Nipple Fluid Carcinoembryonic Antigen and Prostate-Specific Antigen in Cancer-Bearing and Tumor-Free Breasts

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Purpose: **Mammograms and breast examinations are established methods for early breast cancer detection. Routine mammography screening reduces breast cancer mortality among women ages ≥ 50 years, but additional screening methods are needed. We and others have found high levels of carcinoembryonic antigen (CEA) and prostate-specific antigen (PSA) in nipple aspirate fluids (NAFs), but the usefulness for these biomarkers for early breast cancer detection is unknown.**

Patients and Methods: **NAFs from one or both breasts of 388 women were analyzed for CEA, PSA, and albumin levels. The study included 44 women with newly diagnosed invasive breast cancers, 67 women with proliferative breast lesions (ductal and lobular carcinoma in situ and atypical ductal hyperplasia), and 277 controls without these breast lesions. Analyses were conducted using the $\log_{10}$-transformed CEA and PSA levels to normalize the distributions of these tumor markers.**

Results: **Nipple fluid CEAs are significantly higher for cancerous breasts than tumor-free breasts (median 1,830 and 1,400 ng/mL, respectively; $P < .01$). However, at 90% specificity of the assay (CEA = 11,750 ng/mL), the corresponding sensitivity for cancer detection is 32%. CEA levels are not significantly different for breasts with proliferative lesions compared with tumor-free breasts. Nipple fluid PSAs do not differ by tumor status. Analyses of NAF albumin-standardized CEAs and PSAs yield similar results. Nipple fluid CEA and PSA titers are correlated in the affected and unaffected breast of women with unilateral lesions.**

Conclusion: **Nipple fluid CEAs are higher for breasts with untreated invasive cancers, but the test sensitivity is low. Nipple fluid PSA titers do not seem to be useful for breast cancer detection.**

J Clin Oncol 19:1462-1467.

BREAST CANCER is the most common neoplasm among women in the United States and other industrialized nations.[1-3] Mammography and physical examination are the standard methods for early breast cancer detection. Mammography screening reduces breast cancer mortality among women age greater than 50 years and may be beneficial to younger women.[4-7] However, additional methods are needed for early breast cancer detection. Breast nipple fluid aspiration is a relatively simple, noninvasive, and inexpensive method of obtaining biologic samples for tumor biomarker studies.[8-10]

Carcinoembryonic antigen (CEA) was identified in 1965 as the first human cancer-associated antigen.[11] Serum CEA levels are used clinically to assess and monitor tumor burden in patients with cancers of the breast, colon, or other sites.[12-14] For unknown reasons, CEA titers in nipple aspirate fluids (NAFs) from normal breasts are typically more than 100 times higher than corresponding serum CEAs.[10] Likewise, prostate-specific antigen (PSA) has been detected at high levels in nipple fluids from cancer-free breasts, despite barely detectable PSA levels in sera of most women.[15,16] To further evaluate the clinical usefulness of these biomarkers for breast cancer detection, we examined nipple fluid CEA and PSA levels from breasts with cancer or proliferative lesions that increase cancer risk (ductal and lobular carcinoma in situ and atypical ductal hyperplasia) compared with tumor-free breasts.

PATIENTS AND METHODS

Between 1993 and 1998, we enrolled study subjects in ambulatory clinics and mammography suites at five Boston hospitals (Dana-Farber Cancer Institute, Faulkner Hospital, Beth-Israel Hospital, Brigham and Women's Hospital, and Massachusetts General Hospital). Their physicians gave permission for our research nurse to explain the study and offer participation to eligible women. Signed consent for NAF collection was obtained from 1,314 women who also completed a brief questionnaire on breast cancer risk factors and history of neoplasia. Available medical records were abstracted for relevant mammographic and clinical data, including newly diagnosed breast neoplasms. Pregnant or lactating women and those with bleeding tendencies, scarred nipples, bloody breast discharges, or prior breast cancer were excluded from the study.

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Submitted May 12, 2000; accepted October 25, 2000.

Supported in part by a Department of Defense Army Grant, grant no. DAMD17-97-1-7108 and The Starr Foundation. F.P.L. is a Harry and Elsa Jiler American Cancer Society Clinical Research Professor.

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0732-183X/01/1905-1462

Table 1. Selected Characteristics of 388 Study Subjects, by Clinic Status

Characteristics of Participants	Subjects*							
	Preoperative Breast Cancer (n = 44)		DCIS, LCIS, and ADH (n = 67)		Tumor-Free Controls (n = 277)		Total (N = 388)	
	No.	%	No.	%	No.	%	No.	%
Age at NAF collection								
≤ 50	25	57	45	67	214	77	284	73
> 50	19	43	22	33	63	23	104	27
Menstrual status								
Premenopausal	19	43	33	51	159	60	211	56
Perimenopausal	11	25	9	14	35	13	55	15
Postmenopausal	14	32	23	35	72	27	109	29
Parity								
Nulliparous	9	21	16	25	65	24	90	23
Parous	34	79	49	75	210	76	293	77
Current tobacco use								
Smoker	7	17	10	16	49	18	66	18
Nonsmoker	35	83	54	85	217	82	306	82

*Missing data on subjects: menstrual status, 13 women; parity, five; and current tobacco use, 16.

Abbreviations: DCIS, ductal carcinoma-in-situ; LCIS, lobular carcinoma-in-situ; ADH, atypical ductal hyperplasia.

NAFs were successfully obtained from one or both breasts of 449 (34%) of the 1,314 women. Three hundred eighty-nine subjects produced sufficient NAFs for CEA, PSA, and albumin analyses; some cases were described in earlier preliminary reports.[10,15] Two hundred eighty-four subjects in this study (73%) were ≤ age 50, 211 (56%) were premenopausal, 293 (77%) were parous, and 66 (18%) were active smokers (Table 1). The series was sorted into three subgroups based on clinical status at the time of NAF collection: 44 women with newly diagnosed, untreated unilateral invasive breast cancer; 67 women with proliferative breast lesions associated with increased cancer risk (42 untreated and 15 recently treated for ductal and lobular carcinoma in situ and atypical ductal hyperplasia); and 277 women without history of these breast lesions. All diagnoses of neoplasia were based on pathology reports, but data were not collected for tamoxifen use by these affected women. The 67 subjects with proliferative breast lesions were not further divided by histologic subgroups because of small numbers and similar patterns of nipple fluid CEA and PSA levels, regardless of treatment status. One healthy woman who provided nipple fluid samples 1 year apart with CEAs > 480,000 ng/mL was excluded from analysis; these titers were more than five times higher than other CEAs in the series.

NAFs were collected using previously described methods.[10,15] In brief, NAFs were expressed from the nipple by manual compression of the breast or by use of a suction cup placed over the nipple. Available NAFs were collected into capillary tubes, and viscous samples were diluted up to 10-fold with 1× phosphate-buffered saline before centrifugation and storage of the supernatant at −70°C. Quantitative CEA assays were performed using the commercial immunoenzymometric assay kit, AIA-PACK CEA (Tosoh Medics, Foster City, CA) to detect the 180 kDa CEA glycoprotein. In addition, both free 33 kDa and bound 100 kDa forms of PSA were assayed using the AIA-PACK PSA (Tosoh Medics).[10,15] CEA and PSA results were also standardized to the corresponding nipple fluid albumin levels assayed in duplicate by colorimetric reaction with Bromcresol green (Sigma Diagnostics, St Louis, MO).

Medians and ranges of nipple fluid CEA, PSA, and albumin-standardized CEA and PSA levels were computed separately for breasts with cancer, proliferative lesions or no history of these lesions (controls). For statistical analyses, the CEA, PSA, CEA/albumin, and PSA/albumin levels were normalized by $\log_{10}$ transformations (Fig 1). Only results for the affected breast were analyzed for women with breast cancer or proliferative lesions. For bilateral NAFs obtained from tumor-free subjects, paired CEA, PSA, and corresponding albumin standardized results were averaged. Univariate logistic regression analysis was used to obtain the unadjusted odds ratios of the relationship between breast tumor status and $\log_{10}$-transformed biomarker titers. Multivariate logistic regression method was used to examine these relationships after accounting for potential confounders and

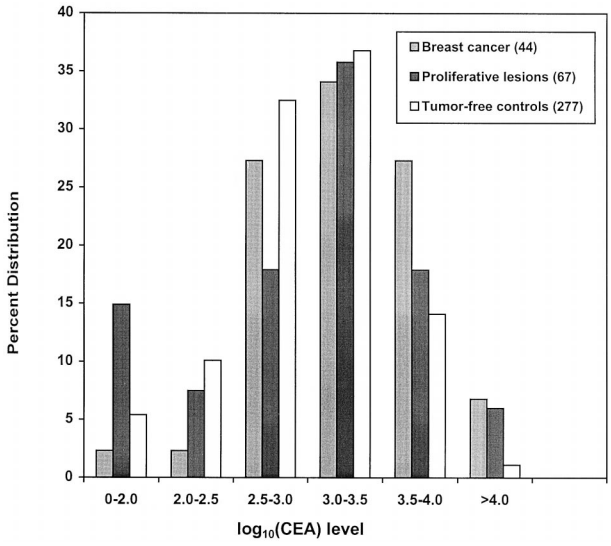

Fig 1. Percent distribution of breast nipple fluid $\log_{10}$ CEAs for breasts of 44 women with cancer, 67 with proliferative lesions, and 277 without history of these breast lesions (controls).

Table 2. CEAs and PSAs and Albumin-Standardized CEAs and PSAs in NAF Samples of 44 Women With Preoperative Unilateral Breast Cancer, 67 with Proliferative Lesions (DCIS, LCIS, or ADH), and 277 Tumor-Free Controls

Biomarker, by Clinical Status	Biomarker Titer		OR			
	Median	Range	Unadjusted	*P*	Adjusted*	*P*
CEA, ng/mL†						
Preoperative breast cancer	1,830	0-19,200	4.1	< .01	5.4	.01
Proliferative breast lesions	1,400	0-87,000	1.7	.06	1.7	.08
Tumor-free breasts, controls‡	1,060	0-33,800	1.0		1.0	
CEA per mg albumin						
Preoperative breast cancer	45	0-900	2.3	.02	2.4	.01
Proliferative breast lesions	28	0-4,360	1.2	.51	1.2	.50
Tumor-free breasts, controls‡	29	0-1,300	1.0		1.0	
PSA, ng/mL§						
Preoperative breast cancer	67	0-13,000	1.4	.20	1.7	.06
Proliferative breast lesions	22	0-33,100	0.8	.41	0.8	.34
Tumor-free breasts, controls‡	49	0-9,480	1.0		1.0	
PSA per mg albumin						
Preoperative breast cancer	1.4	0-350	1.1	.70	1.3	.44
Proliferative breast lesions	0.7	0-1,000	0.6	.16	0.6	.15
Tumor-free breasts, controls‡	1.3	0-580	1.0		1.0	

*Adjusted for age, smoking, and menstrual status.

†Undetectable CEAs in fluids from one cancerous breast, six with proliferative breast lesions, and eight tumor-free breasts.

‡No history of breast cancer or proliferative lesions in either breast.

§Undetectable PSAs in fluids from 11 cancerous breasts, 20 with proliferative breast lesions, and 57 tumor-free breasts.

Abbreviations: DCIS, ductal carcinoma-in-situ; LCIS, lobular carcinoma-in-situ.

interactions (age, smoking, and menstrual status).[10,17,18] The data were analyzed using the SAS program for microcomputer, and the *P* values were based on two-sided tests of significance.[19]

RESULTS

CEA levels in NAFs of the 388 subjects ranged from 0 to 87,000 ng/mL (Table 2). Corresponding albumin-standardized CEAs ranged up to 4,360 ng/mg albumin in NAFs. Median nipple fluid CEA titers were 1,830 ng/mL for the 44 cancerous breasts, 1,400 ng/mL for the 67 breasts with proliferative lesions, and 1,060 ng/mL for breasts of 277 tumor-free controls. CEAs and albumin-standardized CEAs were significantly higher for cancerous breasts than tumor-free breasts (unadjusted odds ratios [OR] = 4.1 and 2.3, respectively; $P = .0002$ and $P = .02$). Adjustment for potential confounding factors (age, smoking, and menstrual status) did not substantially alter the results. As a candidate biomarker for breast cancer, the sensitivity of nipple fluid CEA was 48% when specificity was 75% (CEA = 2,140 ng/mL). At 90% specificity (CEA = 11,750 ng/mL), the corresponding sensitivity was 32%. CEA for breasts with proliferative lesions were marginally higher than CEAs for tumor-free breasts (OR = 1.7; $P = .06$) but not after standardization for NAF albumins (OR = 1.2; $P = .5$).

Nipple fluid PSAs ranged from undetectable to 33,100 ng/mL, and the albumin-standardized PSAs ranged up to 1,000 ng/mL albumin (Table 2). Both measures of PSAs were slightly higher for cancerous breasts than tumor-free breasts, but the differences were not significant ($P = .2$ and $P = .7$, respectively). PSAs and albumin-standardized PSAs did not differ for breasts with proliferative lesions compared with tumor-free breasts.

Bilateral NAFs obtained from 205 women were analyzed for correlations in CEA and PSA titers within paired breast samples. Paired CEAs and PSAs were highly correlated among 142 subjects without breast tumors (Pearson coefficient r = 0.50 to 0.78 and $P < .05$ for both crude and albumin-standardized CEA and PSA titers). However, these biomarker levels were also highly correlated for paired NAFs of 31 subjects with unilateral untreated breast cancer and an opposite tumor-free breast ($r \geq 0.70$, $P < .01$ for both crude and albumin-standardized levels) (Table 3). In the 32 women with unilateral proliferative breast lesions, paired CEAs were moderately correlated (r = 0.37 to 0.52, $P < .05$), and the paired PSAs were less so (r = 0.20 and 0.07, $P > .05$ for crude and albumin-standardized PSAs, respectively). These results suggest that systemic host factors influence nipple fluid CEA and PSA levels.

DISCUSSION

In 1958, Papanicolaou[20] described the use of a suction device to collect breast nipple fluid for breast cancer diagnosis by cytologic examination. However, the finding

Table 3. Correlations of CEA and PSA Titers in NAFs From 63 Breasts With Cancer or Proliferative Lesions Compared With Contralateral Tumor-Free Breasts

Breast Pairs	Median Biomarker Titer (ng/mL)			
	Affected Breasts	Opposite Tumor-Free Breasts	r*	P
Cancerous breast *v* opposite tumor-free breast (n = 31)				
CEA	2,650	1,620	0.71	< .01
CEA/ng albumin	48	40	0.74	< .01
PSA	90	106	0.70	< .01
PSA/ng albumin	1.6	1.5	0.73	< .01
Breast with proliferative lesions *v* opposite tumor-free breast (n = 32)				
CEA	1,430	1,800	0.52	< .01
CEA/ng albumin	26	31	0.37	.04
PSA	43	98	0.20	.26
PSA/ng albumin	0.8	1.8	0.07	.67

*Pearson coefficient.

has been difficult to reproduce.[21] Another study reported that women with cytologic atypia in nipple fluid epithelial cells had a higher breast cancer rate over the ensuing 10 to 18 years.[22] Obstacles to NAF cytology studies include failure to obtain fluid from many subjects, low yield of breast epithelial cells, and difficulty in identifying cancer cells.[21,22] Successful NAF collection is associated with premenopausal status, early age at menarche, wet-type ear cerumen, non-Asian ethnic origin, parity, and breast feeding.[17,23,24] Our success rate for obtaining NAFs (34%) is lower than figures reported by other investigators (> 95% by one group).[8] The reason is that we were allowed to collect NAFs from five hospitals with the understanding that morbidity would be minimal among study subjects recruited at time of mammography. Thus, our research nurse was instructed to stop collection efforts if the subject complained of discomfort.

Diverse biochemical constituents of NAFs have also been studied, but most reports have focused on CEA and PSA.[21,25-27] CEA is a serologic marker used for initial cancer staging, monitoring response to therapy, and detecting relapse of diverse carcinomas.[12-14] PSA is used for early detection and follow-up of prostate cancer.[28,29] Previous reports have described higher CEA and PSA levels in NAFs than sera, whereas CEA and PSA are barely detectable in human breast milk.[10,15] In the present study, we assessed the usefulness of nipple fluid CEAs and PSAs as biomarkers for breast cancer detection. Results show that nipple fluid CEAs and albumin-standardized CEAs are significantly higher in the unilateral untreated cancerous breast of 44 patients compared with the breasts of the 277 tumor-free controls (Table 2). The differences persist after adjustment for potential confounders (age, smoking, and menstrual status). However, sensitivity of nipple fluid CEAs is only 32% when specificity is established at 90%, thus limiting the usefulness of the assay for early breast cancer detection. The CEA test characteristics are roughly comparable to 50% sensitivity and 90% specificity reported for noninvasive clinical breast examinations and inferior to the 80% sensitivity and 90% specificity of mammography.[30,31] Moreover, our analyses excluded a tumor-free outlier with more than 480,000 ng/mL in nipple fluids collected on two separate occasions from her right breast; follow-up revealed that this breast has remained tumor-free, but two fibroadenomas were diagnosed in her left breast that had CEAs of only 3,500 ng/mL.

In Japan, studies of spontaneous bloody nipple discharges found that higher CEA levels are associated with the presence of breast cancer.[32-36] In one study, 33 of 44 patients with cancer-associated nipple discharges had CEAs more than 400 ng/mL compared with seven of 33 of those with nipple discharge caused by noncancerous condition. The corresponding sensitivity and specificity for breast cancer detection were 75% and 79%, respectively.[33] Discordance between these studies and our data might also be in part caused by differences in CEA assays, as well as biologic determinations of CEA levels in nipple fluids and abnormal bloody nipple discharges.

Some published reports of NAF biochemical constituents have standardized the results to NAF albumin or total protein level, but others have not.[15,17,25-27] We analyzed the crude CEA and PSA levels as well as the corresponding albumin-standardized titers and obtained essentially similar results. Our nipple fluid PSA and albumin-standardized PSA titers do not differ for breasts with cancer or prolifer-

ative breast lesions compared with tumor-free breasts. However, Sauter et al[17] have reported that nipple fluid PSA titers are lower in breasts with cancer or precancerous mastopathy than in normal breasts. Some NAFs of their cancer and mastopathy case were collected from mastectomy specimens that might have been diluted with peripheral blood with low PSA levels.[8,17,37]

In our study, bilateral NAF samples were obtained from 31 women with unilateral breast cancer, 32 women with a unilateral proliferative lesion, and 142 women with both tumor-free breasts. The CEAs, PSAs, and the corresponding albumin-standardized CEAs and PSAs for paired samples tend to be correlated, despite the presence of a neoplasm in only one breast (Table 3). PSAs in NAFs from 32 breasts with proliferative breast lesions are somewhat lower than corresponding PSAs in NAFs from the opposite tumor-free breasts, but the difference is not significant ($r = 0.2$, $P = .26$). In aggregate, these findings suggest that nipple fluid CEAs and PSAs are partially determined by unknown systemic host influences on both breasts. Possible biologic mechanisms might include the subjects' state of hydration (dehydration would be associated with higher concentrations of nipple fluid CEA or PSA) or genetically determined regulation of CEA and/or PSA secretion into breast ductules. If so, these systemic influences would diminish the usefulness of nipple fluid tumor biomarkers for early breast cancer detection.

Studies are needed to explain the high CEA and PSA levels in NAFs and the wide range of these biomarker levels in normal and tumor-bearing breasts. Elevated nipple fluid CEA levels are correlated with breast cancer in our series, but the biomarker has low sensitivity and limited clinical utility. Noninvasive, inexpensive nipple fluid CEA analyses might be used in conjunction with other assays for early cancer detection, particularly molecular genetic methods for detection of the cancer cells observed in nipple fluid by Papanicolaou and other investigators.

ACKNOWLEDGMENT

We thank Anastasia Satterfield, Nina Cardoza, Christine Henault, and Peter Gudrais for laboratory and data management support and Donna Joseph, Miram Hawkes, and Anna Andrade for sample collection.

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