Carcinoembryonic Antigen in Breast Nipple Aspirate Fluid

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Abstract

New diagnostic tools are needed to complement mammography and physical examinations for early detection of breast cancer, particularly among younger women. We evaluated the tumor biomarker, carcinoembryonic antigen (CEA), in 215 nipple aspirate fluid (NAF) samples collected from one or both breasts of 147 women, ages 27–87 years. Most subjects were recruited at the time of mammography examination. The 215 nipple fluid CEAs range from undetectable levels to 8400 ng/ml (median, 1100 ng/ml). Normal serum CEA levels are less than 6 ng/ml. There are no significant differences between the CEAs in fluid from normal breasts (112 samples) and breasts with various histories of tumors (103 samples). Analyses for determinants of CEA in fluids from normal breasts show higher levels among current smokers (P = 0.03) and marginal elevations among nulliparous women (P = 0.07). CEAs in these samples are not correlated with age, menopausal status, current hormone use, prior breastfeeding, or family history of breast cancer. Follow-up studies of these women and comparisons of CEAs in fluids from normal and cancer-containing breasts will help clarify whether this biomarker is useful for risk assessment or early cancer detection.

Introduction

One woman in nine in the United States eventually develops breast cancer (1). Mammograms and physical examinations are the standard methods for early breast cancer detection. Routine mammography screening has been shown to reduce breast cancer mortality among women ages 50 years and over and probably among women in their 40s (2–4). However, accuracy of mammography is lower among young women, and additional early detection methods are needed (5).

For several decades, cells and the supernatants from NAFs3 have been examined for evidence of early breast cancer and biomarkers of high cancer risk. Early studies focused on cytological examination of epithelial cells in NAF for dysplasia and carcinoma (6–12). Other reports have described various biochemical constituents of NAF, including immunoglobulins, lipids, cholesterol, fatty acids, lactose, hormones, and growth factors (9, 13–17). Recently, we and others reported high levels of PSA in NAF (18, 19).

CEA was identified in 1965 as the first human cancer-associated antigen and serological tumor biomarker (20). CEA is a secreted protein, and elevated serum CEAs are found in patients with diverse forms of advanced cancers, particularly carcinomas of the colon, breast, and lung (21). CEA is elevated in serum of 40–50% of patients with metastatic breast cancer, and is used both in initial tumor staging and monitoring of response to treatment (22). CEA is detectable immunohistochemically in breast cancer cells, whereas most of the normal and benign tumor tissues stain weakly or not at all with anti-CEA antibodies (23). CEA has been detected in foamy macrophages and intraluminal material of nonneoplastic lobules and ducts adjacent to the CEA-positive cancerous tumors (23, 24). The physiological role of CEA in breast nipple fluid and the clinical significance of high NAF concentrations have not been studied previously. This study describes the range of CEA levels in NAFs and several correlates of CEA elevations.

Materials and Methods

Subjects. Participants were recruited between 1993 and 1996 in ambulatory clinics and mammography suites of four Boston hospitals (Dana-Farber Cancer Institute, Faulkner Hospital, Beth-Israel Hospital, and Brigham and Women’s Hospital). Excluded from the study were lactating women and those with bleeding tendencies, scarred nipples, local infections, or spontaneous bloody nipple discharge. Informed consent was obtained to perform breast nipple aspiration on 474 women who responded to a brief questionnaire on demographic characteristics, breast diseases, and breast cancer risk factors. Their mammography and breast biopsy results were obtained from a review of available medical records.

Specimen Collection Procedures. NAFs were collected using techniques described previously (7). In brief, nipples were cleansed with alcohol swabs to remove cellular debris. The large majority of samples were collected by placing over the nipple a small plastic suction cup attached to a 20-ml syringe, and applying suction for several seconds. Recently, we found that NAFs can also be successfully collected from women who can manually express fluid from their breasts. Droplets of

3 The abbreviations used are: NAF, nipple aspirate fluid; CEA, carcinoembryonic antigen; PSA, prostate-specific antigen.
nipple fluid were collected into microcentrifuge tubes. The volume of NAFs varied from 0–280 µl, usually 5–20 µl. Nipple suction was well tolerated by nearly all subjects and was stopped if substantial discomfort was encountered. No serious complications occurred. As control specimens, 20 breast milk samples were obtained from the Regional Milk Bank of The Medical Center of Central Massachusetts.

**Laboratory Methods.** NAFs were transported on ice to the laboratory within 8 h after collection. NAFs were usually viscous and were diluted up to 10-fold in 1 X PBS and centrifuged. The supernatant was aliquoted for storage at −70°C. Quantitative CEA assays were performed using the commercially available immunoenzymometric assay kit AIA-PACK CEA (Tosoh, Foster City, CA), which has a lower detection limit of 0.1 ng/ml. Due to high CEA levels in most NAFs, samples were diluted (1:200) before analysis with CEA Sample Diluting Solution (Tosoh, Deerfield, IL). Reproducibility of the assay was examined in 21 samples by repeating the NAF dilution and CEA analysis steps. The replicate error accounts for 1% of the total CEA variation. To demonstrate detection of the M₆ 180,000 CEA glycoprotein, Western blots were performed using purified human CEA (Calbiochem Novabiochem, San Diego, CA) as a standard protein and a mouse monoclonal anti-CEA antibody (IgG1 subclass; Pierce, Rockland, IL). Western blots of 20 NAFs showed the expected 180,000 CEA glycoprotein along with CEA cross-reacting species of lower molecular weight (Fig. 1). In addition, CEA titers were normalized to albumin levels, which were determined in duplicate by colorimetric reaction with Bromcresol green (Sigma Diagnostics, St. Louis, MO). Albumin levels vary more widely in NAFs (range, 2–100 mg/ml; median, 29 mg/ml) than in serum (39–50 mg/ml).

**Data Analysis.** The 215 NAFs were considered the units of analysis unless otherwise noted. Based on a review of questionnaires and available medical records, breasts were classified as normal when there was no history of cancer, precancerous lesions (dextral carcinoma *in situ*, lobular carcinoma *in situ*, or atypical duct hyperplasia), or nonneoplastic conditions (Table 1). Median, log₁₀ of the mean, and range of CEA levels were analyzed for all samples and subsets of NAFs. The variability of replicate CEA determination was estimated from the variance components of a general linear model. The correlation of CEA levels between the right and left breasts was estimated using the Pearson coefficient. Associations of CEA levels with factors such as age, parity, lactation history, menstrual status, smoking history, family history, and diagnoses were estimated using Kendall’s Tau- b and associated hypothesis tests (25). To account for multiple collections and bilateral NAF samples, statistical inferences regarding the effect of such factors as smoking and parity were adjusted for multiple measurements in applicable subjects by using the average CEA level per subject (26).

**Results**

NAFs were obtained from one or both breasts of 147 of the 474 women (31%). Donors were 27–87 years of age (median, 44 years), and all but five were Caucasian. Similar to other reports, our rates of successful collection declined with age: 47% before age 40, 38% at ages 40–49, 22% at ages 50–59, and 14% thereafter (27). Ninety-seven of the 147 women (66%) were premenopausal, 42 (29%) had a first-degree relative with breast cancer, 29 (20%) were active smokers, and 20 (14%) were taking hormones for contraception or menopause (8–10). Eight women were receiving cancer therapy or were within 6 months of completing treatments. One hundred seven (73%) had one or more completed pregnancies, and 70 (65%) of these mothers breastfed their infants.

Nipple fluids were obtained from both breasts of 56 women, and 9 provided serial samples. A total of 215 NAFs were collected from 112 breasts without any history of tumors (normals), 4 from breasts with newly diagnosed breast cancers, 16 from breasts after excision of various *in situ* and invasive neoplasms, and 83 from pre- or postoperative breasts with benign diseases (fibrocystic disease, cyst, fibroadenoma, papilloma, and sclerosing adenosis).

<table>
<thead>
<tr>
<th>Clinical status at NAF collection</th>
<th>No. of samples</th>
<th>Median CEA (ng/ml)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative breast invasive carcinoma</td>
<td>4</td>
<td>2000</td>
<td>800–6400</td>
</tr>
<tr>
<td>Postoperative breast neoplasia (carcinoma, DCIS, LCIS, ADH)</td>
<td>16</td>
<td>1400</td>
<td>&lt;100–3770</td>
</tr>
<tr>
<td>Preoperative nonneoplastic conditions</td>
<td>43</td>
<td>1500</td>
<td>&lt;100–6630</td>
</tr>
<tr>
<td>Postoperative nonneoplastic conditions</td>
<td>40</td>
<td>800</td>
<td>&lt;100–8440</td>
</tr>
<tr>
<td>Normal breast</td>
<td>112</td>
<td>1100</td>
<td>&lt;100–7530</td>
</tr>
<tr>
<td>Total</td>
<td>215</td>
<td>1100</td>
<td>&lt;100–8400</td>
</tr>
</tbody>
</table>

* No statistically significant differences between CEA from normal breasts, as compared with breasts with various abnormalities (P > 0.05 for each of the four disease categories when compared with normals).

* NAF collected before surgical excision of cancer.

* NAF collected after excision of cancer, DCIS (dextral carcinoma *in situ*), LCIS (lobular carcinoma *in situ*), ADH (atypical duct hyperplasia).

* Non-neoplastic conditions include fibroadenoma, fibrocystic disease, cyst, sclerosing adenosis, papilloma.

* No history of cancer or other listed lesions.
levels are highly correlated in 18 paired samples collected from normal breasts \((r = 0.61, P = 0.01)\) and 20 paired samples from one normal breast and a breast treated previously for benign disease \((r = 0.68, P = 0.001)\).

Unlike NAF, 15 of 20 undiluted breast milk samples tested have no detectable CEA \((<0.1 \text{ ng/ml})\), and the other 5 breast milks have CEA levels of 0.1–2.0 \text{ ng/ml} (data not shown). Western blots of breast milk do not exhibit the Mr 180,000 CEA glycoprotein but show the same CEA cross-reacting species detected in Western blots of NAFs (Fig. 1).

Table 1 shows CEA levels by clinical status of the breast at NAF collection (preoperative breast cancer, postoperative breast neoplasia, pre- and postoperative benign masses, and normals). CEA levels in 112 NAFs from normal breasts range from undetectable to 7500 \text{ ng/ml} (median, 1100 \text{ ng/ml}). Among the 103 breasts with various histories of tumors, NAFs from 4 newly diagnosed cancer-bearing breasts have the highest CEA levels (median, 2000 \text{ ng/ml}), but the excess is small and nonsignificant. In addition, no significant CEA elevations are found in NAFs from breasts that had been treated for benign tumors, in situ and invasive carcinomas, as well as breasts containing masses that eventually proved benign.

The 112 NAFs from normal breasts of 93 women were examined for associations between CEA levels and donor characteristics. The 18 current smokers have significantly higher CEA levels when compared with nonsmokers \((P = 0.03; \text{ Table 2})\). In addition, CEA levels were moderately decreased among 68 parous women when compared with the nulliparous \((P = 0.07)\). CEA levels showed no association with subjects’ age at study, menopausal status, hormone use at the time of fluid collection, prior breastfeeding, or family history of breast or ovarian cancer in a first-degree relative (data not shown).

Discussion

In addition to developing cytological diagnosis for carcinoma of the cervix, Papanicolaou studied other body fluids for cytological evidence of cancer (6). In 1958, he described the collection of NAFs with a suction device and reported finding atypical cells in 27 of 45 NAFs collected from patients bearing breast cancers (6). In a prospective study of 2701 women, Wrensch et al. (12) report the association between cytological atypia in NAF cells and breast cancer development up to 15 years later. NAF cytology has not proven useful in cancer diagnosis, however, because of its low predictive value. Major technical limitations are low NAF volumes, paucity of breast epithelial cells, and difficulties in distinguishing cancer from dysplastic cells (12).

NAF supernatants have been examined for a variety of biochemical constituents. Some display much higher levels in NAF as compared with serum, whereas others do not. We have recently described high PSA levels (median, 55 ng/ml) in NAFs that do not correlate with personal or family history of breast cancer (18). Sauter et al. (19) also found elevated PSA levels in NAFs from cancer-free subjects, which vastly exceed PSAs in fluids collected from their mastectomy specimens containing invasive cancer. The discordant results might be due to differences in the collection and assay techniques. Among other NAF constituents, levels of cholesterol, estrogen, and gross cystic disease fluid protein are at least 10-fold higher than subjects’ corresponding blood levels (9, 13–16, 28). Total protein and IgA levels are elevated to lesser extents, whereas albumin, IgG, and IgM levels are lower in NAFs for unknown reasons (9). Because breasts are secretory glands, concentrations of various NAF constituents might be determined by uptake from blood, synthesis and degradation by breast epithelial cells, secretion into breast ductules and resorption, and processing by macrophages and other cells in NAFs (9). These mechanisms might also affect levels of CEA and other tumor biomarkers in NAF.

We examined CEA levels in NAFs to determine whether this tumor biomarker might be useful for breast cancer detection. Study subjects differed with regard to their past history of breast tumors, clinical status at NAF collection, and risk factors for breast cancer. Median CEA level in NAFs of normal breasts (1100 ng/ml) is 200-fold higher than the normal serum CEA (<6 ng/ml); CEA concentrations of 1100 ng/ml in serum are virtually diagnostic of extensive cancer and poor prognosis (22). CEA levels are high in NAFs from normal breasts and concordant in bilateral samples of normal breast pairs. We found no significant differences between nipple fluid CEA levels for normal breasts and breasts with various breast tumors. However, only four NAFs from cancer-containing breasts were available for study because samples were collected primarily in mammography units. CEAs for these cancerous breasts are slightly higher than levels for normal breasts, but the difference is small and nonsignificant.

Our search for determinants of nipple fluid CEAs for normal breasts revealed higher titers in active smokers. Smoking moderately increases serum CEA levels by unknown mechanisms and may have a similar effect in breast fluid (29). Smoking is an important risk factor for many cancers, but its role in breast cancer development remains uncertain (30, 31). CEA levels in NAF do not rise with age, as reported with serum CEA (29). In addition, CEA levels tend to be slightly higher in nulliparous women than in parous women.

Several Japanese studies evaluated CEA levels in spontaneous pathological nipple discharge, which differs from our NAFs collected by suction or manual breast compression. Using a semiquantitative dot-immunobinding assay (32, 33), CEAs from spontaneous nipple discharges were elevated (>400 ng/ml in their assays) in 74% of one series of patients with palpable breast cancer and 76% of patients with nonpalpable breast cancer but in only 24% of patients without cancer. The reported sensitivity, specificity, and accuracy of the CEA nipple discharge were 76, 79, and 78%, respectively (33). The differences in CEAs between the Japanese studies and our series may be due to differences in ethnicity, assay techniques, and pathogenesis of the abnormal discharges. The explanation can be examined by international exchange of samples for duplicate analyses. The Japanese finding of higher CEA in nipple discharge samples from cancerous breasts, if confirmed, may be useful for cancer detection in the few women with spontaneous nipple discharge.

In this study, we found high CEA levels in NAF and identified several determinants of elevated titers. Follow-up of

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**Table 2**  Effect of parity and current smoking on CEA levels in nipple aspirate collected from normal breasts

<table>
<thead>
<tr>
<th>Subject characteristics</th>
<th>No. of subjects</th>
<th>CEA (ng/ml) Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smokers(^b)</td>
<td>18</td>
<td>1600</td>
<td>170–6600</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>69</td>
<td>800</td>
<td>&lt;100–7500</td>
</tr>
<tr>
<td>Parous</td>
<td>68</td>
<td>1000</td>
<td>&lt;100–5800</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>24</td>
<td>1400</td>
<td>&lt;100–7500</td>
</tr>
</tbody>
</table>

\(^a\) Subjects were 93 women who produced NAF from normal breasts. Average CEA levels were used for subjects who provided paired or multiple samples. Missing are data on smoking by six women and parity of one woman.

\(^b\) \(P = 0.03\) for smoking; \(P = 0.07\) for parity.
our series might show whether high CEAs are predictive for future breast cancer development. In addition, a case-control study comparing CEA levels in nipple fluids from normal and cancerous breasts can determine the usefulness of this biomarker for early cancer detection. If nipple fluid CEAs have acceptable predictive values, this low-cost, noninvasive test might be an adjunct to mammography for early cancer detection.

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