Atypia and Ki-67 Expression from Ductal Lavage in Women at Different Risk for Breast Cancer

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Abstract

Purpose: **Ductal lavage provides adequate material and detects atypical cells from ducts in women at increased risk of breast cancer, but the clinical significance of this finding is unclear. We studied the prevalence and predictors of atypia in addition to the proliferation-associated antigen Ki-67 expression in ductal lavage done in women at different risk of breast cancer.**

Results: **Ductal lavage was attempted in 202 women at increased risk and in 16 at average risk. Lavage could not be done in 20 women at increased risk because of anatomic impediments. Seven average-risk women (44%) had samples with inadequate cytology versus 30 women at higher risk (16%; $P = 0.014$). Atypia was observed in two average-risk women [22%; 95% confidence interval (95% CI), 3-60%]. The prevalence of atypia was 33% in women with a 5-year risk of ≥1.3% according to the Gail model (25 of 75; 95% CI, 23-45%), 36% in women with an increased probability of or ascertained BRCA mutation (9 of 25; 95% CI, 18-57%), and 52% in women with contralateral breast cancer (27 of 52; 95% CI, 38-66%). Ki-67 expression measured in a consecutive series of 80 women at increased risk was higher in atypical samples ($P = 0.0001$) and was positively associated with total cell count per slide ($P = 0.002$).**

Conclusions: **Atypia is frequent in women at increased risk of breast cancer but it can also be found in average-risk women. Ki-67 expression is associated with atypia and cell yield and it might be assessed as a surrogate biomarker in early-phase chemoprevention trials.** (Cancer Epidemiol Biomarkers Prev 2006;15(7):1311–5)

Introduction

Breast carcinogenesis is a multistep process of the epithelium lining the ductal system and lobules and is associated with increasing molecular and morphologic changes, including cellular atypia with atypical hyperplasia (1). Several prospective studies have shown that atypical hyperplasia is a nearly obligate precursor of cancer and is associated with a higher risk of developing breast cancer (2, 3).

Attempts to improve early detection of breast cancer and to provide individualized breast cancer risk assessment will greatly benefit from the use of noninvasive methods to sample cellular material from the target tissue. Although the Gail risk model has been validated and proved accurate in estimating a woman's high risk of developing breast cancer, this tool has a modest discriminatory utility for an individual subject (4). A recent prospective study by Fabian et al. (5) has shown that the presence of atypia on random periareolar fine-needle aspirations in high-risk women, as assessed by the Gail model, is associated with a 5-fold increased risk of developing breast cancer compared with high-risk women without atypia. Likewise, Wrensch et al. (6, 7) have shown in prospective studies that women with atypia in nipple aspirate fluid have a greater risk of developing breast cancer compared with women without atypia.

Effective preventive measures for breast cancer have recently been accomplished. Tamoxifen provided an 86% reduction of invasive breast cancer risk in women with biopsy-proven atypical hyperplasia (8) and the incidence of biopsy-proven atypical hyperplasia was lowered by 36% in the same study, an effect which occurred in conjunction with a 49% reduction in invasive breast cancer (9). These results suggest that the development or regression of atypical hyperplasia in high-risk women is an attractive end point for breast cancer risk reduction clinical trials that aim to test the effectiveness of new preventive agents in a cost-effective manner. Such a clinical trial design could considerably accelerate the development of new agents to treat breast premalignant conditions as it would involve hundreds, and not thousands, of high-risk women and could be conducted over 2 to 3 years (1).

Ductal lavage is a new technique for collecting breast cells from apparently normal breasts. It provides more abundant cell material than nipple aspiration fluid and has shown its ability to find atypical cells in nearly 25% of at-risk women with fluid-yielding ducts and with no clinical evidence of disease (10). However, the clinical significance of these findings is still unclear. Specifically, it is unknown whether the finding of atypia indicates preneoplastic or early neoplastic changes in a single fluid-yielding duct or whether it underlies an increased risk of breast cancer in the whole gland (field cancerization), irrespective of the presence of fluid-yielding duct. The latter explanation would be in line with the 25% prevalence of intraepithelial neoplasia in autoptic studies in young women deceased from accidental causes (11). This difference has important clinical implications as ductal lavage is already on the market as a screening tool and as a diagnostic method used to detect early disease in women with fluid-yielding ducts. In addition, it is unknown whether the presence of atypia in ductal lavage is associated with different risks of developing breast cancer. Such an association would greatly support the use of ductal lavage as a risk assessment method in high-risk subjects.

In the present single-institution study, we estimated the prevalence of atypia from ductal lavage in average-risk women

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and in women at high risk of breast cancer for different reasons, including women with a personal history of contralateral breast cancer. Furthermore, we studied which factors predicted the presence of atypia and Ki-67-expressing cells in the cannulated duct. We chose the expression of Ki-67 as an additional biomarker because change in this proliferation antigen predicts the clinical response (12, 13) and the outcome to hormonal (14) and chemotherapeutic agents (15) in preoperative trials of breast cancer. In addition, Ki-67 has a different expression in normal versus premalignant versus malignant epithelial cells (16-18). Only 80 subjects underwent testing for Ki-67 because the decision to use it as a risk marker was taken after the study had already been commenced.

Materials and Methods

Study Objectives and Outcomes. The aims of the study were to assess the prevalence of atypical cells in samples obtained by ductal lavage in women with different levels of breast cancer risk and to study which factors were associated with the finding of atypia and Ki-67 expression in epithelial cells from ductal lavage.

Participants. Women at increased risk for breast cancer, whose mammograms and clinical breast examinations were nonsuspicious, underwent ductal lavage via a prevention research program at the European Institute of Oncology. The first group of women at increased risk included premenopausal and postmenopausal women with a 5-year risk of ≥1.3% according to the updated version of the Gail model (19). This lower cutoff relative to the original threshold of 1.66% was defined to account for the lower incidence of breast cancer in Italy compared with the SEER data (20). The second group included women with documented genetic mutation in the *BRCA1* or *BRCA2* gene or with a probability of ≥10% of carrying a mutation according to the Berry-Parmigiani model (21). The third group consisted of a consecutive series of women with a personal history of contralateral breast cancer. Those who exhibited a nipple fluid discharge had contributed to the original multicentric trial (10) and most of these women underwent ductal lavage under general anesthesia at the time of breast cancer surgery in the opposite breast. A control group included asymptomatic women at no increased risk of breast cancer who had volunteered to undergo ductal lavage to assess the prevalence of atypical cells. Sixty percent of these average-risk women to whom the procedure was proposed declined mainly because of fear of pain or discomfort. Exclusion criteria were the following: history of lactation within the past 12 months; current or past pregnancy within the past 12 months; surgery for benign disease near the nipple to be studied; presence of breast implant; active infections or inflammation in the breast; chemotherapy or hormone therapy within the past 6 months; and known allergy to local anesthesia. The study was conducted at the European Institute of Oncology, Milan, Italy, after Institutional Review approvals in accordance with Good Clinical Practice procedures. All subjects provided their written informed consent. Complete medical history and clinical examination were obtained for all subjects.

Study Procedures. Before the ductal lavage procedure, a systematic search for the presence of a fluid yielding duct was carried out in all subjects. This was done using either a breast aspirator device or through manual compression. When nipple discharge was present, cannulation of the fluid-yielding duct was first attempted to perform ductal lavage. At variance with a previous study (10), however, ductal lavage was attempted in all subjects irrespective of the presence of a fluid-yielding duct. Thus, ductal lavage was done by gently probing different orifices even in the absence of a nipple discharge or in different orifices from the fluid-producing duct until initial cannulation was anatomically impeded, participants experienced discomfort, or there was resistance to fluid infusion.

Ductal lavage was done according to the previously described procedures (10), with some modifications. Briefly, EMLA cream (i.e., 2.5% lidocaine-2.5% procaine; Astra Zeneca, Milan, Italy) was applied topically and then covered with an occlusive dressing for ~1 hour before the procedure. Nipple aspiration following breast massage was carried out by placing a suction cup (Firstcyte Aspirator, Cytyc Health Corporation, Boxborough, MA) fitted with a 20-mL syringe over the nipple and applying a small amount of suction. Ductal lavage was attempted immediately after nipple treatment on all ducts that yielded. When necessary, ductal orifices were enlarged with dilators to facilitate cannulation. A separate microcatheter (Firstcyte Microcatheter, Cytyc Health) was used for each duct cannulation. After the insertion, a total of 1 to 2 mL of 1% lidocaine was infused intraductally and followed by the infusion of ~10-mL saline solution in four to five pulses. After breast massage, the ductal fluid was recovered and immediately placed into a 15-mL empty tube, labeled with subject ID and date, and sent to the laboratory for cytologic analysis.

Cytologic Processing and Examination. Samples were centrifuged at 1,700 rcf for 7 minutes (Megafuge 2.0, Heraeus Instruments GmbH, Hanau, Germany) and the pellet suspended. An appropriate preservative solution (50% alcohol) for clear samples, Esposti's preservative solution (45% methanol, 45% distilled water, 10% glacial acidic acid) for cloudy samples, was added to obtain a total volume of 6 mL. After 30 minutes, the specimens were centrifuged again and the sediment resuspended. At least six slides were prepared by means of a cytocentrifuge (Cytospin 3, Shandon Lipshaw, Pittsburgh, PA); each slide was labeled with the subject ID, coupled with a dual cuvette (Dual Cytocuvette, Bio-Chem, Milan, Italy) filled with different aliquots (100 μL per upper chamber and 200 μL per lower chamber) and centrifuged at 450 rpm for 10 minutes. Finally, slides were air-dried; three slides were stained with the Papanicolaou stain and three slides stained with H&E by means of an automatic stainer (Autostainer XL, Leica Instruments GmbH, Nussloch, Germany). Slides were then mounted with an automatic mounter (Tissue-Tek, Sakura Finetek Europe B.V., Zoeterwoude, the Netherlands) using a film (Coverslipping Film, Sakura Finetek Europe) with xylene as the mounting medium; this film can be rapidly removed, when necessary, with acetone solution for 2 to 3 minutes.

Samples were first examined by a cytoscreener (L.C.) and then reviewed by a pathologist (C.C.). The cytologic diagnostic categories were those described in the guidelines for nonoperative diagnostic procedures and reporting in breast cancer screening (22): inadequate (C1), hypocellular specimen (<10 epithelial cells per slide) or artifacts which do not allow any evaluation (poor quality preparation, excessive secretion, or large presence of blood or anucleated squamous cells); negative (C2), adequate specimen with normal epithelial cells isolated or in sheets; atypical, nonsuspicious for malignancy (C3), adequate specimen containing ductal epithelial cells, in stratified sheets or papillary-like aggregates, with some nuclear and cytoplasmic atypia, foam cells, inflammatory cells, and secretion, if any, in the background; atypical, suspicious for malignancy (C4), adequate specimen with slight or few atypical epithelial cells with nuclear and/or cytoplasmic alterations, associated or not with microcalcifications, cellular debris, RBC, and pigmented histiocytes; malignant (C5), adequate specimen with frankly malignant epithelial cells.

A cell count was done on all ductal lavage samples rendered adequate (>10 ductal epithelial cells per slide) by one reviewer (L.C.). The cell count included the total number of cells and,

more specifically, the number of ductal epithelial cells. One slide from each sample was used for the cell count, counting only cells from the upper cytospin material which contained 100 μL of ductal lavage material.

Ki-67 Immunostaining and Evaluation. A consecutive series of 80 women with adequate ductal lavage were immunostained for Ki-67 after cytologic examination. Only 80 subjects underwent testing for Ki-67 because the decision to use it as a risk biomarker was taken after the study had initiated. Ki-67 immunoreactivity was assayed using the MIB-1 (Dako monoclonal antibody, Glostrup. Denmark) and a Dako Autostainer-Universal Staining System (Dako Cytomation). The antibody was diluted 1:200 and antigen retrieval was done using EDTA (pH 8) at 99°C for 30 minutes. The sample was then incubated with the antibody for 30 minutes at room temperature. Immunoreactivity was evaluated as the percentage in all adequate specimens by counting all positive epithelial cell nuclei (single lying or in aggregates) over the total number of epithelial cells (at least 200 epithelial cells). Only one operator (C.C.) evaluated all samples for Ki-67 immunoreactivity.

Statistical Analysis. The proportion of inadequate cytology was calculated over the total of ductal lavages by risk group. The proportions of atypical cytology (C3-C5) and normal cytology (C2) were then calculated excluding the samples with inadequate cytology, and the exact confidence intervals were estimated. Tests for differences in the proportion of atypia among the different risk groups were done using the Fisher's exact test. Predictors of atypia and Ki-67 expression were determined using logistic regression models. Univariate analysis was done, then the variables significantly associated with the presence of cytologic atypia or KI-67 expression were included simultaneously in a single model and interactions between the predictors were tested. Finally, in the resulting model, the other variables were included separately to test for possible associations. Statistical significance was defined as $P < 0.05$. All tests were two sided.

Results

Subject Characteristics. From November 1999 to May 2002, 202 women at increased risk and 16 women at average risk for breast cancer underwent ductal lavage. The procedure could not be done in 20 subjects at increased risk because of anatomic impediments, including narrow orifice, high resistance to fluid infusion, or introflexed nipple. The remaining 182 women at increased risk for breast cancer consisted of 28 unaffected women with strong family history for breast and ovarian cancer leading to a ≥10% probability of carrying *BRCA-1* or *BRCA-2* mutation (including 6 women with positive genetic testing), 91 women at increased risk according to the Gail model, and 63 women with a history of breast cancer in their opposite breast.

The main characteristics of the 182 at increased-risk subjects assessable for analysis are summarized in Table 1. Mean age (±SD) was 46 ± 8 years; mean body mass index was 24 ± 8 kg/m^2; and ~80% of the women were premenopausal. First-degree family history of breast cancer was present in 54% of the subjects. For the women in the average-risk group, the mean age was 42 ± 7 years and the mean body mass index was 24 ± 4 kg/m^2.

Prevalence of Atypia. Samples with inadequate cytology were more frequent in women at average risk for breast cancer (7 of 16, 44%) than in women at increased risk (30 of 182, 16%; $P = 0.014$). The proportion of samples with inadequate cytology was similar in the three groups of women at increased risk (Gail, *BRCA*, and contralateral breast cancer). In our sample, we found no other predictors of inadequate cytology. When considering only samples with adequate cytology, the proportion of samples with atypical cytology was 22% [2 of 9; 95% confidence interval (95% CI), 3-60%] in women at average risk, 33% (25 of 75; 95% CI, 23-45%) in women at increased risk according to the Gail model, 36% (9 of 25; 95% CI, 18-57%) in women with a higher probability of mutation of *BRCA1* or *BRCA2* or with a documented mutation in one of these two genes, and 52% (27 of 52; 95% CI, 38-66%) in women with a personal history of breast cancer.

Among the 61 samples with atypia observed in high-risk women for breast cancer, only 6 (10%) cases were suspicious for malignancy (C4), and these were all women with contralateral breast cancer. In addition, one of the two samples with atypia found in average-risk women was suspicious for malignancy (C4). Table 2 shows that, although there was no evidence that overall the risk group is a predictor of atypia ($P = 0.1$), there is suggestive evidence that the prevalence of atypia in women with a personal history of breast cancer is higher than in women at increased risk of cancer according to the Gail model [odds ratio (OR), 2.16; 95% CI, 1.04-4.46]. Age,

Table 1. Main characteristics of the women at increased risk for breast cancer who underwent ductal lavage (n = 182)

Mean age ± SD (y)	46 ± 8
Risk group	
High risk (Gail model*)	91 (50%)
High risk (BRCA mutations†)	28 (15%)
Contralateral breast cancer	63 (35%)
Mean ± SD body mass index (kg/m^2)	23.5 ± 8.2
Nipple discharge	
No	88 (48%)
Yes	94 (52%)
Height (cm)	
<161	67 (38%)
161-166	54 (30%)
>166	56 (32%)
Smoking status	
Never	98 (55%)
Ex	49 (27%)
Current	32 (18%)
Age at menarche (y)	
<12	48 (27%)
12-13	93 (52%)
>13	37 (21%)
Menopausal status	
Pre	143 (80%)
Post	36 (20%)
Parity	
0	37 (21%)
1	58 (32%)
2+	85 (47%)
Age at first pregnancy (y)	
<25	32 (23%)
25-30	56 (39%)
>30	54 (38%)
Breastfeeding	
No	29 (20%)
Yes	114 (80%)
Hormone replacement therapy	
No	164 (90%)
Yes	18 (10%)
Oral contraceptives	
Never	81 (45%)
Ex	77 (43%)
Current	21 (12%)
First-degree family history of breast cancer	
No	83 (46%)
Yes	97 (54%)

NOTE: For some women, information on some of the variables was missing.
*Premenopausal and postmenopausal women with a 5-year risk of ≥1.3% according to the updated version of the Gail model.
†Women with documented genetic mutation in the *BRCA1* or *BRCA2* gene or with a probability of ≥10% of carrying a mutation according to the Berry-Parmigiani model.

Table 2. Association between risk group and cytologic atypia in nipple duct lavage in women at increased risk of breast cancer

Risk group	Normal (N = 91)	Atypical (N = 61)	OR (95% CI)*	P †
High risk (Gail model)	50 (67%)	25 (33%)	Reference	0.10
High risk (BRCA)	16 (64%)	9 (36%)	1.13 (0.44-2.90)	
Contralateral breast cancer	25 (48%)	27 (52%)	2.16 (1.04-4.46)	

NOTE: Atypia includes C3 to C5 categories (see Materials and Methods). A total of 30 women were excluded because of inadequate cytology.
*From a logistic regression model with the presence of atypia as outcome measure and risk-group as predictor. Potential predictors listed in Table 1 were not associated with cytologic atypia (all $P > 0.1$). Total cell count was not a predictor of atypia ($P = 0.01$).
†Likelihood ratio test for the inclusion of the variable in the model.

body mass index, height, smoking status, age at menarche, menopausal status, age at first pregnancy, history of lactation, hormone replacement therapy and oral contraceptive use, and cell count per slide were not predictors of atypia (all $P > 0.1$). Family history of breast cancer was not associated with atypia in our group of women at increased risk of breast cancer (OR, 1.06; 95% CI, 0.56-2.06).

Predictors of Ki-67 Expression. The levels of Ki-67 expression were assessed in 80 consecutive subjects (53%) and ranged from 0% to 10% (median 1%). The median values were 2% (interquartile range, 2-4%) and 0% (interquartile range, 0-1%) in subjects with or without atypical cells, respectively. Table 3 shows that atypical cytology (P = 0.0001) and a higher total cell count per slide (P_{trend} = 0.002) were significant predictors of Ki-67 expression. Expression of Ki-67 was higher in samples with atypia (91%) than in samples with normal cytology (45%; OR, 11.7; 95% CI, 2.9-46.8). The proportion of samples showing any Ki-67 expression increased with the total cell count per slide, increasing from 40% in samples in the lowest tertile to 62% and 88% in samples in the second and third tertiles, respectively. Interestingly, none of the nine adequate samples from average-risk women showed Ki-67 expression in their ductal lavage epithelial cells. The proportion of samples showing Ki-67 expression was not significantly associated with the menstrual cycle (62% in samples taken in the first 2 weeks and 66% in the last 2 weeks; P = 0.8). Risk group, age, body mass index, height, smoking status, age at menarche, menopausal status, age at first pregnancy, history of lactation, hormone replacement therapy and oral contraceptive use, and ductal cell count per slide were not independent predictors of Ki-67 expression (all $P > 0.1$).

Discussion

Since its initial publication (10), ductal lavage has received increasing attention as a result of its ability to obtain valuable cellular material from the fluid-yielding ducts of the breast in a relatively noninvasive manner. However, the significance of the high rate of atypia (~25%) found in women at increased risk is still unclear as no data are available on the association between atypia in ductal lavage and subsequent risk of breast cancer (23). Moreover, the sensitivity and specificity of cellular atypia in ductal lavage in detecting early disease are unknown. In an attempt to provide insight into these important issues, we compared the prevalence of atypia in women in different risk groups. In addition, we studied the association between atypia and Ki-67 expression as the change in this cell proliferation biomarker predicts the clinical response and the outcome of breast cancer in a preoperative setting (12-15) and might therefore serve as a surrogate biomarker in phase I/II chemoprevention trials.

Our results indicate that (*a*) the frequency of inadequate cytology is higher in average-risk than in high-risk women; (*b*) atypia is a frequent finding in high-risk women, particularly those with contralateral breast cancer, but can be found also in average-risk women undergoing ductal lavage; and (*c*) Ki-67 expression is significantly and positively associated with the presence of atypia and with total cell count.

Overall, 61 (40%) women of the high-risk group had atypia in ductal lavage, including 6 (4%) cases of suspicious atypia, a figure which is slightly higher compared with the previous multicentric study (10). The reason for the higher rate of atypia found in the present study in high-risk women is unclear, but it could be due to the different selection criteria, including a higher proportion of contralateral breast cancer and/or the presence of a single investigator who did all the ductal lavage procedures. Two of nine (22%) average-risk women also had atypia, including one case suspicious for malignancy. To our knowledge, this is the first report of average-risk women undergoing ductal lavage. Notably, 60% of the average-risk women to whom the procedure was proposed declined mainly because of fear of pain or discomfort. Because of the limited number of average-risk women, our results should be considered exploratory and require confirmation. However, they suggest that atypia is not a specific marker of increased risk as assessed by the current predictive models. These results are in line with those recently reported in a smaller study by Maddux et al. (24), in which the rate of atypia was similar in women with 5-year Gail score of ≥1.7% compared with women with Gail score of <1.7%. Because these predictive models may not be useful to predict breast cancer risk in an individual subject, prospective studies are necessary to determine the association between atypia in ductal lavage and subsequent risk of developing breast cancer.

The prevalence of atypia was similar in women at increased risk according to the Gail model or because of genetic reasons. The latter group was heterogeneous. It included women at increased risk of carrying a mutation in *BRCA1* or *BRCA2* according to their family history and women who were tested and found positive for mutations in one of these two genes. Despite the limited number of women found positive for mutations, our results are in line with those of other authoritative recent studies which have already analyzed the prevalence of atypia in samples of *BRCA1* or *BRCA2* mutation carriers (25-27).

Table 3. Predictors of Ki-67 expression in ductal lavage in a consecutive series of 80 women at increased risk of breast cancer

	No expression Ki-67* = 0 (N = 29)	Expression Ki-67 > 0 (N = 51)	OR (95% CI) †	P ‡
Atypia				
No	26 (55%)	21 (45%)	Reference	0.0001
Yes	3 (±9%)	30 (91%)	11.7 (2.9-46.8)	
Total cell count per slide§				
Tertile I (≤23,030)	15 (60%)	10 (40%)	Reference	0.002
Tertile II (23,031-91,640)	11 (38%)	18 (62%)	2.3 (0.6-8.0)	
Tertile III (>91,640)	3 (12%)	22 (88%)	10.6 (2.2-51.2)	

*No cell expressing Ki-67.
†From a logistic regression model with the presence of Ki-67 expression as outcome measure and atypia and cell count per slide as predictors. No other variable including ductal cell count per slide significantly improved the model (all $P > 0.1$).
‡Likelihood ratio test for the inclusion of atypia in the model and test for trend for the inclusion of total cell count per slide in the model.
§Total cell count per slide was unknown in 1 woman; therefore, the estimated ORs and P values derive from a model applied to 79 women.

The high rate of atypia we observed in high-risk individuals is consistent with the contention that premalignant and early malignant changes are present in a high proportion of unaffected women, a finding supported by previous autoptic data in women, ages 25 to 50 years, who had died from accidental causes, where the prevalence of intraepithelial neoplasia and invasive cancer was ~25% (11). Whereas the proportion of women with abnormal changes who are likely to progress to clinical disease is unknown, some average-risk women will eventually develop breast cancer. Indeed, the attributable risk based on known risk factors, such as age at first pregnancy, family income, and family history of breast cancer, accounts for only 40% of all breast cancers in the general U.S. population (28). Altogether, the results underline the need for a prudent attitude when treating atypia in the clinical setting because it is unknown how many of these cases will progress to overt cancer, thus resulting in unnecessary diagnostic workup and surgical procedures with potential risks, including adverse events, distress, increased costs, and overtreatment.

Of particular note is the finding that Ki-67 was differently expressed in normal versus atypical cytologic samples. Specifically, Ki-67-expressing cells were present in 91% of the samples with atypia versus 45% of those without atypia. In a recent cross-sectional study (26) in the tissue containing atypical hyperplasia, women who subsequently developed cancer had median levels of Ki-67 of 3.82% (interquartile range, 0.85-11.28%) versus 0.77% (interquartile range, 0.04-1.72%; $P < 0.001$) in women who did not progress to cancer. These observations suggest that Ki-67-expressing cells might be associated with an increased risk of developing subsequent breast cancer, but prospective studies are needed to confirm this association. The positive association between Ki-67 expression and cell count might underlie a true biological effect. Indeed, a higher cell yield as a predictor of a higher proliferative potential is in line with the notion that nipple discharge per se is associated with a statistically significantly higher risk of subsequent breast cancer, possibly as a result of an increased proliferative activity in the fluid-yielding duct (6). Because Ki-67 is an objective and quantitative biomarker, there is great interest in defining its role as a risk biomarker and as a surrogate measure of intervention efficacy in chemoprevention trials. Our data show that the levels of expression of Ki-67 are quite low even in the presence of atypia, ranging from a median of 0% (interquartile range, 0-1%) in normal cells to 2% (interquartile range, 2-4%) in atypical samples. However, these levels are similar to those observed in apparently normal histologic samples which were associated with subsequent cancer (29) or low-grade invasive breast cancer (30). Moreover, similar values were observed by Khan et al. (31) using fine-needle aspirates in high-risk women.

We conclude that atypia is frequent in high-risk women undergoing ductal lavage, particularly those with contralateral breast cancer, but it can even be found in average-risk women. Ki-67 expression is associated with atypia and cell yield and it might be assessed as a surrogate biomarker in early-phase chemoprevention trials.

References

1. O'Shaughnessy JA, Kelloff GJ, Gordon GB, et al. Treatment and prevention of intraepithelial neoplasia: an important target for accelerated new agent development. Clin Cancer Res 2002;8:314–46.
2. Dupont WD, Page DL. Risk factors for breast cancer in women with proliferative breast disease. N Engl J Med 1985;312:146–51.
3. Page DL, Schuyler PA, Dupont WD, Jensen RA, Plummer WD, Jr., Simpson JF. Atypical lobular hyperplasia as a unilateral predictor of breast cancer risk: a retrospective cohort study. Lancet 2003;361:125–9.
4. Rockhill B, Spiegelman D, Byrne C, Hunter DJ, Colditz GA. Validation of the Gail et al. model of breast cancer risk prediction and implications for chemoprevention. J Natl Cancer Inst 2001;93:358–66.
5. Fabian CJ, Kimler BF, Zalles CM, et al. Short-term breast cancer prediction by random periareolar fine-needle aspiration cytology and the Gail risk model. J Natl Cancer Inst 2000;92:1217–27.
6. Wrensch MR, Petrakis NJ, King EB, et al. Breast cancer incidence in women with abnormal cytology in nipple aspirates of breast fluid. Am J Epidemiol 1992;135:130–4.
7. Wrensch MR, Petrakis NL, Miike R, et al. Breast cancer risk in women with abnormal cytology in nipple aspirates of breast fluid. J Natl Cancer Inst 2001; 93:1791–8.
8. Tan-Chiu E, Wang J, Costantino JP, et al. Effect of tamoxifen on benign breast disease in women at high risk for breast cancer. J Natl Cancer Inst 2003;95:302–7.
9. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 1998;90:1371–88.
10. Dooley WC, Ljung BM, Veronesi U, et al. Ductal lavage for detection of cellular atypia in women at high risk for breast cancer. J Natl Cancer Inst 2001;93:1624–32.
11. Nielsen M, Jensen J, Anderson J. Precancerous and cancerous breast lesions during lifetime and at autopsy. Cancer 1984;54:612–5.
12. Chang J, Powles TJ, Allred DC, et al. Prediction of clinical outcome from primary tamoxifen by expression of biologic markers in breast cancer patients. Clin Cancer Res 2000;6:616–21.
13. Decensi A, Robertson C, Viale G, et al. A randomized trial of low-dose tamoxifen on breast cancer proliferation and blood estrogenic biomarkers. J Natl Cancer Inst 2003;95:779–90.
14. Makris A, Powles TJ, Allred DC, et al. Changes in hormone receptors and proliferation markers in tamoxifen treated breast cancer patients and the relationship with response. Breast Cancer Res Treat 1998;48:11–20.
15. Vincent-Salomon A, Rousseau A, Jouve M, et al. Proliferation markers predictive of the pathological response and disease outcome of patients with breast carcinoma treated by anthracycline-based preoperative chemotherapy. Eur J Cancer 2004;40:1502–8.
16. Selim AG, El-Ayat G, Wells CA. Expression of c-erbB2, p53, Bcl-2, Bax, c-myc and Ki67 in apocrine metaplasia and apocrine change within sclerosing adenosis of the breast. Virchows Arch 2002;441:449–55.
17. Shoker BS, Jarvis C, Clarke RB, et al. Estrogen receptor-positive proliferating cells in the normal and precancerous breast. Am J Pathol 1999;155:1811–5.
18. Khan SA, Sachdeva A, Naim S, et al. The normal breast epithelium of women with breast cancer displays an aberrant response to estradiol. Cancer Epidemiol Biomarkers Prev 1999;8:867–72.
19. Gail MH, Brinton LA, Byar DP, et al. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. J Natl Cancer Inst 1989;81:1879–86.
20. Rosemberg J, Chia YL, Plevritis S. The effect of age, race, tumor size, tumor grade, and disease on invasive ductal breast cancer survival in the U.S. SEER database. Breast Cancer Res Treat 2005;89:47–54.
21. Parmigiani G, Berry D, Aguilar O. Determining carrier probabilities for breast cancer-susceptibility genes BRCA1 and BRCA2. Am J Hum Genet 1998;62:145–58.
22. Robinson IA, Blackham RB. Is there a better way to assess performance in breast cytology? Cytopathology 2001;12:227–34.
23. Hollingsworth AB, Singletary SE, Morrow M, et al. Current comprehensive assessment and management of women at increased risk for breast cancer. Am J Surg 2004;187:349–62.
24. Maddux AJ, Ashfaq R, Naftalis E, Leitch AM, Hoover S, Euhus D. Patient and duct selection for nipple duct lavage. Am J Surg 2004;188:390–4.
25. Kurian AW, Mills MA, Jaffee M, et al. Ductal lavage of fluid-yielding and non-fluid yielding ducts in BRCA 1 and BRCA 2 mutation carriers and other women at high inherited breast cancer risk. Cancer Epidemiol Biomarkers Prev 2005;14:1082–9.
26. Higgins SA, Matloff ET, Rimm DL, Dziura J, Haffty BG, King BL. Patterns of reduced nipple aspirate fluid production and ductal lavage cellularity in women at high risk for breast cancer. Breast Cancer Res 2005;7:R1017–22.
27. Mitchell G, Antill YC, Murray W, et al. Nipple aspiration and ductal lavage in women with a germline BRCA 1 or BRCA 2 mutation. Breast Cancer Res 2005;7:R1122–31.
28. Madigan MP, Ziegler RG, Benichou J, Birne C, Hoover RN. Proportion of breast cancer cases in the United States explained by well-estabilished risk factors. J Natl Cancer Inst 1995;87:1681–5.
29. Shaaban AM, Sloane JP, West CR, Foster CS. Breast cancer risk in usual ductal hyperplasia is defined by estrogen receptor-α and Ki67 expression. Am J Pathol 2002;160:597–604.
30. Trihia H, Murray S, Price K. Ki-67 expression in breast carcinoma. Its association with grading systems, clinical parameters, and other prognostic factors—a surrogate marker? Cancer 2003;97:1321–31.
31. Khan QJ, Kimler BF, Clark J, et al. Ki-67 Expression in benign breast ductal cells obtained by random periareolar fine needle aspiration. Cancer Epidemiol Biomarkers Prev 2005;14:786–9.

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