Association of Abnormal Nipple Aspirate Cytology and Mammographic Pattern and Density

Marion M. Lee, Nicholas L. Petrakis, Margaret R. Wrensch, Eileen B. King, Rei Miike, and Edward Sickles
Department of Epidemiology and Biostatistics, School of Medicine, University of California, San Francisco, San Francisco, CA 94143-0560

Abstract
The pattern and density of mammograms have been shown to be associated with proliferative histopathology and an increased risk of breast cancer. We recently reported the risk of breast cancer among women from whom no fluid could be obtained, and had three times greater risk than women with normal cytology (8). A subsequent analysis of these data showed that the increased risk was strongly associated with prior benign biopsy (9). Our findings of an association of breast cancer risk with cytological epithelial atypia, coupled with studies by others indicating a relationship of breast cancer risk and mammographic readings, stimulated us to investigate whether an association exists between atypia in NAF3 and the pattern and density of mammograms.

Introduction
An association between the P2 and DY mammographic patterns and severe proliferative epithelial changes in breast biopsies was reported by Wellings and Wolfe (1). They found that the proportion with high grade atypical lobules and the degree of fibrosis increased with severity of mammographic classification (i.e., Wolfe scales N1, P1, P2, to DY). These findings were confirmed by a number of other investigators who also found that mammographic parenchymal patterns (2–5) and mammographic densities (3, 6, 7) were associated with proliferative histopathology and indicative of breast cancer risk, particularly among premenopausal women. We recently reported the risk of breast cancer in a cohort of 2343 women who underwent nipple aspiration cytology 10–18 years earlier. Women with nipple aspirate cytological diagnoses of atypia had five times higher risk of breast cancer than women from whom no fluid could be obtained, and had three times greater risk than women with normal cytology (8). A subsequent analysis of these data showed that the increased risk was strongly associated with prior benign biopsy (9).

Methods
A total of 2712 women between the ages of 25 and 65 came to the University of California San Francisco mammography clinic between 1988 and 1990. This clinic is a referral, not a screening mammography, center. Women were excluded from the study either because they had previous breast cancer (n = 437) or had language problems (n = 146). Of the 2129 eligible women contacted, 1134 (53%) volunteered for the study. Reasons for refusal were anxiety about undergoing mammographic examination, disinterest in filling out the brief (20 min) questionnaire, or concern about the nipple aspiration procedure.

Participants completed a self-administered, structured questionnaire asking their age, ethnicity, menopausal status, age at menarche, oral contraceptive use, breast feeding patterns, parity, history of tobacco and alcohol use, weight, height, and first-degree family history (mother, sister, daughter) of breast cancer.

Nipple aspiration was attempted following mammography using the nipple aspirator and techniques previously described (8, 10). Fluid was collected from the surface of the nipple and prepared for cytological examination using methods described by King et al. (11). We obtained nipple aspirate fluid adequate for cytological evaluation from 463 of the 1134 women (41%). Cytological diagnoses were reported as either normal, epithelial hyperplasia, or atypia.

To examine the association of mammographic and cytological findings, we compared mammograms from all...
women who yielded nipple aspirate fluid with cytological diagnoses \((n = 463)\) with a sample of those from whom no NAF could be obtained \((n = 125/671)\). Women who did not yield fluid were frequency-matched to the women with cytological findings by age \((\pm 5\text{ years})\), parity \((\text{yes}/\text{no})\), and weight \((\pm 20\text{ lb})\).

All mammograms were reviewed blindly as to cytology outcome in a standardized fashion by Dr. Edward Sickles, and were classified by Wolfe parenchymal patterns as N1, P1, P2, DY, and by the percentage area of density \((<25\%, 25-49\%, 50-74\%, \geq75\%)\) \((6)\). In view of the relatively small sample size, the mammographic diagnoses were collapsed into two groups, low and high density \((\text{i.e.}, \text{areas of density less than and greater than or equal to 50\%}, \text{respectively})\).

Bivariate analyses were performed to examine the association between breast fluid cytologic findings and individual breast cancer risk factors, including mammographic patterns, density, and calcifications. We similarly examined associations of other breast cancer risk factors with mammographic readings to control for possible confounding.

We computed multivariate logistic regression analysis to estimate odds ratios for those factors associated with both cytological and mammographic findings by age \((\pm 5\text{ years})\), parity \((\text{yes}/\text{no})\), and weight \((\pm 20\text{ lb})\). All mammograms were reviewed blindly as to cytology outcome in a standardized fashion by Dr. Edward Sickles, and were classified by Wolfe parenchymal patterns as N1, P1, P2, DY, and by the percentage area of density \((<25\%, 25-49\%, 50-74\%, \geq75\%)\) \((6)\). In view of the relatively small sample size, the mammographic diagnoses were collapsed into two groups, low and high density \((\text{i.e.}, \text{areas of density less than and greater than or equal to 50\%}, \text{respectively})\).

Bivariate analyses were performed to examine the association between breast fluid cytologic findings and individual breast cancer risk factors, including mammographic patterns, density, and calcifications. We similarly examined associations of other breast cancer risk factors with mammographic readings to control for possible confounding.

We computed multivariate logistic regression analysis to estimate odds ratios for those factors associated with both cytological and mammographic findings by age \((\pm 5\text{ years})\), parity \((\text{yes}/\text{no})\), and weight \((\pm 20\text{ lb})\). All mammograms were reviewed blindly as to cytology outcome in a standardized fashion by Dr. Edward Sickles, and were classified by Wolfe parenchymal patterns as N1, P1, P2, DY, and by the percentage area of density \((<25\%, 25-49\%, 50-74\%, \geq75\%)\) \((6)\). In view of the relatively small sample size, the mammographic diagnoses were collapsed into two groups, low and high density \((\text{i.e.}, \text{areas of density less than and greater than or equal to 50\%}, \text{respectively})\).

Bivariate analyses were performed to examine the association between breast fluid cytologic findings and individual breast cancer risk factors, including mammographic patterns, density, and calcifications. We similarly examined associations of other breast cancer risk factors with mammographic readings to control for possible confounding.

We computed multivariate logistic regression analysis to estimate odds ratios for those factors associated with both cytological and mammographic findings by age \((\pm 5\text{ years})\), parity \((\text{yes}/\text{no})\), and weight \((\pm 20\text{ lb})\). All mammograms were reviewed blindly as to cytology outcome in a standardized fashion by Dr. Edward Sickles, and were classified by Wolfe parenchymal patterns as N1, P1, P2, DY, and by the percentage area of density \((<25\%, 25-49\%, 50-74\%, \geq75\%)\) \((6)\). In view of the relatively small sample size, the mammographic diagnoses were collapsed into two groups, low and high density \((\text{i.e.}, \text{areas of density less than and greater than or equal to 50\%}, \text{respectively})\).

Bivariate analyses were performed to examine the association between breast fluid cytologic findings and individual breast cancer risk factors, including mammographic patterns, density, and calcifications. We similarly examined associations of other breast cancer risk factors with mammographic readings to control for possible confounding.

We computed multivariate logistic regression analysis to estimate odds ratios for those factors associated with both cytological and mammographic findings by age \((\pm 5\text{ years})\), parity \((\text{yes}/\text{no})\), and weight \((\pm 20\text{ lb})\). All mammograms were reviewed blindly as to cytology outcome in a standardized fashion by Dr. Edward Sickles, and were classified by Wolfe parenchymal patterns as N1, P1, P2, DY, and by the percentage area of density \((<25\%, 25-49\%, 50-74\%, \geq75\%)\) \((6)\). In view of the relatively small sample size, the mammographic diagnoses were collapsed into two groups, low and high density \((\text{i.e.}, \text{areas of density less than and greater than or equal to 50\%}, \text{respectively})\).

Bivariate analyses were performed to examine the association between breast fluid cytologic findings and individual breast cancer risk factors, including mammographic patterns, density, and calcifications. We similarly examined associations of other breast cancer risk factors with mammographic readings to control for possible confounding.

We computed multivariate logistic regression analysis to estimate odds ratios for those factors associated with both cytological and mammographic findings by age \((\pm 5\text{ years})\), parity \((\text{yes}/\text{no})\), and weight \((\pm 20\text{ lb})\). All mammograms were reviewed blindly as to cytology outcome in a standardized fashion by Dr. Edward Sickles, and were classified by Wolfe parenchymal patterns as N1, P1, P2, DY, and by the percentage area of density \((<25\%, 25-49\%, 50-74\%, \geq75\%)\) \((6)\). In view of the relatively small sample size, the mammographic diagnoses were collapsed into two groups, low and high density \((\text{i.e.}, \text{areas of density less than and greater than or equal to 50\%}, \text{respectively})\).
Therefore, we limited the number of variables in the model to include age, previous biopsy, BMI, calcification, and mammographic density (Table 5). In multivariate analyses, women with high density mammograms were 4.4 times more likely to have cytological atypia than women with low density mammograms (95% CI, 0.9–21.5; P < 0.01). The CI was wide because of small numbers (i.e., 17 cases of atypia).

Although the logistic analysis indicates an independent effect of BMI on cytology, in which women with a BMI greater than 25 were three times more likely to have cytological atypia in their breast fluid than were leaner women, the associations of atypia, BMI, and mammographic density are complex. Analysis of these three variables in Table 6 shows that among women with BMI ≥25, there is a positive association between atypical cytology and high mammographic density. No such association was seen among women with BMI <25. Also, there is a positive association between atypical cytology and high BMI among women with high density mammograms that is not present in women with low density mammograms. Finally, mammographic density is very strongly inversely related to BMI in women with normal cytology but not in those with atypia.

When women from whom no NAF was obtained were used as the referent group in another logistic analysis, the results were very similar (Table 5). The odds ratio for atypia with mammographic density was 4.6. These data also indicate that women with previous biopsy were 3.7 times more likely to have atypia than to have not yielded fluid.

### Discussion

Similar to our findings, a few studies (13–17) have reported associations of mammographic parenchymal patterns and some known breast cancer risk factors, showing that age, parity, and BMI were related significantly to Wolfe’s mammographic parenchymal patterns, which are highly correlated with mammographic density.

Several studies have examined associations of mammographic patterns with histopathology with somewhat inconsistent results (2, 13, 18–20). Urbanski et al. (14) demonstrated a weak relationship between the extent of mammographic dysplasia and histological evidence of epithelial atypia and carcinoma in situ among women aged 50 or less. In contrast, Arthur et al. (18) recently reported no correlation between Wolfe patterns and histological evidence of epithelial hyperplasia, atypia, or in situ carcinoma. Their failure to confirm this association indicates that the high risk of cancer associated with P2 and DY patterns might be due to factors other than epithelial abnormalities. As Arthur et al. reported (18), variation in the Wolfe pattern was related to the distribution of the fibrous and adipose tissue in the breast interlobular stroma but not to epithelial parenchymal content. Bear in mind that mammographic density is different from Wolfe pattern, even if they correlate.
A retrospective study conducted by Helvie et al. (19) showed a direct correlation of mammographic abnormalities with histological findings of atypical hyperplasia. Their classification of mammographic abnormalities was not based on Wolfe’s parenchymal patterns but on broad categories, such as microcalcifications, nodular capacity, etc. Clustered microcalcifications were found to be the most frequent mammographic abnormality directly correlated with atypical hyperplasia at histological examination.

Boyd et al. (20) recently showed that women with mammographic densities greater than 75% of breast volume had a 9.7 times greater risk of developing carcinoma in situ or atypical hyperplasia, a 12.2 times greater risk of hyperplasia without atypia, and a 3.1 times greater risk of nonproliferative breast disease than women showing no mammographic densities. Mammographic evidence of calcification at the biopsy site was strongly associated with high risk histological changes.

Our findings on nipple aspirate cytology and mammographic density are similar to those of Boyd et al., although of a lower order of magnitude. King et al. (21) previously showed that women with nipple aspirates of breast fluid were four to five times more likely to have atypia in nipple aspirates of breast fluid than either women with normal cytology findings or women from whom fluid could not be aspirated. Nipple aspirate cytology in nipple aspirates of breast fluid. Am. J. Epidemiol., 135: 130-141, 1992.

Since we are measuring biological parameters, the fact that the participants were volunteers is unlikely to have introduced any bias into our results. Mammograms were reviewed blindly as to the cytology outcome, so we are confident that no observer bias was present.

The relationship of BMI, mammographic density, and breast fluid cytology is puzzling. As we showed in our stratified analyses, the apparent association of atypical cytology and high mammographic density is both large and significant in women with BMI ≥25, but is minor and nonsignificant in women with BMI <25.

Despite the small number of subjects and other limitations, we believe further investigation is warranted on the role of nipple aspirate of breast fluid cytology as an adjunct to screening for breast cancer risk. Particularly in premenopausal women, the identification of women at increased risk for breast cancer might be enhanced using nipple aspiration in conjunction with mammography. Studies to evaluate the predictive value of breast fluid cytological findings along with other screening techniques are needed. This information may help to further identify high risk women and provide a basis for designing improved breast cancer screening programs.

Acknowledgments
The authors wish to thank Christine Choy, Jill Obata, Betty Chang-Lee, Florence Lee, and Maureen Morris for their assistance.

References
Association of abnormal nipple aspirate cytology and mammographic pattern and density.

M M Lee, N L Petrakis, M R Wrensch, et al.


Updated version  Access the most recent version of this article at: http://cebp.aacrjournals.org/content/3/1/33

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, use this link http://cebp.aacrjournals.org/content/3/1/33. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.