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Biologic markers of breast cancer in nipple aspirate fluid and nipple discharge are associated with clinical findings☆

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Abstract

Background: The aim of this prospective study was to assess predictive markers in nipple aspirate fluid (NAF) and pathologic nipple discharge (PND) collected prior to excisional breast biopsy, as well as clinical factors available prior to biopsy, with histopathologic results in women with a radiographically suspicious and/or palpable breast lesion. *Methods*: 208 NAF samples from 191 women were evaluated for the following candidate predictive proteins and cellular markers: prostate-specific antigen (PSA), human glandular kallikrein 2 (hK2), basic fibroblast growth factor (bFGF), S phase fraction (SPF), DNA index, and cytology. Clinical factors included whether or not the lesion was palpable, menopausal status, history of pregnancy, history of birth control or hormone replacement use, and PND. *Results*: Considering all women, bFGF ($p = 0.005$) and SPF (0.031) were associated, and abnormal cytology approached an association ($p = 0.056$) with the presence of breast cancer. Women with PND were less likely to have breast cancer (4 vs. 37%, $p < 0.001$) or palpable lesions (10 vs.43%, $p < 0.001$), were younger, had lower PSA levels ($p = 0.046$), and were more likely to have atypical NAF cytology ($p = 0.002$). Excluding PND, increased age, postmenopause (both $p < 0.01$), high bFGF ($p = 0.004$) and low PSA ($p = 0.05$) were associated with cancer. The best breast cancer predictive model included cytology, bFGF, and age (88% sensitive and 57% specific). When the data were divided by menopausal status, the optimal models to predict breast cancer, which included NAF hK2 or PSA and age, were 100% sensitive and 41% specific in pre- vs. 93% sensitive and 12% specific in postmenopausal women. *Conclusion*: NAF and clinical biomarkers are sensitive predictors of whether a breast contains cancer, and may ultimately help guide treatment. Future studies to determine the optimal combination of predictive markers are warranted.

Keywords: Breast cancer; Nipple aspirate fluid; Predictive markers; Proteins; Prostate-specific antigen; Human glandular kallikrein 2; Basic fibroblast growth factor; S phase fraction; DNA index; Cytology; Hormone replacement therapy

1. Introduction

Mammography and physical examination are the only generally accepted screening tools available for breast cancer. Both are limited by the need to perform an invasive diagnostic procedure (needle or surgical biopsy) to determine if the breast contains atypia or cancer. Moreover, needle biopsies are limited by sampling error (12% of fine needle and 3% of core needle biopsies are interpreted as benign when the lesion is malignant [1,2]), while excisional biopsy requires surgery, is costly, and raises concerns regarding cosmesis. Breast nipple aspiration, which provides nipple aspirate fluid (NAF), is noninvasive, inexpensive, and provides both cells and extracellular fluid from the breast ductal and lobular epithelium. Ductal and lobular epithelia are the source of 99% of breast cancers [3]. The

Abbreviations: BCP, birth control pill; bFGF, basic fibroblast growth factor; FD, fiberoptic ductoscopy; hK2, human glandular kallikrein 2; HRT, hormone replacement therapy; NAF, nipple aspirate fluid; PND, pathologic nipple discharge; PSA, prostate-specific antigen; SPF, Sphase fraction

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purpose of this prospective study was to assess if biomarkers in NAF can determine the benign or malignant nature of both nonpalpable and palpable breast lesions.

We previously demonstrated [4] that NAF cytology was highly ($p = 0.002$) associated with the presence of breast cancer, that malignant NAF cytology was 7% sensitive (7/97 cases with histologic evidence of cancer has malignant cytology) and 100% specific (all 7 cases of malignant cytology came from breasts with cancer) for the presence of cancer in the breast after excisional biopsy [5], and that NAF cytology and clinical parameters which are available prior to surgery can be used to develop a sensitive model to predict which women have residual breast cancer [6]. The cases in the current study include undiagnosed palpable and nonpalpable lesions of any etiology, with nipple aspiration performed prior to excisional biopsy or mastectomy. They include women with and without pathologic nipple discharge (PND) from one breast, not both, which may or may not have been bloody. The sensitivity and specificity of NAF cytology and other NAF biomarkers has not been previously evaluated in this population.

We have shown that increased DNA index is associated with atypical and malignant NAF cytology ($p = 0.0002$). We employed image analysis (IA) to determine whether DNA index (ploidy) and S-phase fraction (SPF) were predictors of breast cancer [4].

NAF contains highly concentrated proteins secreted from the ductal and lobular epithelium. We have recently found that two human glandular kallikreins, hK2 and hK3 (also known as prostate-specific antigen, PSA) are coexpressed in breast tumors and in NAF, and that lower levels of hK2, hK3, and a lower ratio of hK2/PSA in NAF were associated with breast cancer [7].

Basic fibroblast growth factor (bFGF) is an important angiogenic factor [8,9] which is elevated in various body fluids of patients with cancer [10,11]. A preliminary report found that bFGF levels in NAF were higher in women with breast cancer than in normal subjects [12]. This was confirmed in a larger study [13], in which we found that using bFGF alone, a logistic regression model to predict which women had breast cancer was 89.9% sensitive and 69.0% specific in predicting which women had breast cancer.

Thousands of women undergo invasive biopsy procedures each year based upon findings on mammogram and/or breast exam. Individual NAF biomarkers have demonstrated breast cancer predictive ability. Our objective is to assess if multiple NAF biomarkers, each promising when analyzed alone, in combination with clinical parameters, can determine the benign or malignant nature of both nonpalpable and palpable breast lesions in women with or without PND. If we can develop a sensitive and specific predictive model for the presence of malignancy in the breast, then findings in NAF may allow the subject to forego an invasive diagnostic procedure and proceed directly to prevention strategies or to definitive surgery, as indicated.

2. Materials and methods

2.1. Subjects

Institutional Review Board approval was obtained to collect breast fluid from women 18 years of age or older scheduled for diagnostic breast surgery. Women were prospectively enrolled between 2000 and 2004. All subjects enrolled for whom biomarker data are available are included in this study. This population included women with a suspicious breast lesion identified on an imaging study, women with a solid palpable breast mass, and women with unilateral single duct pathologic nipple discharge. This study included two types of specimens: PND and NAF, the latter which was collected using a modified breast pump from women without PND. These women may have undergone needle biopsy, but could not have undergone surgical biopsy prior to NAF collection. Subjects could not have been receiving chemotherapy or radiation therapy at the time of nipple aspiration. Subjects must have had at least one breast that had not received prior radiation. Subjects were recruited from the breast evaluation centers within the Thomas Jefferson University and University of Missouri Health Systems, where subjects are seen with clinical breast disease.

Two hundred three women signed informed consent to participate in the study. NAF was successfully collected from 191 of these 203 women (94%). NAF was collected from both breasts of 17 women, providing a total of 208 samples for analysis. The 191 women were aged 20 to 83 years (mean 51.7, median 51.0). Eighty-seven (46%) women were pre- and 104 women were postmenopausal, 166 (87%) were Caucasian and 19 (10%) were African American. The subjects were evaluated for the following biologic markers: PSA, hK2, bFGF, SPF, DNA index, and cytology.

2.2. Aspiration technique

Nipple fluid was aspirated by a trained physician or nurse clinician using a modified breast pump [4]. The pump is composed of a No. 4 endotracheal tube adapter attached to a 10 cc syringe. The breast nipple was cleansed with alcohol, 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 quantity of fluid varied from 1 μL to 200 μL.

2.3. Specimen preparation

Every NAF sample collected was of sufficient volume for evaluation. Samples were collected in 50 μL capillary tubes (generally 1–5 μL per tube). Immediately after collection, half of the NAF was transferred to eppendorf tubes containing 1 mL of 3% polyethylene glycol in ethanol–isopropranol and cytocentrifuged onto ten glass slides for cytology, S phase fraction and DNA index studies. The