Prostate-specific Antigen Production in the Female Breast: Association with Progesterone[1]

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Abstract

Prostate-specific antigen (PSA) is produced by the female
breast. Prior *in vitro* evidence suggests that PSA
expression in breast epithelial cells is regulated by
androgens and progestins but not estrogens. The purpose
of this study was to determine whether (*a*) PSA
expression in breast nipple aspirate fluid (NAF) and in
serum is influenced by progesterone (PG); (*b*) the ability
to obtain NAF decreases with repeated breast aspirations;
and (*c*) PSA in NAF correlates with abnormal NAF
cytology. Eight pre- and three postmenopausal women
with no breast cancer risk factors were enrolled in a pilot
study and had NAF and serum collected every 3–4 days
for a month to evaluate the influence of serum PG,
luteinizing hormone, estradiol, and follicle-stimulating
hormone on PSA in serum and in NAF. NAF was
obtained in 99% (112 of 113) of aspiration visits. Median,
mean, and peak NAF but not serum PSA levels were
higher in pre- than in postmenopausal subjects. NAF
PSA levels were associated with the rise or peak in serum
PG in seven of eight premenopausal women (seven of
seven with a PG surge) and in zero of three
postmenopausal women. Considering all 11 women, there
was an association between NAF PSA and PG ($P =$
0.005) but not luteinizing hormone, estradiol, or follicle-
stimulating hormone. NAF volume did not significantly
change over time. Atypical hyperplasia (9%) and
hyperplasia without atypia (36%) were identified in the
NAF of a subset of the subjects. Median, mean, and peak
levels of NAF PSA ($P =$ 0.05, 0.05, and 0.10, respectively)
were higher in subjects with normal *versus* hyperplastic
cytology. PSA production in the breast increases in
association with PG. With aspiration every 3–4 days,
NAF volume does not significantly decrease over time.
NAF cytology and PSA levels in NAF may help identify
women at increased breast cancer risk. Changes in
biomarkers of breast cancer risk in NAF (including PSA
and cytology) may predate mammographic abnormalities.
NAF may, therefore, be useful as a breast cancer
screening tool for young women who are not
recommended to undergo mammography and as an
adjunct to screen women who have mammograms
performed.

Introduction

Serum PSA[3] has become a useful marker of disease in the
prostate gland, with high levels being suggestive of cancer. This
diagnostic tool is especially predictive of disease if previously
normal levels are abnormal at the time of measurement. *In vitro*
studies to elucidate the mechanism by which PSA is involved
in the development and or progression of prostate cancer iden-
tified the influence of androgens on PSA production (1). With
the recent identification of PSA production by the female breast
(2), as well as the correlation of PSA levels in both breast tissue
(3) and in fluid obtained from the nipple (4) with a woman's
risk of breast cancer, we hypothesized that PSA might be
influenced by one or more steroids in the pituitary-ovarian axis.

Fluid secreted by the ductal epithelium of the breast in
nonlactating women has been of interest to investigators for
many years, either for use as a diagnostic screening tool or to
evaluate response to therapy. The advantages of using this fluid
are obvious, including the presence of shed ductal epithelial
cells, the cells from which the vast majority of breast cancers
form, as well as proteins secreted from the ductal epithelium
into the fluid, which are concentrated and, therefore, far easier
to measure than serum levels. The limitations of the nipple
aspirate samples encountered by other investigators relate pri-
marily to the inability to obtain the NAF reliably and the lack
of cellularity in a percentage of the fluid samples (5). Our
preliminary work led to modifications in both the breast aspi-
ration device used and in the preparation of the breast prior to
aspiration, both of which dramatically increased our success.
We are now able to obtain NAF in virtually all subjects and
cellular samples in over 50% of subjects (6). Samples of low
cellularity are also informative because these subjects have
been shown to have the lowest risk of future breast cancer.
Nonetheless, questions remain regarding nipple aspiration, in-
cluding whether repeat aspirations spaced close together will
decrease the amount of fluid collected with each attempt.

In an attempt to evaluate the role of pituitary/ovarian
steroids on PSA production in the breast, to assess the influence
of repeat aspirations on the ability to obtain and the yield of

NAF, and to determine the incidence of cytological hyperplasia and atypia in a group of subjects with normal breast cancer risk, we recruited 11 women of normal breast cancer risk to undergo regular nipple aspirations and phlebotomy for a full menstrual cycle (premenopausal subjects) or for 30–35 days (postmenopausal subjects).

Materials and Methods
Subjects

Eleven female subjects (8 pre- and 3 postmenopausal), ages 30–65 years, were recruited between June and October 1996 to a pilot study approved by the Fox Chase Institutional Review Board. All subjects were categorized as being of normal breast cancer risk, defined as having no first-degree relatives with breast cancer, all prior mammograms read as normal by a radiologist (only subjects 50 years of age or older had undergone prior mammography), a normal breast exam by one of the authors (E. R. S.), and no prior breast biopsies. Each subject underwent breast aspiration and had 8 ml of blood withdrawn every 3–4 days. For premenopausal subjects, aspirations were begun the first day the individual noted menstrual flow, continued through the entire cycle, and ended with at least one aspiration after the next cycle of menstrual flow was noted. For postmenopausal subjects, aspirations were performed for 30–35 days. Nine of the subjects underwent 11–14 aspirations, whereas two subjects underwent only 5 aspirations, due to scheduling conflicts which prevented their continuing the study for the planned 10–14 visits.

Aspiration Technique

After informed consent was obtained, nipple fluid was aspirated using a modified breast pump (4). The breast nipple was cleansed with alcohol, and the plunger of the aspiration device was withdrawn to the 7-ml level and held for 15 s. Fluid in the form of droplets was collected in capillary tubes. The procedure was repeated twice.

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

Cytology

Specimen Preparation. The NAF was collected in 50-μl capillary tubes, rinsed into a container with 1 ml of Cytospin Collection Fluid containing 3% polyethylene glycol in ethanol-isopropanol (Shandon Lipshaw, Pittsburgh, PA), and transported to the cytology laboratory for processing.

The specimen was cytocentrifuged onto 10 glass slides. Three of the slides were used for cytological examination. If the slides contained <10 epithelial cells, two additional slides were examined. The remaining slides (five or seven) were stored for biomarker studies. The slides selected for cytological examination were washed twice in 95% ethanol for 5 min each, rehydrated in tap water, and stained by the Papanicolaou method.

Specimen Interpretation. The Papanicolaou-stained smears were examined by a cytopathologist (H. E.) experienced with breast cytology. Each specimen was designated as containing no or few epithelial cells (class I), normal epithelial cells (class

IIA), hyperplastic epithelial cells without atypia (class IIB), atypical epithelial cells (class III), or malignant cells (class IV), using criteria described previously (6).

Nipple Aspirate and Serum PSA

NAF was extracted from glass capillaries as described previously (4). The sample was analyzed for total protein with the bicinchoninic acid method (Pierce Chemical Co., Rockford, IL). PSA in both NAF and serum was then analyzed using a highly sensitive and specific immunofluorometric procedure (7). The PSA assay uses a mouse monoclonal anti-PSA capture antibody coated to polystyrene microtiter wells, a biotinylated monoclonal detection antibody, and ALP-labeled streptavidin. In the assay, 100 μl of sample were incubated with the coating antibody in the presence of 50 μl of assay buffer containing the detection antibody. After incubation for 1 h and six washes, the ALP-labeled streptavidin conjugate was added for 15 min, followed by six washes. The activity of ALP is then measured by addition of the substrate 5-fluorosalicylphosphate, incubation for 10 min, and addition of an EDTA-Tb^{3+} solution to form a ternary fluorescent complex between the released 5-fluorosalicylate, Tb^{3+}, and EDTA. The fluorescence is measured in the time-resolved fluorometric mode. The interassay variability in both serum and NAF PSA was generally <5%. Because NAF levels of PSA are often far more concentrated than serum levels, the NAF samples were prediluted 100-fold prior to PSA analysis. In previous studies evaluating PSA levels in NAF, we found that our results in a given sample were equivalent at serial dilutions between 20- and 200-fold.

Serum FSH, LH, E2, and PG

All serum samples were analyzed for all four hormones. FSH and LH were assayed with the Access Immunoassay Analyzer (Sanofi Diagnostics Pasteur, Montreal, Quebec, Canada). The lowest detectable level of FSH and LH that was distinguishable from zero with 95% confidence was 0.2 units/liter, and imprecision was <10% for each hormone across the assay range. E2 was measured by a solid-phase, chemiluminescence enzyme immunoassay system (Immulite, Diagnostic Products Corporation, Los Angeles, CA). Detection limit and coefficient of variation were 0.044 nmol/liter and <10%, respectively. PG was measured with the Ciba-Corning ACS PG chemiluminescence immunoassay (Ciba-Corning Canada, Markham, Ontario, Canada). The ACS PG assay has a minimum detectable concentration of 0.35 nmol/liter and coefficients of variation of <10% within the measuring range.

Statistical Analysis

Pearson's product moment (r) and Spearman's rank correlation (s) coefficients (8) were used to evaluate the correlation of PSA levels in NAF with serum levels of PSA, PG, E2, LH, and FSH. These correlation coefficients were also used to evaluate factors such as age and menopausal status that might influence the ability to obtain NAF.

Differences in NAF volume over time were evaluated by the Wilcoxon matched-pair signed-rank test. The Mann-Whitney U test was used to assess differences between groups (either menopausal status or normal versus abnormal cytology) in total volume or decline in total volume. Subjects were divided by menopausal status (pre- versus postmenopausal) and by cytological class: I and IIA versus IIB and III (no subject had class IV cytology). The Mann-Whitney U test was also used to determine whether the median, mean, or peak NAF PSA values

Table 1   PSA results in subjects of normal breast cancer risk

Age	Menses	PG surge	NAF PSA (ng/g)			Serum PSA (ng/liter)			PG range (nmol/liter)	E2 range (pmol/liter)	LH range (units/liter)	FSH (units/liter)
			Median	Mean	Range	Median	Mean	Range				
Premenopausal												
30	Irregular	No	714	575	121–1,027	0	0	0	1.1–3.4	73–266	0.5–27.7	11–112
31	Regular	Yes	625	680	320–1,544	9.9	34.8	3–128	1.4–36.4	73–608	1.1–17.5	2.7–9.7
36	Regular	Yes	1,863	11,350	1,319–26,237	3	3.5	2.3–5	2.2–97.35	613	13–100	11.3–23
37	Regular	Yes	387	484	123–1,216	2.9	2.7	1–4.4	0.53–17.9	73–1,512	2.9–10	1.9–8.7
37	Regular	Yes	188	264	79–741	0.5	0.4	0–1.4	1.8–56.2	111–642	2.5–8.7	6.2–13.7
44	Regular	Yes	2,769	5,469	608–13,465	0.5	0.4	0–1.4	1.72–53.9	73–515	8.2–75.6	5–19.5
44	Regular	Yes	5,767	8,726	3,227–22,097	4.8	5.1	1.5–15	0.44–48.9	94–1,296	0.9–31.4	2.6–11.8
45	Regular	Yes	36	251.7	0.4–2,093	0	0	0	1.8–39.48	100–776	4.1–55.7	7.2–29.3
Postmenopausal												
57	N/A[a]	No	112	174	12–524	0	0	0	1.7–2.1	73–93	31.1–37.5	72.5–84
57	N/A	No	70	56.6	35–352	0	0	0	0.8–1	73–93	39.6–43.7	53.5–101
67	N/A	No	66	66.4	7.6–172	14	13.8	1.9–27	1.4–2.5	73–93	31–37.4	32–87

[a] N/A, not available.

were different for pre- and postmenopausal women and whether there was a difference between cytology groups I/IIA and IIB/III.

Least squares regression was used to create a model that best explained the association between PG and NAF PSA. Associations with NAF PSA included change in PG level (PG surge), peak PG, PG value in the serum sample collected 3–4 days prior to the PSA-NAF sample, and the combination of the latter two associations.

Results

Information regarding parity and lactation was available from 10 of the 11 subjects. Three of seven (43%) premenopausal women and one of three (33%) postmenopausal women had one or more live births. One of the three premenopausal women who bore children and the postmenopausal woman who bore children nursed their children. Neither parity nor lactation appeared to influence PSA, PG, LH, FSH, or E2 levels.

The yield of NAF per attempt ranged from 1 to 60 μl and was not significantly influenced by age, menopausal status, or number of prior aspirations. Median aspiration volumes ranged from 10.5 to 28.5 μl (only one subject had a median volume of >17.5 μl) for premenopausal subjects and from 9 to 14 μl for postmenopausal subjects.

Median, mean, and peak NAF PSA levels were higher in seven of eight, eight of eight, and eight of eight premenopausal subjects ($P = 0.05$, 0.01, and 0.01, respectively) than in any of the 3 postmenopausal subjects (Table 1). On the other hand, although the median values of serum PSA were generally higher in the pre- than in the postmenopausal subjects, the highest median serum PSA (14 ng/liter) was in a postmenopausal woman, and levels of serum PSA were not significantly different between the groups.

Correlation of PSA in NAF with Serum PSA, PG, LH, FSH, and E2. In premenopausal subjects with regular menstrual cycles, nipple aspirate PSA peaked in conjunction with either the peak of serum LH (which coincided with a rise in PG) or with the PG peak (Table 2 and Fig. 1). There was no relationship between levels of serum LH or PG and NAF PSA in the premenopausal subject with an irregular menstrual cycle who lacked a PG peak, nor was there a relationship in any of the postmenopausal subjects (Fig. 2). There was a peak in serum PSA in four of seven premenopausal subjects with regular menstrual cycles (Table 2), but these peaks appeared to follow

Table 2   Day of peak values of PSA, PG, and LH[a]

Age	NAF PSA	Serum PSA	PG	LH
Premenopausal				
31	10	22	14	10
36	11	15	18	11
37	15	None	15	12
37	36	4	36	18
44	16	None	16	12
44	17	8	17	8
45	13	None	19	13
30	None	None	None	None
Postmenopausal				
57	None	None	None	None
57	None	None	None	None
67	None	None	None	None

[a] Indicates day of menstrual cycle for premenopausal subjects or, in postmenopausal subjects, days counted from first aspiration, with the first aspiration designated day 1.

the PG peak by 1–4 weeks. No association was identified between levels of either serum FSH or E2 and levels of NAF or serum PSA.

When all subjects were considered, there was a correlation of NAF PSA with PG ($r = 0.21$, $P = 0.025$; $s = 0.27$, $P = 0.005$). When subjects were separated according to menopausal status, in the premenopausal subjects, there was an association of NAF PSA with both PG ($r = 0.16$, $P = 0.1$; $s = 0.19$, $P = 0.09$) and LH ($r = 0.41$, $P = 0.0003$). Spearman's coefficient did not demonstrate a correlation between NAF PSA and LH. In postmenopausal subjects, no associations were found between NAF PSA and any of the four serum markers (PSA, PG, LH, and FSH). When the correlation of NAF PSA with the serum markers was evaluated for each subject separately, there was an association between NAF PSA (Table 3) and PG (r or s of >0.5) in five of eight premenopausal and in zero of three postmenopausal subjects. Among the three premenopausal subjects without a strong association, one lacked a PG surge, whereas the second ($s = 0.80$) and third ($r = 0.95$) had strong associations with LH. There was a strong association between NAF PSA and both LH and PG in two subjects. Thus, the levels of PG and/or LH were strongly associated with NAF PSA in seven of eight premenopausal subjects (all premenopausal women with a PG surge) and in zero of three postmenopausal subjects. In all cases, the LH peak was associated with a rising
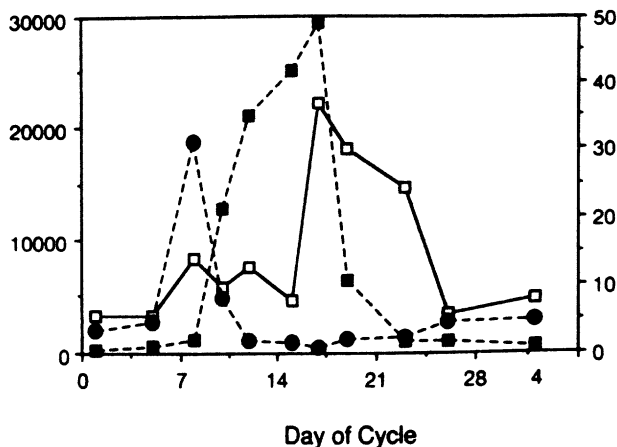
Fig. 1. Nipple aspirate levels of PSA (□, *PSA-NAF ng/g total protein*) compared to serum PG (■, *PROG nmol/L*) and LH (●, *LH 1 U/liter*) in a representative premenopausal subject with a PG surge.
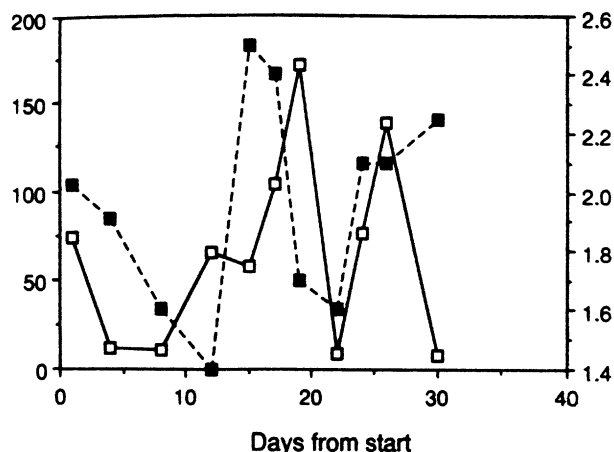

Fig. 2. Nipple aspirate levels of PSA (□, *PSA-NAF ng/g total protein*) compared to serum PG (■, *PROG nmol/L*) in a postmenopausal subject.

PG level. PSA levels in serum were inversely correlated with NAF PSA in one premenopausal subject, E2 correlated with NAF PSA in the individual without a PG surge, and FSH correlated with NAF PSA in one premenopausal subject.

Least squares regression was used to create a model that best explained the association between PG and NAF PSA. In five of eight pre- and zero of three postmenopausal subjects, an adequate model could be created whereby a significant association between PG and NAF PSA was found. For two subjects, the best associations ($P = 0.02$ and 0.04) were found comparing the NAF PSA to the PG from the previous visit (3–4 days earlier). For two different subjects, the best associations ($P = 0.0003$ and 0.0004) were found comparing PG and NAF PSA collected on the same day (peak PG corresponding to peak NAF PSA). In the fifth subject, the PG surge ($P = 0.01$) best explained the association between PG and NAF PSA.

Ability to Obtain NAF. Two factors were considered regarding NAF yield. The first was the ability to obtain a sample, regardless of volume. Of 113 aspiration visits made by 11 subjects, on only 1 occasion was fluid not obtained. Clearly, the ability to obtain a NAF sample, regardless of volume, did not decrease with time. The second was whether NAF volume decreased with time. There was not a significant decline in fluid volume over time in either pre- or postmenopausal subjects, whether considering all aspirations, the first *versus* the second aspiration in each subject, the first *versus* the the third aspiration, the first *versus* the last aspiration, the second *versus* the third aspiration, or the second *versus* the last aspiration. Age also did not significantly influence NAF volume.

Differences in PSA Based on Cytological Diagnosis. NAF cytology from three of eight (38%) pre- and two of three (67%) postmenopausal subjects with normal clinical risk for breast cancer contained hyperplasia without atypia (two postmenopausal subjects) or atypical hyperplasia (one premenopausal subject). Median, mean, and maximum PSA levels were higher ($P = 0.05, 0.05,$ and 0.10) in subjects with normal cytology.

Discussion

The breast ducts of adult nonpregnant women secrete small amounts of fluid (9). 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 (10). This fluid contains several types of cells, including exfoliated breast epithelial cells (11). Because breast cancer develops from ductal and lobular epithelium, NAF is a potentially useful epidemiological and clinical research tool. 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.

It is known that the production of PSA in the male prostate is regulated by androgens (1), and we have preliminary evidence from PSA levels in female serum that PSA in the female breast may be regulated by hormone(s) of the pituitary-ovarian-adrenal axis (12). Nonetheless, because serum PSA cannot be measured in a large proportion (50–80%) of female subjects and because serum levels of PSA in females may be influenced by organs other than the breast, such as the endometrium (13), we sought a reliable measure of PSA production in the breast. We had previously demonstrated that PSA is concentrated in breast fluid, that levels of PSA in nipple aspirate fluid were measurable in all of the women we studied, and that levels of PSA in NAF correlated inversely with the risk of breast cancer (4). We, therefore, sought to determine whether levels of PSA in the NAF of women of clinically normal breast cancer risk would correlate with one or more hormones produced by the pituitary-ovarian-adrenal axis. We also sought to confirm our previous success rate in obtaining NAF, to determine whether there is a change in either the yield or ability to obtain NAF over time, and to determine whether cytology, a cellular marker of breast cancer risk which we have identified in NAF, would be evaluable in this population.

Wrensch *et al.* (14) evaluated NAF cytology in a cohort of white female volunteers whom they aspirated and followed for 18 years. In this population, they demonstrated that subjects with NAF that contained normal cytology, hyperplasia without atypia, or atypical hyperplasia have a relative risk of breast cancer similar to subjects who have a biopsy with similar diagnoses. They also reported that subjects with low cellularity in the NAF and subjects in whom NAF was not obtained had the lowest risk of breast cancer. Thus, risk assessment is possible in all women who undergo nipple aspiration, even if they yield few breast epithelial cells or even if no NAF is obtained.

Age	Serum PSA		PG		E2		LH		FSH	
	r	s	r	s	r	s	r	s	r	s
Premenopausal										
30	N/A[b]	N/A	0.14	-0.02	0.75	0.78	0.20	0.08	0 12	0 08
31	-0.39	**-0.66**	0.38	0.52	-0.09	0.20	**0.70**	**0.63**	0 10	-0 13
36	-0.01	0.46	-0.58	0.10	N/A	N/A	0.44	0.80	0 45	0 10
37	0.46	0.40	**0.92**	**0.73**	-0.08	0.28	-0.60	-0.48	-0.19	-0.05
37	-0.10	-0.10	**0.90**	0.33	0.11	0.14	-0.23	-0.21	-0.44	-0.33
44	-0.25	-0.29	0.39	0.56	-0.24	-0.37	-0.15	-0.34	-0.38	-0.25
44	-0.24	0.10	0.86	**0.90**	0.49	0.41	0.40	0.60	-0.28	0.10
45	N/A	N/A	-0.09	0.25	-0.31	-0.20	**0.95**	-0.12	**0.89**	-0.01
Postmenopausal										
57	N/A	N/A	-0.01	0.09	N/A	N/A	-0.09	-0.03	0.11	0.05
57	N/A	N/A	0.33	0.25	N/A	N/A	-0.04	0.14	0.06	0.23
67	0.21	0.26	0.12	0.16	0.05	0.10	0.36	0.31	-0.32	-0.20

Table 3 Pearson (r) and Spearman (s) correlations with NAF PSA[a]

[a] Significant correlations (r or s) between NAF PSA one of the other variables (serum PSA, E2, LH, or FSH) are illustrated by boldface numbers.
[b] N/A, not available.

Noncellular markers in NAF can be used to complement cellular results and are are always evaluable, so long as the protein of interest can be detected. In our hands, 1 μl of NAF is more than sufficient to detect and quantify PSA.

One of the potential difficulties with repeat nipple aspiration is that there might be a decrease in the fluid volume over time. Petrakis et al. (15) found that aspirations performed monthly for 9–12 months did not lead to decreased volume over time, although the intervention of soy extract from months 4 to 9 appears to have influenced the results. Our findings demonstrate that nipple aspiration performed every 3–4 days for a month does not lead to a significant decrease in NAF volume over time. One would expect that, as aspirations are spaced at increasingly closer intervals, however, a point would eventually be reached whereby the NAF volume would decrease.

Although PSA is a valuable marker of disease in the male prostate, the biological function of PSA is still not clearly defined. It has been suggested that PSA, a serine protease, is a growth factor regulator that enzymatically digests IGFBP-3 to release insulin-like growth factor-I or enzymatically activating latent human transforming growth factor-β (16). Others data suggest that PSA is a regulator of IGFBP-2 and IGFBP-3 in patients with prostate cancer (17). Our results substantiate earlier findings (12) that PSA levels in female breast cancers are associated with PG but not estrogen receptors and in vitro studies showing that PSA production in breast cancer cell lines is mediated through the action of PG, androgen, mineralocorticoid, and glucocorticoid but not estrogen receptors (18). These latter data are consistent with the finding that all of the above receptors except the estrogen receptor bind to the same hormone response element on DNA (19).

PSA values in NAF peaked at a rise in or peak of serum PG. This is not unexpected, given the fact that PSA production has been shown to be mediated by the action of the PG receptor. Serum levels of PSA (Tables 1 and 2) were routinely measurable in half (four of eight) of the premenopausal subjects and in one of three postmenopausal subjects. All premenopausal subjects with measurable levels of serum PSA had an identifiable peak value, although in the postmenopausal subject, a peak value was not identified. The peak in serum PSA followed the peak in serum PG by 1–4 weeks. This is consistent with our previous report (12). This delayed rise in serum PSA may account for our not finding a relationship between PG and

serum PSA, for, unlike the earlier report, we do not have data over a sufficient time span to detect such an association.

Unlike Petrakis et al. (15), who identified hyperplasia in only 1 of 24 (4%) women of normal breast cancer risk aspirated monthly for 3 months, we found cytological changes in 5 of 11 (45%; hyperplasia in 4 and atypical hyperplasia in 1) women who underwent aspiration 5–14 times. We also found an association between normal cytology and higher PSA values, consistent with our earlier study (4), which demonstrated that low PSA levels in NAF were associated with increased breast cancer risk. When they performed monthly aspirations 6–9 more times while the subjects were on or after they had stopped taking a soy extract, 7 of 24 (29%) were found on one or more occasions to have hyperplasia in the NAF. Although they proposed that the apparent increase in the incidence of hyperplasia was due to the estrogenic influence of the soy extract, our data would suggest that their results may also reflect the fact that more samples from a given subject provide a better reflection of the morphology of the entire breast.

The report by Wrensch et al. (14) determined that hyperplasia and atypical hyperplasia found in the NAF of normal volunteers indicates a relative risk of breast cancer of 2.5 and 4.9, compared to subjects in whom NAF is not obtained. We identified hyperplasia or atypical hyperplasia in 45% of clinically normal risk subjects. Two of the three women were less than 50 years old and had not undergone prior mammography. The subject with atypical hyperplasia is 30 years old and may not have been recommended to undergo mammography for another 20 years, barring the palpation of a suspicious breast mass. In the cytological evaluation of 177 NAF samples from subjects of various breast cancer risk categories (no increased risk to recently diagnosed breast cancer in the aspirated breast), we found that atypical hyperplasia but not hyperplasia without atypia was significantly associated with increased breast cancer risk (6). Combining the findings of Wrensch with our report, it appears that atypical hyperplasia in NAF is clearly associated with increased breast cancer risk, whereas the implications of hyperplasia without atypia are less certain. The subject with atypical hyperplasia is now in a clinic for women at high risk for breast cancer and will have increased breast cancer surveillance.

On the basis of our results, we conclude that nipple aspiration can be repeated at least as often as every 3 days without a significant decrease in the fluid yield; that levels of PSA in the

breast (as reflected in the fluid secreted by the breast) increase in association with increases in serum PG; that cytological changes, including those known to increase breast cancer risk, can be identified in the NAF of women with clinically normal breast cancer risk; and that PSA levels in the NAF of normal risk women are generally lower if cellular hyperplasia or atypia is present. Future studies with a larger sample size should be performed to confirm these findings.

Mammograms miss 10-40% of early breast cancers (20). Markers of risk identified in NAF may predate abnormalities identified by mammography or breast self-examination. This suggests that nipple aspiration may be useful as a tool to screen for breast cancer in young women for whom mammography may not be recommended and as an adjunct to screen women who are undergoing routine mammography.

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