

Epithelial cells and abnormal cytology in nipple aspirate fluid and subsequent breast cancer risk: a historic prospective study

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Introduction

Background

Breast cancer is the second leading cause of cancer death in women in the United States [1]. Of the 200,000 women who will be diagnosed with breast cancer this year, approximately 40,000 will die of this disease. Determining who is at risk for breast cancer has proven to be an inexact science, despite advances in imaging technologies. At present, the only high-risk individuals identifiable with a biomarker are individuals with a strong familial history of breast cancer, and these comprise about 5% of all breast cancer cases [2]. For the remaining 95%, well-established hormone-related breast cancer risk factors account for only a slight increase in risk. This leaves a deficit of factors for breast cancer risk assessment.

Since 95% of breast tumors begin in the lining of the milk ducts, exploration of these ducts might be a means of identifying abnormal cells that could progress to cancer. Past studies have shown that women with abnormal cytology in nipple aspirate fluid (NAF) have an increased relative risk (RR) of breast cancer when compared to women with normal cytology in NAF and women from whom NAF was attempted but not obtained [3, 4]. In addition, a recent study found that women with epithelial cells present in NAF, regardless of cytological category, were more likely to develop breast cancer than women without NAF or NAF that did not contain epithelial cells [5]. This study analyzed NAF results from a group of women seen by Dr. Otto Sartorius in his Santa Barbara breast clinic between 1970-1991 (N=2,480). Our analysis is an aggregate of two subgroups: women with questionnaire data (n=712) and those with NAF visits beginning in 1988 (n=238), the year in which cancer case information was uniformly collected in

the state of California. The purpose of this paper is to present the results of a historic prospective study determining 1) whether or not the type of epithelial cells present in NAF (normal, hyperplasia or atypia) influenced subsequent breast cancer development and 2) whether or not there was an increased risk of breast cancer development if epithelial cells were present in NAF, regardless of cytological category.

Materials and Methods

Study Population

The study principal investigator (P.I.) had access to a cohort of 3,203 women seen by Dr. Otto Sartorius in his Santa Barbara breast clinic between 1970-1991. The women were self-referred or referred by physicians. Dr. Sartorius performed all NAF collection during this period of time and utilized one pathologist to determine cytological diagnoses for all specimens. NAF collection/classification information and covariate information (specifically age) used in this analysis was abstracted from the subjects medical records by an R.N. abstraction team in Santa Barbara, California.

Inclusion/Exclusion Criteria

All women seen by Dr. Sartorius between 1970-1991 were eligible for inclusion in the study (N=3,203). Women who were diagnosed with breast cancer at initial visit with Dr. Sartorius or within six months of initial visit with Dr. Sartorius were excluded from the study. Women were also excluded who did not have NAF collection attempted by Dr. Sartorius.

The final model (N=946) is composed of women who completed questionnaire data and/or women who were seen by Dr. Sartorius after 1/1/1988 (Table 1). This is due to the fact that the California Cancer Registry (CCR) did not begin uniformly collecting

data on breast cancer cases in California until 1988. A comparison of the group of women not in the model versus the women in the final model is shown in Table 2.

Follow-Up Protocol

Beginning in October 2005, all women were mailed structured questionnaires and consent forms requesting information on personal and family history of breast cancer and reproductive factors associated with an increased breast cancer risk. After two mailings and no response we attempted to contact the women by telephone. Several methods were used to update the addresses of the non-responders, including requests to the California Department of Motor Vehicles (DMV), California Vital Statistics, and several internet search engines. A modified proxy questionnaire was sent to next-of-kin for subjects identified as deceased. Follow-up ended in June, 2006.

Nipple Aspiration Technique and Cytologic Classification

Nipple aspirate was obtained using the technique developed by Dr. Sartorius and has been described elsewhere [6, 7]. The clinical database used for this study contained a variety of cytologic classifications. These classifications were condensed and categorized as either 1) normal cells 2) hyperplasia or 3) atypical hyperplasia by our study pathologist (EK). For this analysis, the most severe epithelial changes observed in the fluid specimens were used for the date of age at study entry.

Data Analysis

This paper evaluates breast cancer risk in relation to NAF cytology results which were categorized as follows: no fluid obtained, insufficient specimen, normal/benign, hyperplasia, atypia, and unable to classify. For women with multiple NAF results, the most severe result was used.

Two logistic regression models were used in the analyses presented here. The first model used the “no fluid obtained” group of women as the referent and calculated the odds ratio for women with insufficient specimens, normal, or hyperplasia/atypia combined. The decision to use the nonyielders as the referent is consistent with past studies and the hypothesis that women without NAF secretions are at a lower risk of developing breast cancer than women who produce breast fluids [3]. The second model tested the hypothesis that the presence of epithelial cells may be a risk factor for breast cancer. In this case the no fluid and insufficient specimen groups were combined as the referent. All other women comprised the non-referent group (normal, hyperplasia, atypia, and unable to classify). Both models adjusted for age at the time of the NAF visit.

The PROC LOGISTIC procedure in SAS version 9.1 [8] was used to generate the odds ratios for these analyses.

Results

Description of the cohort and follow-up

Of the 946 women in the final model, 712 completed questionnaire data and 238 were seen by Dr. Otto Sartorius after 1/1/1988. The women with completed follow-up were between ages 16-87 at study entry, with a mean age of 42.6. The mean age at breast cancer diagnosis was 60.8 years with a range of 34-86. As of June 2006, 93 of 946 (10%) women had developed breast cancer since enrollment in the study (Table 2). Of the breast cancer cases reported, 75 were self-reported and the remaining 18 were confirmed via the CCR.

Breast cancer incidence by cytologic diagnosis

In our review of the type of epithelial cells present in NAF and subsequent breast cancer development, a progressive increase in breast cancer cases was seen with each progressive category of cytology (Table 3). Breast cancer incidence was 9% (63 of 714) in women from whom no fluid could be obtained, 11% (2 of 19) in women with insufficient specimens, 12 % (11 of 89) in women with normal epithelial cells, and 14% (17 of 124) in women with hyperplasia/atypia. Women with insufficient specimens were 1.4 times more likely to develop breast cancer than women from whom no fluid could be obtained (95% CI, 0.3-6.4). Women with normal epithelial cells in NAF were 1.7 times more likely to develop breast cancer than women who did not yield fluid (95% CI, 0.9-3.5). Women with proliferative epithelial cells in NAF (hyperplasia/atypia) were 2 times more likely to develop breast cancer than women from whom no fluid was obtained (95% CI, 1.2-3.6, p value = 0.02).

Breast cancer incidence by presence or absence of epithelial cells

An increased risk of breast cancer was also found in our comparison of solely the presence of epithelial cells in NAF, regardless of cytological category (Table 3). Women with any epithelial cells in NAF were 1.8 times more likely to develop breast cancer than women from whom fluid could not be obtained or women with fluid from NAF that did not contain epithelial cells (95% CI, 1.1-3.0, p value = 0.01).

Discussion

The progression of breast epithelium from normal cells to proliferative cells with an increasing degree of severity is an important concept in the study of breast cancer pathogenesis. Most women do not have NAF evaluated prior to the development of breast cancer; therefore studies such as ours are important in establishing a carcinogenic

continuum. Breast biopsies are performed once a woman is symptomatic, limiting their ability to provide predictive value in determining who is at risk for breast cancer. The use of NAF is a relatively inexpensive, non-invasive route for evaluating breast epithelium, the site of 90% of diagnosed breast cancer cases.

The findings reported in this study are consistent with the findings of other recent studies [3-5]. Wrensch et al. [4] found a 2-5 fold greater risk of breast cancer development in women with proliferative epithelial cells in NAF when compared to women with no NAF or normal epithelial cells in NAF. Buehring et al. [5] found women with any epithelial cells in NAF had a 2-fold greater risk of breast cancer development when compared to women from whom fluid could not be obtained or women with NAF fluid that did not contain epithelial cells. These findings confirm that women with epithelial cells in NAF, normal or abnormal, are at a greater risk of developing breast cancer. Past studies have shown that younger women are more likely to yield NAF fluid for evaluation [7, 9]. These women are less likely to benefit from current breast cancer screening modalities, making NAF an important adjunct to presently employed screening modalities.

There were limitations involved in the conducting of this study. First, due to the inability to accurately track breast cancer cases prior to 1988, we were unable to use information from the cohort without questionnaire data prior to 1988. In addition, the women in the study were visiting a breast clinic and the majority presented with breast symptoms for evaluation. Therefore, this cohort is likely to represent a high risk group and the results cannot be generalized to the population at large. Finally, the ethnic make-up of this cohort was predominantly white; therefore the relative risks found may not be

applicable to other ethnicities with varying physiologic factors influencing breast epithelium.

Conclusions

These results support our hypothesis that 1) women with abnormal epithelial cells in NAF have an increased risk of breast cancer when compared to women with no NAF or normal epithelial cells in NAF and 2) women with epithelial cells present in NAF have an increased risk of breast cancer when compared to women with no NAF fluid or NAF with epithelial cells present. As early detection of breast cancer is critical for a cure, being able to identify women at high risk for breast cancer would justify closer follow-up and the use of multiple methods to ensure early detection. Future studies are necessary to explain the association between our findings and increased breast cancer risk. It is necessary to test breast fluids for specific biomarkers, especially carcinogenic agents, in order to further the research on etiologic factors involved in breast carcinogenesis and true breast cancer prevention.

List of Abbreviations

CCR – California Cancer Registry

CI – Confidence interval

DMV – Department of Motor Vehicles

NAF – nipple aspirate fluid

OR – odds ratio

PI – principal investigator

RR – relative risk

Authors' Contributions

KB conceived of the study and set up the design and coordination of the research team. She also reviewed and interpreted all statistical analyses and drafted the original manuscript. MM performed all statistical analyses. TR and JS participated in the design of the study and also performed statistical analyses. MW participated in the study design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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Tables 1-3

Table 1. Final selection of eligible women in used regression model, Sartorius Cohort 1970-1991.

	N=3203	Total number in tracking database
		-159 No cytology results at all
		-200 Not NAF or malignant results
		-364 w/ breast cancer w/in 6 months of most severe NAF result
TOTAL # ELIGIBLE	2480	w/NAF result and no breast cancer w/in 6 months of most severe NAF result visit (note: this includes 9 women w/ breast cancer dates unknown)
		<i>Subset of women used in this analysis:</i>
		712 Women w/ qx data
		238 Women seen 1/1/1988 or after
		950
		-3 "Unable to classify" NAF result
		-1 Age missing (NAF date missing)
Total number in model	946	
Total number not in model	1534	

Table 2. Comparison between women in the model versus those not in the regression model, Sartorius Cohort 1970-1991.

Basic stats	Not in model N=1534		In model N=946	
Age at NAF visit:				
Mean	43.9*		42.6	
Median	42		41	
Std Err	0.39		0.42	
Min-max	16-89		16-87	
Age at breast cancer diagnosis:				
N	81	(9 missing age at dx)	93	
Mean	62.0		60.8	
Median	62		62	
Std Err	1.5		1.27	
Min-max	30-89		34-86	
Breast cancer	90	6%	93	10%
NAF Cytology				
No fluid	929	61%	714	75%
Insufficient specimen	45	3%	19	2%
Normal	232	15%	89	9%
Hyperplasia	234	15%	97	10%
Atypia	81	5%	27	3%
[Unable to classify]	13	1%		
*N=1525, 9 missing NAF age				

Table 3. Odds ratio of breast cancer risk by cytological categories and presence of epithelial cells in NAF, Sartorius Cohort 1970-1991.

Breast cancer by cytology	In model						
	N=946	# w/ breast cancer	n	%	OR	95% CI	p value
No fluid		63	714	9%	1.0	<i>(referent)</i>	
Insufficient specimen		2	19	11%	1.4	0.32-6.4	0.64
Normal		11	89	12%	1.7	0.87-3.5	0.12
Hyperplasia or atypia		17	124	14%	2.0	1.1-3.6	0.02
Any epithelial cells							
No fluid/insufficient specimen		65	733	9%	1.0	<i>(referent)</i>	
Unable to classify, normal, hyperplasia, atypia		28	213	13%	1.8	1.1-3.0	0.01