a cohort of 480 high-risk women and found that only atypical cytology and risk based on the Gail model statistically significantly and independently predicted short-term (up to 5 years) breast cancer risk in a multivariate analysis. The RRs of breast cancer associated with atypical cy-

tology detected by fine-needle aspiration were very similar to those associated with atypical hyperplasia observed in breast biopsy specimens *(51–61)* and those associated with a cytologic diagnosis of atypical hyperplasia in nipple aspirates of breast fluid, reported here and in our previous study *(1)*. In summary, both histologic and cytologic proliferative findings in breast epithelial cells, whether detected in biopsy specimens, fine-needle aspirates, or nipple aspirates, are associated with increased risk of breast cancer.

There are advantages and disadvantages to both periareolar fine-needle aspiration and nipple aspiration in obtaining specimens for cytologic analysis. Periareolar fine-needle aspiration has a major advantage because, unlike nipple aspiration, material for cytologic analysis can theoretically be obtained from all women. However, the major disadvantage of fine-needle aspiration is that it is an invasive procedure that is more likely to involve complications and to require physician services, making it unsuitable for large-scale use in clinically "normal" women. In contrast, nipple aspiration of breast fluid is a simple, noninvasive method for obtaining breast duct fluid from women in the general population who are neither pregnant nor lactating. In our experience with more than 8000 women, we have received very few complaints about this procedure and have had no untoward effects from nipple aspiration when it was performed in accordance with the technique described by Sartorius et al. *(30)*.

Approximately 40% of our cohort did not yield breast fluid upon three attempts at nipple aspiration. Some researchers *(23,27,64)* have reported success in obtaining nipple aspirates from higher proportions of women by taking samples from the women on several visits over a period of 1–2 weeks. However, despite variations in rates of obtaining fluid, our results suggest that whether or not breast fluid is obtained from women might reflect potentially pathogenetic variations in breast physiology *(11,65)*. For example, we found that women with normal nipple aspirate fluid cytology had a 20% (group 2) to 60% (group 1) increase in breast cancer risk compared with nonyielders. This finding suggests that the metabolic characteristics that lead to lower secretory activity of the breast epithelium resulting in a decreased likelihood of yielding breast fluid upon nipple aspiration also may be protective against breast cancer. Whether such putative protective factors result from diminished hormonal stimulation or diminished responsiveness to hormonal stimulation is conjectural but may warrant further investigation.

Nipple aspirate cytology has several potential uses in research on breast cancer detection and prevention. For example, results from our studies of the two groups of women clearly demonstrate that nipple aspirate cytology is an independent and relatively strong predictor of breast cancer risk, especially among women who have had a previous breast biopsy for benign breast disease. Studies of biochemical and molecular markers in breast duct fluids obtained by nipple aspiration may discover markers that might be more sensitive predictors of breast cancer risk than conventional cytology. Moreover, nipple aspirate cytology provides a more complete picture of the natural history of proliferative changes in the breasts of women in the general population who do not have apparent breast disease than breast biopsy, which is performed only in response to abnormalities found at physical examination or detected by mammography. Studies that combine the results of nipple aspiration cytology with observations of areas of high radiographic density in mammograms may further facilitate the detection of disease in women at high risk for breast cancer *(20)*. Finally, nipple aspirate cytology and duct fluid analysis may find use by providing intermediate endpoints in studies of breast cancer pathogenesis and in clinical trials of new chemopreventive agents against breast cancer and benign breast disease. We strongly support the efforts of clinical investigators to find applications for this easy-to-perform, noninvasive procedure.

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NOTES

[1]*Editor's note:* SEER is a set of geographically defined, population-based central cancer registries in the United States, operated by local nonprofit organizations under contract to the National Cancer Institute (NCI). Registry data are submitted electronically without personal identifiers to the NCI on a biannual basis, and the NCI makes the data available to the public for scientific research.

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