Ki-67 Expression in Benign Breast Ductal Cells Obtained by Random Periareolar Fine Needle Aspiration

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Abstract

Ki-67 expression in ductal cells obtained by random periareolar fine needle aspiration (RPFNA) is currently being used as a response biomarker in phase II breast cancer chemoprevention trials; however, Ki-67 in RPFNAs has not been well studied as a risk predictor for cancer, which would support its use as a response indicator. We examined the expression of Ki-67 in RPFNAs specimens with hyperplasia ± atypia obtained from 147 women at high risk for development of breast cancer. Median Ki-67 was 1.4% (range 0–24%). Ki-67 was higher in specimens from women <50 versus those ≥50 (median 2% versus 0.6%; P = 0.006) and from premenopausal women versus postmenopausal women (P = 0.037); however, hormone replacement therapy (predominately low-dose estrogen without progestins) had no effect. By univariate analysis, Ki-67 was positively correlated with ductal cell number (P = 0.001) and hyperplasia with atypia (P = 0.007). By multivariable analysis, the proportion of ductal cells expressing Ki-67 was again predicted by cell number, which, in turn, was predicted by cytologic atypia. The association of Ki-67 expression with cytologic atypia, a known risk factor for development of breast cancer, provides preliminary justification for its use as a response biomarker in phase II chemoprevention trials. (Cancer Epidemiol Biomarkers Prev 2005;14(4):786–9)

Introduction

Risk biomarkers that are subject to modulation are used as response indicators in early prevention trials (1–3). We have previously shown that specimens obtained from high-risk women by random periareolar fine needle aspiration (RPFNA) and assessed as hyperplasia ± atypia are associated with a 5-fold increase in the short-term risk of subsequent development of ductal carcinoma in situ or invasive cancer (4). RPFNA cytology is being used as a primary response end point in phase II prevention trials; however, intra- and inter-interpretive variance can substantially increase study subject number requirements. Quantitative biomarkers, such as Ki-67, minimize interpretive variance, reducing the number of subjects required for a prevention study (3). The rationale for use of Ki-67 in proliferative breast disease as a risk biomarker stems from the hypothesis that the majority of breast cancers evolve from proliferative lesions (5–8), and a cross-sectional study of Ki-67 in benign biopsies where Ki-67 was higher in hyperplastic foci from women who later developed cancer than from women who had not developed cancer (medians 3.8% versus 0.8%; ref. 9). The observation that early reduction in Ki-67 predicts clinical response to tamoxifen in cancer treatment trials provides the rationale for use of Ki-67 as a response biomarker (10, 11). In the absence of prospective trials assessing Ki-67 in benign breast disease and subsequent breast cancer incidence, a positive association between RPFNAs atypia and Ki-67 would provide preliminary support for use of Ki-67 as a risk and response biomarker in early prevention trials (1, 7).

Materials and Methods

Study Cohort. The study cohort consisted of high-risk women undergoing baseline eligibility assessment for one of several phase II chemoprevention trials at the University of Kansas Medical Center Breast Cancer Prevention Center. Women eligible for RPFNAs were those whose 5-year Gail risk was ≥1.7%, whose relative risk of developing breast cancer was at least five times that of the general population for their age based on a 5-year Gail risk assessment, whose prior breast biopsy had exhibited atypical hyperplasia or lobular carcinoma in situ, who were known to carry a BRCA1/2 mutation, or who had a prior contralateral treated breast cancer. Women must have a normal mammogram at the time of aspiration and have been at least 6 months from change in hormone replacement therapy (HRT), ingestion of any selective estrogen receptor modulator or aromatase inhibitor, and 1 year from pregnancy, lactation, or any prior chemotherapy.

Eligibility Criteria. Ki-67 assessment was attempted only for specimens estimated to have sufficient (≥500) ductal cells on each of the slides designated for cytology and Ki-67.

Gail Risk Calculation. The projected probability of developing in situ or invasive cancer at 5 years was calculated for each subject according to the Gail model estimated at the time of aspiration (http://bcra.nci.nih.gov/brc/; ref. 12).

RPFNA Procedure. Subjects with a prior history of breast cancer (invasive or ductal carcinoma in situ) had RPFNAs done only on the uninvolved breast. RPFNAs were done as previously described (4). For premenopausal women, all RPFNAs were done on days 1 to 12 (follicular portion) of the menstrual cycle.

Specimen Handling. Material from all breast aspiration sites for each woman was pooled in a 15 mL conical tube containing 9 mL CytoLyt (Cytvy, Boxborough, MA) and 1 mL of 10% neutral buffered formalin. Conical tubes were placed on a Verimix Rocker (Barnstead International, Dubuque, IA) at low speed. Cells were then washed with CytoLyt, processed to a pellet, placed in PreservCyt (Cytvy) for 48 hours, and then
Table 1. Correlation of Ki-67 values with demographic and cytologic characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>Median (%)</th>
<th>Range (%)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>147</td>
<td>1.4</td>
<td>0-23.6</td>
<td>0.006</td>
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<tr>
<td>Age</td>
<td></td>
<td>2.0</td>
<td>0-23.6</td>
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<td>&gt;50 y</td>
<td>58</td>
<td>0.6</td>
<td>0-16.0</td>
<td></td>
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<tr>
<td>Menopause/HRT</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pre</td>
<td>67</td>
<td>2.0</td>
<td>0-15.8</td>
<td>0.037</td>
</tr>
<tr>
<td>Post, HRT</td>
<td>47</td>
<td>1.0</td>
<td>0-23.6</td>
<td></td>
</tr>
<tr>
<td>Post, no HRT</td>
<td>33</td>
<td>1.0</td>
<td>0-12.0</td>
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<td>5-y Gail</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>&lt;Median of 2.2%</td>
<td>71</td>
<td>2.2</td>
<td>0-23.6</td>
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<tr>
<td>&gt;Median of 2.2%</td>
<td>71</td>
<td>1.0</td>
<td>0-16</td>
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<tr>
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<td>1.1</td>
<td>0-23.6</td>
<td>0.007</td>
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<td>Benign</td>
<td>73</td>
<td>1.0</td>
<td>0-23.6</td>
<td>0.028</td>
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<td>Indeterminate/ atypia</td>
<td>74</td>
<td>1.6</td>
<td>0-16.0</td>
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<td>Masood score</td>
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<td>≤12</td>
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<td>0-12.0</td>
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<td>13</td>
<td>29</td>
<td>1.2</td>
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<td>14</td>
<td>47</td>
<td>1.4</td>
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<td>≥15</td>
<td>34</td>
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<td>0-13.4</td>
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<td>Cell number per slide</td>
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<td>1.0</td>
<td>0-23.6</td>
<td>0.001</td>
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<td>1,000-4,999</td>
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<tr>
<td>≥5,000</td>
<td>29</td>
<td>2.4</td>
<td>0-16.0</td>
<td></td>
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</tbody>
</table>

* Mann-Whitney test or Kruskal-Wallis test for distribution of Ki-67 values.

Gail risk could not be calculated for five subjects.

processed to three to four slides using a standard Thin Prep 2000 (Cytyc) nongynecologic protocol. At least two slides were Papanicolaou stained, with one used for morphology and one for Ki-67 staining.

RNase-Free Papanicolaou Staining Procedure. Slides for both cytomorphology and Ki-67 were Papanicolaou stained under RNase-free conditions with hematoxylin, OG-6, and EA-65 (all from Richard Allen Scientific, Kalamazoo, MI) and were prepared on the ThinPrep Processor.

Cytomorphology Assessment. Cytomorphology was assessed by a single cytopathologist (C.M. Zalles) and classified by three different methods: traditional (13), Masood semiquantitative index (14), and the 1996 National Cancer Institute Consensus Panel Criteria (15). The traditional method classifies preparations as nonproliferative, apocrine metaplasia, epithelial hyperplasia, hyperplasia with atypia, or carcinoma (13). The Masood index scoring system assigns a score of 1 to 4 to each of six morphologic characteristics: cellular arrangement, cellular pleomorphism, prevalence of myoepithelial cells, anisonucleosis, nucleoli, and chromatin clumping. A score of 6 to 10 is generally associated with nonproliferative specimens, 11 to 14 with hyperplasia without atypia, 15 to 18 with hyperplasia with atypia, and 19 to 24 with malignancy (14). National Cancer Institute Consensus Panel Criteria classify specimens as benign, atypical/indeterminate, suspicious/probably malignant, malignant, and unsatisfactory (15). Cytologic assessments were made without knowledge of the results of the Ki-67 assessment.

Ki-67 Assessment. Only slides containing >500 epithelial cells were processed and stained for Ki-67. Antigen retrieval was done with 10 mmol/L citrate buffer (pH 6) in a BioCare (Walnut Creek, CA) decloaking chamber for 2 minutes at 120°C. Slides were then stained with MIB-1 monoclonal antibody (M7240; DakoCytomation, Carpenteria, CA) at a 1:20 dilution in a Dako Autostainer. A categorical estimate of the number of ductal epithelial cells present on the Ki-67 slide was made as 500 to 1,000; 1,000 to 5,000; or >5,000.

Hyperplastic cell clusters were preferentially assessed and the number of cells with unequivocal nuclear staining out of 500 cells assessed was recorded. Slides were hand scored by two readers and assessments recorded on separate case report forms; however, in case of a difference between the two readers, the scores were averaged. As we have previously reported, agreement between two readers using this method was excellent (Cronbach’s α = 0.99; ref. 16).

Statistical Analysis. Frequencies of categorical variables were assessed using χ² analysis. Continuous variables were assessed using the Mann-Whitney nonparametric test. Multivariate analyses were done using stepwise linear regression.

Results

One hundred and forty-seven high-risk women who underwent RPFNA between March 2003 and August 2003 and had sufficient ductal cells (≥500) in the slide designated for Ki-67 were included in this analysis. Median age was 49 years (range 28-78 years). Eighty-nine (60%) women were <50 years of age. Sixty-seven (45%) women were premenopausal and 80 (55%) were postmenopausal. Among postmenopausal women, 47 (59%) women were on some form of HRT, including 26 on estrogen alone, 4 on estrogen plus progestin, 11 on estrogen plus progesterone (3 with testosterone as well), 5 on poorly absorbed vaginal estrogens, and 1 on a soy supplement.

Ki-67 Expression. For the entire cohort, the median level of Ki-67 expression was 1.4% (range 0-23.6%) as shown in Table 1. There was excellent agreement and low interobserver variance between the two readers for the 147 specimens (R² = 0.99).

Correlation of Ki-67 Expression with Demographic Factors and Gail Risk. Median Ki-67 was 2.0% in specimens from women <50 years of age compared with 0.6% in specimens from women ≥50 years (P = 0.006, Mann-Whitney test). Median Ki-67 was 2% among specimens from premenopausal women compared with 1% in specimens from postmenopausal women (P = 0.037). There was no difference in Ki-67 expression for specimens from postmenopausal women receiving HRT versus those not on HRT, but only 23% of women on HRT were on estrogen plus a progestin. Lastly, there was a marginally significant difference for Ki-67 expression between specimens from women <50 years of age compared with 0.6% in specimens from women ≥50 years (P = 0.037). Median Ki-67 was 2% among specimens from premenopausal women compared with 1% in specimens from postmenopausal women (P = 0.037). There was no difference in Ki-67 expression for specimens from postmenopausal women receiving HRT versus those not on HRT, but only 23% of women on HRT were on estrogen plus a progestin.

Cytologic assessments were made without knowledge of the results of the Ki-67 assessment.

Figure 1. Frequency of specimens exhibiting Ki-67 expression greater than or equal to specific percent positive values as a function of cytotology (hyperplasia versus hyperplasia with atypia). This shows that a greater proportion of specimens classified as hyperplasia with atypia exhibited specific levels of Ki-67 staining than did specimens classified as hyperplasia.
Correlation of Ki-67 Expression with Cytologic Morphology and Cell Number

Traditional Cytologic Assessment. Ki-67 was higher in women classified as having RPFNA epithelial hyperplasia with atypia than those with hyperplasia without atypia (Fig. 1). Median Ki-67 was 2.8% in specimens characterized as hyperplasia with atypia compared with 1.1% in specimens of hyperplasia ($P = 0.007$; Table 1).

Masood Score and Consensus Panel. Median Masood score for the study cohort was 14, with a range of 10 to 17. As shown in Table 1, median Ki-67 was 0.8% for Masood scores <12, and 1.2%, 1.4%, and 2.6% for Masood scores of 13, 14, and $\geq 15$, respectively (Fig. 2). Globally, these values are statistically significantly different ($P = 0.025$ by Kruskal-Wallis test) with a specific pairwise comparison being significant for $\leq 12$ versus $\geq 15$ ($P = 0.002$; Fig. 2). Specimens classified as indeterminate/atypia had a significantly higher Ki-67 than those who were benign ($P = 0.028$).

Cell Number on Slide. For the 77 specimens with 500 to 1,000 cells in the Ki-67 slide, the median Ki-67 was 1.2%; for the 41 specimens with 1,000 to 5,000 cells, Ki-67 was 2.2%; and for the 29 specimens with >5,000 cells, Ki-67 was 2.4%. The distribution of Ki-67 values was statistically significantly different between the three groups ($P = 0.001$).

Despite the general correlations of Ki-67 with cytomorphology, there was still considerable heterogeneity. For example, 21% of specimens with both evidence of atypia and >5,000 cells per slide exhibited no Ki-67 staining.

Prediction of Ki-67 Expression: Multivariable Analysis. Regression analysis was conducted to ascertain which of the above variables, associated with Ki-67 by univariate analysis (Table 1), would best predict for Ki-67 expression. The variables examined in the regression analysis were age, menopause status, HRT use, Gail risk, cell category number, and cytomorphology assessment by traditional, National Cancer Institute Consensus Panel criteria, and Masood score. Only cell number category was a significant ($P = 0.004$) determinant of Ki-67 in the multivariable analysis. However, if cell number was excluded from the regression analysis, traditional cytomorphology category was the only variable to be associated with Ki-67 expression ($P = 0.027$). Cytomorphology and cell count were highly associated in univariate analysis ($P = 0.002$).

Discussion

The positive association between RPFNA hyperplasia with atypia, an established risk biomarker, and Ki-67 expression provides preliminary evidence for use of Ki-67 in benign breast tissue as a risk indicator and response biomarker. A prospective study with adequate numbers of both premenopausal and postmenopausal women would be required for definitive evidence that proliferation (Ki-67) is indeed a risk factor that might complement cytomorphology assessment.

Our values for Ki-67 in cytology specimens with hyperplasia are similar to those reported by other authors for histologic preparations (9, 17-20). Further, our study results were similar to those reported by others in which higher Ki-67 values were reported in premenopausal than in postmenopausal women (19). All of our specimens from premenopausal women were obtained during the follicular phase. Higher values might be anticipated had premenopausal women with proliferative breast changes been sampled during the luteal phase of the menstrual cycle (20). Given the small size of this pilot study, it is not clear whether the association between cytomorphology and Ki-67 holds for both premenopausal and postmenopausal women. Perhaps the greatest use for Ki-67 assessment in benign breast tissue will not be for risk stratification but as a response biomarker in early phase prevention trials (2, 3). For the majority of our ongoing pilot and phase II prevention trials in which Ki-67 is the primary response biomarker, we now require a 2% level of Ki-67 staining for a woman to enter the treatment portion of the study. This seems logical given that cytologic atypia is associated with a median value Ki-67 of 2.8%. Our prior studies indicate that 70% of high-risk women will exhibit proliferative cytology and our current study indicates that 40% of high-risk women with proliferative cytology (57% with atypia, 27% with hyperplasia alone) can be expected to exhibit this ≥2% level of Ki-67 staining.

In summary, our observation that increased Ki-67 expression is associated with the established risk biomarker of RPFNA evidence of cytologic atypia provides preliminary support for its current use as a risk biomarker and response end point in early breast cancer chemoprevention trials.

References


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