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Biologic markers of breast cancer in nipple aspirate fluid and nipple discharge are associated with clinical findings[1]

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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, which included NAF hK2 or PSA and age, were 100% sensitive and 41% specific in pre- vs. 93% sensitive and 12% specific in predicting breast cancer 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.

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Condensed abstract: This prospective study demonstrates that combining predictive markers and clinical factors available prior to surgery may eventually allow us to minimize the number of diagnostic breast biopsies.

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Keywords

breast cancer; nipple aspirate fluid; predictive markers; proteins

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 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

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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.

MATERIALS AND METHODS

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.

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 sec. Fluid in the form of droplets was collected in capillary tubes. The quantity of fluid varied from 1 μl to 200 μl.

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 remainder was snap frozen at −80 °C until analysis of extracellular biomarkers (PSA, hK2, and bFGF). For the analysis of extracellular markers, the portion of the capillary tube containing the sample was introduced into a 1.7 mL eppendorf tube and 100 μL of a 0.1 mol/L solution of sodium bicarbonate (pH 7.8) was added. The capillary tube was then crushed by using a glass rod and the mixture was vortexed to disperse the sample. The crushed capillary tube was left in the bicarbonate buffer overnight at 4 °C to allow proteins adherent to the glass to go into solution. The mixture was centrifuged at 14,000 g for 5 min and the supernatant used without further dilution.

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Biomarker Analysis: Cellular Markers (cytology, SPF, DNA index)

Cytology—Cytologic review was performed on three slides stained using the Papanicolaou method. The slides were examined without the knowledge of clinical or pathologic findings. All slides were evaluated by a single cytopathologist (H.E.). Each specimen was classified as representing inadequate epithelial cells for evaluation, benign epithelial cells, atypical cells, or carcinoma.

SPF, DNA index—A standardized quantitative DNA staining kit (Feulgen kit, Tripath, Burlington, NC) was used following the manufacturer's instructions. In brief, after rehydration the slides were placed in 5N HCl, transferred to the staining solution (Schiff's reagent), rinsed, dehydrated and mounted with synthetic resin. We used normal human cultured lymphocytes that were also stained with Feulgen as controls in order to establish the normal diploid value (2c) with each stain batch.

For interpretation of Feulgen stained specimens, a Fairfield DNA Ploidy System for image analysis (Fairfield Imaging Ltd., Nottingham, UK) was used. This system, employing a light microscope, a multicolor solid state camera and a computer, has the ability to process cell images and calculate cell cycle and DNA index parameters [14]. The parameters calculated were: DNA index (ratio between DNA content of specimen/DNA content of control specimen), SPF, and percent of hypertetraploid (HT) cells. Hypertetraploid cells are a subset of aneuploid cells in which the DNA content is more than twice the content of a control specimen. All epithelial cells were measured if under 100 on a slide (minimum required=10 cells), or at least 100 cells if more were present on the slide. Most specimens contained 10–30 measurable cells. All specimens were evaluated in a blinded fashion by a single pathologist (A. K-S.).

Biomarker Analysis: Extracellular Markers (PSA, hK2, bFGF)

The NAF samples vary both in their total protein concentration and in the volume in the capillary tube used for marker analysis. For this reason, it has been our practice to determine the concentration of a given protein based on total NAF protein, after controlling for the degree to which the NAF was diluted prior to analysis. Total protein was measured using the bicinchoninic acid method (Pierce Chemical Co., Rockford, IL). Both PSA and hK2 were analyzed using time-resolved immunofluorometric assays developed by us [7]. PSA has a detection limit of 1 ng/L, and hK2 a detection limit of 6 ng/L. The PSA assay has less than 0.2% cross reactivity with hK2. The coefficients of variation for both the hK2 and hK3 assays were < 10% within the measurement range. bFGF was analyzed using an ELISA kit from R&D Systems (Minneapolis, MN), following the manufacturer's instructions. The kit utilizes a quantitative sandwich enzyme immunoassay technique. The detection limit of the kit is 10 ng/L. All values are recorded per gram total NAF protein.

Statistical analysis

For purposes of analysis, some biomarker values (PSA, hK2, and bFGF) were log-transformed [log (biomarker value + 1) for inclusion of samples where biomarker = 0] to enable us to apply parametric statistics. The unpaired t-test was applied when comparing two independent groups. Chi Square analysis was applied to categorical data (such as cytology, menopause status, PND, palpable masses, cancer status). Logistic regression was applied to identify clinical and biologic parameters (from those measured in this study) likely to predict the presence of cancer. Since it is more important not to miss cancer than to do a biopsy unnecessarily, if we could not achieve both high sensitivity and specificity, we optimized sensitivity. Descriptive statistics were calculated and all analyses were conducted using the software SigmaStat for Windows version 2.03S (SPSS, Inc., Chicago, IL 60606), or S-Plus for Windows version 6.2 (Insightful, Seattle, WA 98109). The significance level was set at alpha = 0.05.

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RESULTS

Biomarkers in Women Scheduled for Diagnostic Biopsy which are Associated with Breast Cancer

We evaluated a number of clinical factors and biomarkers previously reported in NAF to be associated with breast cancer. The clinical factors included menopausal status, ever use of birth control pills (BCP) and hormone replacement therapy (HRT), and number of previous pregnancies. Premenopause ($p<0.001$) and BCP use, controlling for age ($p=0.006$), were significantly associated with a lower incidence of breast cancer (Table 1), whereas HRT use was associated with a higher incidence of breast cancer ($p=0.011$). The number of previous pregnancies and whether or not the participant had a family history of breast cancer were not associated with a higher breast cancer incidence.

One microliter NAF was sufficient for the analysis of each biomarker. Biomarkers included PSA, hK2, bFGF, SPF, DNA index, and cytology. Lower bFGF ($p=0.005$) and lower SPF ($p=0.031$) were associated with a decreased risk of having breast cancer (lower section of Table 2). The relationship between normal (inadequate epithelial cells for evaluation or benign epithelial cells) cytology and a lower risk of having breast cancer approached significance ($p<0.056$) (upper section of Table 2).

Differences in Clinical Factors and Biomarker Expression Based on whether the Lesion Removed was Palpable or Not

The prevalence of cancer (Table 2) was not significantly different in palpable compared to nonpalpable lesions (34/101, 34% vs. 25/107, 23%, $p=0.135$). Conversely, the prevalence of PND was lower (10/101, 10% vs. 46/107, 43%, $p<0.001$) in palpable than in nonpalpable lesions (Table 3). No other associations between biomarkers and palpable vs. nonpalpable lesions was found.

Differences in Clinical Factors and Biomarker Expression Based on whether or not the Subject Presented with PND

Cancer was relatively uncommon (2/56, 4% of subjects) in women presenting with PND who required surgical excision (Table 3), whereas 57/152 (37%) subjects without PND requiring surgery were found to have breast cancer. The difference was significant ($p<0.001$). The women with PND were younger (age < 49, $p=0.002$) and more likely to be premenopausal ($p<0.001$) than other subjects in the study.

The expression of some biomarkers was also different in women with PND (Table 3). There was a higher proportion of atypical specimens, but no malignant specimens, in women with PND ($p=0.002$). PSA was lower in samples from women with PND ($p=0.046$). None of the other biomarkers evaluated (bFGF, hK2, DNA index, or SPF) were associated with PND.

Clinical Factors and Biomarker Expression in Women Without PND Requiring Breast Biopsy

Based on the differences outlined above, it appears that women without PND are quite different from those with PND, and are far more likely to have breast cancer. We therefore determined which clinical factors and biomarkers predicted breast cancer in women requiring surgery who did not present with PND (Table 4). Premenopause and young age were significantly related to a lower risk of breast cancer. Low bFGF ($p=0.004$) and high PSA ($p=0.05$) were associated with a lower risk of breast cancer, while the association of normal cytology ($p=0.066$) with a lower risk of breast cancer approached significance.

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Logistic Regression

Clinical variables (whether or not the lesion was palpable, menopausal status, number of pregnancies, family history of breast cancer, age, and PND), and biomarkers (PSA, bFGF, SPF, and cytology) were evaluated individually among 208 samples for their ability to predict whether or not a woman requiring breast surgery had cancer (Table 5). Sensitivity, specificity, positive and negative predictive value were determined. The factors with sensitivity > 80% included age ≥ 49, postmenopause, PSA ≤ 400 ng/g or ≤ 120 ng/g for postmenopausal subjects, hK2 ≤ 40 ng/g, and fourth root bFGF ≤ 3 ng/g. Factors with specificity > 90% were cytology (atypical or malignant) and DNA index ≥ 1.3. The optimal model for cancer detection, which included NAF cytology, bFGF, and age in 101 samples, was 88% sensitive and 57% specific in predicting if a subject's breast contained cancer. When we considered only premenopausal women, the optimal model included PSA ≤ 120 ng/g and/or hK2, and age in 52 samples, and was 100% sensitive and 41% specific. For postmenopausal subjects, the optimal model included PSA ≤ 120 ng/g and/or hK2 and age in 69 samples, providing a sensitivity of 93% and specificity of 12%.

Since women requiring surgery for PND appear to have a different clinical and biomarker profile, and are less likely to have breast cancer than our other subjects who underwent surgery, we then conducted similar sensitivity, specificity, positive and negative predictive and logistic regression analyses among 152 samples (Table 6). The factors with sensitivity > 80% included age ≥ 49, postmenopause, PSA ≤ 401 ng/g, PSA ≤ 120 ng/g for postmenopausal subjects, hK2 ≤ 45 ng/g, and fourth root bFGF ≤ 3 ng/g. Factors with specificity > 90% were cytology (atypical and malignant) and DNA index ≥ 1.3. The optimal model for cancer detection, which included age, PSA, hK2, bFGF and SPF in 38 samples, was 82% sensitive and 51% specific in predicting if a subject's breast contained cancer. When we considered only premenopausal women, the optimal model included PSA ≤ 120 ng/g, hK2, and age in 27 samples, and was 71% sensitive and 43% specific. For postmenopausal subjects, the optimal model included PSA ≤ 120 ng/g and/or hK2 and age in 54 samples, providing a sensitivity of 91% and specificity of 39%.

DISCUSSION

Non- and minimally invasive intraductal methods for the early detection of breast cancer are under active investigation. These include nipple aspiration, ductal lavage, and breast ductoscopy. Of these, nipple aspiration is the only totally noninvasive approach, does not require an expensive catheter to perform the procedure, and provides both ductal epithelial cells and concentrated secreted proteins from the ductal epithelium which are not diluted by lavage fluid. A limitation of all three intraductal approaches is the mixed and limited cellularity of the material collected, making analyses of cellular DNA, RNA and protein difficult to obtain, and if results are obtained, difficult to interpret. This is why we chose to analyze biomarkers which were either secreted proteins or interpretation of cell nuclear changes on a cell-by-cell basis and did not require physical separation of epithelial from non-epithelial cells. A strength of NAF is the concentrated secreted proteins present, whereby one microliter was sufficient for the analysis of each biomarker.

The association between higher bFGF in NAF and breast cancer has been reported by ourselves and others [12,13]. In the current study which focused on women requiring breast surgery, we observed higher levels of bFGF in women with cancer than in subjects with benign disease, with mean levels 8.7 fold higher in women with breast cancer. Mean SPF was significantly higher (41.6 vs. 4.8 ng/g) in women with breast cancer vs. those with benign disease. When women with PND were excluded, mean bFGF remained significantly higher (39.2 vs. 3.3 ng/g).

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We previously demonstrated the PSA levels in NAF [7] were inversely associated with the presence of breast cancer. As such, low levels were associated with breast cancer. In our prior studies, we included both women before and after diagnostic breast biopsy. To minimize the influence of surgery on PSA (and other biomarker) levels, we excluded women who had undergone recent diagnostic breast surgery. Inconsistent with our earlier studies, PSA levels were not significantly higher in women with benign disease than in women with breast cancer. This inconsistency was clarified when we split our samples into breasts with vs. without PND (Table 3). We anticipated that in breasts with PND PSA levels would be higher, since the percentage of samples from breasts with cancer was lower (4 vs. 37%) than in the non-PND group, but they were not (302.9 vs. 2044.1 ng/g). This suggests that papillomas and other lesions causing PND do not secrete high levels of PSA into breast ductal fluid. In women without PND (Table 4), median PSA levels were 9.9 fold higher in women with benign breast lesions ($p=0.05$). The other kallikrein analyzed, hK2, was not differentially expressed in the NAF of women with benign vs. malignant disease.

As in prior NAF studies [4,6,15,16], malignant cytology was only observed in women with breast cancer. Malignant cytology was a very specific, but not very sensitive, method to detect breast cancer. Atypical NAF cytology was just as frequent in women with benign disease as in women with breast cancer. We recently reported our cytologic findings in FD specimens [17] and found that atypia was as frequent in benign as in cancerous specimens. A multicenter trial [18] which assessed ductal lavage cytology also did not clarify the importance of atypical specimens collected using that intraductal approach. Thus, unlike malignant cytology, atypical cytology does not provide a clear indication of whether or not a breast contains cancer.

SPF but not DNA index in NAF was higher in samples from breasts with cancer than those without when considering all samples ($p=0.031$), but not when analyzing only samples from women without PND, perhaps because of reduced sample size. One of the limitations of image analysis markers is that they can only be performed on NAF samples with adequate epithelial cells.

We evaluated a number of clinical variables, including age, ever use of BCPs, ever use of HRT, whether or not the lesion was palpable, and whether there was breast fluid discharged from the nipple of a single breast spontaneously. We observed, as has been reported by many others, that increasing age is linked to increased breast cancer risk [19]. The association of ovarian hormone use, whether BCPs and HRT, has been studied by many investigators. In summary, there is no evidence for an increased risk of breast cancer with BCP use [20], whereas at least some preparations of combination estrogen and progestin-containing HRT do appear to increase breast cancer risk [21]. We observed a decreased risk of breast cancer among women who had ever used BCPs, controlling for age, and an increase in risk among women using HRT.

Palpable breast lesions have different features than nonpalpable. Palpable cancers, independent of tumor size, are more likely to metastasize than nonpalpable cancers [22]. Less is known about the molecular differences between palpable and nonpalpable breast cancers which might be important in determining methods of breast cancer detection and targets for therapy. We did not find a significant difference in the percentage of specimens with cancer among palpable vs. nonpalpable lesions, nor in the expression of biomarkers. On the other hand, the fraction of women requiring surgery with PND who had a palpable mass was significantly lower than other women requiring breast surgery, indicating that PND does not usually present as a breast mass.

Most often the cause of PND is a papilloma, but cancer is another possible etiology, which is why women presenting with PND require lesion removal to exclude malignancy [23]. In the

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current study, women with PND were younger and more often premenopausal. Cancer was more prevalent among women requiring breast surgery for reasons other than PND. Although malignant cytology was found only in breasts with cancer, atypical cytology was more frequent in breasts with PND than those without, consistent with previous reports of atypia in fluid from ducts containing papillomas [17].

We combined our biologic and clinical markers to identify the optimal breast cancer predictive models. Each of these markers can be assessed in the absence of surgery. The best model was 88% sensitive and 57% specific in predicting which women had breast cancer. When we looked at predictive models based on menopausal status, we were able to increase sensitivity (100% for pre- and 93% postmenopausal), but with lower specificity. One of the limitations of our data set is that not all subjects had all biomarkers analyzed, due primarily to the identification of new biomarkers at various time points during the trial. As such, the number of subject samples which met criteria for logistic regression analyses is a subset of the enrolled subjects. Nonetheless, the encouraging sensitivity and specificity findings suggest that combining individually promising biologic and clinical markers can be used in combination to optimize breast cancer prediction, and warrants further investigation both to validate these findings, and to find additional biomarkers to obtain a sensitivity which approaches 100%.

References

1. Shah VI, Raju U, Chitale D, Deshpande V, Gregory N, Strand V. False-negative core needle biopsies of the breast: an analysis of clinical, radiologic, and pathologic findings in 27 concecutive cases of missed breast cancer. Cancer 2003;97(8):1824–31. [PubMed: 12673707]
2. Choi YD, Cho YH, Lee JH, Nam JH, Juhng SW, Choi C. Analysis of fine needle aspiration cytology of the breast: a review of 1,297 cases and correlation with histologic diagnoses. Acta Cytol 2004;48 (6):801–6. [PubMed: 15581165]
3. Winer, EP.; Morrow, M.; Osborne, CK.; Haris, JR. Cancer of the Breast. In: DeVita, VT.; Hellman, S.; Rosenberg, SA., editors. Cancer: Principles & Practice of Oncology. 6. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1651-1716.
4. Sauter ER, Ross E, Daly M, Klein-Szanto A, Engstrom PF, Sorling A. Nipple aspirate fluid: a promising non-invasive method to identify cellular markers of breast cancer risk. Br J Cancer 1997;76(4):494–501. [PubMed: 9275027]
5. Sauter ER, Ehya H, Babb J, Diamandis E, Daly M, Klein-Szanto A, et al. Biological markers of risk in nipple aspirate fluid are associated with residual cancer and tumour size. Br J Cancer 1999;81(7): 1222–7. [PubMed: 10584885]
6. Sauter ER, Ehya H, Mammen A, Klein G. Nipple aspirate cytology and pathologic parameters predict residual cancer and nodal involvement after excisional breast biopsy. Br J Cancer 2001;85(12):1952–7. [PubMed: 11747339]
7. Sauter ER, Welch T, Magklara A, Klein G, Diamandis EP. Ethnic variation in kallikrein expression in nipple aspirate fluid. Int J Cancer 2002;100(6):678–82. [PubMed: 12209605]
8. Folkman J, Klagsbrun M. Angiogenic factors. Science 1987;235(4787):442–7. [PubMed: 2432664]
9. Folkman J, Shing Y. Angiogenesis. J Biol Chem 1992;267(16):10931–4. [PubMed: 1375931]
10. Nguyen M, Watanabe H, Budson AE, Richie JP, Hayes DF, Folkman J. Elevated levels of an angiogenic peptide, basic fibroblast growth factor, in the urine of patients with a wide spectrum of cancers. J Natl Cancer Inst 1994;86(5):356–61. [PubMed: 7508518]
11. Nguyen M. Angiogenic factors as tumor markers. Invest New Drugs 1997;15(1):29–37. [PubMed: 9195287]
12. Liu Y, Wang JL, Chang H, Barsky SH, Nguyen M. Breast-cancer diagnosis with nipple fluid bFGF. Lancet 2000;356(9229):567. [PubMed: 10950239]
13. Hsiung R, Zhu W, Klein G, Qin W, Rosenberg A, Park P, et al. High basic fibroblast growth factor levels in nipple aspirate fluid are correlated with breast cancer. Cancer J 2002;8(4):303–10. [PubMed: 12184408]

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14. Sudbo J, Kildal W, Risberg B, Koppang HS, Danielsen HE, Reith A. DNA content as a prognostic marker in patients with oral leukoplakia. N Engl J Med 2001;344(17):1270–8. [PubMed: 11320386]
15. Krishnamurthy S, Sneige N, Thompson PA, Marcy SM, Singletary SE, Cristofanilli M, et al. Nipple aspirate fluid cytology in breast carcinoma. Cancer 2003;99(2):97–104. [PubMed: 12704689]
16. King EB, Chew KL, Petrakis NL, Ernster VL. Nipple aspirate cytology for the study of breast cancer precursors. J Natl Cancer Inst 1983;71(6):1115–21. [PubMed: 6581355]
17. Sauter ER, Ehya H, Schlatter L, MacGibbon B. Ductoscopic cytology to detect breast cancer. Cancer J 2004;10(1):33–41. [PubMed: 15000493]discussion 15–6
18. Dooley WC, Ljung BM, Veronesi U, Cazzaniga M, Elledge RM, O'Shaughnessy JA, et al. Ductal lavage for detection of cellular atypia in women at high risk for breast cancer. J Natl Cancer Inst 2001;93(21):1624–32. [PubMed: 11698566]
19. Ries, LAG.; Kosary, CL.; Hankey, BF.; Miller, BA.; Clegg, L.; Edwards, BK. SEER Cancer Statistics Review 1973–1996. Bethesda MD: National Cancer Institute; 1999. NIH publication No 99-2789
20. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53,297 women with breast cancer and 100,239 women without breast cancer from 54 epidemiological studies. Lancet 1996 Jun 22;347 (9017):1713–27. [PubMed: 8656904]
21. Chlebowski RT, Hendrix SL, Langer RD, Stefanick ML, Gass M, Lane D, et al. Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial. JAMA 2003;289(24):3243–53. [PubMed: 12824205]
22. Chao C, Edwards MJ, Abell T, Wong SL, Simpson D, McMasters KM. Palpable breast carcinomas: a hypothesis for clinically relevant lymphatic drainage in sentinel lymph node biopsy. Breast J 2003;9 (1):26–32. [PubMed: 12558667]
23. Shen KW, Wu J, Lu JS, Han QX, Shen ZZ, Nguyen M, et al. Fiberoptic ductoscopy for patients with nipple discharge. Cancer 2000;89(7):1512–9. [PubMed: 11013365]

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
S phase fraction

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TABLE 1

Clinical Factors in Breasts of Women with a Suspicious Mammogram, Pathologic Nipple Discharge and/or Palpable Lesion Requiring Biopsy

Clinical Factors	N	Cancer		No Cancer		P value
		N	%	N	%	
Menopause	208					
Pre	87	10	11	77	89	<0.001
Post	121	49	40	72	60	
Birth control pills	207					
Yes	149	34	23	115	77	0.006
No	58	25	43	33	57	
Hormone replacement therapy	208					
Yes	86	33	38	53	62	0.001
No	122	26	21	96	79	
Family History	206					
Yes	43	17	40	26	60	0.118
No	163	42	26	121	74	
Previous pregnancies[1]	208					
Yes	174	46	26	128	74	
No	34	13	38	21	62	0.408

[1]: Information was missing regarding birth control use for one subject, and regarding family history of breast cancer for two subjects.

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TABLE 2

Predictive Markers in Breasts of Women with a Suspicious Mammogram, Pathologic Nipple Discharge and/or Palpable Lesion Requiring Biopsy[1]

Predictive Markers	Cancer		No Cancer		N	Mean	SEM[2]	Median	P value
	N	%	N	%					
Cytology	59		149		208				
Inadequate	34	**58**	103	69					
Benign	20	34	36	24					
Atypical	3	5	10	7					
Malignant	2	3	0	0					**0.056**
Palpable	59		149		208				
Yes	34	34	67	66	101				
No	25	23	82	77	107				0.135
PSA(ng/g)[2]					121				
Cancer					37	1477.4	898.6	19.0	
No Cancer					84	1464.6	655.2	57.6	0.180
hK2(ng/g)[2]					106				
Cancer					36	37.4	22.5	0.0	
No Cancer					70	42.1	17.2	0.0	0.463
bFGF (ng/g)[2]					101				
Cancer					34	41.6	17.4	3.5	
No Cancer					67	4.8	2.3	0.0	**0.005**
SPF[2]					66				
Cancer					18	16.2	5.6	5.8	
No Cancer					48	7.9	1.7	4.3	**0.031**
DNA Index					65				
Cancer					18	1.2	0.1	1.08	
No Cancer					47	1.1	0.0	1.05	0.383

[1]: not all biomarkers were analyzed in all subjects

[2]: bFGF: basic fibroblast growth factor; ng/g: hK2: human glandular kallikrein 2; nanogram of biomarker/gram total protein; PSA: prostate specific antigen; SEM: standard error of the mean; SPF: S phase fraction

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TABLE 3

Clinical Factors and Predictive Marker Expression in Breasts of Women Requiring Biopsy That Did or Did not Present with Pathologic Nipple Discharge (PND)

	PND				No PND				P value
	N	N (%)			N	N (%)			
Cancer	56	(100)			152	(100)			
Yes		2 (4)				57 (37)			**<0.001**
No		54 (96)				95 (63)			
Cytology	56	(100)			152	(100.0)			
Inadequate		30 (54)				107 (70.0)			**0.002**
Benign		17 (30)				39 (26.0)			
Atypia		9 (16)				4 (2.6)			
Malignant		0 0				2 (1.4)			
Menopause	56	(100)			152	(100)			
Pre		36 (64)				51 (34)			**<0.001**
Post		20 (36)				101 (66)			
Palpable	101	(100)			107	(100)			
Yes		10 (10)				46 (43)			
No		91 (90)				61 (57)			
		Mean	**SEM**[1]	**Median**		**Mean**	**SEM**[1]	**Median**	
Age	56	46.8	1.5	47.0	152	53.6	1.2	53.0	**0.002**
bFGF(ng/g)[1]	29	12.0	7.1	0.0	72	19.3	8.3	0.0	**0.603**
PSA(ng/g)[1]	40	302.9	100.4	12.5	81	2044.1	782.9	59.1	**0.046**
hK2(ng/g)[1]	31	18.5	7.8	0.0	75	49.6	18.9	0.0	**0.301**
DI[1]	28	1.06	0.0	1.05	38	1.12	0.1	1.1	**0.427**
%DI >2	28	0.0	0.0	0.0	38	1.7	1.5	0.0	**0.719**
SPF[1]	28	6.7	1.9	4.3	38	12.7	3.1	5.3	**0.432**

[1]: bFGF: basic fibroblast growth factor; DI: DNA Index; hK2: human glandular kallikrein 2; ng/g: nanogram of biomarker/gram total protein; PSA: prostate specific antigen; SEM: standard error of the mean; SPF: S phase fraction

[2]: % DI> 2: percentage of epithelial cells with more than twice the normal DNA content as measured by the DNA index

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TABLE 4

Biomarker Expression in Breasts of Women without Pathologic Nipple Discharge (PND) Requiring Breast Biopsy

Value	Cancer +				Cancer −				Total	P value
	N	N (%)			N	N (%)				
Cytology	57	(100.0)			95	(100)			152	**0.066**
Inadequate Benign Atypia Malignant		34 (60.0) 19 (33.0) 2 (3.5) 2 (3.5)				73 (77) 20 (21) 2 (2) 0 (0)				
Menopause	57				95				152	**0.002**
Pre		10 (20)				41 (80)			51	
Post		47 (47)				54 (53)			101	
		Mean	**SEM**[1]	**Median**		**Mean**	**SEM**[1]	**Median**		
Age	57	60.7	1.6	59.0	95	49.3	1.4	49.0		<**0.001**
bFGF(ng/g)1	32	39.2	18.1	3.5	40	3.3	1.8	0.0		**0.004**
PSA(ng/g)1	35	1562	949	20.0	46	2411	1181	197.5		**0.05**
hK2(ng/g)1	35	38.5	23.1	0.0	40	59.4	29.3	3.5		0.59
DI1	17	1.2	0.1	1.1	21	1.1	0.0	1.1		0.27
% DI > 22	17	3.3	3.3	0.0	21	0.4	0.4	0.0		0.25
SPF1	17	16.5	6.0	5.0	21	9.7	2.9	5.6		0.29

[1]: bFGF: basic fibroblast growth factor; DI: DNA Index; hK2: human glandular kallikrein 2; ng/g: nanogram of biomarker/gram total protein; PSA: prostate specific antigen; SEM: standard error of the mean; SPF: S phase fraction

[2]: % DI >2: percentage of cells with more than twice the normal DNA content as measured by the DNA index

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TABLE 5

Summary of Results for Individual Factors as Predictors of Cancer Status[1]

Factor Predicting Cancer	N	Sensitivity	Specificity	PPV[2]	NPV[2]	Accuracy
Palpable	208	57.6	55.0	33.7	76.6	55.8
Pathologic nipple discharge	208	3.4	63.8	3.6	62.5	46.6
Age ≥ 49	208	88.1	54.4	43.3	92.0	63.9
Postmenopause	208	83.1	51.7	40.5	88.5	60.6
Fewer than 4 Pregnancies	208	78.0	28.9	30.3	76.8	42.8
History of BCP Use[2]	207	57.6	22.3	22.8	56.9	32.4
History of HRT[2]	208	55.9	64.4	38.4	78.7	62.0
Family History of Cancer	206	28.8	82.3	39.5	74.2	67.0
Cytologic Atypia	208	5.1	93.3	23.1	71.3	68.3
Cytologic Cancer	208	3.4	100	100	72.3	72.6
PSA ≤ 400 ng/g[2]	121	83.8	33.3	35.6	82.4	48.8
PSA ≤ 120 ng/g[2]						
premenopausal subjects	52	50.0	50.0	11.5	88.5	50.0
postmenopausal subjects	69	87.1	36.8	52.9	77.8	59.4
hK2 ≤ 40 ng/g [2]	106	88.9	20.0	36.4	77.8	43.4
bFGF ng/g [fourth root ≤3 ng/g][2]	101	91.2	1.5	32.0	25.0	31.7
DNA Index (DI) ≥ 1.3	65	5.6	95.7	33.3	72.6	70.8
S Phase Fraction > 2	66	66.7	45.8	31.6	78.6	51.5

[1]: The fraction of false positive samples (1-Specificity) and false negative samples (1-Sensitivity) can be readily calculated. Information was missing regarding birth control use for one subject, and regarding family history of breast cancer for two subjects.

[2]: BCP: birth control pills; bFGF: basic fibroblast growth factor; hK2: human glandular kallikrein 2; HRT: hormone replacement therapy; NPV: negative predictive value; PPV: positive predictive value; PSA: prostate specific antigen

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TABLE 6

Summary of Results for Individual Factors as Predictors of Cancer Status for Breasts of Women without Pathologic Nipple Discharge[1]

Factor Predicting Cancer	N	Sensitivity	Specificity	PPV[1]	NPV[2]	Accuracy
Palpable	152	59.6	40	37.4	62.3	47.4
Age ≥ 49	152	87.7	50.5	51.5	87.3	64.5
Postmenopause	152	82.5	43.2	46.5	80.4	57.9
Fewer than 4 Pregnancies	152	77.2	29.5	39.6	68.3	47.4
History of BCP Use[2]	152	59.6	20	30.9	45.2	34.9
History of HRT[2]	152	54.4	57.9	43.7	67.9	56.6
Family History of Cancer[3]	150	29.8	80.6	48.6	65.2	61.3
Cytologic Atypia	152	3.5	97.9	50	62.8	62.5
Cytologic Cancer	152	3.5	100	100	63.3	63.8
PSA < 401 ng/g[2]	81	85.7	39.1	51.7	78.3	59.3
PSA < 120 ng/g[2]						
premenopausal subjects	27	50.0	66.7	30.0	82.4	63.0
postmenopausal subjects	54	86.2	44.0	64.1	73.3	66.7
hK2 ≤ 45 ng/g[2]	75	88.6	22.5	50	69.2	53.3
bFGF [fourth root ≤ 3 ng/g][2]	72	93.8	0	42.9	0	41.7
DNA Index ≥ 1.3	38	5.9	95.2	50	55.6	55.3
S Phase Fraction > 2	38	64.7	47.6	50	62.5	55.3

[1]: The fraction of false positive samples (1-Specificity) and false negative samples (1-Sensitivity) can be readily calculated.

[2]: BCP: birth control pills; bFGF: basic fibroblast growth factor; hK2: human glandular kallikrein 2; HRT: hormone replacement therapy; NPV: negative predictive value; PPV: positive predictive value; PSA: prostate specific antigen

[3]: Information was missing regarding family history of breast cancer for two subjects.