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C-reactive Protein in Nipple Aspirate Fluid Associated With Gail Model Factors

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Background: The majority of breast cancers originate in the epithelial lining of the breast ductal system. Premalignant cell damage in this lining may produce biochemical signals that deliver inflammatory proteins to the site. The presence of C-reactive protein (CRP) in nipple aspirate fluid (NAF) may reflect an inflammatory state indicative of a premalignant breast microenvironment. This study ascertained CRP's presence in NAF and evaluated if risk factors, as identified by the Gail model, were associated with NAF CRP levels among healthy women. **Design:** NAF CRP levels were assayed in 59 women. **Results:** CRP was present

in NAF and significantly ($p = .04$) and positively related to breast cancer risk as predicted by the Gail model. **Conclusion:** CRP is differentially present in NAF and varies by Gail model risk factors. CRP in NAF holds promise as a noninvasive biomarker that detects a precarcinogenic breast ductal microenvironment and may contribute to the diagnosis of breast cancer early in the course of the disease when prognosis is most favorable.

Key words: biomarkers; breast cancer; C-reactive protein; Gail model; inflammatory proteins; precancerous

Breast cancer is the most commonly diagnosed cancer in women in the United States. Women without a history of breast cancer have a 1 in 8 lifetime risk of developing this disease (American Cancer Society, 2005). Early detection of a precancerous state is key to reducing breast cancer incidence and related morbidity and mortality. Yet mammography—which, along with clinical and self-breast examination, is the screening modality currently available—has poor specificity and sensitivity for precancerous states,

detects changes in the breast only after cellular malignancy has been initiated, and typically fails to identify 20% to 40% of breast cancers (Institute of Medicine, 2000). Clearly, improved diagnostic technologies efficient in the detection of premalignant changes are needed.

Noninvasively acquired nipple aspirate fluid (NAF), a physiological substrate that is present in all patent ductal microenvironments, holds promise as a clinical diagnostic tool to detect precarcinogenic states in epithelial cells (Dooley et al., 2001; Sauter, Ehya, Schlatter, & MacGibbon, 2004; Shao & Nguyen, 2001; Wrensch et al., 2001). The epithelial cell lining of the terminal duct lobular unit (TDLU) operates as a “protein factory” (Romagnolo & DiAugustine, 1994), synthesizing proteins and delivering them into the breast fluids that subsequently bathe the ductal lining (Mallon et al., 2000). As the ductal epithelial cells persist in the proliferative growth associated with carcinogenesis, proteins (and potentially inflammatory

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proteins) become concentrated in this mammary ductal fluid (Sauter, Shan, Hewett, Spookman, & Du Bois, 2005). Furthermore, at the interepithelial cell tight junction, protective serum proteins, such as immunoglobulins, and toxic and mutagenic substances move into the fluid, resulting in a bioactive, pooled substrate reflective of and interacting with the microenvironment (Mallon et al., 2000). At this cell surface, the origin of most breast carcinomas (Mallon et al., 2000; Sukumar & McKenzie, 1996), molecular and cellular changes in the microenvironment stimulate immune processes, resulting in a local inflammatory response (Mallon et al.).

Breast epithelial-cell carcinogenesis typically follows the transformation of the cell by carcinogenic stimuli (Russo et al., 1993). Once a cell has deviated from its normal function, it emits signals recognized as foreign by the inflammatory-response detection mechanisms (Boehm & Hahn, 2005). Several studies have further demonstrated that tumor cells in the breast (Barth, Ebrahimade, Ramaswamy, & Moll, 2002; Chauhan et al., 2003) can induce the inflammatory cascade. Indeed, Eskin (1999) hypothesizes that benign cellular change in the breast intralumen result "from inflammatory or traumatic conditions, notably in connective tissues" (p. 65). These experiments demonstrate that inflammation is related to early carcinogenic changes. Markers of this inflammatory response, then, may be indicative of breast cancer risk.

C-reactive protein (CRP), an acute-phase protein that is synthesized in the liver, circulates routinely in the vasculature at low levels, although a standard normal level remains unknown (Salazar-Mather & Hokeness, 2003). Considered a classic marker for inflammation (Gabay & Kushner, 1999), CRP acts as a surveillance molecule for altered body cells, rapidly elevates 1,000-fold within hours after tissue damage, and remains elevated in the presence of continued inflammation (Abbas & Lichtman, 2001). The protein, a serum biomarker in other cancers (Nielsen, Christensen, Sorensen, Moesgaard, & Brunner, 2000) and an indicator of cancer disease prognosis in some sites, has been associated in serum with breast cancer (Robertson et al., 1991), and increased levels of serum CRP have been detected in more advanced stages of breast malignancy. This finding has led to the proposed use of CRP as a biomarker of disease prognostics (Mortensen & Rudczynski, 1982). The proliferative changes that occur in the ductal epithelium before carcinoma *in situ* are associated with activation of the inflammatory cascade, resulting in

differential levels of CRP pooled in NAF. Therefore, if CRP is quantitatively responsive to the magnitude of early cellular or tissue damage before malignancy, it may be a candidate biomarker of the activation of the inflammatory process in premalignant stages.

Prior research suggests that biochemical analysis of NAF holds promise for detecting premalignant changes in the breast microenvironment and evaluating breast cancer risk. For example, researchers who have studied the protective effect of breastfeeding on breast tissue found anticarcinogenic molecules such as carotenoids in nipple aspirates postlactation (Harding et al., 2000). Other investigators have examined proteomic profiles of ductal fluid to detect a relation between NAF and breast cancer (Hsiung et al., 2002; Varnum et al., 2003). In a 2002 study, Sauter and colleagues determined that NAF contained proteins secreted from ductal and lobular epithelial cells and were able to identify differential expression of NAF proteins from normal breasts and breasts with cancer.

Although in the past NAF research has been impeded by minute samples and low patient tolerance, Varnum and colleagues (2003) recently described a method involving the use of a patented suction apparatus (Covington & Morton, 2003) combined with techniques designed to increase patient acceptability and improve NAF yield. Furthermore, Zangar, Varnum, Covington, and Smith (2004) have developed techniques for proteomic analysis that are effective with minute (less than 5- μ l) samples. Sauter and colleagues (1997) concluded that analysis of NAF is "a promising non-invasive method to identify cellular markers of breast cancer risk" (p. 494).

The purpose of this study is to determine if one such protein, CRP, is present in NAF, which can be obtained noninvasively in an ambulatory or home setting. Furthermore, this study evaluates the relation between the level of CRP in NAF and breast cancer risk as predicted by the Gail model. The aims of this study are as follows: (a) ascertain if CRP is present in NAF, (b) determine the relation between CRP in NAF and that in serum, and (c) evaluate if risk factors, as identified in the Gail model, are associated with the NAF CRP levels among healthy women with no evidence of breast cancer. The hypotheses are that the CRP level in NAF (a) is detectable, (b) has a unique differential presence and does not merely mirror (is not significantly related to) serum CRP, and (c) is positively related to the Gail-model risk score.

Methods

Design

This article describes a descriptive and correlational study evaluating the presence of CRP in NAF and the relation between NAF CRP levels and the Gail model breast cancer risk scores in women from Kenya and Detroit. The Kenyan setting was selected to provide a wider range of variability on reproductive factors that influence breast cancer risk, such as age at first pregnancy, gravidity, and breastfeeding duration in comparison to the U.S. setting. This study is a corollary study to an NAF breast cancer prevention study conducted by one of the coauthors, Chandice Covington, in 2001–2002. It was approved by Wayne State University in Detroit and by the Kenya Medical Research Institute in Kenya.

Participants and Sampling Plan

The parent study focused on healthy women, 30 to 65 years of age, who were not currently pregnant, taking exogenous hormones, or lactating. To be eligible to participate in either the Kenyan or Detroit sample, women could not have any other major chronic diseases or evidence of breast cancer as determined by history and clinical examination.

The U.S. sample was selected from an existing volunteer pool of women previously identified by the Community Outreach Core through the Karmanos Cancer Institute in Detroit. Kenyan participants were recruited by local community nurses from two villages in coastal Kenya and the town of Mombasa after being informed of the study by flyer advertising and referral.

This corollary study used 59 samples from the parent study in which research nurse time allowed for sufficient NAF samples to be obtained to complete additional analysis for the variables of interest. Volume of total NAF ranged from 5 μ l to 100 μ l, although this is not a true indication of potential NAF volume, as time availability for the aspirations was constrained in both settings. A total of 39 samples from Detroit and 20 from Kenya were analyzed. Because of evolving laboratory analysis techniques evaluating small fluid samples, 36 cases were analyzed using milliliters (ml) of NAF and 26 using milligrams (mg) of NAF, totaling 62 analyses. Three of these cases were redundantly analyzed using both ml and mg, which explains the disparity between the number of cases analyzed and the number of NAF samples analyzed (i.e., 59).

Participants ranged in age from 31 to 64 years, with a mean age of 44; there was no significant difference between the settings for age, as shown in Table 1. Education, parity, and length of time breastfeeding all differed significantly between the two settings, with Detroit women being more educated on average by 7 years and Kenyan women having significantly more pregnancies and time spent breastfeeding. The Kenyan group was 100% African; the Detroit group was 34% African American and 66% Caucasian.

Measurements

The Gail model. Currently, the only available breast cancer risk prediction tool for an individual woman, the Gail model, is not a physiologic tool but rather an epidemiologic one. It was developed by Gail and colleagues at the National Cancer Institute in 1989 and uses statistical methods applied to data from the Breast Cancer Detection and Demonstration Project, a mammography screening project conducted in the 1970s and 1980s, to predict breast cancer risk (Gail et al., 1989). The Gail model is used to quantify an individual woman's risk of developing breast cancer based on her personal and family medical history.

The original Gail model, which predicts risk of in situ and invasive cancer in White women, was reformulated to include risk of invasive cancer by race, and researchers currently use this version. Both versions were used in the Breast Cancer Prevention Trials (Bondy, Lustbader, Halabi, Ross, & Vogel, 1994), and predictive validity was substantiated for risk of breast cancer occurrence in 1-year, 5-year, and lifetime scenarios (Bondy et al., 1994; Costantino et al., 1999; Spiegelman, Colditz, Hunter, & Hertzmark, 1994). The model uses five significant predictors of a woman's lifetime breast cancer risk (current age, age at menarche, number of breast biopsies, age at first live birth, and number of first-degree relatives with breast cancer) to yield a woman's cumulative breast cancer risk during the next 5 years and a lifetime risk-prediction score.

This tool is the sole risk-prediction tool available that has established reliability and validity for assessing a woman's individual risk of breast cancer (Bondy et al., 1994; Costantino et al., 1999; Spiegelman et al., 1994). The model has been criticized for not including the protective effects of cumulative pregnancies and months of lactation in the calculations (Euhus, Leitch, Huth, & Peters, 2002; Fentiman,

Table 1. Comparison of the Two Participant Groups: U.S. (Detroit) Participants and Kenyan Participants

Variable	<i>M</i> ± <i>SD</i>	Student's <i>t</i>	<i>p</i>
Age (years)			
Combined	44.0 ± 8.9		
Detroit	43.0 ± 5.9		
Kenya	45.0 ± 6.8	-38.55	.156
Gravidity			
Combined	4.7 ± 3.1		
Detroit	4.5 ± 1.2		
Kenya	7.4 ± 2.9	-4.93	.043
Age at first pregnancy (years)			
Combined	23.3 ± 4.4		
Detroit	24.6 ± 5.3		
Kenya	18.2 ± 3.6	-37.16	.001
Cumulative breastfeeding (months)			
Combined	49.2 ± 59.5		
Detroit	18.0 ± 31.1		
Kenya	102.0 ± 60.4	-31.21	.001
Time since weaning last baby (years)			
Combined	20.6 ± 13.5		
Detroit	25.6 ± 14.5		
Kenya	18.7 ± 13.1	-10.48	.001

NOTE: Significance levels correspond to Student's *t* test for the Detroit and Kenya independent samples, *df* = 57.

2001; Key, Verkasalo, & Banks, 2001). Rockhill, Spiegelman, Byrne, Hunter, and Colditz (2001) studied 1,354 breast cancer patients to evaluate the Gail model's predictive ability and found agreement between observed (*O*) and expected (*E*) cancers with an overall *E/O* ratio of 0.94 (95% confidence interval equal to 0.89–0.99) in the population studied. In the current study, the Health Factor Interview, developed by the parent study team, was conducted by trained research nurses to obtain historical personal and medical data related to diet, exercise, lifestyle, and social and behavior information as well as the gynecologic and obstetric data needed to ascertain risk using the Gail model.

CRP assays. Serum was collected by venipuncture and was analyzed for CRP levels. Blood samples were centrifuged immediately after collection, and the plasma was withdrawn and stored in amber microtubes at -70°C . Serum CRP was assessed with a standard CRP ELISA kit (KMI Diagnostics) by Dr. Richard Zangar's laboratory at Pacific Northwest National Laboratory in Richland, Washington.

The NAF was collected and stored in microtubes at -70°C . Analysis was performed with a protein assay protocol for small samples that was developed by Dr. Zangar's laboratory using the standard CRP ELISA

kit. The protocol was adapted for clinical laboratory use by modifying it to address the small volumes of the NAF samples and to achieve reasonable quantitative detection of low levels ($<1\ \mu\text{g/ml}$) of CRP in the NAF samples. Briefly, these modifications include diluting the samples and determining the protein concentration. The protein concentration was then used to normalize sample loading. In addition, the standard curve for the CRP ELISA was modified to better differentiate values in the low CRP ranges, as were expected in the NAF samples. The first-run analysis of NAF CRP was based on protein concentration in micrograms (μg) of CRP per milligrams (mg) of NAF, whereas a later set of analyses used μg of CRP per ml of NAF because of evolving laboratory-analysis techniques to evaluate small fluid samples.

Procedure

Because of the high rate of illiteracy in Kenya, the research nurse read the consent form to each potential Kenyan participant in her language to ensure comprehension. The nurse then documented that the woman agreed to participate, and the participant signed her name or mark. In the United States, the research nurse obtained written informed consent from each participant.

Research nurses in both settings conducted the Health Factor Interview with each participant to determine breast cancer risk factors for the Gail model assessment. A research nurse practitioner then conducted a clinical breast examination to rule out obvious clinical evidence of breast disease.

NAF was collected from participants using an aspirator and the method developed by Covington and Morton (2003). Past studies established the ability of a research team to successfully aspirate NAF in 80% of participants from a general population of healthy, low-risk women ages 18 to 65 (Covington, Mitchell-Gielegem, Lawson, Ito, & Grubbs, 2001; Varnum et al., 2003). The technique includes the use of heat, massage, and gentle aspiration.

In Kenya, the NAF was collected in a community health center clinic. In Detroit, it was collected in the lab setting located in the Karmanos Cancer Center. The Gail model assessment was conducted and the risk score calculated via the National Institutes of Health (NIH) computer program based on the data collected from the Health Factors Interview (National Institutes of Health, 2005).

Data Transformation and Analysis

Data were entered into a spreadsheet, cleaned, and examined for normal distribution. NAF and serum CRP data were normally distributed; however, the Gail risk score data were not, having a slight tail to the right. We hypothesized a positive relation between the variables of interest based on the literature on inflammation and breast pathology, and therefore, we chose a bivariate correlation using a one-tail analysis to conduct the analysis, with an a priori alpha level set at .05. All analyses were performed using Statistical Package for the Social Sciences (SPSS) version 14.0 software.

To establish a standard score across values of NAF CRP measured in mg or ml of NAF, we determined an adjusted CRP value for all cases in consistent ml of NAF units. First, because there were 3 cases measured in both units, a prediction equation was generated, predicting ml from mg. Based on these 3 cases, the conversion factor for generating the value of μg of CRP per ml of NAF was calculated at 0.11947 ($\text{mg of NAF} \times 0.11947 = \text{ml of NAF}$). This adjusted score was generated for all 26 cases in which the original analysis used mg of NAF, resulting in 59 cases for analysis. Table 2 presents the values for serum CRP and the original and adjusted CRP NAF values. One NAF sample had an

extreme value (Case 536) at 6.2 μg of CRP per mg of NAF and was treated as an outlier. This value was winsorized to 0.1 above the next highest value, which resulted in the 6.2 being recoded to a 1.6. Nonparametric correlation using Spearman's rho was conducted because of the distribution of the Gail risk-score data.

Results

The first hypothesis, that CRP is present and detectable in NAF, was supported for all cases, with a mean of 0.0701 $\mu\text{g}/\text{ml}$ for the entire sample (range = 0.0004–0.2355; median = 0.0524; see Table 2). The difference between the mean amounts of CRP in NAF between the groups was statistically significant, as determined by Student's *t* test ($t = 20.519$, $df = 57$, $p = .001$, two-tailed), with the U.S. participants having a higher mean (Table 3). The difference between the means by race (African + African American vs. Caucasian) was also significant ($t = 20.66$, $df = 57$, $p = .001$, two-tailed).

The second hypothesis predicted a differential presence of CRP in NAF that did not merely reflect serum CRP levels. The mean serum CRP level was 2.23 μg with a range of 0 to 10.3 and a median of 1.3 μg . Serum CRP is normally reported by reference laboratories as less than 5 $\mu\text{g}/\text{ml}$. As shown in Table 3, the mean serum CRP levels, as opposed to the mean NAF CRP levels, were not significantly different by site or race. Serum CRP was not significantly related to NAF CRP ($r = .143$).

The third hypothesis predicted a positive relation between NAF CRP levels and Gail risk score. A positive relation was evident between the NAF CRP values and the 5-year Gail risk scores ($r = .28$, $p = .04$) and between the CRP values and the lifetime Gail risk scores ($r = .23$, $p = .04$).

Discussion

The majority of malignant breast lesions arise from the intraluminal epithelial cells (Sukumar & McKenzie, 1996). This mammary microenvironment is thus of interest in the search for early biomarkers of malignancy in the breast. The findings from our study suggest that CRP may have a future role in this capacity. The presence of CRP in the NAF from the breast ductal microenvironment is a significant finding in that this acute-phase protein is not believed to be generated and released until after activation of the

Table 2. C-reactive Protein (CRP) Levels in Serum and Nipple Aspirate Fluid (NAF) in Original Units and Levels of NAF CRP Adjusted to Standardize Units to $\mu\text{g/ml}$

ID	CRP Serum $\mu\text{g/ml}$	CRP NAF $\mu\text{g/ml}$	CRP NAF $\mu\text{g/mg}$	CRP NAF $\mu\text{g/ml}$ Adjusted	ID	CRP Serum $\mu\text{g/ml}$	CRP NAF $\mu\text{g/ml}$	CRP NAF $\mu\text{g/mg}$	CRP NAF $\mu\text{g/ml}$ Adjusted
2	1.3	0.0094		0.0094	66	1.9	0.0958		0.0958
3	2.3	0.1481		0.1481	67	0.9	0.0660		0.0660
5		0.0415		0.0415	70	0.9	0.1032		0.1032
10	4.0	0.0912		0.0912	72	1.2	0.0492		0.0492
12	1.5	0.0410		0.0410	73		0.0505		0.0505
13	1.3	0.0524		0.0524	74	4.3	0.1138	1.0	0.1138
15		0.0531		0.0531	75	3.6	0.0973		0.0973
19	2.7	0.0816		0.0816	502	9.5		0.5	0.0597
20	1.1	0.0004		0.0004	504	1.5		0.3	0.0358
22	0.6	0.0300		0.0300	505	1.6		0.4	0.0478
24	0	0.0351		0.0351	508	1.1		0.2	0.0239
25	10.3	0.1245		0.1245	509			0.6	0.0717
26	0.6	0.1382		0.1382	510	1.0		0.3	0.0358
28	1.6	0.0963	0.4	0.0963	512			0.2	0.0239
30	2.2	0.0903		0.0903	514	5.5		1.5	0.1792
33		0.0945		0.0945	517	0.3		0.3	0.0358
35	0.7	0.0505		0.0505	519	4.3		0.7	0.0836
36	0.3	0.0775		0.0775	520	0.9		0.2	0.0239
37	0.5	0.1686		0.1686	524	0.3		0.3	0.0358
39	0.6	0.0312		0.0312	525	0.9		0.2	0.0239
41	0.8	0.0698		0.0698	526	0.7		0.5	0.0597
44	0.9	0.0876	0.7	0.0876	528	6.2		0.4	0.0478
46	5.2	0.2355		0.2355	530			0.3	0.0358
48	0	0.1153		0.1153	531	0		0.2	0.0239
49	2.6	0.0197		0.0197	533	6.8		0.9	0.1075
51		0.1108		0.1108	535	0		0.3	0.0358
53	7.7	0.1101		0.1101	536	5.1		6.2 ^a	0.7407 ^a
62	1.0	0.0312		0.0312	537	1.0		0.2	0.0239
63	1.6	0.0454		0.0454	539	1.4		0.3	0.0358
					544	1.4		0.3	0.0358

a. Outlier was winsorized for analysis to 1.6, with a corresponding 0.1912 μg CRP/ml NAF adjusted.

Table 3. Comparison of Levels of CRP in NAF and Serum by Site and Race

Variable	$M \pm SD$	Student's t	p
CRP in NAF by site			
Detroit	0.0790 \pm 0.0492		
Kenya	0.0560 \pm 0.0469	20.519	.001
CRP in NAF by race			
African + African American	0.0530 \pm 0.0451		
Caucasian	0.0850 \pm 0.4870	20.662	.001
CRP in serum by site			
Detroit	2.2130 \pm 2.2700		
Kenya	2.2190 \pm 2.3600	0.086	.932
CRP in serum by race			
African + African American	2.2140 \pm 2.0900		
Caucasian	2.3460 \pm 2.4110	-2.484	.170

NOTE: Significance levels correspond to Student's t test for independent samples, $df = 57$.

inflammatory cascade. Furthermore, this protein's presence in NAF did not simply reflect its presence systemically in this sample, as serum CRP was not significantly related to NAF CRP.

Recent research has demonstrated that there are, indeed, extrahepatic sites of CRP synthesis—for example, in the smooth-muscle cells of diseased coronary artery bypass grafts (Jabs, Theissing, et al., 2003), in the human coronary artery (Calabro, Willerson, & Yeh, 2003), in plaques found in coronary artery disease (Yasojima, Schwab, McGeer, & McGeer, 2001), and in lung epithelium (Gould & Weiser, 2001). In a 2003 study investigating extrahepatic production of CRP, Jabs, Logering, and colleagues (2003) assessed CRP in the serum and urine of kidney-transplant patients. With *in vivo* and *in vitro* experimentation, they determined that during transplant rejection, the urinary CRP did not correlate with serum CRP values, thus concluding that the kidney allografts were the source of CRP production and not the distant hepatic cells. Furthermore, Wei and colleagues (2001) experimentally demonstrated CRP synthesis in the rat lacrimal and salivary glands—apocrine glands working like the breast apocrine unit. Therefore, it is plausible that extrahepatic mechanisms result in CRP synthesis at the distal site of the breast epithelium. Mammary ductal epithelial cell production of CRP, in the presence of premalignant changes, may be a local inflammatory defense mechanism. Whereas the mammary cell in lactogenesis produces varied proteins (Romagnolo & DiAugustine, 1994), little is known about the dormant breast's capability to synthesize proteins. However, Varnum and colleagues (2003) report 64 proteins residing in the NAF of nonlactating women with no known breast cancer disease.

The relation between the levels of CRP in the NAF and 5-year Gail risk scores reflects a general schema that risk is reflected by an internal state of inflammatory changes in the mammary microenvironment. CRP presence in the NAF may thus reflect the early development of proliferative changes in the ductal epithelium before carcinoma *in situ* and invasive carcinoma. Based on this relation, further research to validate NAF CRP as a noninvasive marker of the breast microenvironment is warranted. Among important additional evaluative steps would be establishing predictive validity between NAF CRP obtained noninvasively and confirmation via pathology of hyperplasia or cancer tissue removed during fine-needle biopsy, tissue biopsy, or surgery.

The Gail risk score is currently the only breast cancer risk prediction tool available to clinicians, and it does not include data on biological specimens. Perhaps documentation of inflammatory proteins, specifically CRP, could be used to augment the predictive power of the current Gail model with an additional predictive factor. In a 2005 prospective observational study, Tice and colleagues evaluated epithelial cells in 6,904 NAF samples for premalignant cytological changes. Breast cancer cases were identified through follow-up and cancer-registry linkage. These researchers added the premalignant cytologic variable to the set of Gail-model predictor variables for calculating breast cancer risk and created a second model of prediction of risk. They concluded that the addition of NAF cytology had the potential to improve the Gail prediction model of breast cancer incidence. The addition of biochemical markers such as CRP, in which changes occur before cytologic changes (Black, Kushner, & Samols, 2004), could further improve the prediction potential of this model.

Further research with different populations and larger sample sizes must be completed before it can be determined that NAF evaluation provides reliable information about premalignant shifts in the ductal milieu. In addition, the significant differences in levels of NAF CRP by setting and race found in the current study may reflect reproductive or racial differences and require further investigation with a sample large enough to have adequate power to address these possible mediating and moderating influences on NAF CRP.

The detection of premalignant changes based on the presence of inflammatory proteins is analogous to the Pap-test detection of premalignant changes of cervical cells and could become a clinical screening tool. The identification of protein biomarkers for breast cancer risk would allow health care providers to increase surveillance of the women identified by this method and might lead to prophylactic treatment (Tice et al., 2005). Such a novel and promising screening tool developed to augment the current procedures, namely, mammography, clinical breast exam, and self-breast exam, has the potential to extend the sensitivity and specificity of diagnostic strategies to promote women's health and prevent the sequelae of late-diagnosed breast cancer.

References

- Abbas, A., & Lichtman, A. (2001). *Basic immunology: Functions and disorders of the immune system*. Philadelphia, PA: W. B. Saunders.

- American Cancer Society. (2005). *Cancer facts and figures 2005*. New York: Author.
- Barth, P. J., Ebrahimade, S., Ramaswamy, A., & Moll, R. (2002). CD34+ fibrocytes in invasive ductal carcinoma, ductal carcinoma in situ and benign breast lesions. *Virchows Archive*, 440(3), 298-303.
- Black, S., Kushner, I., & Samols, D. (2004). C-reactive protein. *Journal of Biological Chemistry*, 279, 48487-48490.
- Boehm, J. S., & Hahn, W. C. (2005). Understanding transformation: Progress and gaps. *Current Opinion in Genetics and Development*, 15, 13-17.
- Bondy, M., Lustbader, E., Halabi, S., Ross, E., & Vogel, V. (1994). Validation of a breast cancer risk assessment model in women with a positive family history. *Journal of the National Cancer Institute*, 86, 620-625.
- Calabro, P., Willerson, J. T., & Yeh, E. T. H. (2003). Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells. *Circulation*, 108, 1930-1932.
- Chauhan, H., Abraham, A., Phillips, J. R., Pringle, J. H., Walker, R. A., & Jones, J. L. (2003). There is more than one kind of myofibroblast: Analysis of CD34 expression in benign, in situ, and invasive breast lesions. *Journal of Clinical Pathology*, 56(4), 271-276.
- Costantino, J., Gail, M., Pee, D., Anderson, S., Redmond, C., & Benichou, J. (1999). Validation studies for models projecting the risk of invasive and total breast cancer incidence. *Journal of the National Cancer Institute*, 91, 1541-1548.
- Covington, C., Mitchell-Gielegem, A., Lawson, D., Ito, I., & Grubbs, C. (2001). Presence of carotenoid, an anti-carcinogenic marker, in nipple aspirates postlactation. In D. S. Newburg (Ed.), *Bioactive components of human milk* (pp. 143-152). New York: Kluwer Academic, Plenum Publishers.
- Covington, C. Y., & Morton, K. (2003). Nipple fluid aspiration method and aspirator cup. (U.S. Patent No.: 6471660; 6517513).
- Dooley, W. C., Ljung, B. -M., Veronesi, U., Cazzaniga, M., Elledge, R. M., O'Shaughnessy, J. A., et al. (2001). Ductal lavage for detection of cellular atypia in women at high risk for breast cancer. *Journal of the National Cancer Institute*, 93, 1624-1632.
- Eskin, B. A. (1999). Fibrocystic disease of the breast. In B. A. Eskin, S. Asbellm, & L. Jardimem (Eds.), *Breast disease for primary care physicians* (pp. 65-73). New York: Parthenon.
- Euhus, D. M., Leitch, A. M., Huth, J. F., & Peters, G. N. (2002). Limitations of the Gail model in the specialized breast cancer risk assessment clinic. *Breast Journal*, 8, 23.
- Fentiman, I. S. (2001). Fixed and modifiable risk factors for breast cancer. *Current Opinion in Oncology*, 13, 420-425.
- Gabay, C., & Kushner, I. (1999). Acute-phase proteins and other systemic responses to inflammation. *New England Journal of Medicine*, 340, 448-454.
- Gail, M., Brinton, L., Byar, D., Corale, D. K., Green, S. B., Schairer, C., et al. (1989). Projecting individualized probabilities of developing breast cancer for White females who are being examined annually. *Journal of the National Cancer Institute*, 81, 1879-1886.
- Gould, J. M., & Weiser, J. N. (2001). Expression of C-reactive protein in the respiratory tract. *Infection and Immunity*, 69, 1747-1754.
- Harding, C., Osundeko, O., Tetlow, L., Faragher, E. B., Howell, A., & Bundred, N. J. (2000). Hormonally regulated proteins in breast secretions have markers of target organ sensitivity. *British Journal of Cancer*, 82, 345-360.
- Hsiung, R., Zhu, W. Z., Klein, G., Qin, N. Y., Rosenberg, A., Park, P., et al. (2002). High basic fibroblast growth factor levels in nipple aspirate fluid are correlated with breast cancer. *Cancer Journal*, 8, 303-310.
- Institute of Medicine. (2000). *Developing technologies for early detection of breast cancer*. Washington, DC: IOM Committee for the Early Detection of Breast Cancer Workshop.
- Jabs, W. J., Logering, B. A., Gerke, P., Kreft, B., Wolber, E. M., Klinger, M. H. F., et al. (2003). The kidney as a second site of human C-reactive protein formation in vivo. *European Journal of Oncology*, 33, 152-161.
- Jabs, W. J., Theissing, E., Nitschke, M., Bechtel, J. F. M., Duchrow, M., & Mohamed, S. (2003). Local generation of C-reactive protein in diseased coronary artery venous bypass grafts and normal vascular tissue. *Circulation*, 108, 1428-1431.
- Key, T. J., Verkasalo, P. K., & Banks, E. (2001). Epidemiology of breast cancer. *Lancet*, 358, 1389-1399.
- Mallon, E., Osin, P., Nasiri, N., Blain, I., Howard, B., & Gusterson, B. (2000). The basic pathology of human breast cancer. *Journal of Mammary Gland Biology*, 5, 139-154.
- Mortensen, R. F., & Rudczynski, A. B. (1982). Prognostic significance of serum CRP levels and lymphoid cell infiltrates in human breast cancer. *Oncology*, 39(3), 129-133.
- National Institutes of Health. (2005). *National Cancer Institute: The Gail Model Breast Cancer Risk Assessment Tool*. Accessed May 3, 2005, at <http://bcra.nci.nih.gov/brc/q1.htm>
- Nielsen, H. J., Christensen, I. J., Sorensen, S., Moesgaard, F., & Brunner, N. (2000). Preoperative plasma plasminogen activator inhibitor type-1 and serum C-reactive protein levels in patients with colorectal cancer. *Annals of Surgical Oncology*, 7, 617-623.
- Robertson, J. F. R., Pearson, D., Price, M. R., Selby, C., Pearson, J., Blamey, R. W., et al. (1991). Prospective assessment of the role of 5 tumor markers in breast cancer. *Cancer Immunology, Immunotherapy*, 33, 403-410.
- Rockhill, B., Spiegelman, D., Byrne, C., Hunter, D. J., & Colditz, G. A. (2001). Validation of the Gail et al. model of breast cancer risk prediction and implications for chemoprevention. *Journal of the National Cancer Institute*, 93, 358-366.

- Romagnolo, D., & DiAugustine, R. P. (1994). The mammary gland: Protein factory of the future. *Environmental Health Perspectives*, 102, 644-646.
- Russo, J., Calaf, N., Sohi, N., Tahin, Q., Zhang, P. L., Alvarado, M. E., et al. (1993). Critical steps in breast carcinogenesis. *Annals of the New York Academy of Sciences*, 698, 1-20.
- Salazar-Mather, T., & Hokeness, K. (2003). Calling in the troops: Regulation of inflammatory cell trafficking through innate cytokine/chemokine networks. *Viral Immunology*, 16, 291-306.
- Sauter, E. R., Ehya, H., Schlatter, L., & MacGibbon, B. (2004). Ductoscopic cytology to detect breast cancer. *Cancer Journal*, 10(1), 33-41.
- Sauter, E. R., Ross, E., Day, M., Klein-Szanto, A., Engstrom, P. F., Sorling, A., et al. (1997). Nipple aspirate fluid: A promising non-invasive method to identify cellular markers of breast cancer risk. *British Journal of Cancer*, 76, 494-501.
- Sauter, E. R., Shan, S., Hewett, J. E., Spookman, P., & Du Bois, G. C. (2005). Proteomic analysis of nipple aspirate fluid using SELDI-TOF-MS. *International Journal of Cancer*, 114, 791-796.
- Sauter, E. R., Zhu, W., Fan, X. J., Wassell, R. P., Chervoneva, I., & Du Bois, C. G. (2002). Proteomic analysis of nipple aspirate fluid to detect biologic markers of breast cancer. *British Journal of Cancer*, 86, 1440-1443.
- Shao, Z., & Nguyen, M. (2001). Nipple aspiration in diagnosis of breast cancer. *Seminars in Surgical Oncology*, 20, 175-180.
- Spiegelman, D., Colditz, G., Hunter, D., & Hertzmark, E. (1994). Validation of the Gail et al. model for predicting individual breast cancer risk. *Journal of the National Cancer Institute*, 86, 600-607.
- Sukumar, S., & McKenzie, K. (1996). Breast cancer prevention strategies for the twenty-first century. *Molecular Medicine Today*, 2, 453-459.
- Tice, J. A., Miike, R., Adduci, K., Petrakis, N. L., King, E., & Wrensch, M. R. (2005). Nipple aspirate fluid cytology and the Gail model for breast cancer risk assessment in a screening population. *Cancer Epidemiology Biomarkers & Prevention*, 14, 324-328.
- Varnum, S. M., Covington, C. C., Woodbury, R. L., Petris, K., Kangas, L. J., Abdullah, M. S., et al. (2003). Proteomic characterization of nipple aspirate fluid: Identification of potential biomarkers of breast cancer. *Breast Cancer Research and Treatment*, 80(1), 87-97.
- Wei, W., Parvin, M. N., Tsumura, K., Akamatsu, T., Tada, J., Kanamori, N., et al. (2001). Induction of C-reactive protein, serum amyloid P component, and kininogens in the submandibular and lacrimal glands of rats with experimentally induced inflammation. *Life Sciences*, 69, 359-368.
- Wrensch, M., Petrakis, N., Miike, R., King, E. B., Chew, K., Neuhaus, J., et al. (2001). Breast cancer risk in women with abnormal cytology in nipple aspirates of breast fluid. *Journal of the National Cancer Institute*, 93, 1791-1798.
- Yasojima, K., Schwab, C., McGeer, E. G., & McGeer, P. L. (2001). Generation of C-reactive protein and complement components in atherosclerotic plaques. *American Journal of Pathology*, 158, 1039-1051.
- Zangar, R., Varnum, S. M., Covington, C. Y., & Smith, R. D. (2004). A rational approach for discovering and validating cancer markers in very small samples using mass spectrometry and ELISA microarrays. *Disease Markers*, 20(3), 135-148.