Prostate-specific Antigen Levels in Nipple Aspirate Fluid Correlate with Breast Cancer Risk[1]

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Abstract

Despite the fact that breast cancer is the most common non-cutaneous cancer and a leading cause of cancer deaths in women, accepted markers of breast cancer risk miss up to 40% of these tumors. Moreover, screening methods involving the analysis of tissue or cells are limited by the need for a surgical biopsy. Nipple aspiration is a quick, efficient, noninvasive method to obtain breast epithelial cells, the cells at risk for transformation to carcinoma. Prostate-specific antigen (PSA), a protein thought to be specific to the prostate but recently found in a subset of breast tumors, has been correlated with improved survival. The purpose of this study was to measure PSA in a group of women with increasing breast cancer risk (no risk or family history of breast cancer, precancerous mastopathy, and invasive cancer) and determine if PSA correlates with risk.

Nipple aspirate fluid was obtained from the intact breast and from surgical specimens using a modified breast pump. PSA was then measured in the fluid using a highly sensitive and specific immunofluorometric procedure. PSA was found at levels ranging from 0–13,423 ng/g of total protein, and there was a significant relationship between PSA level and breast cancer risk ($P = 0.001$). That is, all women with no risk factors and 90% of those with a family history had high PSA levels, whereas 68% of subjects with precancerous mastopathy or invasive cancer had low PSA levels. PSA was higher in premenopausal subjects ($P = 0.002$). After adjusting for the effect of menopausal status, there remained a significant association between PSA and breast cancer risk. These findings suggest that PSA in nipple aspirate fluid may be a useful marker of breast cancer risk.

Received 3/19/96; revised 9/4/96; accepted 9/5/96.

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[1] Supported in part by a grant from the Aaron Gold Cancer Prevention Research Fund.

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Introduction

Breast cancer is the most common cancer (other than skin cancer) among women in the United States. Forty-five thousand women in the U.S. die yearly from the disease. The only well-established procedures to screen patients for breast cancer are physical examination and mammography. Unfortunately, physical examination does not identify a significant number of early breast cancers, and mammograms miss 10–40% of early breast cancers (1). Additional screening tools are needed to identify women who are more likely to develop the disease.

Efforts to evaluate the breast directly either through evaluation of tissue, individual cells, or extracellular fluid have been hindered because the analysis of these specimens generally required a surgical biopsy. A noninvasive method for evaluating the breast would be beneficial. NAF[3] is a quick, repeatable, noninvasive method of obtaining both breast epithelial cells and extracellular fluid.

PSA was isolated from seminal plasma in 1971 and from prostatic tissue in 1980. Until recently, PSA was thought to be produced exclusively by the epithelial cells lining the acini and ducts of the prostate gland (2). This opinion has recently been challenged, because ultrasensitive immunological assays for PSA have demonstrated that PSA is frequently present in female breast tumors (3). The protein identified in the female breast as PSA has the same size (M_r 33,000) as free PSA found in male serum (2). Molecular characterization of breast tumor PSA mRNA using reverse transcription-polymerase chain reaction and nucleic acid sequencing has shown that the mRNA in the breast is identical in sequence to PSA mRNA in prostatic tissue (4). When PSA was measured in a group of breast carcinomas, patients whose tumors contained measurable levels had an increased disease-free and overall survival (5), suggesting that PSA was a marker of good prognosis for women with breast cancer.

Because of the attractiveness of nipple aspiration for evaluating the breast, we have enrolled women of various risk categories in an attempt to identify markers of breast cancer risk in NAF. With the recent finding that PSA may be a marker of good prognosis for women with breast cancer, we hypothesized that PSA levels would be higher in NAF from women with no breast cancer risk factors and would decrease as breast cancer risk increased. The results of our investigation, in which we compared PSA levels in NAF from women of all breast cancer risk categories, form the basis of this report.

Materials and Methods

Between April 1995 and January 1996, 54 women (aged 30–65 years for women with an intact breast and 30–72 years for women who underwent mastectomy) with either invasive car-

[3] The abbreviations used are: NAF, nipple aspirate fluid; PSA, prostate-specific antigen; PM, precancerous mastopathy.

Table 1 Clinical characteristics of subjects who underwent nipple aspiration obtained from a health history questionnaire

Risk	Mean age (yrs)	Previous pregnancy			Oral contraceptives[a]			Hormone therapy[b]			Menopausal status	
		Y(%)	N	N/A[c]	Y(%)	N	N/A	Y(%)	N	N/A	Pre-(%)	Post-
No risk/family history	43.6	10 (67)	5	1	12 (75)	4	0	2 (13)	14	0	13 (81)	3
Precancerous mastopathy	47.8	6 (67)	3	1	4 (50)	4	2	2 (22)	7	1	4 (40)	6
Invasive cancer	52	19 (90)	2	6	7 (54)	6	14	4 (31)	9	14	13 (48)	14

[a] If yes, indicates that the subject received birth control pills at some time in her life.
[b] If yes, indicates that the subject received estrogen ± progesterone as replacement therapy or to control menstrual irregularities at some time in her life.
[c] Y(%), number and percentage of individuals whose response was yes; N, number of individuals who responded no; N/A, number of individuals who did not respond to the question.

Table 2 Patient number, subjects with low *versus* high PSA values, and PSA values (ng/g total protein)[a] in nipple aspirate fluid *versus* breast cancer risk, menopausal status, and oral contraceptive use

	Patient no.	PSA		
		Low (%)	High	Median (range)
Breast cancer risk[b]				
No risk/family history	16	1 (6)	15	640.5 (67–13,423)
Precancerous mastopathy	10	8 (80)	2	83 (5–4,802)
Invasive cancer	27	17 (63)	10	56 (0–5,378)
Menopausal status[c]				
Premenopausal	30	9 (30)	21	469 (0–11,708)
Postmenopausal	23	17 (74)	6	65.4 (0–13,423)
Oral contraceptive use[d]				
Ever	23	6 (26)	17	599.1 (0.7–13,423)
Never	14	8 (57)	6	122.5 (0–11,708)

[a] High PSA defined as greater or equal to the median of 197 ng/g total protein.
[b] χ^2 analysis: $P < 0.001$.
[c] χ^2 analysis: $P = 0.002$. We classified a subject as postmenopausal if she had undergone surgical removal of both ovaries or clinical symptoms of menopause associated with the absence of menstrual flow for at least 3 months.
[d] χ^2 analysis: $P = 0.059$. Total subjects are <53 because some subjects did not provide this information.

Table 3 Results of final logistic regression model of the probability of having a low PSA value among 53 subjects

Variable	Parameter estimate	SE	*P*	Odds ratio (95% confidence interval)
Cancer *vs.* no cancer[a]	3.19	1.119	0.005	24.3 (2.7,217.7)
Menopause				
Post- *vs.* Pre-[b]	1.56	0.718	0.03	4.8 (1.2,19.4)

[a] Cancer = precancerous mastopathy or invasive cancer; no cancer = no risk or family history of breast cancer. "No cancer" is the referent group.
[b] Premenopausal is the referent group.

cinoma, PM (atypical hyperplasia or *in situ* carcinoma), a first degree relative with breast cancer, or no risk factors (defined as a woman with none of the previously listed risks) participated in a protocol approved by the Fox Chase Institutional Review Board. Clinical characteristics of the enrolled subjects, obtained from a health history questionnaire, are listed in Table 1. There are missing data on oral contraceptive use and hormone replacement therapy because some of the subjects did not provide this information in the questionnaire which they filled out.

If subjects were eligible for two risk categories (for example, if they had a family history of breast cancer and were diagnosed with PM), they were placed in the category with greater breast cancer risk (in this example, PM). All eligible candidates who consented to participate were enrolled in the study. Subjects were divided into pre- and postmenopausal categories. We classified a subject as postmenopausal if she had undergone surgical removal of both ovaries or if she had undergone clinical symptoms of menopause associated with the absence of menstrual flow for at least 3 months. Subjects who had been pregnant and/or lactated within the preceding 2 years were not eligible, due to the known influence of pregnancy and lactation on both the cellularity and the protein composition of NAF (6, 7). Subjects who had received prior irradiation to the breast for any reason or had received systemic chemotherapy were excluded because it is unknown if these treatments influence the contents of nipple aspirate secretions. Asians, as well as women under 30 or greater than 65 years, were excluded because the ability to obtain NAF from the intact breast in these groups is known to be low (8). We relaxed the age limit in women undergoing mastectomy because: (*a*) there is no data outlining the NAF yield from a breast which has just been removed; and (*b*) our preliminary attempts to obtain NAF in mastectomy specimens from women up to 72 years of age were successful. Aspiration visits spaced 2 weeks apart were performed by a trained physician or nurse clinician, and each subject underwent 1, 2, or 3 aspirations. To insure consistency, when NAF was obtained at more than one aspiration, fluid from the first aspiration was used for PSA analysis.

Aspiration Technique. After written informed consent was obtained from each subject, NAF was obtained using a modified breast pump. The device consists of a 10-ml syringe attached to the end of a no. 4 endotracheal tube over which is placed a respiratory humidification adapter. Each of these pieces is inexpensive and readily available in any hospital where mechanical respiratory support is provided.

For subjects without a diagnosis of invasive breast cancer, the patient was seated in a comfortable position, and the breast nipple was cleansed with alcohol. After the alcohol had evaporated, a warm moist cloth was placed on each breast. After 1–2 min, the cloths were removed, the patient compressed her breast with both hands, and the plunger of the syringe was withdrawn to the 7-ml level and held for 15 s or until the patient experiences discomfort.

For subjects with invasive cancer, the mastectomy specimen was aspirated immediately after removal from the chest wall. The aspiration of mastectomy specimens was performed in fashion similar to aspiration of the intact breast, with the exception that warm cloths were not used on the mastectomy specimens. In general, a similar amount of breast massage was required to obtain fluid from the surgical specimens as from the intact breast. In all cases, fluid in the form of droplets was collected in capillary tubes. The quantity of fluid varied from 1 to 200 μl. The negative pressure produced was well tolerated. The procedure was repeated once on the same breast. Aspiration was then performed on the opposite breast, if present. If no

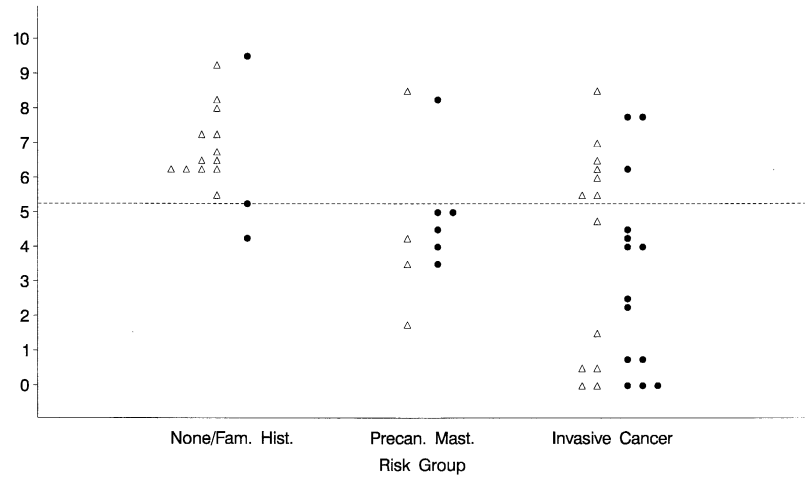

Fig. 1. Log (PSA + 1) in NAF *versus* risk (no risk/family history, PM, and invasive cancer) and menopausal status (△, premenopausal; ●, postmenopausal). ---, Log(1 + median).

fluid was obtained from either breast, or if the only breast which could be sampled (due to previous mastectomy) yielded no fluid, the patient was designated a nonsecretor.

Fluid for PSA analysis was obtained from the right breast for all subjects with no risk or with a family history of breast cancer, unless fluid was not obtained, in which case fluid from the left breast was analyzed. For subjects with PM (ductal carcinoma *in situ,* atypical hyperplasia, or lobular carcinoma *in situ*), a sample from the uninvolved breast was analyzed. For subjects with invasive cancer, the breast with invasive cancer was aspirated.

Occasionally, keratin plugs rather than NAF were obtained after suction was completed. The plugs were removed with an alcohol swab, and suctioning repeated. At times, suctioning must be performed two or three times to remove all of the plugs. Fluid frequently could then be obtained. To obtain additional fluid, the nipple can be gently compressed between two fingers. One or two additional droplets of fluid often appeared.

PSA. Glass capillaries containing the NAF were cut to obtain a small piece that contained the fluid specimen (1–5 μl volume). This piece of glass was placed in a 5-ml plastic tube containing 200 μl of a 0.1 M Na_2HCO_3 solution. The capillary tube was crushed with a stainless steel spatula to release the NAF. After vortexing, the resulting diluted NAF extract was analyzed for total protein with the bicinchoninic acid method (Pierce Chemical Co., Rockford, IL) and for PSA using a highly sensitive and specific immunofluorometric procedure (9).

Statistical Analysis. Due to the skewed distribution of PSA values measured in the NAF, PSA measurements were categorized as high (greater than or equal to the median) *versus* low (less than the median). The median value was 197 ng/g total protein. χ^2 and/or generalized Fisher's exact tests (10) were used to test the null hypothesis that the distribution of high/low PSA values in the NAF was independent of specific factors. These factors included risk group (no risk or family history, PM, or invasive cancer), menopausal status (premenopausal *versus* postmenopausal), oral contraceptive use (ever used *versus* never used), hormone replacement therapy (ever used *versus* never used), and pregnancy history (≥1 pregnancy *versus* no pregnancies).

A logistic regression model (10) was fit to the data to test the significance of the relationship between subject age and the probability of a high PSA value. Additionally, multivariable logistic regression methods using a backwards variable reduction procedure were used to examine the significance of the relationship between risk and PSA after accounting for the effect of potential confounders.

Results

We were able to obtain NAF specimens in 53 of 54 women (26 of 27 subjects with intact breasts and 27 of 27 surgical specimens). The relationship between PSA and a number of clinical variables was evaluated. The distribution of high *versus* low PSA values were not significantly related to hormone replacement therapy or prior pregnancy (data not shown). Significant relationships between PSA and clinical variables are indicated in Table 2. All but one of the low PSA values were found in subjects with biopsy-proven precancer or invasive cancer. The relationship between PSA and breast cancer risk was highly significant ($P < 0.001$).

The PSA level in nipple aspirate specimens in pre- and postmenopausal women was compared (Table 2). High PSA values were more often found in subjects who had not undergone either natural or surgical menopause ($P = 0.002$). There was also a relationship between age and PSA, with a greater probability of a low PSA with increasing age ($P = 0.02$).

The influence of oral contraceptive usage (past or present) on PSA was evaluated (Table 2). A borderline significant relationship ($P = 0.059$) between PSA levels and a history of oral contraceptive use was observed.

A series of multivariable logistic regression models were fit to the PSA data to evaluate the significance of the relationship between risk group and PSA after adjusting for confounders (Table 3). The initial model included as covariates all factors that were associated with PSA at the $\alpha = 0.1$ level. These factors were age, risk, menopausal status, and oral contraceptive use. A backwards elimination variable reduction technique was used.

The findings indicate that after adjusting for the effect of the other term in the model, both risk group ($P = 0.005$) and menopausal status ($P = 0.03$) are significantly related to the probability of having a low PSA measurement. Postmenopausal women were much more likely to have a low PSA value than premenopausal women (odds ratio, 4.8). Women with PM or invasive cancer were much more likely to have a low PSA measurement than women with no risk or a family history (odds ratio, 24.3). Furthermore, these analyses indicate that after

adjusting for menopausal status, there was no significant difference in the probability of having a low PSA value between women with invasive cancer and with PM. The distribution of log (PSA + 1) in NAF *versus* risk group and menopausal status is illustrated in Fig. 1.

Discussion

The breast ducts of adult nonpregnant women secrete small amounts of fluid (11). This fluid does not escape because the nipple ducts are occluded by smooth muscle contraction, dried secretions, and keratinized epithelium. Breast fluid can be obtained by nipple aspiration in a significant proportion of women without spontaneous nipple discharge with the use of a modified breast pump. This fluid contains several types of cells, including exfoliated breast epithelial cells (12). Because breast cancer develops from ductal and lobular epithelium, NAF is a potentially useful epidemiological and clinical research tool (6). A major limitation of the technique has been the lack of ability to obtain NAF in all women, and when fluid was obtained, it frequently contained few or no breast epithelial cells.

The highest yield of NAF is from women aged 30–55 years with early onset of menarche, non-Asian ethnicity, and prior parity and/or lactation. In premenopausal Caucasian and African-American women over the age of 30, this procedure has yielded NAF in over 50% (13). With a definition of ≥ 10 epithelial cells as an adequate aspiration, King et al. (14) obtained an adequate specimen in 42% of women whom they aspirated. The nipple aspiration technique has been used without significant side effects in over 7000 women (13).

Through a variety of modifications in the original technique, we are now able to obtain NAF in well over 90% of the time from the intact breast and from surgical specimens. We were able to obtain NAF 100% of the time from the intact breast of women who underwent the procedure at three visits and in all of our surgical specimens. The amount of pressure required to obtain fluid from the surgical specimen was similar to that applied to the intact breast. In an attempt to identify markers of breast cancer risk in NAF, we have evaluated a number of cellular and noncellular markers. Diamandis *et al.* (3) found PSA in 30% of breast tumors. In a separate study, they found that the presence of PSA in breast cancers was a marker of good prognosis (5).

The contralateral breast was aspirated in subjects with PM, whereas the ipsilateral breast was aspirated in subjects with invasive cancer. This is because many subjects with PM had already undergone mastectomy or irradiation to the involved breast. Thus, only the contralateral breast was available for aspiration. It has been established through numerous studies that the incidence of bilaterality in subjects with atypical lobular hyperplasia and lobular carcinoma *in situ* is up to 90%, whereas the incidence of bilaterality in subjects with atypical ductal hyperplasia and ductal carcinoma *in situ* is up to 30% (15, 16). We, therefore, felt that it was reasonable to aspirate the contralateral breast in subjects with PM. The ipsilateral breast was aspirated in subjects with invasive cancer because it was the breast with proven disease and was available to be aspirated.

The relationship which we identified between PSA and menopausal status suggests that PSA levels may be under the influence of one or more female hormones. This is consistent with previous reports, which indicate that PSA production is associated with the presence of the progesterone receptor (17), that tissue from the normal female breast of subjects receiving progestin-containing oral contraceptives has easily measurable amounts of PSA (18), and that after pregnancy the normal breast produces PSA and secretes it into the milk of lactating women (19).

The significant relationship between PSA and breast cancer risk points to the fact that PSA may have potential as a marker of breast cancer risk. PSA may prove useful as an adjunct to mammography and physical examination to screen for the development of primary or recurrent breast cancer. Of course, because of our limited sample size, the implications of our findings await confirmation in a larger patient population.

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