Circulating Testosterone and Prostate-Specific Antigen in Nipple Aspirate Fluid and Tissue Are Associated with Breast Cancer

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Preliminary evidence has associated testosterone and prostate-specific antigen (PSA) with breast cancer. Our objective was to determine whether *a*) testosterone levels in nipple aspirate fluid (NAF), serum, or breast tissue are associated with breast cancer; *b*) testosterone levels in serum are associated with levels in NAF; *c*) PSA in NAF, serum, or breast tissue is associated with breast cancer; and *d*) serum PSA is associated with NAF PSA levels. We obtained 342 NAF specimens from 171 women by means of a modified breast pump. Additionally, we collected 201 blood samples from 99 women and 51 tissue samples from 41 subjects who underwent surgical resection for suspected disease. Women currently using birth control pills or hormone replacement therapy were excluded from the study. Controlling for age and menopausal status, serum testosterone was significantly increased in women with breast cancer ($p = 0.002$). NAF and serum testosterone levels were not associated. Neither NAF nor tissue testosterone was associated with breast cancer. Controlling for menopausal status and age, NAF PSA was significantly decreased in women with breast cancer ($p < 0.001$). We did not find serum PSA to be associated with breast cancer, although we found an indication that, in postmenopausal women, its levels were lower in women with cancer. Serum PSA was associated with NAF PSA in postmenopausal women ($p < 0.001$). PSA levels in cancerous tissue were significantly lower than in benign breast specimens from subjects without cancer ($p = 0.011$), whereas levels of PSA in histologically benign specimens from subjects with cancer were intermediate. Our results suggest that serum testosterone is increased and NAF PSA is decreased in women with breast cancer, with PSA expression being higher in normal than in cancerous breast tissues. NAF and serum PSA levels in postmenopausal women are correlated, suggesting that as laboratory assessment of PSA becomes more sensitive, serum PSA may become useful in identifying women with breast cancer. *Key words*: breast cancer, nipple aspirate fluid, prostate-specific antigen, sex hormones, testosterone. *Environ Health Perspect* 110:241–246 (2002). [Online 5 February 2002]

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Breast cancer continues to be a significant health threat, with 180,000 new cases and over 41,000 deaths occurring in the United States in 2000. Both environmental and heritable factors are important in breast disease development (*1*). The current generally accepted surveillance techniques of mammogram and breast exam miss up to 40% of early breast cancers. New methods of early detection and prevention are sorely needed. Nipple aspiration, which noninvasively provides both breast epithelial cells (the cells that cause breast cancer) and proteins secreted from the breast epithelium, has the potential to be an important new surveillance tool.

The androgen testosterone is associated with breast cancer risk. Testosterone levels in serum have long been known to increase with worsening breast cancer risk and disease. This association has been consistently demonstrated in case–control and prospective studies in postmenopausal subjects (*2–8*), whereas the results in premenopausal women have been inconsistent (*5*). These studies of serum and plasma extended evidence of increases in urinary levels of testosterone in women with breast cancer (*9–11*). Testosterone is also found in nipple aspirate

fluid (NAF) (*12*) and may be a valuable marker of risk. The advantage of NAF over serum is that many proteins are highly concentrated compared to corresponding serum and are breast specific, whereas levels in serum may be contributed by many organs.

Testosterone is thought to exert a variety of effects on the breast. These include *a*) preferential binding to circulating binding proteins, thus increasing free estradiol available to breast cells; *b*) increasing total estrogen levels through aromatic conversion of testosterone; *c*) direct stimulation of breast cells through androgen receptors; and *d*) stimulation of other growth factors (*2*). We therefore hypothesized that NAF testosterone may prove to be a marker of breast cancer.

Prostate-specific antigen (PSA), also known as human kallikrein 3 (hK3), is a member of the kallikrein family of glycoproteins. It was originally thought to be produced only by the prostate (*13*). Functionally, PSA acts to liquefy seminal coagulum (*14*). PSA is the best screening marker available for prostate cancer (*13*). PSA is now known to exist in nonprostatic tissues, including the breast, lung, ovary, uterus, and pancreas (*15–17*). We have determined in a small

cohort that PSA levels in NAF are inversely associated with breast cancer (*18*), suggesting that PSA may be a useful marker to screen women at risk for the disease. The usefulness of PSA in the detection and surveillance of prostate cancer is in the fact that it is easily measurable in male serum. Unfortunately, quantifying serum PSA in women has been difficult because the level of PSA is often below the detection limit of available methods. New methods have recently been developed to detect PSA levels as low as 1 ng/L (*19*), levels often found in female serum. These more sensitive techniques hold the promise that serum PSA may become useful in assessing risk or in the diagnosis of breast cancer (*20*).

Prostatic PSA is hormonally controlled (*21*). Breast PSA is also under hormonal control (*22,23*). PSA production is inducible in cultured T47D breast cancer cells by the addition of steroid hormones, including androgens and progestins, to the culture media (*23–25*).

In a study by Yu et al. (*26*), carcinomas, benign breast disease, and normal breast tissues were extracted and evaluated for PSA expression. Benign and normal specimens were collected from women without evidence of cancer. Specimens with benign breast disease had significantly higher levels of PSA than did carcinoma specimens. What was not evaluated was PSA expression in histologically benign tissue adjacent to carcinoma. There is ample evidence to support the notion that field cancerization exists in the breast. According to this hypothesis, specific aberrations may be present in both cancer and normal-appearing adjacent tissue. The latter changes reflect damage due to long-term carcinogenic exposure, and they precede morphologically detectable neoplastic transformation. It has been demonstrated that chromosomal alterations (*27*), inhibition of apoptosis (*28*), *HER-2/neu* amplification (*29*), and *p53* mutations (*30*) occur in histologically benign tissue adjacent to breast carcinomas. Skip lesions have been identified in mammary Paget disease, which is best

explained by the field defect hypothesis (*31*). Our intention was both to validate the earlier findings in normal and malignant breast tissue and to compare PSA expression in these specimens with expression in histologically benign tissues adjacent to carcinomas, which were not evaluated in the earlier study. We felt that this was important to give some insight into whether alterations in PSA expression occur globally in the breast as a field phenomenon or, rather, occur specifically with phenotypic evidence of cellular degeneration to cancer.

Because earlier studies demonstrated that serum levels of testosterone were associated with breast cancer (*3,7,32*), as well as evidence that testosterone was measurable in NAF (*12*), we first sought to determine if testosterone levels in NAF, serum, or tissue were associated with breast cancer. We next sought to confirm our earlier findings (*12*) that NAF PSA was associated with breast cancer and to investigate the association between serum PSA and breast cancer. We also evaluated the correlation of NAF and serum levels of both testosterone and PSA. Finally, we analyzed testosterone and PSA in benign, cancer adjacent, and cancerous tissues to test the hypothesis that there is a field effect in breast cancer.

No single marker in clinical use is sufficiently sensitive and specific to detect breast cancer. Therefore, multiple markers will be required to optimize our ability to detect disease at its earliest stage. By expanding the number of known markers of breast cancer available in NAF, serum, and/or tissue, we can add to the current regimen of detection and surveillance methods and perhaps reduce the staggering incidence and annual death rate produced by this disease.

Materials and Methods

Subject and specimen accrual. Between 1995 and 2000, informed consent was secured from 233 women who enrolled in an Institutional Review Board-approved study. The women were between the ages of 24 and 80 years, with 84% whites and 49% premenopausal. Four percent of premenopausal subjects were taking oral contraceptives, and 15% of postmenopausal women were on hormone replacement therapy at the time of enrollment. We categorized the women into one of seven risk groups: 1) no risk factors, 2) history of a first degree relative with breast cancer, 3) history of cancer in the breast contralateral to that being studied, 4) biopsy-proven atypical hyperplasia, 5) biopsy-proven lobular carcinoma *in situ*, 6) biopsy-proven ductal carcinoma *in situ*, or 7) biopsy-proven invasive carcinoma. Subjects who fit into two risk groups were assigned to the category of greater risk. Because few women were in risk

categories 2–5, we collapsed categories 0–5 ("noncancer") and 6 and 7 ("cancer") for all analyses.

We attempted to obtain NAF and serum specimens from all subjects. Some subjects agreed to provide one or the other specimen, whereas some subjects provided both. We collected 342 NAF specimens from 171 women and 201 blood samples from 99 women. In addition, we obtained 51 tissue samples from 41 subjects who underwent surgical resection of suspected disease. We successfully collected serum from all of the subjects and NAF from 99% of the subjects who agreed to provide these specimens. Tissue was obtained when available from all subjects who underwent breast surgery after pathologic review in which tissue was classified as benign (including fibroadenomas, fibrocystic disease, and histologically normal tissue), cancer adjacent, or cancerous.

We recorded NAF testosterone and PSA measures as coming from either the right or the left breast. Some subjects had measures from one side only, whereas others had measures from both sides. For subjects who had measures from both sides (8 for NAF testosterone and 17 for NAF PSA), we randomly selected one side for the analyses (along with the corresponding side-specific cancer risk

assessment). If multiple testosterone or PSA measures were available from serum or for NAF from the selected side, we collapsed them into a single value (their median). For the tissue analyses, some women with cancer had measures available for both cancerous and cancer-adjacent tissue. In those cases, we only used the cancer-adjacent tissue data. We used only one measurement per woman in each analysis.

Nipple aspiration technique. We performed nipple aspiration using a modified breast pump after obtaining informed consent. The technique has previously been described (*18*). Briefly, we warmed the breasts with moist towels. The subject then massaged her breasts for approximately 2 min. We cleansed her nipples with a mild soap followed by alcohol. We then placed a suction device first over the right breast (if present) and then over the left breast. Suction was created using a 10 cc syringe, which we held for 10–15 sec or until the participant experienced discomfort. We collected the fluid, which appeared in the form of droplets, into glass capillary tubes.

Testosterone assays of NAF, serum, and tissue. We prepared NAF, serum, and tissue specimens in a fashion similar to that described for PSA (see below). For measuring

Table 1. NAF and serum testosterone and PSA among cancer and noncancer specimens.

Age (years)	Noncancer		Cancer	
	Subjects (*n*)	Median (range)	Subjects (*n*)	Median (range)
Testosterone in NAF (µg/g)				
Premenopausal				
< 42	3	7.0 (3.5–14.5)	6	16.5 (10–62)
≥ 42	8	13.0 (0–192)	7	11.5 (4–53)
Postmenopausal				
< 60	3	6.0 (1–25)	8	8.5 (3–12)
≥ 60	0	NA	16	11.0 (0–229)
Total	14	11.0 (0–192)	37	11.0 (0–229)
Testosterone in serum (ng/mL)*				
Premenopausal				
< 42	7	49.0 (37–59)	6	64.5 (47–108)
≥ 42	10	55.0 (36.5–68)	8	60.0 (40–78)
Postmenopausal				
< 60	6	38.0 (27–54)	11	58.0 (44–93)
≥ 60	1	62.0 (62–62)	16	59.5 (32–156)
Total	24	50.5 (27–68)	41	59.0 (32–156)
NAF PSA (ng/g) **				
Premenopausal				
< 42	16	982.0 (19–8,176)	23	37.0 (0–9,812)
≥ 42	21	2037.5 (1–4,569)	20	110.8 (0–5,684)
Postmenopausal				
< 60	16	163.0 (31–13,423)	16	69.0 (0–2,522)
≥ 60	1	340.0 (340–340)	38	31.5 (0–12,408)
Total	54	851.0 (1–13,423)	97	49.0 (0–12,408)
Serum PSA (ng/L)				
Premenopausal				
< 42	12	0.3 (0–11)	6	4.5 (0–21)
≥ 42	11	1.0 (0–24)	9	2.0 (0–13)
Postmenopausal				
< 60	9	8.0 (0–63)	11	0.0 (0–19)
≥ 60	2	1.0 (0–2)	17	0.0 (0–34)
Total	34	1.0 (0–63)	43	1.0 (0–34)

NA, not available.
*$p = 0.002$; **$p < 0.001$ (comparison of cancer vs. noncancer across all four menopause/age strata).

testosterone levels, we used a commercially available kit on an ACS180 Immunochemistry Analyzer (Bayer Diagnostics, Tarrytown, NY).

PSA studies of NAF, serum, and tissue. We determined NAF total protein content using a Pierce BCA Protein Assay Reagent Kit (Rockford, IL) and analyzed PSA as previously described (*19*). Briefly, this procedure combines a time-resolved immunofluorometric assay with two monoclonal antibodies and has a detection limit of 1 ng/L. For NAF, the capillary tubes containing the specimen were broken in half and placed in 400 μL of a 0.1 M $NaHCO_3$ solution, pH 7.80. We then crushed the capillary tube with a steel spatula to release the NAF. Breast tissue was snap frozen in liquid nitrogen, pulverized, and homogenized. The specimen was then centrifuged, and the cytosol extract was collected using lysis buffer as previously described (*26*). We then aliquoted the serum samples, leaving behind the cellular fraction. The coefficients of variation for both the PSA and testosterone assays were < 10% within the measurement range. We stored samples for analysis at –80°C and batched them for analysis. We performed all analyses under the supervision of a laboratory administrator with over 20 years of experience. Technicians were blinded to the risk group of each sample.

Statistical analyses. In assessing the association of NAF and serum testosterone and PSA with breast cancer, we had planned to control for potential confounders, including menopausal status, age, oral contraceptive use, and hormone replacement therapy.

However, in our data only a small number of women were currently on birth control pills (*n* = 4) or hormone replacement therapy (*n* = 12). This made it impossible to control for these factors via statistical adjustment. We therefore decided to exclude those few women on birth control pills or hormone replacement therapy from our analyses of NAF and serum testosterone and PSA.

We compared the cancer and noncancer groups with respect to NAF or serum testosterone and PSA, using the exact version of the stratified Wilcoxon two-sample test. Menopausal status and age formed the strata (with age dichotomized at the median, separately for the premenopausal and postmenopausal groups). The stratified Wilcoxon test is the nonparametric equivalent of an analysis of variance model, with main effects for menopause and age (but no interaction terms) included. It is a distribution-free test; that is, it does not require an assumption of normality for the testosterone and PSA measures. We measured the association between the levels of testosterone or PSA in NAF and serum with the nonparametric Spearman correlation coefficient. Finally, we compared testosterone and PSA levels across various types of tissues (benign vs. cancer-adjacent vs. cancerous) using the nonparametric Kruskal-Wallis test. Because of the small sample size for which tissue measures were available, these analyses did not control for any other factors. We used SAS 8.0 (SAS Institute, Inc., Cary, NC) and StatXact-4 (Cytel Software Corporation, Cambridge, MA) for the analyses.

Results

We first present results from the investigation of the association of NAF and serum testosterone and PSA with breast cancer status (cancer vs. noncancer). These analyses control for menopausal status and age. However, because of the small number of older postmenopausal women without cancer, we are not confident that our results can be extrapolated to that group. We next present the correlations between the NAF and serum measures, separately for premenopausal and postmenopausal women. The final set of results pertains to the comparison of cancerous, cancer-adjacent, and normal tissue with respect to testosterone and PSA levels. Those analyses do not control for any covariates.

Testosterone in NAF and serum. Testosterone levels in NAF ranged from 0 to 229 μg/g, with a median value of 11 μg/g (Table 1). Controlling for menopausal status and age, NAF testosterone was not associated with breast cancer (*p* = 0.597). Serum levels of testosterone were uniformly measurable, unlike PSA (see below). Values ranged from 21 to 156 ng/mL (Table 1). Controlling for menopausal status and age, we found a statistically significant difference in serum testosterone between women with and without cancer (*p* = 0.002), with serum testosterone levels higher in women with cancer across all strata (Figure 1). In post hoc testing, the difference in serum testosterone between women with and without cancer was not significant in premenopausal women (*p* = 0.059) but was significant in postmenopausal women (*p* = 0.015). The significant difference was mainly attributable to the postmenopausal group < 60 years of age. Only one noncancer subject was ≥ 60 years old, so we are uncertain if a difference exists in the latter group.

We found no statistically significant correlation between NAF and serum testosterone in either 15 premenopausal women or in 11 postmenopausal women who had both measures available. In both premenopausal and postmenopausal groups, however, we found an indication of an inverse association (Spearman correlations of –0.44, *p* = 0.105, in premenopausal women and –0.38, *p* = 0.252, in postmenopausal women).

PSA in NAF and serum. PSA in NAF ranged from 0 to 13,423 ng/g of total protein (Table 1). Controlling for menopausal status and age, we found a significant difference in NAF PSA levels between the cancer and noncancer groups (*p* < 0.001; Table 1). PSA levels in NAF were substantially lower in breasts with cancer than in breasts without cancer across all strata defined by menopausal status and age (Figure 2). In post hoc testing, the difference in NAF PSA between women with and without cancer
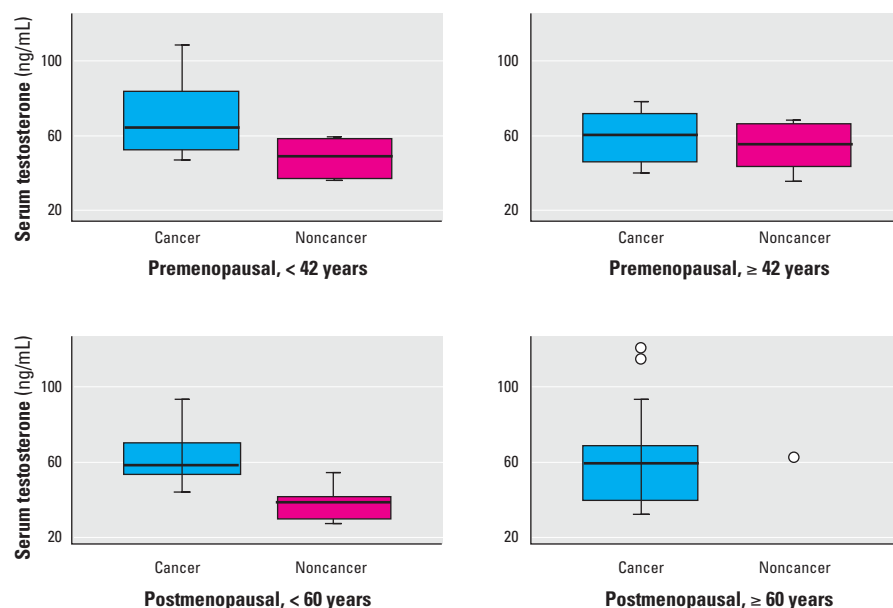

Figure 1. Box plots of serum testosterone levels for the cancer and noncancer groups by menopausal status and age. Boxes indicate 25th and 75th percentiles, solid lines inside boxes indicate median; error bars indicate minimum and maximum, and circles indicate outliers. One extreme observation (156 ng/mL) has been omitted from the postmenopausal ≥ 60 years group.

was significant in premenopausal women ($p < 0.001$) but not in postmenopausal women ($p = 0.059$). The latter results are inconclusive for older postmenopausal women because only one NAF PSA measure was available from a noncancerous breast.

Although NAF has the theoretical advantage over serum of being breast specific, we were not sure which physiologic fluid would prove to be the better source of PSA. We therefore wondered if serum PSA was routinely detectable in women, if it was associated with NAF PSA, and whether it was associated with breast cancer. Serum PSA levels ranged from 0 to 63 ng/L (with undetectable levels in 39% of the samples). Controlling for menopausal status and age, we did not find a significant difference in serum PSA levels between women with and without cancer ($p = 0.744$). Post hoc analysis suggests, however, that in premenopausal women serum PSA tends to be higher in women with cancer ($p = 0.156$), whereas in postmenopausal women serum PSA is higher in those without cancer ($p = 0.044$; see medians in Table 1). The apparent different direction of the association is suggestive of an interaction based upon a subject's menopausal status, but our sample size is too small for any reliable conclusions regarding this matter.

We evaluated the association between serum and NAF PSA levels in 25 premenopausal and 20 postmenopausal women who had both serum and NAF measures available. This association was high and significant in the postmenopausal group (Spearman correlation coefficient of 0.71, $p < 0.001$), but not in the premenopausal group (Spearman correlation coefficient of 0.18, $p = 0.385$).

Testosterone and PSA in tissue. Tissue levels of PSA ranged from 1 to 1,369 ng/g and were significantly different in the different tissue types ($p = 0.024$; Table 2). Although the PSA levels in cancer-adjacent tissue were not significantly different from the PSA levels in benign tissue ($p = 0.172$) or cancerous tissue ($p = 0.068$), PSA was higher in benign tissue from women without cancer than in cancerous specimens ($p = 0.011$). Tissue levels of testosterone ranged from 2 to 88 mg/g and were not significantly different in cancerous, cancer-adjacent, and benign breast tissue ($p = 0.787$; Table 2).

Discussion

Despite intense research and prevention efforts, breast cancer remains the most prevalent cancer in U.S. women. The best current surveillance regimen (mammogram, breast self-exam, and physical exam by a health care provider) still misses a significant percentage of early cancers. New detection methods to identify patients with the disease must be developed. Nipple aspiration shows promise as a valuable adjunct to existing procedures. In addition, advances in laboratory techniques may bring value to known markers previously thought worthless for the diagnosis and surveillance of breast cancer. More valuable than any one of these tests is the combination of results. Thus, new markers must always be sought.

Nipple aspiration is a quick and painless method of obtaining breast epithelial cells and the fluid they secrete. In our quest to develop new candidate markers in NAF, we evaluated testosterone, knowing its proven association in serum with postmenopausal breast cancer. We confirmed that, controlling for age and menopausal status, the levels of circulating testosterone were significantly higher in women with breast cancer compared to women without cancer. We observed this association in both premenopausal and postmenopausal women, although we did not have enough women ≥ 60 years of age without cancer to make accurate inferences for this group. We were not able to demonstrate an association between circulating testosterone and NAF testosterone levels. Likewise, we did not observe an association between NAF or tissue testosterone levels and breast cancer. However, all of these analyses may have limited power due to the small sample size.

A limitation of our study is that we measured only total testosterone. Whether the free or albumin-bound fraction is more relevant biologically is a matter of debate (*2,33*). We elected to analyze total testosterone in order to be able to compare our results with other reports because most have elected to analyze the total hormone. Of note, one of the studies that did find significantly higher levels of circulating testosterone in premenopausal women with breast cancer evaluated the free fraction (*32*).

In this study, we confirmed our previous findings that NAF PSA is inversely associated with breast cancer (*18*). We also determined that levels of PSA in NAF are associated with PSA levels in serum in postmenopausal women. Markers in NAF and serum have been compared in the past, showing increases in both serum and breast
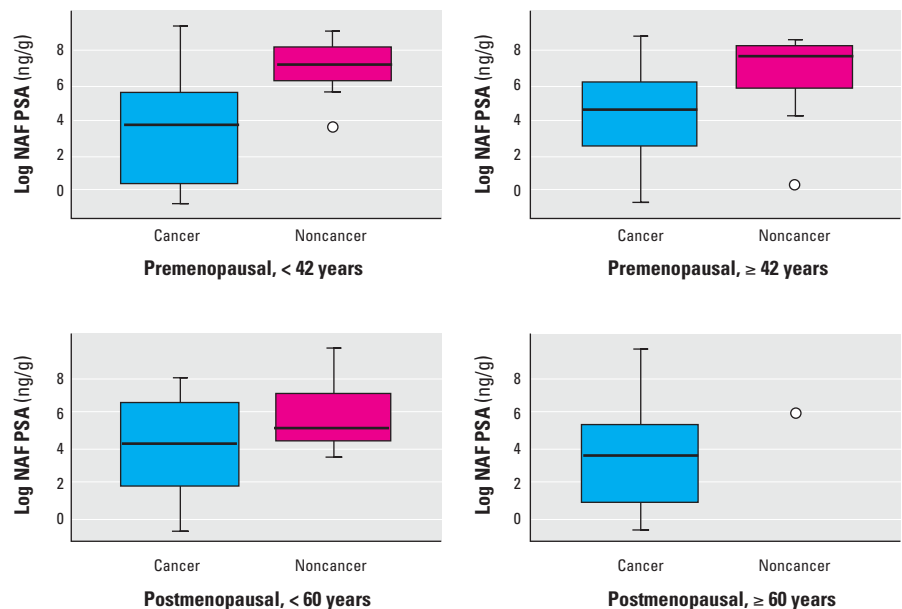

Figure 2. Box plots of log NAF PSA levels for the cancer and noncancer groups by menopausal status and age. Boxes indicate 25th and 75th percentiles, solid lines inside boxes indicate median; error bars indicate minimum and maximum, and circles indicate outliers.

Table 2. Testosterone and PSA in tissue with and without breast cancer.

Specimen type	Subjects	Median (range)
Testosterone (mg/g)		
Benign tissue	5	5 (3–13)
Cancer-adjacent tissue	10	6 (2–88)
Cancer	11	5 (2–54)
PSA (ng/g)*		
Benign tissue	7	88.0 (7–598)
Cancer-adjacent tissue	18	26.5 (1–3,733)
Cancer	16	5.0 (0–1,369)

*$p = 0.024$ (Kruskal-Wallis test).

fluid levels of lactogenic hormones in women with cystic breast disease (*34*). This comparison is of interest to this investigation in hopes that an association between NAF and serum levels of PSA would extend to an association between circulating PSA levels and breast cancer. Thus, serum PSA could possibly become as helpful to screen women for breast cancer as it has to screen men for prostate cancer.

We did not find a significant overall association between serum PSA and breast cancer, although this may be due to the fact that this association appears to be different in premenopausal and postmenopausal women. Among premenopausal women we observed a nonsignificant trend toward higher serum PSA in women with cancer, whereas among postmenopausal women serum PSA levels were significantly higher in those without cancer. PSA levels in the blood of normal men are lower than in men with prostate cancer, yet gland levels are higher in normal males (*35*). It appears that PSA expression in premenopausal women may mimic the pattern in males, with breast-specific (both NAF and tissue) PSA being higher and serum PSA being lower in normal women than in those with breast cancer. On the other hand, there is a direct relationship in postmenopausal women, with lower PSA levels in both NAF and serum being found in those with breast cancer. This difference is likely due to the influence of ovarian hormones and the relative levels of PSA found in the breast of premenopausal versus postmenopausal women, but larger studies are needed to clarify this.

PSA is highly concentrated in NAF, often at concentrations much higher than in serum (up to 10,000-fold) and is readily measured with currently available assays. The fact that NAF PSA also appears to be associated with breast cancer makes it a potentially valuable marker for this disease.

In a recent report, Zhao et al. (*36*) evaluated the association of PSA in NAF with breast cancer. The investigators were successful in collecting NAF from 34% of subjects, and had adequate NAF to perform analyses in 29.6% of subjects who consented to undergo aspiration. The authors observed similar levels of PSA in tumor-free breasts and in those recently diagnosed with breast cancer. Zhao et al. (*36*) speculated that the difference we observed (*18*) was due to hemodilution in the NAF collected from mastectomy specimens resulting in lower PSA levels. We are not sure why the results of the two studies differ, but we do not believe it is due to hemodilution. We routinely evaluate our NAF specimens for the presence of red blood cells. Thirty-two of the 110 NAF specimens in this study obtained

from subjects with breast cancer contained red blood cells. We found no statistically significant difference in NAF PSA levels in the specimens with versus without blood from subjects with breast cancer, whether we considered the overall sample or premenopausal or postmenopausal women separately. When we eliminated specimens containing blood and compared PSA levels in women with and without breast cancer, controlling for menopausal status and age, we found that PSA was significantly lower in NAF from the breasts with cancer than from the breasts without cancer ($p < 0.001$). We therefore speculate that the difference in the findings may lie in the population studied, because we were able to collect NAF from 99% of the subjects who enrolled in this study, whereas NAF was obtained from 34% of those enrolled in the other study (*34*). Because NAF was obtained from only a subset of subjects in the other study, the results may not reflect the entire enrolled population. Further studies will help to determine the usefulness of NAF PSA to predict which women have or will develop breast cancer.

The concept of field cancerization is well demonstrated in the aerodigestive tract, where cigarette smoking puts all areas exposed to the carcinogens in tobacco smoke at risk. Although the environmental carcinogens responsible for inducing and potentiating the development of breast cancer are not well defined, there is increasing evidence that, with perhaps the exception of cancers that develop in women who have mutations in high penetrance susceptibility genes such as *BRCA1* and *BRCA2*, environmental carcinogens are important to the development of cancers of the breast (*37,38*). We evaluated cancerous breast tissue, histologically benign tissue adjacent to cancer, and benign breast tissue from subjects without cancer. We observed that PSA was significantly higher in the benign tissue from women without breast cancer than it was in cancerous tissue. The difference in the median level of PSA (88 in benign vs. 5 ng/g in cancer) was quite striking. Although the PSA expression in the histologically benign but cancer-adjacent tissue was not statistically greater than in malignant tissues, the median values (26 in cancer adjacent vs. 5 ng/g in cancer) suggest that the difference might be significant if the analysis were not limited by sample size. These preliminary findings suggest that PSA alterations may occur as a field effect, in response to a carcinogenic insult.

As more candidate markers are studied, nipple aspiration shows increasing potential as a helpful adjunct to existing breast cancer screening. Just as interesting is the development of new markers in serum to enhance the predictive value of the armament of tests

currently performed. Serum levels of testosterone, especially in postmenopausal women < 60 years of age, may prove to be an important marker of breast cancer. To date, PSA has not yet proven itself in this medium, but may become useful as detection techniques improve.

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