Association of Abnormal Nipple Aspirate Cytology and Mammographic Pattern and Density

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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 found that epithelial atypia in nipple aspirate fluid obtained 10–18 years earlier was associated with an increased risk of breast cancer. In the present study we examined the association between the cytology of nipple aspirate fluid and mammographic patterns in 588 volunteers recruited from the mammography clinic at the University of California. Nipple aspirate fluid cytology was classified according to the most severe epithelial change present and mammograms were classified by the Wolfe method and the percentage area of density. A direct relationship was found between mammographic density and cytological abnormality. When controlled for age, body mass index, previous biopsy, and calcification, the odds ratios of high density mammograms (over 50%) with nipple aspirate fluid cytological atypia was 4.4 (95% confidence interval, 0.9–21.5; \( P = 0.08 \)) when normal cytology was the referent. These preliminary findings indicate that highly dense mammograms are associated with cytological atypia and are consistent with studies reporting an association of histological hyperplasia and atypical hyperplasia with severe mammographic findings. If confirmed by further studies, nipple aspirate cytology may be a useful adjunct to mammographic patterns in the prediction of breast cancer risk, especially among premenopausal women.

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).

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 NAF and the pattern and density of mammograms.

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

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3 The abbreviations used are: NAF, nipple aspirate fluid; CI, confidence interval; BMI, body mass index.
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 \( \pm 5 \) years, parity \( \text{yes/no} \), 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, D1, and by the percentage area of density \( \langle 25\% \), 25–49\%, 50–74\%, \geq 75\% \) \( (6) \). In view of the relatively small sample size, the mammographic diagnoses were collapsed into two groups, low and high density (i.e., areas of density less than and greater than or equal to 50\%, 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 findings and mammographic readings. The 95\% CIs were computed using methods described by Schlesselman \( (12) \). Age was categorized into 2 groups \( \langle 50 \), \geq 50 \) to improve the fit to logistic models.

We analyzed the data by both mammographic density and Wolfe pattern. Parenchymal mammographic patterns and percentage density classifications were very highly correlated \( r = 0.98 \); \( P < 0.0001 \). Wolfe et al. \( (6) \) and Saftlas et al. \( (7) \) proposed that density is a more useful predictor for breast cancer risk than parenchymal patterns. Because density showed a stronger effect in our analysis, we are presenting only the data pertaining to mammographic density.

### Results

Table 1 shows personal characteristics of the entire sample of women who volunteered for our study compared with 588 women whose mammograms were used in this analysis. The two groups were very similar in terms of age, educational level, ethnic or racial distribution, percentage with family history of breast cancer, percentage parous, and percentage with history of previous breast biopsy. The only difference was in NAF status, due to the selection criteria described above.

The distribution of NAF cytology findings by mammographic density is shown in Table 2. The percentage of women with high density mammograms increased with increasing cytological abnormality.

In bivariate analyses, women with older age, previous biopsy, high mammographic density, or mammographic calcifications were more likely to have cytological diagnoses of atypia than normal cytology or to have not yielded fluid (Table 3). Associations of cytology with parity, BMI, and family history of breast cancer were compatible with chance in this group of women \( P > 0.10 \).

Age, parity, BMI, and calcifications in mammograms were found to be significantly associated with mammographic density in our sample (Table 4). Women with a history of biopsy or family history of breast cancer were more likely to have dense mammograms but not significantly so.

In the first logistic model of cytological outcome with age, parity, BMI, calcification, previous biopsy, family history of breast cancer, and mammographic density, neither parity nor family history showed an effect on cytology.
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 cytologic atypia than women with low density mammograms (95% CI: 0.9–21.5; P < 0.05). The CI was large 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 cytologic 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 atypia and high mammographic density. No such association was seen among women with BMI <25. Also, there is a positive association between atypia 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 breast 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 found that nipple aspirate cytology with atypia, it is the first to examine the association of abundance Lee, and Maureen Morris for their assistance.

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References


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