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Breast Cancer Incidence in Women with Abnormal Cytology in Nipple Aspirates of Breast Fluid

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This is a prospective study of breast cancer risk in relation to nipple aspirate fluid cytology in 2,701 volunteer white women from the San Francisco Bay Area first enrolled between 1973 and 1980. The women were not pregnant or lactating and were free of breast cancer within 6 months of entry into the study. The breast cancer status of this cohort was determined between June 1988 and April 1991. Follow-up was complete for 87% (n = 2,343) of the cohort, representing 29,961 person-years and an average of 12.7 years of follow-up. The overall breast cancer incidence was 4.4% (104 of 2,343) and rose with fluid cytology findings as follows: no fluid obtained, 2.6% (9 of 352); unsatisfactory specimen, 4.8% (15 of 315); normal cytology, 4.3% (56 of 1,291); epithelial hyperplasia, 5.5% (18 of 327); and atypical hyperplasia, 10.3% (6 of 58). Relative risks for breast cancer and their 95% confidence intervals were estimated by Cox regression, adjusting for age and year of entry. Compared with the relative risk for women who yielded no fluid, relative risks were: unsatisfactory specimen, relative risk (RR) = 1.4 (95% confidence interval (CI) 0.6–3.3); normal cytology, RR = 1.8 (95% CI 0.9–3.6); epithelial hyperplasia, RR = 2.5 (95% CI 1.1–5.5); and atypical hyperplasia, RR = 4.9 (95% CI 1.7–13.9). These findings were strongest for and were mainly confined to women aged 25–54 years. Women with atypical hyperplasia and a first-degree family history of breast cancer were six times more likely to develop breast cancer than were women with atypical hyperplasia but without a family history of breast cancer (95% CI 1.0–30.2). These findings provide strong support for our hypothesis that hyperplasia and atypical hyperplasia diagnosed in nipple aspirates of breast fluid are associated with an increased risk of breast cancer. *Am J Epidemiol* 1991;135:130–41.

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For the past 18 years, we (1–4) have investigated the epidemiologic, biochemical, and cytologic features of breast fluid obtained by nipple aspiration and the interre-

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Abbreviations: CI, confidence interval; RR, relative risk; SEER, Surveillance, Epidemiology, and End Results; SMSA, Standard Metropolitan Statistical Area.

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lations of these components of breast fluid to benign breast disease and breast cancer. The simple, noninvasive technique of nipple aspiration has permitted us to evaluate biochemical constituents of nipple aspirate fluid and the status of exfoliated breast epithelial cells in a high proportion of adult nonpregnant women. Four factors have been consistently associated with increased ability to obtain breast fluid: 1) age 35–50 years; 2) early age at menarche; 3) non-Asian versus Asian ethnicity; and 4) history of parity and/or lactation (4). These and other aspects of the epidemiology, cytopathology, and biochemistry of nipple aspirate fluid were recently reviewed (2–5). Recent evidence suggests that dietary fat intake may influence breast fluid secretion (6).

On the basis of the most severe epithelial changes observed, we have classified the cytology of nipple aspirate fluid samples as hyperplasia, moderate hyperplasia, atypical hyperplasia, or malignant (5–8). In an earlier study, King et al. (9) reported a significant concordance of cytopathologic findings in nipple aspirates with the histopathologic findings from biopsy specimens. However, while demonstrating an association of atypical hyperplasia and breast cancer, that study could not directly address whether cytopathologic changes detected in nipple aspirate fluid preceded the development of benign or malignant breast disease. Except for a preliminary positive assessment (10), the prognostic value of nipple aspirate cytology has not been determined.

To our knowledge, this paper presents the results of the first prospective study of the risk of breast cancer by nipple aspirate fluid cytologic diagnosis in a cohort of 2,701 women who underwent nipple aspiration of breast fluid 10–18 years previously.

MATERIALS AND METHODS

Description of cohort

Study subjects were 2,701 white women who participated in our breast fluid studies between 1973 and 1980, who lived in the San Francisco Bay Area, were neither pregnant nor lactating at entry into the study, were free of breast cancer, and did not develop breast cancer within 6 months of entry. Their characteristics have been described elsewhere (11). Briefly, the women were volunteers from various outpatient clinics at the University of California, San Francisco (35 percent), the Breast Cancer Detection and Diagnosis Project of the American Cancer Society/National Cancer Institute at the Breast Screening Center of Northern California, Merritt Hospital, Oakland, California (59 percent), and other community sources, such as local health fairs and screening programs (6 percent). The women were self-referred or were referred by physicians. Potential risk factors for breast cancer were assessed with a structured questionnaire and included age, family history of breast cancer, parity, ethnicity, other demographic factors, and reproductive and medical histories.

Nipple aspirate fluid was obtained by the method of Sartorius et al. (12). A small plastic cup attached to a 10-cc syringe by a short plastic tube was placed over the nipple, which had been previously cleaned with a detergent. While the subject gently compressed the breast with both hands, the syringe was retracted to the 5–6 ml mark. If fluid did not appear at the nipple surface within 5 seconds, the plunger was withdrawn to the full 10 ml mark and held for an additional 10–15 seconds. If no fluid appeared within this time, the subject was designated as a nonyielder. The fluid appearing at the nipple surfaces was collected in capillary tubes and processed for cytology by the techniques previously described by King et al. (5, 9). The cytologic diagnosis of epithelial and other cell types was noted by our project pathologist (E. B. K.) on standardized coding forms using criteria described in detail elsewhere (9). Each breast fluid specimen was classified according to the most severe epithelial change present, i.e., normal, mild hyperplasia, moderate hyperplasia, or atypical hyperplasia. For this report, mild and moderate hyperplasia were combined into a single category, designated hyperplasia.

Follow-up methods

Beginning in June 1988, all women were mailed a structured questionnaire inquiring about personal history of breast cancer and breast procedures (such as biopsy, fine needle aspiration, and mastectomy), family history of breast cancer, parity, menstrual status, etc. Several methods were used to trace the nonresponders. These included requests to the California Department of Motor Vehicles; the regional San Francisco Bay Area Tumor Registry, a member of the Surveillance, Epidemiology, and End Results (SEER) Program (13); the California Automated Mortality Linkage Information System (CAMLIS); the National Death Index; the Health Care Financing Administration; and individual sources such as physicians or neighbors. Next-of-kin of deceased women were sent a modified questionnaire. Follow-up ended in April 1991.

Ascertainment and validation of breast cancer incidence

Breast cancer status was initially ascertained through self-reports or next-of-kin reports of breast cancer or of malignant findings in biopsy or mastectomy, and subsequently by pathology reports and slides from hospitals and medical care providers for all identified biopsies and mastectomies. Breast cancer incidence was also determined from death certificate designation and the San Francisco Bay Area Tumor Registry. Slides and pathology reports were reviewed by our project pathologist (E. B. K.) and recorded and coded on a standardized form, using definitions consistent with those of others and based on our experience (9, 14–16).

Data analysis

This paper compares breast cancer incidence in five groups of women by cytologic diagnoses made on nipple aspirates of breast fluid: nipple aspiration attempted, fluid not obtained (referent group); specimen obtained, but unsatisfactory for cytologic diagnosis; normal cytology; epithelial hyperplasia without atypia; and epithelial atypical hyperplasia. Women who did not yield nipple aspirate fluid were chosen as the referent group. This decision was based on our hypothesis that breast fluid secretion is related to the risk of breast cancer and that nonyielders of nipple aspirate fluid are presumed to have the lowest risk (2). The data were analyzed by age at time of nipple aspiration and by family history of breast cancer, as follows: 1) women of all ages; 2) women in the age group 25–54 years at entry into the study; 3) women aged 55 years or older at entry into the study; and 4) women of all ages with and without a first-degree family history of breast cancer.

Logistic regression analysis (17) was used to compute the odds ratios of developing breast cancer in women with differing cytologic diagnoses compared with nonyielders. In the initial analysis, we controlled for age and year at attempted breast fluid aspiration. A Cox model (18) (life table methods) was then used to compare the distributions of time to breast cancer development (controlling for age at entry into the study) in women with different cytologic diagnoses compared with nonyielders. Separate models were used that combined the categories of unsatisfactory specimen and normal cytology. Further analyses also controlled for parity (nulliparous vs. parous), place of examination, and first-degree family history of breast cancer. Separate logistic and Cox models were fit using either the most severe cytologic findings from any visit for women who had more than one visit or using results from the first visit only. We reconstructed missing data on parity status at the time of entry into the study or on the date of the most severe cytologic diagnosis by using information from the follow-up questionnaire. We used reports of no first-degree relative with breast cancer on the follow-up questionnaire to replace missing family history data from the study entry questionnaire. Statistical Analysis System (SAS) (19) was used for data management, and BMDP (20) was used for logistic regressions (BMDPLR) and Cox regressions (BMDP2L). Because of the great similarity of the results obtained by these regression techniques, we have presented

only the results obtained by the Cox regression.

In addition to internal comparisons of breast cancer risk within the cohort of women, using data from the SEER registry we compared the observed number of invasive breast cancer cases among white women in the cohort with the number of breast cancer cases expected if the women had risk comparable with white women in the San Francisco-Oakland Standard Metropolitan Statistical Area (SMSA) for the time period under study. Under the null hypothesis that the incidence of breast cancer is the same in women irrespective of cytologic diagnosis, we would expect each woman to have the same age-dependent risk of developing breast cancer at any age. We assumed this risk to be equal to the average annual age-specific incidence rate of breast cancer among white females in the San Francisco-Oakland SMSA for three time periods. We used rates for 1973–1977 for our years of follow-up 1973–1977 (21), rates for 1978–1981 for our years of follow-up 1978–1984 (21), and rates for 1985–1987 furnished by the Northern California Cancer Center for our years of follow-up 1985–1990. Each woman's probability of not developing breast cancer during the entire period from entry into the study through the end of 1990 was computed as the cumulative product of her probabilities of not developing breast cancer at each year. Her probability of not developing breast cancer in any year was taken as one minus the probability of developing breast cancer at her age during that year. Age was incremented 1 year for each year of follow-up, and each woman's expected risk of breast cancer was taken as one minus her cumulative risk of not developing breast cancer over the entire study period. The number of women expected to develop breast cancer was then the sum of each woman's risk over all women.

RESULTS

Description of the cohort and follow-up

Baseline nipple aspirate fluid findings, demographics, and risk factor data for the women in whom follow-up was completed and for those not traced are shown in table 1. Overall, follow-up was completed for 87 percent (2,343 of 2,701) of the women. The women with completed follow-up were more likely to be ages 35–70 years at entry, to have a first-degree relative with breast cancer, and to be parous. A total of 29,961 person-years of follow-up, an average of 12.7 years, was completed on these women. Among the 1,991 women from whom nipple aspirate fluid was obtained, cytologic diagnoses were based on the most severe finding

TABLE 1. Comparison of characteristics of women at entry into the study by follow-up status, San Francisco Bay Area, 1973–1991

Factor	% who completed follow-up* (n = 2,343)	% not traced (n = 358)
Cytologic diagnosis		
No breast fluid	15	16
Unsatisfactory	13	13
Normal	55	55
Hyperplasia	14	15
Atypical hyperplasia	2	2
Age (years)		
18–24	3	13
25–34	14	39
35–44	28	20
45–54	31	14
55–64	17	8
≥65	7	6
Year of entry		
1973–1974	24	34
1975 or later	76	66
Parity		
Nulliparous	26	36
Parous	74	52
Missing information†	0.2	11
First-degree relative with breast cancer		
No	86	88
Yes	13	9
Missing information†	0.1	3
Place of examination		
UCSF‡	30	64
BCDDP‡	65	23
Other	5	13

* Comparisons of women who were and those who were not successfully traced differed significantly ($p < 0.05$) for all factors presented except cytologic diagnosis.

† Proportions of women with missing information differ because we obtained pertinent data on follow-up questionnaires.

‡ UCSF, University of California, San Francisco; BCDDP, Breast Cancer Detection and Demonstration Project, Breast Screening Center of Northern California, Merritt Hospital, Oakland, California.

in the specimens obtained at a single visit for 1,792 (90 percent) women; at two visits for 159 (8 percent) women; and at three or more visits for 40 (2 percent) women. Nipple aspirate fluid satisfactory for cytologic diagnosis was obtained from 80 percent of women who were age 25–54 years, but from only 50 percent of women aged 55 or over.

As of April 1991, 104 of 2,343 (4.4 percent) of the women had developed breast cancer since they were originally enrolled in the study. Of the 104 cases, six (5.8 percent) were solely self-reported and two (1.9 percent) were identified only on death certificates; the remaining 92.3 percent of cases were confirmed either through the tumor registry or through pathology review by the project pathologist. A total of 10.6 percent (11 of 104) of identified breast cancers were carcinoma in situ.

Breast cancer incidence by cytologic diagnosis

All ages. A progressive increase in incidence and relative risk of breast cancer was found by severity of cytologic diagnosis (table 2). Women from whom fluid was obtained that was unsatisfactory for diagnosis or contained normal cytology were 1.4 and 1.8 times more likely to develop breast cancer than were women from whom nipple aspirate fluid could not be obtained (relative risk (RR) for the two categories combined = 1.7, 95 percent confidence interval (CI) 0.8–3.4). Women with epithelial hyperplasia were approximately 2.5 times more likely to develop breast cancer than were women who did not yield fluid (95 percent CI 1.1–5.5) and 1.4 times more likely to develop breast cancer than were women with normal cytologic findings (95 percent CI 0.8–2.4). Women with atypical hyperplasia were 4.9 times more likely to develop breast cancer than were nonyielders of fluid (95 percent CI 1.7–13.9) and 2.8 times more likely to develop breast cancer than were women with normal cytologic findings (95 percent CI 1.2–6.4).

As shown in figure 1, the increased cumulative breast cancer incidence among women with atypical hyperplasia compared with women who did not yield fluid or with women with hyperplasia or normal findings was apparent at all time intervals since entry into the study. The cumulative incidence of breast cancer in women with hyperplasia also was consistently higher than it was in women with normal cytology or in those who did not yield fluid.

Ages 25–54 years versus ages 55 and older. We examined the association of breast cancer risk and cytology in two separate age groups because over all ages there was a highly significant ($p < 0.01$) interaction between age and cytology on breast cancer risk. The increase in breast cancer incidence and relative risk with abnormal cytologic findings was more pronounced in women who were aged 25–54 years compared with women who were age 55 years or older at the time of specimen collection (table 3). Among the former, those with normal cytologic findings or with fluid unsatisfactory

TABLE 2. Breast cancer incidence and adjusted relative risks of breast cancer by cytologic diagnosis in nipple aspirates of breast fluid from white volunteer women in the San Francisco Bay Area, 1973–1991

Cytologic diagnosis	No. with breast cancer/ no. of women	% with breast cancer	Cox regression	
			Adjusted relative risk*	95% confidence interval
No breast fluid	9/352	2.6	1.0†	
Specimen unsatisfactory	15/315	4.8	1.4	0.6–3.3
Normal	56/1,291	4.3	1.8	0.9–3.6
Hyperplasia	18/327	5.5	2.5	1.1–5.5
Atypical hyperplasia	6/58	10.3	4.9	1.7–13.9
Total	104/2,343	4.4		

* Relative risks adjusted by Cox regression for age and year of specimen collection.
† Referent group.

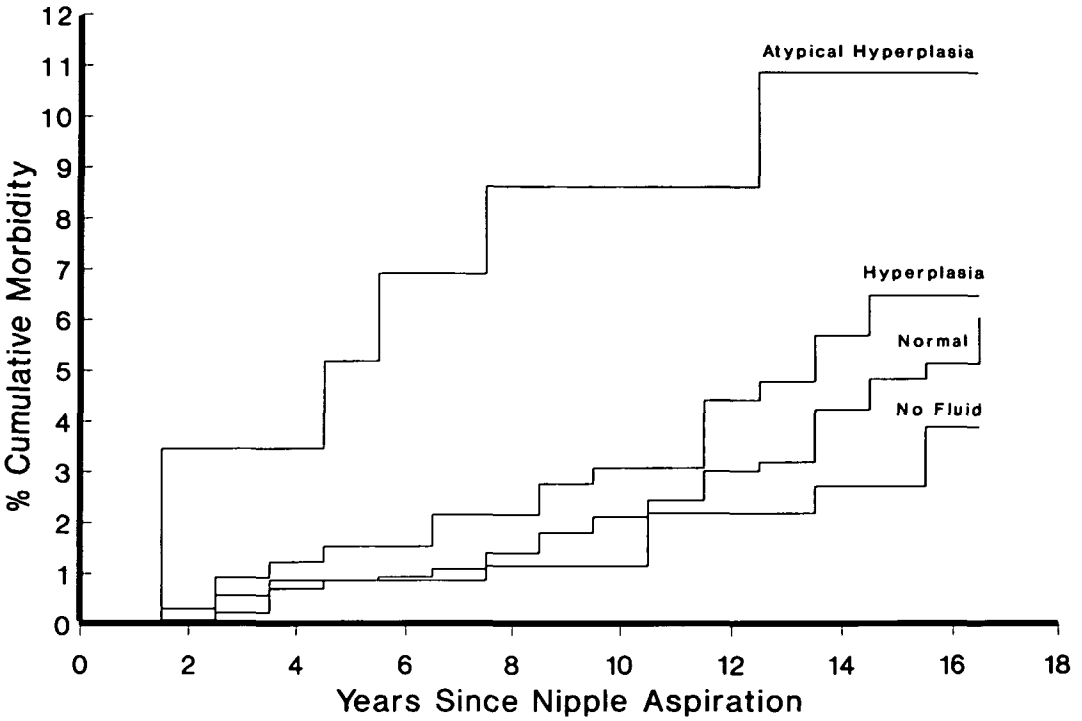

FIGURE 1. Cumulative incidence of breast cancer by cytologic diagnosis in relation to years since nipple aspiration in white volunteer women from the San Francisco Bay Area, 1973–1991.

for cytologic diagnosis were six to seven times more likely to develop breast cancer than were women from whom nipple aspirate fluid could not be obtained (RR for the two categories combined = 6.4; 95 percent CI 0.9–46.4). Women with epithelial hyperplasia were 9.5 times more likely to develop breast cancer than were nonyielders of fluid (95 percent CI 1.3–71.7), and 1.5 times more likely to develop breast cancer than were women with normal cytologic findings (95 percent CI 0.8–2.7). Women with atypical hyperplasia were 16.3 times more likely to develop breast cancer than were nonyielders of fluid (95 percent CI 1.9–139.3), and 2.6 times more likely to develop breast cancer than were women with normal cytologic findings (95 percent CI 1.0–6.5).

In contrast to the increased risks found in younger women, among women age 55 years and over at time of specimen collection, no significant or consistent differences were found in risk of breast cancer by the differing cytologic diagnoses (table 3).

Women with and those without a first-degree family history of breast cancer. Women of all ages with both a first-degree relative with breast cancer and a cytologic diagnosis of hyperplasia or atypical hyperplasia were at particularly increased risk of breast cancer (table 4). Among women with a first-degree family history of breast cancer, women with atypical hyperplasia had 7.2 times the risk of breast cancer as did women with normal cytology (95 percent CI 2.0–26.0). Among women with cytologic atypical hyperplasia, those with a first-degree family history of breast cancer were approximately six times more likely to develop breast cancer than those without such family history (95 percent CI 1.0–30.2). In contrast, a first-degree family history of breast cancer conferred no significant increase in risk of breast cancer among women who did not yield fluid upon nipple aspiration (RR = 1.3, 95 percent CI 0.3–6.3). Because of the small number of women with a family history of breast cancer, it was not feasible to

TABLE 3. Breast cancer incidence and adjusted relative risks of breast cancer by cytologic diagnosis in nipple aspirates of breast fluid from white volunteer women in the San Francisco Bay Area, for women aged 25–54 years and those aged 55 years and over, 1973–1991

Cytologic diagnosis	No. with breast cancer/ no. of women*	% with breast cancer	Cox regression	
			Adjusted relative risk†	95% confidence interval
Women aged 25–54 years				
No breast fluid	1/178	0.6	1.0‡	
Specimen unsatisfactory	8/168	4.8	6.7	0.8–53.4
Normal	41/1,031	4.0	6.4	0.9–46.3
Hyperplasia	16/281	5.7	9.5	1.3–71.7
Atypical hyperplasia	5/51	9.8	16.3	1.9–139.3
Total	71/1,709	4.2		
Women aged ≥55 years				
No breast fluid	8/144	5.6	1.0‡	
Specimen unsatisfactory	7/132	5.3	0.8	0.3–2.2
Normal	15/224	6.7	1.0	0.4–2.3
Hyperplasia	2/41	4.9	0.7	0.1–3.3
Atypical hyperplasia	1/6	16.7	2.6	0.3–20.9
Total	33/547	6.0		

* Women under age 25 years (n = 87) were not included in the table.
† Relative risks adjusted by Cox regressions for age and year of entry.
‡ Referent group.

TABLE 4. Breast cancer incidence and adjusted relative risks by family history of breast cancer and cytologic diagnosis in white volunteer women of all ages in the San Francisco Bay Area, 1973–1991

Risk category	No. with breast cancer/ no of women*	% with breast cancer	Cox regression	
			Adjusted relative risk†	95% confidence interval
No first-degree relative with breast cancer and				
No breast fluid	7/293	2.4	1.0‡	
Specimen unsatisfactory	11/270	4.1	1.4	0.5–3.5
Normal	46/1,132	4.1	1.7	0.8–3.9
Hyperplasia	14/283	5.0	2.3	0.9–5.8
Atypical hyperplasia	3/48	6.3	3.0	0.8–11.5
First-degree relative with breast cancer and				
No breast fluid	2/58	3.5	1.3	0.3–6.3
Specimen unsatisfactory	4/45	8.9	2.3	0.7–7.9
Normal	10/159	6.3	2.5	1.0–6.7
Hyperplasia	4/44	9.1	4.2	1.2–14.4
Atypical hyperplasia	3/10	30.0	18.1	4.6–70.2

* Family history data were missing for one woman, who was then excluded from this analysis
† Relative risks adjusted by Cox regression for age and year at specimen collection.
‡ Referent group.

further stratify the analyses according to ages younger than 55 years versus those age 55 or older.

The results presented here were controlled only for age and year of entry. Similar relative risks were found when the analyses also controlled for parity and place of examination.

Comparison of observed with expected number of breast cancer cases using SEER rates for the San Francisco-Oakland SMSA. As shown in table 5, the white women comprising our cohort were only 3 percent more likely to develop breast cancer than were all white women in the San Francisco-Oakland SMSA during the study period. When the results were broken down by cytologic diagnosis, we found that women with hyperplasia or atypical hyperplasia were more likely than all women to develop breast cancer and that those who did not yield fluid upon nipple aspiration were less likely to develop breast cancer. Of the 10 women with both atypical hyperplasia and a first-degree family history of breast cancer, we observed three cases of breast cancer compared with an expected 0.42 using the SEER rates, giving a ratio of 7.1.

DISCUSSION

In the pathogenesis of breast cancer, breast epithelial cells are believed to progress from their normal state through morphologically identifiable stages of mild hyperplasia, moderate hyperplasia, and atypical hyperplasia to carcinoma in situ and cancer (14, 22, 23). However, it is not established that all breast cancers arise from this sequence of proliferative lesions. It is possible that in susceptible women, inherited or acquired gene mutations or deletions that effect the regulation of differentiation might result in *direct* transformation of normal cells to cancer, without passing through the intermediate stages. Irrespective of these mechanistic considerations, it is now widely accepted that hyperplasia and atypical hyperplasia, when diagnosed in breast biopsies, are morphologic markers that are associated with a two- to fivefold increased risk of breast cancer (14, 22–26). Studies also have shown that increased risks associated with proliferative or atypical histopathologic findings may be modified by other risk factors for breast cancer, particularly a positive family history of breast cancer (15, 25, 26). However, the great majority of women who develop breast cancer have never undergone a breast biopsy, and the status of their breast epithelia prior to the diagnosis of cancer is unknown (27). Of further importance, less than 15 percent of women with breast symptoms or physical findings have ever undergone a breast biopsy (27, 28), and less than 5 percent of these biopsy specimens are diagnosed as atypical hyperplasia (15, 29). Moreover, less than 15 percent of women who develop breast cancer have a history of a previous breast biopsy for benign breast disease (27, 28, 30). It is therefore likely that current estimates of the prevalence of proliferative breast disease and the risk of breast cancer which are based on biopsy studies reflect only the "tip of the iceberg" of the number of women with proliferative epithelial changes within the breast. In addition, because biopsy can only be used when clinically indicated, it provides no information on the status of the epithelium during the earlier preclinical phases of proliferative breast disease. If new measures are to be developed for the primary prevention of breast cancer, it is important to detect and determine the causal factors of these proliferative precursor or marker lesions in women without clinical breast findings. As noted in our introduction and from our findings, the cytologic examination of nipple aspirate fluid offers a simple, noninvasive method for this purpose.

Our findings demonstrate that women in

TABLE 5. Observed number of invasive breast cancer cases in white women volunteers for nipple aspiration of breast fluid compared with number expected on the basis of Surveillance, Epidemiology, and End Results registry rates of breast cancer in white women in the San Francisco-Oakland Standard Metropolitan Statistical Area, 1973–1991*

Cytologic diagnosis	Observed	Expected	Observed/ expected
No fluid obtained	7	13.3	0.53
Specimen unsatisfactory	14	14.4	0.97
Normal	49	48.3	1.01
Hyperplasia	17	11.7	1.45
Atypical hyperplasia	6	2.1	2.86
All	93	89.9	1.03

* See text for detail of assumptions and computational methods.

whom hyperplasia or atypical hyperplasia was diagnosed in nipple aspirate fluids 10–18 years previously experienced a significantly increased risk of breast cancer compared with women who did not yield fluid. The women with atypical hyperplasia were also significantly more likely to develop breast cancer than were women with normal cytologic findings. The increased risks were mostly confined to women who were aged 25–54 years at entry into the study. Of additional significance was the finding that, compared with women who did not yield nipple aspirate fluid, women who yielded any fluid also had an elevated risk of breast cancer, irrespective of whether the fluid contained normal cytology or was unsatisfactory for diagnosis. This finding was also confined to and most strikingly evident in the age group 25–54 years. These findings suggest that physiologic factors affecting the secretion of breast fluid may be related to breast cancer risk. The lack of any consistent elevated risk associated with abnormal epithelial findings in women aged 55 years or older at entry into the study is likely to result from the normal, age-related process of ductal involution and the accompanying reduction in breast fluid secretion (1–4, 31). Cytologic findings are therefore likely to be more variable and less reliable in older women.

Our ratios of observed to expected cases in the San Francisco Bay Area SEER registry were 2.9 for women with atypical hyperplasia and 7.1 for women with both atypical hyperplasia and a first-degree family history of breast cancer. These ratios were somewhat lower in magnitude than, but similar in direction to, those reported for breast biopsy by Dupont and Page and colleagues (15, 16). These investigators found a relative risk of breast cancer of 5.1 with atypical hyperplasia in biopsy and of 8.9 in women with both atypical hyperplasia in breast biopsy and a family history of breast cancer compared with the overall risk to women in the Atlanta, Georgia, SEER registry. In both studies, women with atypical hyperplasia and a first-degree relative with breast cancer had an especially high risk of breast cancer, but the number of women in our study with both atypical hyperplasia and a family history of breast cancer who developed breast cancer was small, 3 of 10 (30 percent), compared with 10 of 39 (26 percent) by Dupont and Page (15).

Several cautions should be considered in evaluating our results. Because, to our knowledge, this is the first prospective study of breast cancer risk associated with nipple aspirate cytology, additional work and experience in this area will be needed to confirm and extend these findings. Second, our present results probably should not be generalized to the population at large because our study population was not randomly selected, but was largely composed of volunteers and a smaller proportion of women with breast complaints. However, the comparison of breast cancer risk for white women in our cohort with that of all white women in the San Francisco Bay Area indicates that our population did not differ much in breast cancer risk from other women in the area. Finally, because this report was confined to white women, the relative risks associated with epithelial abnormalities may differ or may be modified by different behavioral, life-style, or physiologic factors in other ethnic groups. Despite these caveats, the subjects in this study are more likely to be representative of the general population than are subjects in studies based on women who have undergone breast biopsy.

Because findings from nipple aspirate cytology are often compared with findings based on breast biopsy, it is useful to discuss some differences and potential biases that may influence the results from these methods of investigation. As discussed earlier, it is not known if all breast cancers arise from proliferative epithelial cell precursors. Of the 80 women in our study who developed breast cancer and who had had a nipple aspirate fluid sample satisfactory for cytologic diagnosis, 30 percent (24 of 80) were diagnosed as having epithelial hyperplasia or atypical hyperplasia. In the study of benign biopsy by Dupont and Page and colleagues (15, 16), of the 134 women who developed breast cancer, 77 percent (103 of 134) had

been diagnosed with proliferative epithelium, with or without atypia. This comparison suggests that the finding of proliferative epithelium in nipple aspirates may be less predictive of breast cancer risk than proliferative epithelium in breast biopsies. In the future, newer molecular markers may help us to differentiate proliferative lesions with and those without neoplastic potential.

However, before concluding that a nipple aspirate diagnosis of proliferative epithelium has a lower predictive value than that of breast biopsy, one should note that the follow-up time in our study averaged only 12.5 years, compared with 17 years in the study by Dupont and Page and colleagues. The cumulative incidence of breast cancer from the time of nipple aspiration (figure 1) indicates that up to 17 years after nipple aspiration, the curve for breast cancer incidence in women diagnosed with atypical hyperplasia is almost identical with that found for atypical hyperplasia in the biopsy study by Page et al. (16). Our cohort will need to be revaluated in 5 years to determine if the breast cancer risks will continue to increase in women who had previously received diagnoses of proliferative changes. Finally, the fact that the majority (56 of 80) of the breast cancers among women with satisfactory cytology developed in women with normal cytology might lead to the conclusion that most breast cancers do not arise from proliferative lesions. It should be emphasized that nipple aspiration cytologic diagnoses are based on a very small number of breast epithelial cells. It is likely that, similar to cervical cytology, nipple aspirate diagnoses made on single samples will be subject to a high rate of false negative diagnoses. This might be overcome by the use of repeated sampling.

The opportunistic nature of the clinical findings that lead to breast biopsy is suggested by autopsy studies demonstrating that epithelial proliferation and other benign conditions of the breast are so widely prevalent among Western women that they can almost be considered "normal variants" (32). Many opportunities for selection bias may occur before the decision is made to biopsy a suspicious breast mass. These include practice of breast self-examination; patient anxiety and delay in seeking physician consultation; availability, utilization, and interpretation of mammograms; and experience of the physician and surgeon in managing breast problems. A recent study indicated considerable interobserver variability between experienced breast pathologists in the diagnosis of this high-risk lesion (33). These considerations suggest that current estimates of risk based on the finding of atypical hyperplasia in breast biopsies are obtained from highly selected subsets of women and are likely, to a variable and indeterminate degree, to be "biased." However, the information derived from biopsy studies has had important clinical value for diagnosis, counseling, and prognosis, as well as for many important epidemiologic studies of benign breast disease and breast cancer. For the foreseeable future, histopathologic criteria will remain the "gold standard" for the diagnosis of precursor lesions of breast cancer, although these histologic markers are rapidly being supplemented by new immunohistochemical and molecular markers.

As in biopsy studies, several biases may influence nipple aspirate cytology findings. Because the vast majority of women we studied were volunteers without clinically diagnosed breast disease, "volunteer bias" may have been present. Factors such as publicity, health education, family history of breast cancer, and concerns arising from a prior history of benign breast disease might have influenced a woman's decision to volunteer for nipple aspiration.

The fact that breast fluid cannot be obtained by nipple aspiration from all women in whom it is attempted has also raised concerns about its clinical value. Until this study, we were unable to assess the influence of this factor on breast cancer risk. As discussed earlier, among women under age 55 years at entry into the study, those who yielded any fluid had greater risks of breast cancer than those who did not. Thus, failure to obtain breast fluid may not be a major biasing factor in this age group, but instead may be a physiologic marker indicative of a

normal, low secretory activity. Little is known of the factors that determine the internal secretion of breast fluid in nonpregnant, nonlactating women. Our studies on the biochemistry of breast fluid indicate that many chemical substances of exogenous and endogenous origin, including mutagens and carcinogens, are secreted into the breast duct fluid (2). We have hypothesized that this secretory activity may increase the exposure of the breast epithelia to putative carcinogenic and other chemical substances which may influence the risk of breast cancer (2, 3). This process might increase the risk of initiation and promotion of epithelial cells to premalignant and malignant cells, possibly by activating or inhibiting genes that control cell differentiation and proliferation.

It should be emphasized that as of the end of follow-up in April 1991, only 10.3 percent (6 of 58) of the women with cytologic atypical hyperplasia had developed breast cancer, indicating that additional factors must be involved in susceptibility to the disease. Studies of migrant populations; families with genetic high risk; diet and environmental exposures; and the application of newer molecular probes and genetic markers may help to differentiate those women whose atypia is truly high risk from those whose atypia is not.

The results of this prospective study offer strong support for our hypothesis that cytologic atypical hyperplasia and hyperplasia in nipple aspirates of breast fluid are associated with an increased risk of breast cancer. The identification of these cytologic epithelial changes indicative of high risk for breast cancer may have clinical and epidemiologic applications. For example, these high-risk cytologic lesions may be useful as intermediate outcomes in intervention studies of diet or chemoprevention. At present, we do not advocate the use of this technique as a routine diagnostic procedure. In the future, as clinicians and researchers gain more experience with nipple aspirate cytology, it may become possible to augment clinical counseling of women about their breast cancer risks, incorporating risk estimates based on nipple aspirate cytology. Finally, nipple aspirate cytology permits the investigation of the earlier phases of the natural history of proliferative breast disease in women who have no indication for breast biopsy. Studies of the physiologic factors that regulate breast fluid secretion, biochemistry, and cytology offer many important new opportunities for research on the etiologic factors underlying proliferative breast disease and provide new leads for the prevention of breast cancer.

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