**4. TITLE AND SUBTITLE**

Benign Breast Disease: Toward Molecular Prediction of Breast Cancer Risk

**6. AUTHOR(S)**

Lynn C. Hartmann, M.D.

E-Mail: hartmann.lynn@mayo.edu

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

Mayo Clinic
Rochester, MN  55905

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland  21702-5012

**14. ABSTRACT**

Optimal early detection and prevention strategies for breast cancer are predicated on our ability to identify individuals at significantly increased risk for this disease. The purpose of this Center was to bring molecular risk prediction for breast cancer into the clinical arena. The three areas of scientific inquiry included: (i) Establishment of a tissue repository of benign breast disease; (ii) Assessment of potential biomarkers of risk in this tissue set and (iii) Discovery of new, potentially relevant biomarkers of risk. We developed a cohort of 9,376 women, 758 (8%) of whom have been diagnosed with breast cancer since the time of their benign biopsy. We established our tissue repository of benign breast tissue and have collected the subsequent breast cancer tissue. We assessed the significance of benign histology in predicting the risk of future breast cancer, examining in detail the role of proliferative disease, atypia, papillomas, radial scars and involution. We explored the link between centrosome amplification, COX-2 expression, ER, Ki67 and breast cancer outcomes. Lastly, we characterized the histopathology in a cohort of African-American women that we established with Wayne State University.

**15. SUBJECT TERMS**

benign breast disease, biomarkers, histology, breast cancer

**16. SECURITY CLASSIFICATION OF:**

<table>
<thead>
<tr>
<th>a. REPORT</th>
<th>b. ABSTRACT</th>
<th>c. THIS PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>U</td>
<td>U</td>
</tr>
</tbody>
</table>

**17. LIMITATION OF ABSTRACT**

UU

**18. NUMBER OF PAGES**

150

**19b. TELEPHONE NUMBER (include area code)**

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland  21702-5012

E-Mail: hartmann.lynn@mayo.edu
Table of Contents

Introduction .......................................................................................................................4

Body ...................................................................................................................................... 4-30

Key Research Accomplishments .............................................................................. 31-32

Reportable Outcomes ................................................................................................ 32-35

Conclusions.......................................................................................................................35

Appendices .......................................................................................................................36

Appendix A. Persons receiving pay from the research effort…………………..37-38

Appendix B. Abstracts of presentations.................................................................39-63

Appendix C. Manuscripts.........................................................................................64-150
The purpose of the Center of Excellence was to bring molecular risk prediction for breast cancer into the clinical arena. The three main areas of scientific activity within this proposed Center were:

- the establishment of a large tissue repository from a retrospective cohort of women with benign breast disease (BBD) (1967-91) with complete and long-term clinical follow-up to identify those who developed breast cancer (cases) and those who did not (controls);
- the application of potential biomarkers of risk to this archival tissue set;
- the discovery of new, potentially relevant biomarkers of risk in fresh and frozen specimens of BBD.

We received our notice of funding in December of 2001. May 2002 the funding was released for the non-human subjects portion of the grant. February 2003 DOD approval was received for all portions of the grant. In 2007 we requested and received a no cost extension. The no-cost extension was expanded to the 2008 fiscal year to continue our work with Wayne State in African-American women. The Center included a multi-institutional team [(Mayo Clinic; UCSF; Wayne State)] of basic scientists, pathologists, epidemiologists, clinicians, statisticians, and advocates.

I. Establish a retrospective cohort of BBD and a nested case-control study

A. Development of study cohort
We completed the identification of our study cohort, updated the cohort through use of a mailed questionnaire and chart abstraction, and obtained tissue from Mayo tissue registry and outside institutions. Our approach for each of these tasks and results are as follows:

1. Identification of cohort. Female patients who had a breast biopsy performed between January 1, 1967 and December 31, 1991 at the Mayo Clinic in Rochester, Minnesota (MCR) were identified using the resources of the Division of Medical Information (MIR). These include the surgical index, pathology index, medical index and tissue registry.

   a. Surgical Index. For every surgical procedure performed at the MCR, MIR personnel review the surgical and pathology notes for the type of surgical procedures and diagnosis. The diagnostic data are coded using ICD-9 criteria and entered into a computer database referred to as the Surgical Index. We used the surgical index breast codes to begin our search.

   b. Pathology Index. The Pathology Index, a computerized database maintained by the Mayo Clinic Department of Laboratory Medicine and Pathology since 1985 contains all histologic findings for every surgery performed at the Mayo Clinic. This database was
searched to determine the histologic findings associated with each breast biopsy. The histologic findings of all breast biopsies performed prior to 1985 were abstracted by a trained nurse from pre-1985 Pathology Index maintained on index cards by month and year of procedure. For each woman, the histology from their first breast biopsy was reviewed. If the biopsy indicated a malignancy or was performed as part of a prophylactic mastectomy, the patient was not considered eligible for the study.

c. Medical Index and Tumor Registry. The next step in the screening process used two additional databases, the Medical Index and the Tumor Registry. MIR personnel maintain the Medical Index and Tumor Registry. Since 1975, the Medical Index has electronically captured the diagnostic findings of each patient’s inpatient and outpatient episode of care. The Tumor Registry, begun in 1971, contains the diagnostic findings of any malignancy and routine follow-up of patients with a cancer diagnosis.

d. Cohort details. We identified 12,132 women, age 18 to 85, who had a surgical excision of a benign breast lesion during the 25 year period from January 1, 1967 through December 31, 1991. The median follow-up was 15 years. We excluded 1,047 women who had: a diagnosis of breast cancer or lobular carcinoma in situ at, before, or within six months of their breast biopsy; unilateral or bilateral mastectomy or breast reduction at or before their biopsy; or those who refused the use of their medical records for research. Of the remaining 11,085 women, 1,053 women had no follow-up information after their breast biopsy leaving 10,032 women. Another 945 women had unusable or unavailable biopsy tissue of the benign lesion. The remaining group of 9,087 women comprised our study cohort. A comparison of the 10,032 women who met study criteria and 9,087 women with available tissue show no significant difference in their relative risk of breast cancer (1.59 and 1.56, respectively). 7,260 women were alive at study entry, 1,827 were deceased.

B. Participant follow-up
A study specific questionnaire was mailed to all eligible participants or, in the case of those who were deceased, their next-of-kin. Clinical-epidemiologic measures available from the questionnaires include: age at menarche; age when first child born; number of children; number of children breastfed more than one month; blood relatives with any cancer (degree and maternal/paternal) and ages; menopausal status; reason for menopause; oophorectomy (unilateral, bilateral; if yes, age at); use of birth control/oral contraceptives; if yes, ages taken and type; use of tamoxifen or raloxifene, year started and length of time taken; radiation to chest, if yes, age; number of breast biopsies; indication for breast biopsy; frequency of mammograms since breast biopsy; year of last routine mammogram; breast reduction, if yes, age; breast removal, reason, age, side; diagnosis of breast cancer, if yes, age, chemotherapy (before and/or after surgery), radiation (before/or after surgery), recurrence (date of, location); diagnosis of cancer anywhere other than breast, if yes, age diagnosed; height; weight (current and at age 18); race/ethnicity; use of non-steroidal anti-inflammatory drugs (specific drug, average days per month, average number of pills taken on days used, total number of years taken, age when taken); use of specific supplements (multivitamins, vitamin A, beta carotene, vitamin C, vitamin B, folic acid, vitamin E, calcium, selenium, zinc, garlic, fish oil, soy, black cohosh, shark cartilage); alcohol use, if ever used, age began to use regularly, current use, average consumption; physical activity and its frequency both during most of
adult life and currently. Questionnaire information was obtained on 5,619 (62%) of the women. Of these 604 (11%) were completed by next-of-kin.

C. Validate reported breast cancers
To validate reported breast cancers, charts were obtained for women diagnosed at Mayo Clinic and pertinent records were requested from outside institutions for women diagnosed outside Mayo Clinic. Prior to contacting outside institutions, women were asked to sign a consent form, giving researchers permission to access their outside medical records and tissue, as well as to identify the institution and physician providing care at the time of their cancer diagnosis. In the case of the deceased, next-of-kin were asked to give permission to obtain cancer related records. Once records were received, a trained nurse abstractor abstracted pertinent cancer information into database screens.

D. Match breast cancer cases to controls
For some studies, especially the biomarker studies, we used nested case:control series within our large cohort. To identify the case:control series, we randomly selected cases who developed breast cancer from the cohort, stratified by five-year categories of year of benign biopsy to represent the entire spectrum of the cohort. We individually matched two controls to each case based on age and year of benign biopsy.

E. Test sets for preliminary evaluation of biomarkers
1. Establishment of laboratory procedures. We tested a variety of procedures with a “technical set” of benign breast tissue from women not eligible for our study (e.g. those who had a benign biopsy after a previous cancer). We put the slides in long-term cold room storage to ascertain that labels would continue to adhere and the selected antibody dilution continued to work under those conditions. We then stained slides from the samples in the technical set that had been cold-stored for 3 to 6 months. The staining quality was assessed as “excellent” by our study pathologist.

2. Sets for evaluation of biomarkers.
a. Discovery set. A subset of approximately 125 cases and their two closest controls (matched on age at biopsy and year of biopsy) was chosen from the entire study period, 1967 to 1991, to serve as a test set. This set was used to ascertain a point and interval estimate of the prevalence of a candidate marker among the cases, as well as the risk of breast cancer among those with the candidate marker relative to those without the marker. The first step in constructing this test set was to determine the proportion of cases that occurred in each calendar year (1967 – 1991). The number of cases to be included in the test set for a particular year was that percentage of 125. Once the number to be selected from a particular calendar year was determined, that number was randomly selected from among the cases in that calendar year.

b. Atypia set. Given the high-risk status of women with atypia, we also focused on the atypia group (N = 336) to test the significance of biomarkers in them specifically.
II. Examine the association between select biomarkers in the benign specimens and subsequent risk of breast cancer

A. Obtain tissue
1. Benign breast tissue. Archived benign breast tissue slides and paraffin blocks were obtained from Mayo Clinic Tissue Registry for the study cohort. For the first 25 years of the cohort (1967-1991), 838 women could not be included because their benign tissue was not available. Of those, 616 (73.5%) had their biopsies between 1967-1971. The reason for the large number of missing samples in the 1967-71 time period is that all benign tissue was not routinely preserved during that time. That policy was changed at Mayo and since 1977, ≥ 95% of the BBD samples were available. Of note, for the entire 1967-91 group, the relative risk of breast cancer did not differ significantly between the eligible women and the women for whom we had benign tissue, who made up the study cohort (1.59 and 1.56, respectively).

2. Cancer tissue. We were successful in obtaining cancer blocks on 407/427 (95%) of the women diagnosed at Mayo Clinic and 194/331 (59%) diagnosed outside of the Mayo Clinic. We have slides on an additional 5 (1%) of the women diagnosed at Mayo Clinic and 40 (12%) diagnosed outside of Mayo Clinic. Thus, altogether we have either slides or blocks from the breast cancers for 646 (85%) of the 758 women. No tissue has been obtained for 112/758 (15%) of women diagnosed with cancer. Of these women, we did not request permission on 40/112 (35%) as they or their next-of-kin did not complete the questionnaire. Additionally, 38/112 (34%) of these women or their next-of-kin did not grant permission for their tissue to be released to us. No tissue is available for the remaining 34 women.

B. Characterize benign histology
1. Overall impression and risk. In 2005, we published the first manuscript based on our Center of Excellence work. At that time, we had identified 707 breast cancers in our cohort of 9,087 women and examined the relative risk of breast cancer based on histology and family history. The overall relative risk of breast cancer for the cohort was 1.56 (95% CI 1.45 – 1.68), and this increased risk persisted for at least 25 years after biopsy. The relative risk associated with atypia was 4.24 (95% CI 3.25-5.41), for proliferative changes without atypia it was 1.88 (95% CI 1.66-2.12), and for non-proliferative lesions it was 1.27 (95% CI 1.15-1.41). Family history was a significant risk factor independent of histology. Women with a strong family history had a relative risk of 1.93 (95% CI .58-2.32) for no family history it was 1.18 (95% CI 1.01 – 1.37). No increased risk was found among women with a negative family history and non-proliferative findings.
Table 1: Breast cancer risk based on select clinical-epidemiologic factors

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Women</th>
<th>Person-Years</th>
<th>No. of Observed Events</th>
<th>No. of Expected Events</th>
<th>Relative Risk (95% CI)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>9087</td>
<td>144,881</td>
<td>707</td>
<td>453.0</td>
<td>1.56 (1.45–1.68)</td>
</tr>
<tr>
<td>Age at diagnosis of benign breast disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30 yr</td>
<td>726</td>
<td>13,593</td>
<td>21</td>
<td>11.5</td>
<td>1.83 (1.13–2.80)</td>
</tr>
<tr>
<td>30–39 yr</td>
<td>1115</td>
<td>20,169</td>
<td>71</td>
<td>38.3</td>
<td>1.85 (1.45–2.34)</td>
</tr>
<tr>
<td>40–49 yr</td>
<td>2474</td>
<td>45,780</td>
<td>212</td>
<td>136.3</td>
<td>1.56 (1.35–1.78)</td>
</tr>
<tr>
<td>50–59 yr</td>
<td>2145</td>
<td>34,100</td>
<td>196</td>
<td>125.9</td>
<td>1.56 (1.35–1.79)</td>
</tr>
<tr>
<td>60–69 yr</td>
<td>1639</td>
<td>21,364</td>
<td>142</td>
<td>94.5</td>
<td>1.50 (1.27–1.77)</td>
</tr>
<tr>
<td>≥70 yr</td>
<td>988</td>
<td>9,874</td>
<td>65</td>
<td>46.6</td>
<td>1.40 (1.08–1.78)</td>
</tr>
<tr>
<td>Menopausal status‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal (age &lt; 45 yr)</td>
<td>2948</td>
<td>54,419</td>
<td>169</td>
<td>106.1</td>
<td>1.59 (1.36–1.85)</td>
</tr>
<tr>
<td>Perimenopausal (age 45–55 yr)</td>
<td>2583</td>
<td>45,872</td>
<td>245</td>
<td>153.4</td>
<td>1.60 (1.40–1.81)</td>
</tr>
<tr>
<td>Postmenopausal (age &gt; 55 yr)</td>
<td>3556</td>
<td>44,590</td>
<td>293</td>
<td>193.6</td>
<td>1.51 (1.35–1.70)</td>
</tr>
<tr>
<td>Histologic findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonproliferative disease</td>
<td>6061</td>
<td>99,109</td>
<td>379</td>
<td>297.7</td>
<td>1.27 (1.15–1.41)</td>
</tr>
<tr>
<td>Proliferative disease without atypia</td>
<td>2690</td>
<td>41,610</td>
<td>264</td>
<td>140.2</td>
<td>1.88 (1.66–2.12)</td>
</tr>
<tr>
<td>Atypical hyperplasia</td>
<td>336</td>
<td>4,161</td>
<td>64</td>
<td>15.1</td>
<td>4.24 (3.26–5.41)</td>
</tr>
<tr>
<td>Family history of breast cancer§</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>2668</td>
<td>44,574</td>
<td>171</td>
<td>145.4</td>
<td>1.18 (1.01–1.37)</td>
</tr>
<tr>
<td>Weak</td>
<td>1174</td>
<td>21,472</td>
<td>94</td>
<td>65.9</td>
<td>1.43 (1.15–1.75)</td>
</tr>
<tr>
<td>Strong</td>
<td>966</td>
<td>18,687</td>
<td>110</td>
<td>57.0</td>
<td>1.93 (1.58–2.32)</td>
</tr>
</tbody>
</table>

* Numbers of women, person-years, and events may not sum to overall totals because of rounding.
† The relative risk reflects the observed number of events as compared with the number expected on the basis of Iowa SEER data. All analyses account for the effects of age and calendar period. CI denotes confidence interval.
‡ Menopausal status was categorized according to the age at breast biopsy.
§ Information on family history was available for 4808 of the 9087 women.

Hartmann et al., 2005, NEJM.

Over a 25 year period of time, the proportion of BBD with atypical hyperplasia in our cohort increased from 1.5% in the early part of the study to 5.6% in the latter part of the study (Table 2). Similarly, the proportion of proliferative disease without atypia also increased from 24.6% in the early 70s to 34.9% in the later years (see following table). The increased prevalence of proliferative disease, especially atypia, is attributed to the increased use of mammography leading to findings associated with microcalcifications.
2. Atypia and risk. We have studied our cohort of women with atypia (N=336) in depth. We have found that the risk of breast cancer was elevated for women with atypia and even greater for women with atypia who were under age 45 (RR=7.36). We examined risk by number of foci of atypia and found: 1 focus, RR=2.33; 2 foci, RR=5.41; and for three or more foci, RR=7.96 (see cumulative incidence figure below). Moreover, in the highest risk subgroup of women with three or more foci of atypia and histologic calcifications, the cumulative incidence exceeded 50% after 25 years. This level of risk approaches that reported for carriers of BRCA1/2 mutations. Risk was similar for ductal and lobular types of atypia; family history did not significantly increase risk. Breast cancer risk in women with atypia remained elevated over 20 years.
3. Papillomas. The risk of breast cancer development in patients with papillomas, particularly those with multiple or atypical lesions, has been incompletely defined. We examined the association between breast papillomas and subsequent risk of breast cancer. We found that a single papilloma imparts a cancer risk similar to conventional proliferative fibrocystic disease. The presence of single papilloma with atypia does not modify the risk of atypical ductal hyperplasia/atypical lobular hyperplasia overall. The presence of multiple papillomas, however, increases the risk of breast cancer over that of proliferative fibrocystic disease (RR 3.01, 95% CI 1.10-6.55), even more so in women with multiple papillomas with atypia (RR 7.01, 95% CI 1.919-17.97). Thus, multiple papillomas constitute a proliferative breast disease subset having unique clinical and biologic behavior (Lewis JT et al. An analysis of breast cancer risk in women with single, multiple, and atypical papilloma, Am J Surg Pathol 2006;30:665-672).

4. Radial Scars. The significance of radial scars to subsequent risk of breast cancer has been debated. Radial scars (RS) are benign breast lesions of uncertain etiology. The growth pattern in RS can resemble breast cancer and on mammogram a RS can be difficult to distinguish from breast cancer, prompting biopsy. The literature is mixed about the risk of developing breast cancer following the diagnosis of RS, leading to our interest in examining the significance of RS in the subsequent development of breast cancer. We found no increased breast cancer risk for women with radial scars when compared to the risk already present due to proliferative disease with or without atypia. Breast cancer risk was not affected by the size or number of RS lesions (Berg JC et al. Breast cancer risk in women with radial scars in benign breast biopsies. Breast Cancer Res Treat 2008;108:167-174).

5. Involution. There are very few pathologic features identified thus far that are associated with a reduced risk of breast cancer. In our BBD resource, we studied if regression or involution of a woman’s breast lobules (or terminal duct lobular units, TDLUs) was associated with later risk of breast cancer. The breast is organized into approximately 15-20 major lobes, each made up of lobules that contain the milk-forming acini. As a woman ages, these lobules are supposed to regress or involute with a reduction in the number and size of acini per lobule (see figure 2).

a. Qualitative Involution. Our study pathologist assessed the extent of involution in the background breast tissue of the women in our BBD cohort. Notably, those women who had complete involution of their TDLUs had a significantly lower risk of breast cancer (RR 0.91, 95% CI = 0.75 – 1.10) compared to those with partial (RR 1.47, 95% CI = 1.33 –1.61) or no involution (RR = 1.88, 95% CI = 1.59 – 2.21) (Milanese TR et al. Age-related lobular involution and risk of breast cancer, JNCI 2006;98(22):1600-1607). We found that the presence of complete involution reduced risk even in women who were at high risk because they had atypia or a strong family history of breast cancer. This is a novel finding because the subject of age-related involution in relation to breast cancer risk has not been studied in the human. Importantly, this provides an additional feature to assess on a breast biopsy that allows us to fine-tune risk prediction for women. Secondly, and even more importantly, if the scientific community can determine what controls the
process of age-related breast involution, we may be able to induce it medically and thus, introduce a new “chemoprevention” strategy to offer women.

An editorial by Henson, Tarone and Nsouli accompanied our work in *JNCI* 2006. In this editorial they state “It is truly a remarkable event when traditional pathologic observations lead to new ideas about the prevention of cancer……….Results of the Mayo study provide a new paradigm for breast cancer research and prevention” (Henson DE, Tarone RE, Nsouli H. Lobular involution: the physiological prevention of breast cancer. 2006 JNCI;98(22):1589-90).

Figure 2. Histologic features of lobular involution. (a) A field of normal lobules or TDLUs, each comprised of multiple acini and specialized stroma (inset). (b) An example of complete lobular regression leaving small residual structures, largely depleted of acini (inset).
b. Quantitation of involution. We recently developed more quantitative measurements of
lobular involution status to provide more objective assessment methods and to be able to
more precisely identify the extent of involution (McKian KP et al. A novel breast tissue
feature strongly associated with risk of breast cancer 2009 In press, JCO). To pursue
this work, we identified 85 cases from our BBD cohort and matched 142 controls to them
by age and year of BBD biopsy. One H & E stained slide per subject was scanned into
the computer and analyzed using WebSlide Browser software (Bacus Labs). This
software allows the measurement of structural features (lobular area, acini number) as
visualized by light microscopy (Figure 3). The ten largest normal lobules were assessed
for each patient by one observer without knowledge of case status or previous pathologic
assessment (i.e. qualitative involution or histologic category). If fewer than ten normal
lobules were present, all were assessed. Analysis included (i) counting the number of
individual acini per lobular unit and (ii) delineating the circumference of the lobule to
measure its area in square microns (Figure 3). We defined countable acini as nuclei
forming a distinct circular pattern with or without the presence of a discernible lumen.
Distinct lobules were defined by the presence of intersecting stromal tissue. Abnormal
lobules, namely those that contained large portions of terminal ducts, atypical lobular or
ductal hyperplasia, sclerosing adenosis, large cysts, or proliferative disease without atypia
were not included in the analysis.

We found that this method for quantitating involution status was highly reproducible.
Specifically, a random sample of 82 slides (25 cases and 57 controls) was read by a
second observer using the quantitative, manual method described above. We compared
the acinar count of the first and second readers, and there was strong correlation, r=0.91
(5% CI: 0.87-0.94). We then compared acinar counts to other features, and found a
strong association between involution extent judged qualitatively (none, partial,
complete) and the acinar count and that involution extent was independent of histologic
category (Figure 4A). Women with a positive family history were somewhat less likely to
complete the process of involution (Figure 4A). We also found a strong correlation
between breast cancer risk and acinar count (Figure 4B,C), as women who went on to
develop breast cancer had significantly more acini/lobule (less involution).

We compared the acinar count of cases vs controls. Women who developed BC had
significantly more acini per lobule (24.3) than women who remained unaffected (17.8)
(p=0.0008). Dividing acinar count into categories of ten, we observed a step-wise
increase in risk of BC with increasing numbers of acini/lobule (p=0.0004), as shown in
Figure 4B. We also plotted BC risk by acinar count, demonstrating the continuous nature
of this risk feature (see spline in Figure 4C).
Figure 2. Quantitative LI. 

**a**, Quantification by number of acini per lobule. 

**b**, Quantification by lobular area in $\mu^2$. 

Figure 4. Quantitative LI and cancer risk. (A) Correlations between acinar count, other variables, and case status. (B) Risk associated with quantitative LI as a discrete variable. (C) Risk associated with quantitative LI as a continuous variable.

c. Concordance of involution measures across breast tissue. We found that measurements of lobular involution are highly consistent across multiple areas of a woman’s breast (Vierkant RA et al. Lobular involution: localized phenomenon or field effect? 2008 Breast Cancer Res Treat, epub ahead of print). For this study, the tissue from 15 women who had undergone bilateral prophylactic mastectomy was analyzed. Specifically, we chose a single section of breast tissue from each quadrant of both breasts. An H&E stained section was prepared from each specimen, and the extent of involution was
categorized as none (0%), mild (1-24%), moderate (25-74%) or complete (>75% involuted lobules). Within-woman concordance of involution was calculated using intraclass correlation coefficients (ICCs) which indicated strong correlation among involution measures (Table 3). Correlations were also similar when modeling each breast as an experimental unit, which confirmed that patterns of involution were uniform in the entire field of breast tissue.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treating each woman as the experimental unit</td>
<td></td>
</tr>
<tr>
<td>Intraclass correlation (95% CI)</td>
<td>0.75 (0.59, 0.89)</td>
</tr>
<tr>
<td>Kappa coefficient (95% CI)</td>
<td>0.67 (0.59, 0.75)</td>
</tr>
<tr>
<td>Pairwise comparisons, N (%)</td>
<td></td>
</tr>
<tr>
<td>Perfect matches</td>
<td>341 (81)</td>
</tr>
<tr>
<td>Partial matches</td>
<td>76 (18)</td>
</tr>
<tr>
<td>Non-matches</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Treating each breast as the experimental unit</td>
<td></td>
</tr>
<tr>
<td>Intraclass correlation (95% CI)</td>
<td>0.74 (0.60, 0.85)</td>
</tr>
<tr>
<td>Kappa coefficient (95% CI)</td>
<td>0.66 (0.53, 0.78)</td>
</tr>
<tr>
<td>Pairwise comparisons, N (%)</td>
<td></td>
</tr>
<tr>
<td>Perfect matches</td>
<td>145 (81)</td>
</tr>
<tr>
<td>Partial matches</td>
<td>34 (19)</td>
</tr>
<tr>
<td>Non-matches</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>


C. Perform centromere studies
Most invasive breast cancers, like many other solid tumors, have amplified centrosomes. The extent of centrosome amplification correlates with the levels of chromosomal instability in invasive ductal carcinoma of the breast. Centrosome amplification is also present in ductal carcinoma in situ, but has not been investigated in benign breast lesions. In our pilot study, we investigated the status of centrosomes in benign breast lesions of
various histologies to determine if amplified centrosomes can be detected in the absence of malignancy and invasion, and if any histologic types of benign breast lesions have significant levels of centrosome amplification.

We selected paraffin-embedded tissue blocks from women with non-proliferative lesions, proliferative lesions without atypia, and atypical hyperplasia. We had previously determined the relative risks of developing breast cancer associated with these lesions in our large cohort of women; the relative risk associated with non-proliferative lesions was 1.27 (95% CI 1.15-1.41), 1.88 (95% CI 1.66-2.12) in proliferative lesions without atypia, and 4.24 (95% CI 3.26-5.41) in lesions with atypia. Serial sections were cut to allow for staining with hematoxylin and eosin, gamma tubulin, and cyclin D1 on adjacent slides. The lesions of interest were circled by the study pathologist (DV) on the H&E or cyclin D1 stained slides. These slides were then scanned using a digital imaging system. The corresponding area was marked on the immunofluorescent slide stained with gamma tubulin antibodies to facilitate locating the lesion at high magnification.

Centrosome amplification was seen infrequently in non-proliferative lesions and in proliferative lesions without atypia. However, about 88% of atypical hyperplasia lesions had detectable centrosome amplification and about 30% had moderate to considerable levels of centrosome amplification (see Figures 5 and 6 below). Thus, centrosome amplification is seen more frequently in benign lesions having the highest relative risk of developing breast cancer.

This is the first quantitative demonstration of centrosome amplification in benign lesions. These pilot data demonstrate that centrosome amplification is more prevalent in atypical hyperplasia lesions, and these lesions are associated with the highest relative risk of developing breast cancer (Lingle W et al. Centrosome amplification is greatest in benign breast lesions associated with an increase in risk of cancer. San Antonio Breast Cancer Symposium, annual meeting. 2006, San Antonio, TX).
Figure 5. Immunofluorescence staining for centrosomes (red) in an atypical hyperplasia. Many nuclei (blue) have more than 2 centrosomes associated with them. Normal cells have 1 centrosome during G1 of the cell cycle and 2 centrosomes during G2 of the cell cycle.

Figure 6. Each bar represents the average centrosome number in an individual lesion. More than one centrosome per cell on average is found in 88% of atypical hyperplasia samples, compared to only 9% of the other BBD types. The range is also greater in atypical hyperplasias.

D. Individual biomarkers

1. COX-2. COX-2 is a very important mediator of biologic processes during inflammation and cancer. Through work of our UCSF study team led by Dr. Thea Tlsty and other labs, as described in Section III, we know that COX-2 expression is up-regulated in invasive breast cancer and also in ductal carcinoma in situ. We sought to determine if increased expression occurred a step earlier — namely in women with atypia — and if the presence of high levels of COX-2 would predict which women with atypia would go on to develop breast cancer. In fact, we found that moderate to strong COX-2 expression is associated with a significantly greater likelihood of a subsequent breast cancer in women with atypia. (See figure below). For women whose atypia lesion exhibited negligible (0-1+) staining, their likelihood of developing breast cancer was 13% at 15 years from biopsy, vs. 25% for those with 3+ COX-2 staining (Visscher DW et al. Association between cyclooxygenase-2 expression in atypical hyperplasia and risk of breast cancer. JNCI. 2008;100(6):421-7).

Besides its potential for risk prediction, COX-2 represents a molecular target for chemoprevention strategies. COX-2 inhibitors are available pharmaceutically and in fact,
epidemiologic studies have shown that women who have taken COX-2 inhibitors for arthritis have a lower chance of developing breast cancer.

Figure 7. COX-2 staining in atypia. Visscher DW et al. Association between cyclooxygenase-2 expression in atypical hyperplasia and risk of breast cancer. JNCI 2008;100(6):421-7.

2. ER. The estrogen receptor is essential to mediate the growth regulatory signals of estrogen in normal breast tissue and serves as a therapeutic target and predictive factor in breast cancer. The extent of ER staining in a well-characterized cohort of women with atypia, to our knowledge, has not yet been reported.

We used the Automated Cellular Imaging System III (ACIS) to evaluate the intensity and percent ER staining in 231 women with atypia. This system is able to provide automated quantification of biomarkers. The areas of atypia were identified by the study pathologist and read into ACIS with reports produced for each identified area of interest. The preliminary analysis of the 10 most intensely stained areas showed a mean of 56 percent stained cells (standard deviation 30.78, range 0.00 – 99.99). The mean intensity was 114 (standard deviation 28.97, range 0-206 (possible range 0 -256)). A linear multivariate mixed model examined percent staining and intensity differences based on atypia type (lobular, ductal), cancer status, and year of biopsy after controlling for repeated measures within a woman.
This initial analysis showed a stronger staining intensity and a greater percentage of staining of atypical ductal hyperplasia compared to atypical lobular hyperplasia (intensity: ADH mean of 117.68, standard error 2.5224 and ALH mean of 100.32, standard error 2.4537, p<0.0001; percentage: ADH mean of 64.1887, standard error 2.7860 and ALH mean of 44.2121, standard error 2.6720, p<0.0001) (Barr-Fritcher EG et al. Estrogen receptor expression in atypical hyperplasia and its association with type of atypia and age. 2009; United States and Canadian Academy of Pathology annual meeting). We have completed the ER assessments for all areas of atypia and are currently completing our analysis of these data.

3. **Ki67.** Multiple studies have shown that breast cancers with higher proliferation rates are associated with worse outcome. Ki67 is the best characterized proliferation marker. To our knowledge, there has not been a study of Ki67 in the setting of BBD — to check for association with the later development of breast cancer.

Figure 8: Ki67 staining

Here we show the results in a group of 192 women with BBD with all samples stained for Ki67. 32 women have developed breast cancer.
One of the strengths of our study design, namely that of a retrospective cohort, is that we have long follow up and can thus evaluate potential time-varying risk factors such as proliferation. Those women whose atypias had a higher proliferative component (dotted line) were more likely to develop breast cancer within the first 10 years following their biopsy. Those with fewer proliferating cells were still at increased risk for breast cancer, but their cancers occurred later, after 10 years following the biopsy.

The clinical value of this feature is as follows: if we focus on the first 10 years after a biopsy of atypia, we see that there is a distinct difference in risk between those women with high vs. low proliferation in their sample. If there is low proliferation, the woman’s risk is not increased above that of normal age matched women within the first 10 years. This is exactly the type of information we need to stratify women into different risk groups over different time intervals.
III. Discovery – In Vitro Culturings and Gene Profiling Studies

A. Culture BBD specimens and document their growth characteristics
Preliminary growth curves and characteristic micrographs on 14 samples are shown in the Figure below. Of these 14 cell culture samples, 8 were generated from breast tissue of pre-menopausal women and the remaining 5 from post-menopausal breast tissue. UCSF extracted RNA from these samples and reverse transcribed the first and second strand cDNA using Ambion Message Amp kit.

B. Profile BBD specimens
Epithelial cells were isolated and propagated from disease-free breast tissue and tissue containing BBD to determine the growth kinetics of BBD epithelial cells. All BBD tissue generated two epithelial populations with distinct growth characteristics, similar to epithelial cells generated from disease-free breast tissue. Briefly, the first population of human mammary epithelial cells (HMEC) grows in culture for approximately 10-15 population doublings before reaching an irreversible p16-dependent growth arrest termed P1. The second population, variant HMEC (vHMEC), grew in culture for an additional 40-50 population doublings due to the loss of p16/Rb signaling before reaching a telomere-dependent growth arrest. We hypothesized that the identification of molecular
alterations that accompany the extended proliferative capacity of the vHMEC population prior to telomere attrition and genomic instability may provide potential relevant biomarkers of risk. To this end we analyzed the global transcript levels of nine isogenic HMEC and vHMEC populations. Unsupervised hierarchical clustering analysis identified approximately 1240 genes that significantly differentiated the two populations on the basis of expression patterns. We found that many differentially expressed genes in vHMEC resembled expression of genes in DCIS and invasive cancer. These data support the utility of this model for the discovery of novel biomarkers for risk assessment. We chose a subset of 512 genes that robustly stratified the two groups (figure below). Many of the differentially expressed genes in the variant population are known E2F downstream targets, such as survivin, forkhead D1, BUB1 and Rad51. However, many have no known association with p16/Rb signaling, suggesting that the vHMEC are a unique population of cells.

Figure 11. Profile BBD specimens
1. COX-2. COX-2 was identified as one of the most robustly upregulated genes in vHMEC. The sustained expression of COX-2 in the vHMEC population was an intriguing finding because COX-2 is a stress activated gene that is tightly regulated in normal cells, such that it is only transiently expressed in response to cellular stress. This finding in the vHMEC cells suggests that this subpopulation exhibits a sustained stress response compared to the majority of epithelial cells. We find that HMEC (normal primary cells) are refractory to COX-2 induction in response to exogenous stress induced by inflammatory cytokines, DNA or microtubule damage, and oncogene-induced stress (adjacent figure). This differential induction of COX-2 may reflect a potential for transformation since COX-2 overexpression is accompanied by phenotypes that are critically relevant to cancer development, such as promoting proliferation, invasion and angiogenesis as well as inhibiting apoptosis and immune surveillance. This hypothesis is supported by our observations that forced expression of COX-2 in HMEC by retroviral infection produced enlarged flattened cells that were growth arrested (adjacent figure). Cell morphology and proliferation was not altered in vHMEC constitutively expressing COX-2. We find that the molecular changes underlying the differential phenotypic response to COX-2 overexpression are dependent on p16/Rb signaling. HMEC overexpressing COX-2 resulted in elevated p16, p53 and p21 and downregulation of Rb (see figure). This is in contrast to p16 silenced vHMEC where overexpression of COX-2 did not alter the level of p53 or p21. Thus, in normal cells, COX-2 induces a cell cycle arrest through the upregulation of p16 and p53 to protect cells from inappropriate oncogenic signaling. In cells that have lost p16/Rb signaling, COX-2 overexpression does not induce a growth arrest. We argue that sustained stress activation in the absence of growth arrest defines an aberrant stress phenotype that may set the stage for carcinogenesis.
2. **p16.** Our in vitro model demonstrated an inverse relationship between p16 and COX-2 expression, as shown in this figure. This finding prompted us to determine if loss of p16/Rb was sufficient to induce COX-2 expression. We found that sequence specific silencing of p16 causes COX-2 upregulation and provides cells with a proliferative advantage. Although genetic downregulation of p16 did not result in robust COX-2 upregulation, cells became responsive to exogenous induction of COX-2 by TGF-β, as shown. Since p16 exerts many of its biological effects through Rb, we determined if induction of COX-2 is mediated through Rb. We found that downregulation of Rb by retroviral infection of HMEC with the human papilloma virus E7 (HPV-E7) caused a robust upregulation of COX-2 expression and sensitizes cells to COX-2 induction by exogenous inducers such as TGF-β. The absence of Rb also provided a proliferative advantage. Thus, loss of p16 or Rb causes the upregulation of COX-2 and provides cells with a proliferative advantage, thereby mimicking the aberrant stress phenotype described previously in the vHMEC cells. We next sought to determine if p16/Rb signaling is clinically significant.

The majority of normal breast tissue is devoid of p16 immunostaining. Specifically, we observed that only 10% of disease-free tissue contains >30% of the lobules positive for p16. This is in contrast to either pre-malignant or malignant breast lesions. Twenty seven percent of ADH lesions display heterogeneous immunostaining for p16, a significant upregulation (P=0.05) compared to normal tissue. This level of immunopositivity and heterogeneity is maintained in low, intermediate and high grade DCIS lesions. The level of p16 positivity in invasive tumors is similar to that observed in DCIS. However, in contrast to DCIS, the pattern of p16 staining in invasive tumors is much more homogeneous.

In normal cells overexpression of p16 causes a cell arrest that acts as a protective mechanism in response to diverse cellular stressors or inappropriate mitogenic stimulation. To determine if the upregulation of p16 we observe during pre-malignancy is accompanied by cell arrest, we determined the relationship between p16 and
Figure 15. P16 and Ki67 predict DCIS recurrence. To determine if p16 overexpression coupled with proliferation could stratify recurrent from non-recurrent DCIS we examined 70 cases immunostained for p16 and Ki67.

Correlation between p16 and Ki67.

We reasoned that loss of p16/Rb signaling may cause cells to become refractory to stress-induced growth arrest and may reflect a more aggressive phenotype. To determine if p16 overexpression and Ki67 index labeling could stratify recurrent from non-recurrent DCIS we examined a series of 70 DCIS cases with known outcome. We find that coupling p16 and Ki67 indeed identifies DCIS cases that recur (see figure 15).

To further pursue these observations, we have stained our atypia samples for p16 by immunohistochemistry. These samples have been read by the Tlsty team at UCSF and final analyses are currently underway.

C. Profiling of involuted vs. noninvoluted samples

To assess the feasibility of defining biomarkers from paraffin-embedded samples in our BBD cohort, we obtained RNA from patients identified as noninvoluted (N=8) and involuted (N=6), matched for age of patient and year of biopsy. We performed profiling analyses using DASL whole genome microarray chips. Twelve of these fourteen samples (86%) produced excellent expression results (for each of these twelve, more than 65% of transcripts had a detection-p-value less than 0.05, indicating these transcripts were detected). The remaining two samples produced expression results too poor to be analyzed (for each of these two, more than 50% of transcripts had a detection p-value greater than 0.05 indicating they were not detected). A heatmap of the top 450 differentially expressed gene expression profiles shows both up and down regulated genes which may be useful for segregating the involuted from noninvoluted samples (Figure 16a). We converted the RNA to cDNA and assessed expression levels by quantitative PCR of six transcripts that were significantly differentially expressed by the microarray analysis between involuted and noninvoluted samples. We found that three of these showed...
statistically significant differences (p<0.05) between the two sample groups via PCR (Figure 16b): FANCD2, a homologue of FANCD1/BRCA2 that has been associated with increased risk of sporadic breast cancer (Barroso E, Milne RL, Fernandez LP, Zamora P, Arias JI, Benitez J, Ribas G: FANCD2 associated with sporadic breast cancer risk. Carcinogenesis 2006; 27(9):1930-7), CD34, a marker of hematopoetic stem/progenitor cells, fibroblasts, and vascular cells that shows decreased expression in breast cancer, and WNT10A, a marker previously implicated in embryonic developmental pathways that shows decreased expression in breast cancer (Kirikoshi H, Inoue S, Sekihara H, Katoh M: Expression of WNT10A in human cancer. Int J Oncol 2001; 19(5):997-1001). We evaluated expression of these proteins by IHC and found results consistent with those obtained by quantitative RT-PCR (Figure 16c). Thus, we show that we can successfully identify differentially expressed genes to evaluate as potential biomarkers of involution from FFPE biopsies.

Figure 16. (A) Gene expression fingerprint of RNA derived from FFPE samples of patients identified as showing complete involution (complete, N=4) or no involution (noninvoluted, N=8). (B) Quantitative PCR analysis of three transcripts predicted to distinguish complete involuted vs noninvoluted patient samples. (C) Immunohistochemistry analysis of the three biomarkers in complete involuted and noninvoluted biopsies. Unpublished data.

IV. Compare BBD in African-American vs. Caucasian-American women

A better understanding of breast cancer risk and precursor lesions is particularly important in the African-American population as these women are diagnosed at a younger age and tend to have higher grade and more advanced stages than Caucasian-American women. African-American women also experience a significantly higher mortality from breast cancer than Caucasian-American women. Dr. Hind Nassar has read the histology on African-American women with BBD diagnosed at Wayne State University/Karmanos Cancer Institute between 1/1/1998
and 12/31/2000. All women were between 18 and 85. Exclusions are the same as those for the Mayo cohort and include a history of invasive or in situ breast carcinoma prior to, or within six months of the benign breast biopsy, unilateral or bilateral mastectomy prior or at diagnosis, breast reduction and the following histologies: lipoma, fat necrosis, epidermal cysts, hematoma, accessory structure, phyllodes tumor, lymph node with no breast tissue. The resulting sample included 1,126 African-American women, with a mean age at time of biopsy of 47.8 years (SD = 13.92). Breast cancer has been identified thus far in 32 women (2.8%) at a mean follow-up of 8.9 years (SD = 2.13). Our findings thus far are that African-American women are younger at diagnosis and have more nonproliferative and mass forming lesions than Caucasian-American women. Additionally, lobular involution appears to occur at a slower rate in African-American women than Caucasian-American women.

V. Assess accuracy of Gail model.

The Gail model is currently the main tool used in the clinical setting for risk assessment in patients with atypia. This occurs despite the fact that the Gail model has not been validated in this group of patients. Thus, we evaluated the Gail model in our group of women with atypia. We used this model to predict 5 year and follow-up specific risks for each woman and compared the Gail model predictions to actual observed breast cancers in the group. Considering all women with atypia, the Gail model over-estimated the number of breast cancers that would occur in the first 5 years after biopsy (12.8 predicted, 8 occurred). However, when using all years of available follow-up for the group, the Gail model underestimated the risk of breast cancer in women with atypia (Gail model predicted 31.7 breast cancers while 58 occurred). Additionally, we found the concordance between Gail model individual-specific predicted outcomes and observed outcomes to be 0.50 (95% CI 0.44 – 0.55), no better than chance alone. As shown in the figure below, the Gail model predictions for the 58 women with atypia who developed breast cancer (cases) were superimposed on those of the 273 women who did not develop breast cancer (noncases). This has obvious implications for clinical practice. Healthcare providers should be cautious when using the Gail model in counseling patients with atypia regarding their risk of developing breast cancer.
Figure 17. Distributions of Gail model risk probabilities. Plot contains estimates for individualized risk at the end of the available follow-up. As risk predictions depend on age at BBD, and length of follow-up, the risk predictions were corrected for these factors prior to comparison.

VI. Establish a relational database

We created a Sybase database to track tissue samples (paraffin embedded blocks, tissue slides and pieces of tissue) as they are moved between laboratory locations and to manage the biomarker result data. A web-based interface to this database has been created. All samples (individually or in boxes) are tracked using bar codes. The interface allows users to scan the bar code of the sample labels and enter information as they perform a task such as moving a sample, creating a slide, or entering laboratory results.

The process begins as the information on the paraffin blocks is stored in the database. These blocks are inserted into barcode labeled boxes which are scanned whenever they are moved to a new location. In a processing laboratory, the blocks are cut and the tissue slices are affixed to barcode labeled slides or – for very thick slices – placed into vials. The slides are scanned as they are put into slide boxes. The boxes in turn are scanned as they move to new locations and when identical laboratory procedures (e.g., a tissue stain)
are done to all samples in the boxes. Each activity is stored in the database along with the time of the activity and the identification number of the person who performed the activity.

At the time of sample analysis, the physician, researcher or laboratory technician scans the samples and uses the same web-based application to enter the results of the analysis. All results and transactional information are stored in the database and available for statistical analysis.

The database server software is the current Sybase relational database management system. The data model was created and managed with Sybase PowerDesigner. The users will access the database via the Mayo internal web using programs written in Cold Fusion. The data analysts will access the database using connections to the SAS statistics analysis system. ODBC connections are used to connect web forms and the SAS system to the database.

The components of the database are pictured in the figure below. The central table in the database is the "sample" table which contains links to most of the other tables such as the "patient" table (containing patient information), the "block" table (containing information about the paraffin block from which the sample was cut), the "box" table (showing the current location of the sample), the "stain" table (showing stains done to the sample), the "results" table (containing the results of many types of tests done to each sample) and the "fishrslt" table (a table for FISH results). Most of these secondary tables contain links to descriptive tables, such as the "location" table and the "test_type" table, which manage the drop-down selection boxes in the web pages.
Figure 18: Relational database

```
<table>
<thead>
<tr>
<th>Table</th>
<th>Columns</th>
</tr>
</thead>
<tbody>
<tr>
<td>staff</td>
<td>id, type, code, desc, srt</td>
</tr>
<tr>
<td>box type</td>
<td>id, type, desc, cap, set</td>
</tr>
<tr>
<td>location</td>
<td>id, desc, srt,</td>
</tr>
</tbody>
</table>
VII. Key research accomplishments

- We created a retrospective cohort of women with BBD that can support tissue-based risk prediction strategies. Tissue-based features that are strongly associated with risk of breast cancer may signal processes and/or mediators that are central to the process of breast carcinogenesis.

- We identified the degree of risk associated with the common benign epithelial entities and the extent to which age at biopsy and family history influence the risk of breast cancer in women with proliferative or atypical lesions. The highest risk was among women who had proliferative disease with atypia, especially those of younger age (Hartmann et al., NEJM, 2005).

- We identified a marked increased risk of breast cancer in women with three or more foci of atypia, especially for three or more foci with calcifications. Also, risk was higher in women diagnosed with atypical hyperplasia before age 45. Among women with atypia, risk was not affected by family history (Degnim et al, JCO, 2007).

- We identified that a single papilloma without atypia imparts an increased risk of developing a subsequent carcinoma similar to other forms of proliferative breast disease without atypia. Atypical papilloma, particularly in the setting of multiple papillomas, imparts a breast cancer risk similar to or greater than conventional atypical ductal/lobular hyperplasias (Lewis et al, Am J Surg Pathol, 2006).

- We identified that the extent of lobular involution in breast tissue is an important risk indicator for the development of breast cancer. Increasing degrees of involution result in a significant reduction in breast cancer risk, even in women at “high risk” based on atypia or young age (Milanese et al., JNCI, 2006).

- We found that intense COX-2 expression is associated with a significantly greater likelihood of a subsequent breast cancer in women with atypia and represents one potential molecular target for chemoprevention strategies (Visscher et al., JNCI, 2008).

- We found no increased breast cancer risk for women with radial scars compared to the risk already present due to proliferative disease with or without atypia (Berg et al., Breast Cancer Res Treat, 2008).

- We identified that centrosome amplification is seen more frequently in higher risk benign lesions (e.g. atypia) and is infrequently seen in non-proliferative lesions and in proliferative lesions without atypia (Lingle et al., American Association for Cancer Research, 2005).

- We found the Gail model to predict no better than chance alone the breast cancer risk of women with atypia. The model significantly underestimated lifetime risk of our cohort of women with atypia (Pankratz et al., JCO, 2008).
• We found Ki67, a proliferation marker, to be predictive of risk after a benign 
breast biopsy. Those women who had higher proliferation rates were more likely 
than those with low proliferation to develop breast cancer within the first 10 years 
after benign biopsy. Conversely, if there is low proliferation, breast cancers tend 
to occur later, after 10 years of follow-up (Manuscript under review).

• We have helped to develop a retrospective cohort of African-American women 
with BBD at Wayne State University. Our findings thus far are that African-
American women are younger at diagnosis and have more nonproliferative and 
mass forming lesions than Caucasian-American women. Additionally, lobular 
involution appears to occur at a slower rate in African-American women than 
Caucasian-American women (United States and Canadian Academy of Pathology, 
2009).

• We found that measurements of lobular involution are highly consistent across 
multiple areas of a woman’s breast (Vierkant et al., Breast Cancer Res Treat, 
2008).

• We developed more quantitative measurements of lobular involution status to 
provide more objective assessment methods and to more precisely identify the 
extent of involution (McKian et al., JCO, 2009).

• To continue our work, we received an R01 fall 2008: Risk prediction for breast 
cancer: a tissue based strategy which builds on the Center of Excellence work. 
The R01 funding is for 5 years.

VIII. Reportable outcomes

A. Presentations

Poster presentation at the annual meeting of the American Association for Cancer 
Research, April 2003
• Hartmann LC, Visscher D, Reynolds C, Frost MH, Melton LJ, Vachon C, Tlsty T, 
Hillman D, Johnson JL, Lingle WL, Suman V, Sellers TA. Benign breast disease and 
breast cancer risk.

Poster presentation at the annual meeting of the American Association for Cancer 
Research, Orlando, FL, March 2004
• Hartmann LC, Visscher D, Frost MH, Melton LJ, Vachon C, Couch F, Shridhar 
V, Ghosh K, Degnim A, Hillman D, Suman V, Vierkant RA, Maloney SD, Pankratz VS, 
Tlsty T, Sellers TA, Lingle WL. Benign breast disease and breast cancer risk.

Podium presentation at annual meeting of the United States and Canadian Academy 
of Pathology. February 29, 2005 in San Antonio, Texas
• Lewis, JT, Vierkant RA, Maloney SD, Hartmann LC, Visscher DW. Analysis of 
cancer risk among patients with papillary lesions of the breast
Podium presentation at Society of Surgical Oncology Annual Cancer Symposium, March 3-6, 2005 in Atlanta, Georgia
• Degnim, AC, Visscher D, Frost MH, Melton LJ, Vierkant RA, Maloney SD, Pankratz VS, Sellers TA, Lingle WL, Hartmann LC. Multifocal atypia confers increased risk of breast cancer

Poster presentation at annual meeting of American Association for Cancer Research, April 16-20, 2005 in Anaheim, California

Symposium and poster presentations at the Department of Defense Era of Hope June 10, 2005 in Philadelphia, Pennsylvania
• Pankratz VS, Vierkant RA, Maloney SD, Degnim AC, Hartmann LC. Statistical methods to assess the timing and side of breast cancer relative to benign breast biopsies: implications for potential precursor lesions

Podium presentation at annual meeting of the United States and Canadian Academy of Pathology, Atlanta, GA, Feb. 11-17, 2006
• Milanese TR, Hartman LC, Vierkant RA, Maloney SD, Frost MH, Pankratz VS, Visscher DW. The impact of lobular involution on breast cancer risk.

Poster presentation at annual meeting of the United States and Canadian Academy of Pathology, Atlanta, GA, Feb. 11-17, 2006
• Berg JC, Lewis JT, Maloney SD, Vierkant RA, Hartmann LC, Visscher DW. Analysis of cancer risk in women with radial scars of the breast.

Podium presentation at annual meeting of American Association for Cancer Research, Washington, D.C., April 1-5, 2006
- Pankratz VS, Vierkant RA, Maloney SD, Frost MH, Visscher DW, Hartmann LC. Assessment of the Gail model in a cohort of women with atypical hyperplasia.

Poster presentation at Joint Statistical Meetings, Minneapolis, MN, August 10, 2006.
- Pankratz VS, Vierkant SD, Maloney SD, Hartmann LC. Epidemiologic comparisons of disease incidence among populations: The person-years approach.

- Ghosh K, Hartmann LC, Maloney D, Vierkant RA, Milanese TM, Visscher DW, Pankratz VS, Vachon CM. Mammographic breast density is inversely associated with age-related involution.

Poster presentation at the annual meeting of the American Association for Cancer Research, April 2005
- Lingle W, Negron V, Bruzek A, Murphy L, Riehle D, Vierkant RA, Pankratz VS, Hartmann LC, Visscher DW. Centrosome amplification is greatest in benign breast lesions associated with an increase in risk of cancer.


Podium presentation at San Antonio Breast Cancer Symposium, San Antonio, TX, December 10-14, 2008.
Poster presentation at United States and Canadian Academy of Pathology Annual Meeting, Boston, MA, March 7-13, 2009.

• Barr-Fritcher EG, Hartmann LC, Degnim AC, Anderson SS, Vierkant RA, Frost M, Visscher DW, Reynolds C. Estrogen receptor expression in atypical hyperplasia and its association with type of atypia and age.

Podium presentation at United States and Canadian Academy of Pathology Annual Meeting, Boston, MA, March 2009.


B. Manuscripts


• Pankratz VS, Hartmann LC, Degnim AC, Vierkant RA, Ghosh K, Vachon CM, Frost MH, Maloney SD, Reynolds C, Boughey JC. Assessment of the accuracy of the


**Conclusions**

The Center of Excellence grant provided us the opportunity to establish a large cohort of women with BBD and to establish a tissue and data bank that includes benign tissue, subsequent breast cancer tissue, and clinical-epidemiologic data. We worked with Dr. Nassar to establish a cohort of African-American women at Wayne State University and with Dr. Tlsty’s team at UCSF to advance basic discoveries. We were able to make some substantial contributions to our understanding of features present in breast tissue that are associated with later breast cancer. The most notable of these contributions were the identification of relative risk related to non-proliferative disease, proliferative disease without atypia and proliferative disease with atypia published in the *NEJM* and the significance of involution in relation to breast cancer risk published in *JNCI*. Our data showed that women with atypia have a relative risk of 4.24 compared to age-matched women from the Iowa SEER database. Novel data about involution was particularly striking because of its strong relationship with subsequent breast cancer and the fact that it can be determined using a single slide of tissue. We identified several biomarkers of notable significance in predicting breast cancer risk, including COX-2 and Ki-67. We successfully grew fresh breast tissue and used that tissue to identify new, potential markers of interest. We also successfully profiled tissue that had been stored for up to 25 years in paraffin. We contrasted histology findings between African-American and Caucasian-American women, finding involution to occur at a slower rate in African-American women. We examined the usefulness of the Gail model for women with atypical hyperplasia. We found this model to underestimate the risk of breast cancer in women with atypia. On an individual basis, the Gail model performed no better than chance alone in women with atypia. We successfully obtained an R01 for further risk prediction work. The combination of findings obtained through the Center of Excellence grant provides rich data for future research.
Appendices
Appendix A: Persons receiving pay from the research effort
Collaborators
Lynn C. Hartmann, M.D., P.I.
Thomas Sellers, Ph.D.
Piet De Groen, M.D.
Robert Jenkins, M.D., Ph.D.
Aziza Nassar, M.D.
Carol Reynolds, M.D.
Daniel Visscher, M.D.
Fergus Couch, Ph.D.
Wilma Lingle, Ph.D.
Viji Shridhar, Ph.D.
Thea Tlsty, Ph.D., University of California, San Francisco
Hind Nassar, M.D, Wayne State University, Johns Hopkins
Rouba Ali-Fehmi, Wayne State University

Statistical support
Vera Suman, Ph.D.
Vernon Pankratz, Ph.D.
Rob Vierkant
David Hillman
Shaun Maloney
Stephanie Anderson

Data abstractors, study coordinators, data managers
Marlene Frost, Ph.D.,
Teresa Allers
Betty Anderson, R.N.
Mary Campion, R.N.
Joanne Johnson, R.N.
Melanie Kasner
Margie Loprinzi, R.N.
Lois Penheiter, R.N.
Romayne Thompson
Joel Worra
Appendix B: Abstracts of presentations
Benign Breast Disease and Breast Cancer Risk. LC Hartmann¹, D Visscher¹, C Reynolds¹, MH Frost¹, LJ Melton¹, C Vachon¹, T Tlsty², D Hillman¹, JL Johnson¹, WL Lingle¹, V Suman¹, TA Sellers¹.

¹ Mayo Clinic Cancer Center, Rochester, MN
² University of California, San Francisco, CA

Introduction: Benign breast disease (BBD) is an established risk factor for breast cancer (BC), but only a minority of women with BBD ultimately develop BC. The ability to identify the subset of women at greatest risk for breast cancer at the time of BBD diagnosis would permit more aggressive clinical intervention, including closer surveillance and prevention opportunities. To facilitate this discovery process, we have established a large historical cohort of women with BBD in which we can test more specific means of risk prediction, using clinical, histopathologic and molecular tools.

Methods: The Mayo Clinic Surgical Index was used to identify all women who had an open breast biopsy with benign findings at the Mayo Clinic between 1/1/82 and 12/31/91 (n = 5153). The availability of tissue slides and blocks on these patients was verified through linkage to the Pathology Index. Medical record review was performed to verify eligibility and to identify subsequent occurrences of breast cancer diagnosed or treated at Mayo. A study-specific questionnaire was mailed to collect risk factor data on the cohort and to identify breast cancers diagnosed outside of Mayo.

Results: This 10-year cohort includes 5153 women with 66,290 person years of follow-up (through 2/02). The median age at BBD diagnosis was 54 years (13-94), and 41% were age 50 or less. Some family history of breast cancer was present in 32%, while 17% had an affected first-degree relative. Thus far, 255 women are known to have developed BC. The interval from BBD to BC is: ≤ 5 yrs, 33.7%; 5.1-10 yrs, 34.5 %; 10.1-15 yrs, 27.5%; > 15 yrs, 4.3%. The cancer occurred in the same breast as the BBD in 125 women (49%), the opposite breast in 84 (32.9%), and both breasts in 10 (3.9%). Side of BC is pending for 36 (14%) women. The estimated 5-year, 10-year and 15-year breast cancer incidence rates are 1.8% (95%CI: 1.4-2.1%), 3.6% (95%CI: 3.1-4.2%), and 5.8% (95%CI: 5.1-6.5%), respectively. Incorporating time from BBD to cancer and the side of BBD vs BC, we are exploring a panel of biomarkers as indicators of possible BC precursors or a background field change.

Conclusions: We have assembled a large cohort of patients with BBD with extensive follow-up for breast cancer, excellent participation on a risk factor survey, and sufficient quantities of well-characterized tissues to permit independent evaluation of established and novel molecular markers.

Supported by grants from the national Komen Foundation, the Breast Cancer Research Foundation, and DOD Breast Cancer Center of Excellence award DAMD 17-02-1-0473.

1Mayo Clinic Cancer Center, Rochester, MN
2University of California, San Francisco, CA
3Moffitt Cancer Center, Tampa, FL

Introduction: Benign breast disease (BBD) is an established risk factor for breast cancer (BC), but only a minority of women with BBD ultimately develop BC. To identify the subset of women at greatest risk for breast cancer at the time of BBD diagnosis, we have established a large historical cohort of women with BBD in which we can test more specific means of risk prediction, using clinical, histopathologic and molecular tools.

Methods: The Mayo Clinic Surgical Index was used to identify all women ages 18-85 who had an open breast biopsy with benign findings at the Mayo Clinic between 1/1/67 and 12/31/91. The availability of tissue slides and blocks on these patients was verified through linkage to the Pathology Index. Medical record review was performed to verify eligibility and to identify subsequent occurrences of breast cancer diagnosed or treated at Mayo. A study-specific questionnaire is being used to collect risk factor data on the cohort and to identify breast cancers diagnosed outside of Mayo.

Results: This 25-year cohort includes 11,782 women with 181,284 person years of follow-up. The median age at BBD diagnosis was 50.0 years. Some family history of BC was present in 40% of those surveyed; 21% had an affected first-degree relative. Thus far, 705 women are known to have developed BC, at a median of 9.2 years after their BBD. The interval from BBD to BC is <= 5 years, 27%; 5.1 - 10 years, 26%; 10.1-15 years, 24%; >15 years, 23%. The cancer occurred in the same breast as the BBD in 279 women (40%), the opposite breast in 189 (27%) and both breasts in 18 (3%). Side is pending for 219 (31%). The estimated 5-yr, 10-yr and 15-yr BC incidence rates are 2.0%, 4.1%, and 6.4%, respectively for women with BBD from 1982-1991 (follow-up ongoing for 1967-81 group). The histopathologic review has been completed for 3,004 of the BBD specimens. Non-proliferative disease was found in 65.8%, proliferative disease without atypia in 28.6% and atypia (atypical ductal hyperplasia or atypical lobular hyperplasia) in 3%. Incorporating time from BBD to BC, histology, and side of BBD vs BC, we are exploring a panel of biomarkers as indicators of possible BC precursors or a background field change.

Conclusions: We have assembled a large cohort of patients with BBD with extensive follow-up for breast cancer, excellent participation on a risk factor survey, and sufficient quantities of well-characterized tissues to permit independent evaluation of established and novel molecular markers.

Supported by grants from the national Komen Foundation, the Breast Cancer Research Foundation, and DOD Breast Cancer Center of Excellence award DAMD 17-02-1-0473.
Analysis of Cancer Risk among Patients with Papillary Lesions of the Breast

JT Lewis, RA Vierkant, SD Maloney, LC Hartmann, DW Visscher. Mayo Clinic, Rochester, MN

Background: Papillomas are relatively common breast lesions. Although most are single and histologically bland, they may be multiple and demonstrate varying degrees of atypia. The risk of breast carcinoma development in patients with benign papillary breast lesions is incompletely defined.

Design: Papillary breast lesions were identified in a histopathologically-defined benign breast disease cohort of 8872 patients biopsied between 1967-1991. Cases were subclassified into four groups: single papilloma without atypia, single papilloma with atypia, multiple (>3) papillomas without atypia, and multiple papillomas with atypia. Using Cox proportional hazards regression, the risk of cancer development among these groups was compared to patients with other forms of proliferative breast disease (with or without atypia) and patients with non-proliferative breast changes.

Results: Of the 368 patients diagnosed with a single papilloma without atypia, 35 (10%) developed carcinoma. Eleven (22%) of the 49 women with a single papilloma with atypia subsequently developed carcinoma. Forty-one patients were diagnosed with multiple papillomas without atypia, and six (15%) developed carcinoma. Twelve cases of multiple papillomas with atypia were identified, and 4 (33%) of these developed carcinoma. The relative risk of cancer development is presented in Table 1.

Conclusions: We conclude that the diagnosis of a single papilloma without atypia imparts an increased risk of developing a subsequent carcinoma similar to other non-atypical forms of proliferative breast disease. Atypical papilloma, particularly in the setting of multiple papillomas, imparts a breast cancer risk similar to or greater than conventional atypical ductal/lobular hyperplasias.

<table>
<thead>
<tr>
<th>Diagnosis (N)</th>
<th>Person Years Follow-up</th>
<th>Relative Risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Proliferative (5934)</td>
<td>91129</td>
<td>1.00</td>
</tr>
<tr>
<td>Proliferative without Atypia (2211)</td>
<td>32895</td>
<td>1.60 (1.35, 1.90)</td>
</tr>
<tr>
<td>Proliferative with Atypia (257)</td>
<td>3127</td>
<td>3.59 (2.63, 4.92)</td>
</tr>
<tr>
<td>Single Papilloma without Atypia (368)</td>
<td>4979</td>
<td>1.82 (1.28, 2.58)</td>
</tr>
<tr>
<td>Single Papilloma with Atypia (49)</td>
<td>577</td>
<td>4.88 (2.67, 8.92)</td>
</tr>
<tr>
<td>Multiple Papillomas without Atypia (41)</td>
<td>592</td>
<td>2.81 (1.25, 6.31)</td>
</tr>
<tr>
<td>Multiple Papillomas with Atypia (12)</td>
<td>115</td>
<td>8.66 (3.22, 23.31)</td>
</tr>
</tbody>
</table>

Relative risks were calculated using a Cox proportional hazards regression analysis. Results are adjusted for age.
Podium Presentation: Society of Surgical Oncology Annual Cancer Symposium, March 3-6, 2005, Atlanta, GA.

**Multifocal atypia confers increased risk of breast cancer.**

**Introduction:** We evaluated breast cancer risk in relation to histologic features of atypia in a large retrospective cohort of women with benign breast disease.

**Methods:** Through surgical and pathology indexes, women were identified who had an open breast biopsy with benign findings at our institution between 1/1/67 and 12/31/91. Histologic review of original biopsy slides or tissue blocks was performed by a single pathologist who was blinded to clinical outcome. Histopathologic information was collected, including type of hyperplasia and number of atypical foci. Subsequent breast cancers were identified through a follow-up survey and our Tumor Registry.

**Results:** Of 11,109 eligible women with benign breast biopsies, histologic review and follow-up are completed on 9,874 to date. Of 311 biopsies demonstrating atypical hyperplasia, 43% had only atypical ductal hyperplasia (ADH), 52% had only atypical lobular hyperplasia (ALH) and 4% had both ADH and ALH. Data on number of atypical foci are complete for 300 patients; of these, 58% had one focus of atypia, 26% had two foci, and 16% had 3 or more foci. With 3702 person-years of follow-up in these 311 women, we observed 60 cases of incident breast cancer. Age-adjusted Cox proportional hazard regression analysis showed a significantly increased relative risk (RR) of breast cancer with any atypia compared to 5950 women with non-proliferative disease (NPD) without atypia (see Table), and a trend toward higher risk with ADH versus ALH. Similar analysis showed a significantly increased risk of breast cancer with increasing number of atypical foci (see Table). Women with 3 foci of atypia had an 8-fold greater risk of breast cancer compared to those with NPD (p<0.01) and a three-fold greater risk compared to those with one focus of atypia (p<0.01).

**Conclusions:** In women with atypia on benign breast biopsy, multiple foci of atypia indicate a significantly higher risk of subsequent breast cancer. Ductal atypia may convey a somewhat greater risk of subsequent breast cancer compared to ALH.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Events</th>
<th>RR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histologic Type</td>
<td>Non-Prol Dis W/O Atypia</td>
<td>344</td>
<td>1.0 (Reference)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>ALH</td>
<td>30</td>
<td>3.6 (2.5, 5.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>ADH</td>
<td>26</td>
<td>4.5 (3.0, 6.7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>ALH and ADH</td>
<td>4</td>
<td>5.8 (2.2, 15.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td># of Foci of Atypia</td>
<td>Non-Prol Dis W/O Atypia</td>
<td>344</td>
<td>1.0 (Reference)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>1 focus of atypia</td>
<td>21</td>
<td>2.5 (1.6, 4.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>2 foci of atypia</td>
<td>18</td>
<td>4.9 (3.0, 7.9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>3 or more foci of atypia</td>
<td>19</td>
<td>8.2 (5.1, 13.0)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Benign Breast Disease and Breast Cancer Risk in the Mayo Cohort Study
Lynn C. Hartmann1*, Thomas A. Sellers2, Marlene H. Frost1*, Wilma L. Lingle1α, Amy C. Degnim1β, Karthik Ghosh1Ω, Robert A. Vierkant1π, Shaun D. Maloney1π, V. Shane Pankratz1π, David W. Hillman1π, Vera J. Suman1π, Jo Johnson1*, Cassann Blake1*, Thea Tlsty4, Celine M. Vachon1+, L. Joseph Melton III 1+, Daniel W. Visscher1♦. Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905 (*Division of Medical Oncology; αDivision of Experimental Pathology; βDivision of General Surgery; ΩDivision of General Internal Medicine; δDivision of Biostatistics; δDivision of Epidemiology; δDivision of Anatomic Pathology); εH. Lee Moffitt Cancer Center & Research Institute, 12902 Magnolia Drive, Tampa, FL 33620; βWayne State University, Detroit MI 48202; δUniversity of California, San Francisco, 513 Parnassus Avenue, San Francisco, CA. 94143.

Background: Benign breast disease is a significant risk factor for breast cancer. To optimize management of these women, we need precise risk estimates for them. Moreover, it remains unclear if these lesions represent precursors or generalized risk indicators.

Methods: We identified all women with benign breast disease at the Mayo Clinic between 1967 and 1991. Breast cancer events and risk factors were obtained from the medical record and questionnaires. All benign specimens were reviewed by our breast pathologist. To estimate relative risks, we compared observed breast cancers to those expected using Iowa SEER rates.

Results: This Mayo cohort includes 9,087 women followed for a median of 15 years. The histologies are: non-proliferative (66%); proliferative without atypia (30%) and atypical hyperplasia (4%). 707 breast cancers have developed to date. The relative risk for the cohort is 1.56 (95% CI 1.45-1.68). Increased risk persisted to at least 25 years after biopsy. Atypia conveyed a RR of 4.24 (95% CI 3.26-5.41), vs 1.88 for proliferative changes without atypia and 1.27 (95% CI 1.15-1.41) for non-proliferative lesions. Family history information was available for 4,808 women (53%) and conveyed risk independent of histology, with a RR of 1.93 for a strong family history (95% CI 1.58-2.32) vs. 1.18 (95% CI 1.01-1.37) for no family history. For women with negative family history and non-proliferative findings, we saw no increased risk. In the first 10 years after benign biopsy, an excess of cancers occurred in the same breast, especially in women with atypia, consistent with the presence of precursor lesions.

Conclusions: Proper categorization of women with benign breast disease can differentiate high risk subsets from those at no increased risk.

MULTIFOCAL ATYPIA CONFERs INCREASED RISK OF BREAST CANCER

AC Degnim1, DV Vischer1, MH Frost1, LJ Melton1, RA Vierkant4, SD Maloney1, VS Pankratz1, TA Sellers2, WL Lingle1, T Tlsty3, HBerman3, LCHartmann1

1Mayo Clinic Cancer Center, 2Moffitt Cancer Center, 3University of California San Francisco

Degnim.Amy@mayo.edu

We evaluated breast cancer (BC) risk in relation to histologic features of atypia in a large retrospective cohort of women with benign breast disease.

Through surgical and pathology indexes, women were identified who had a surgical breast biopsy with benign findings at our institution between 1/1/67 and 12/31/91. Histologic review of original biopsy slides or tissue blocks was performed by a single pathologist (DV) who was blinded to clinical outcome. Histopathologic information was collected, including type of hyperplasia and number of atypical foci. Subsequent BCs were identified through a follow-up survey and our Tumor Registry. We compared the observed number of incident BCs among women in our cohort with atypical hyperplasia (AH) to that expected using incidence rates from the Iowa SEER data. Internal comparisons of BC and number of atypical foci were made using Cox proportional hazards regression.

Of 10,032 eligible women with benign breast biopsies, histologic review and follow-up are completed on 9,087. Of 336 biopsies demonstrating AH, 62% had one focus of atypia, 24% had two foci, and 14% had 3 or more foci. We observed 4161 person-years of follow-up and 64 cases of incident BC in these 336 women. Those with AH had a 4.2-fold increased risk of BC compared to Iowa SEER (95% CI 3.3-5.4). Kaplan-Meier estimates show increasing risk of BC with increasing number of atypical foci (Figure 1). Women with 3 foci of atypia had a 9.4-fold increased risk of BC vs. Iowa SEER and a 4.1-fold greater risk vs. those in the cohort with one focus of atypia (p<0.01 for each).

In women with AH on benign breast biopsy, multiple foci of atypia indicate a significantly higher risk of subsequent BC. These results may impact decision-making in women with AH who are at increased risk for BC.

The U.S. Army Medical Research and Materiel Command under DAMD17-02-1-0473-1 supported this work.
TEMPORAL CHANGES IN BENIGN BREAST DISEASE 1967 TO 1991

K. Ghosh MD, MS1, L.C. Hartmann MD1, T. A. Sellers Ph.D2, A. C. Degnim MD1, V. S. Pankratz Ph.D1, C. Blake MD3, T. Tlsty Ph.D4, L. J. Melton III MD1, D. W. Visscher MD1

Affiliations: 1Mayo Clinic, Rochester MN; 2H. Lee Moffitt Cancer Center, Tampa FL; 3Wayne State University, Detroit MI; 4University of California, San Francisco CA

Background: Women with benign breast disease (BBD) are at increased risk of breast cancer (BC). The classic study of BBD by Dupont and Page enrolled women with biopsies in the 1950s-1960s. We sought to assess changes in the nature of BBD over time, utilizing a 25-year cohort of BBD from the late 1960s to the early 1990s.

Methods: Utilizing the Mayo Clinic Surgical and Pathology Indices, women ages 18 to 85 who had benign excisional breast biopsy between January 1, 1967 and December 31, 1991 were identified. The clinical outcome of BC was the endpoint for follow-up for the ‘cases’ and was determined using the Mayo medical record and questionnaire information sent to study participants. Our breast pathologist (DV), blinded to both the initial diagnosis and clinical outcome, performed pathology review.

Results:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>5087 (100%)</td>
<td>971 (19.3%)</td>
<td>1808 (35.6%)</td>
<td>1509 (29.7%)</td>
<td>2205 (43.6%)</td>
<td>2504 (49.5%)</td>
</tr>
<tr>
<td>Breast Cancer Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>707 (14.0%)</td>
<td>122 (12.6%)</td>
<td>190 (10.5%)</td>
<td>112 (7.4%)</td>
<td>138 (6.2%)</td>
<td>145 (5.8%)</td>
</tr>
<tr>
<td>Mean Age at Biopsy (standard deviation)</td>
<td>51.4 (14.3)</td>
<td>47.5 (13.3)</td>
<td>49.3 (13.4)</td>
<td>48.8 (14.3)</td>
<td>53.5 (14.3)</td>
<td>54.1 (14.4)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Prolif.</td>
<td>6061 (66.7%)</td>
<td>713 (73.4%)</td>
<td>1339 (74.1%)</td>
<td>1057 (70.0%)</td>
<td>1469 (64.8%)</td>
<td>1483 (59.2%)</td>
</tr>
<tr>
<td>Prolif.</td>
<td>2690 (29.6%)</td>
<td>245 (25.2%)</td>
<td>435 (24.1%)</td>
<td>411 (27.2%)</td>
<td>728 (31.7%)</td>
<td>871 (34.8%)</td>
</tr>
<tr>
<td>Atypia</td>
<td>336 (3.7%)</td>
<td>13 (1.3%)</td>
<td>34 (1.9%)</td>
<td>41 (2.7%)</td>
<td>98 (4.3%)</td>
<td>150 (6.6%)</td>
</tr>
</tbody>
</table>

Conclusions: This study provides data regarding the changing nature of BBD. The number of women in each 5-year period increased, likely due to growth of clinical practice at Mayo Clinic but may also reflect increased adoption of screening mammography. Within each time-frame, there were over 100 cases of BC, but the proportion of “cases” to “non-cases” decreased with decreasing “years of risk” for women in the latter part of the study. Mean age at biopsy increased from 47.5 to 54.1, and BBD samples from the latter years of the study were more likely to show proliferative change with or without atypia, again likely due to increased use of screening mammography and detection of abnormal calcifications. The stable proportion of women with positive family history (about 20% of whom had a strong family history) is consistent with general breast cancer awareness and screening practices in this population.
BENIGN BREAST DISEASE: EVIDENCE FOR PRECURSOR LESIONS

LC Hartmann, A Degnim, MH Frost, RA Vierkant, SD Maloney, TA Sellers, VS Pankratz, T Tlsty, C Blake, WL Lingle, DW Visscher
Mayo Clinic and Mayo Foundation, Rochester, MN; H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL; University of California, San Francisco, CA; Wayne State University, Detroit, MI. E-mail: hartmann.lynn@mayo.edu

Background: Benign breast disease (BBD) represents a significant risk factor for a later breast cancer (BC) that can occur in either breast. Besides aiding in risk prediction, BBD provides a possible window into a continuum of alterations culminating in BC. Information about time to and side of BC after BBD has not been available for most prior studies of BBD. Such information can help distinguish possible precursor lesions from markers of increased risk.

Methods: We used the Mayo Clinic Surgical Index to identify women ages 18-85 who had BBD between 1-1-67 and 12-31-91. The benign H&E-stained sections were evaluated by our study pathologist (DV). Biopsies were classified into: 1) non-proliferative changes, 2) proliferative changes without atypia (PDWA), and 3) atypical hyperplasia (AH). To estimate relative risks, we compared the observed number of incident BCs in our cohort to that expected, using age- and calendar period-matched incidence rates from the Iowa SEER data as the reference.

Results: This cohort consists of 9087 women who have been followed for a median of 15 years (person years 144,881). The benign histologies include: non-proliferative [n=6061 (66%)], PDWA [n=2690 (30%)] and AH [n=336 (4%)]. 707 breast cancers have occurred to date. The overall relative risk for breast cancer for the entire cohort is 1.56 (95% CI 1.45 – 1.68). Benign histology was a major predictor of risk. AH conveyed a relative risk of 4.24 (95% CI 3.26 – 5.41) vs 1.88 (1.66 – 2.12) for women with PDWA and 1.27 (1.15 – 1.41) for non-proliferative lesions. The table shows median years to BC and side of BC by histologic category for those women who developed BC. There is a greater tendency for BC to develop sooner (p=0.03) and in the ipsilateral breast in women whose BBD contained increasing degrees of proliferation and atypia—consistent with the presence of precursors in these higher risk entities.

Conclusion: Information about side of BC and time to BC in studies of BBD can help to identify probable precursor lesions. Studies based in these lesions can guide our understanding of molecular risk and molecular carcinogenesis.

Sidedness and Timing of Breast Cancers after BBD

<table>
<thead>
<tr>
<th>Benign Histology</th>
<th># of BCs*</th>
<th>Median Yrs to BC (1st -3rd quartile)</th>
<th>Side of BC*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(1st -3rd quartile)</td>
<td>Same (n,%), Opposite (n,%)</td>
</tr>
<tr>
<td>Non-proliferative</td>
<td>379</td>
<td>10.7 (5.4-16.4)</td>
<td>185 (54), 156 (46)</td>
</tr>
<tr>
<td>PDWA</td>
<td>264</td>
<td>11.0 (5.8-16.0)</td>
<td>123 (56), 96 (44)</td>
</tr>
<tr>
<td>AH</td>
<td>64</td>
<td>9.3 (5.7-14.5)</td>
<td>34 (61), 22 (39)</td>
</tr>
</tbody>
</table>

* cancers where both BBD and BC were unilateral events and side for both was known

The U.S. Army Medical Research and Material Command under DAMD17-02-1-0473 supported this work.
STATISTICAL METHODS TO ASSESS THE TIMING AND SIDE OF BREAST CANCER RELATIVE TO BENIGN BREAST BIOPSIES: IMPLICATIONS FOR POTENTIAL PRECURSOR LESIONS

V.S. Pankratz, R.A. Vierkant, S.D. Maloney, A.C. Degnim, L.C. Hartmann
Mayo Clinic, Rochester, MN
pankratz.vernon@mayo.edu

Introduction: Benign breast disease is an important predictor of risk for breast cancer. It may also provide information about a continuum of benign breast alterations culminating in breast cancer. The agreement between side of the benign lesion and subsequent breast cancer provides one means of obtaining evidence for the presence of precursors. However, little data have been reported describing the concordance between side of the benign lesion and the cancer. Also, methods to assess the evidence of this concordance, particularly with regarding the time interval between benign lesion and breast cancer are lacking.

Methods: Extensive follow-up data were obtained from a consecutive series of women undergoing an open breast biopsy with benign findings from 1967 through 1991, including the timing of subsequent breast cancers and the side(s) of benign biopsy and cancer development. A variety of methods to assess concordance between benign lesions and breast cancers were explored. These ranged from the simple (e.g. chi-square tests) to the complex (e.g. survival models). Ultimately, we estimated the relative risk of cancer in the same vs. the opposite breast for five-year time intervals using a survival analysis approach by computing the relative incidence of ipsilateral and contralateral cancers. We calculated the incidence for each of these categories using two observations per person and censoring for the type of cancer that did not occur. Using this method, the relative risks are equivalent to ratios of observed events, as the approach yields identical person years for each event type. We capitalized on this and used properties of the binomial distribution to obtain exact p-values and 95% confidence intervals for these relative risks.

Results: The study has so far followed 9087 eligible women for 144,881 person-years (median 15 years), and 707 breast cancers have been observed to date. 91 of these cases were either missing side information, or had bilateral biopsies or cancers. Most of the unilateral events, 342 of 616 (56%), developed in the same breast as the benign biopsy. During the first ten years, there was an excess of ipsilateral cancers, with relative risks for ipsilateral vs. contralateral of 1.88 and 1.34 for years 0-5 and 6-10, respectively. Additionally, the 35 women with atypia who developed breast cancer within 10 years of their benign biopsy were 2.5 times more likely (p=0.02) to develop cancer in the same breast vs. the opposite breast.

Conclusions: We have examined and used a range of statistical methods to evaluate side-specific breast cancer risk. An excess of breast cancers occurred in the same breast within the first years of follow-up, especially in women with atypia. This suggests that precursors may exist within the spectrum of benign breast disease that can be identified with molecular techniques and targeted with tailored interventions.

The U.S. Army Medical Research and Materiel Command under DAMD17-02-1-0473 supported this work.
**BENIGN BREAST DISEASE AND BREAST CANCER RISK**

LC Hartmann¹, MH Frost¹, K Ghosh¹, A Degnim¹, RA Vierkant¹, SD Maloney¹, VS Pankratz¹, T Tlsty², C Blake³, TA Sellers⁴, WL Lingle¹, LJ Melton¹, D Visscher¹

Mayo Clinic Cancer Center¹, Rochester, MN; University of California², San Francisco, CA; Wayne State University³, Detroit, MI; Moffitt Cancer Center⁴, Tampa, FL. E-mail: hartmann.lynn@mayo.edu

**Background:** Benign breast disease (BBD) represents a significant risk factor for a later breast cancer that can develop in either breast. Questions remain about the degree of risk associated with non-proliferative findings and the degree of interaction between atypia and family history. Having accurate risk estimates is essential to counsel women properly regarding surveillance and risk reduction strategies.

**Methods:** The Mayo Clinic Surgical Index was used to identify all women ages 18-85 who had an open breast biopsy with benign findings at the Mayo Clinic between 1/1/67 and 12/31/91. Our study pathologist (DV) reviewed and classified all benign lesions. Medical records and a study-specific questionnaire were used to collect risk factor data and to identify subsequent breast cancers (BC). To estimate relative risks, we compared the observed number of incident BCs in our cohort to that expected, using age- and calendar period-matched incidence rates from the Iowa SEER data as the reference.

**Results:** This 25-year cohort includes 9,087 women with 144,881 person years of follow-up (median 15 yrs). The mean age at BBD was 51.4 years. Non-proliferative disease was found in 66%, proliferative disease without atypia in 30% and atypia (atypical ductal hyperplasia or atypical lobular hyperplasia) in 4%. Thus far, 707 women are known to have developed BC, at a median of 10.7 years after their BBD.

The overall relative risk for breast cancer in our cohort is 1.56 (95% CI 1.45 -1.68). Benign histology was a major predictor of risk. Atypical hyperplasia conveyed a relative risk of 4.24 (3.26 – 5.41) vs 1.88 (1.66 – 2.12) for women with proliferative disease without atypia and 1.27 (1.15 – 1.41) for non-proliferative lesions. Knowledge of family history allowed further refinement of risk estimates. For women with no family history, the relative risk was 1.18 (1.01 – 1.37) compared to 1.43 (1.15 – 1.75) for women with a weak family history, and 1.93 (1.58 – 2.32) for those with a strong family history. For women with non-proliferative findings and no or weak family history, there was no increased risk. We did not see an interaction between atypia and family history. Women with atypia and no family history had a RR of 2.95 (1.65 – 4.87) vs 4.18 (1.80 – 8.23) for those with a weak family history and 4.0 (2.07 – 6.99) for those with a strong family history. Risk of BC was inversely associated with age at benign biopsy, with younger women demonstrating greater risk than older women (RR for age < 30 = 1.83 vs RR 1.40 for age ≥ 70).

**Conclusions:** Benign breast disease is a major risk factor for a later breast cancer. Within BBD, age at BBD, family history and histology are major predictors of subsequent risk.

*The U.S. Army Medical Research and Material Command under DAMD17-02-1-0473 supported this work.*
Podium Presentation: United States and Canadian Academy of Pathology, Annual Meeting, February 11-17, 2006, Atlanta, GA.

**The Impact of Lobular Involution on Breast Cancer Risk.**
T R Milanese, L C Hartmann, R A Vierkant, S D Maloney, M H Frost, V S Pankratz, and D W Visscher.

*Background:* Lobular involution is a histologic finding that reflects atrophy associated with physiologic aging in the human breast. Based on epidemiologic associations, involution has been hypothesized to have relevance in breast tumorigenesis.

*Methods:* A breast pathologist examined benign breast biopsies of 8,743 women in the Mayo Benign Breast Disease cohort and classified them according to the degree of lobular involution as follows: none (0%), partial (1-74%), or complete (>75%). Each benign biopsy was also evaluated per standard criteria as nonproliferative (NP), proliferative disease without atypia (PDWA), and atypical hyperplasia (AH). Age at biopsy, family history of breast cancer, and development of breast cancer were obtained from medical records or questionnaires (17-year mean follow-up). Associations of involution with other breast cancer risk factors were carried out using chi-square tests and logistic regression analyses. Relative risks of breast cancer were estimated by comparing the number of observed events with the number expected based on rates from the Iowa SEER registry.

*Results:* Distribution of the patients by the three levels of involution was as follows: none-1,628 (18.6%); partial-5,202 (59.5%); and complete-1,913 (21.9%). Increased involution was found to correlate with increased age and decreased family history of breast cancer. The relative risk of breast cancer was significantly lower in patients who had complete (0.91, 95% CI 0.74-1.10) compared to those with partial (1.45, 95% CI 1.32-1.59) or no involution (1.88, 95% CI 1.59-2.21) (P<0.001). Age and family history modified breast cancer risk. In patients with PDWA, the relative risk for women with no involution was (2.94, 95% CI 2.26-3.75), while that for women with complete involution was only (1.11, 95% CI 0.68-1.72) (P<0.001). The relative risks in patients with NP and AH displayed similar associations.

*Conclusions:* The degree of lobular involution correlates inversely with breast cancer risk. It modifies breast cancer risk in patients stratified by age, family history, and type of histology. These data indicate that aberrant or delayed involution is a biologically important constitutional variable in breast cancer biology.
Analysis Of Cancer Risk In Women With Radial Scars Of The Breast
JC Berg, JT Lewis, SD Maloney, RA Vierkant, LC Hartmann, DW Visscher - Mayo Clinic, Rochester, MN

Background: Radial scars (RS) are characterized by an elastotic central core containing entrapped tubules that radiate outward in a stellate manner. The epithelial component may show varying degrees of proliferation. Previous studies have shown that RS impart an increased risk of breast cancer development.

Design: RS were systematically identified in a histopathologically defined benign breast disease (BBD) cohort of 9073 patients biopsied between 1967 and 1991. Overall histology was classified as nonproliferative (NP), proliferative disease without atypia (PDWA), or atypical hyperplasia (AH) per standard criteria. The presence, number and size of RS were recorded for each case. The relative risk of cancer development within the BBD cohort was compared to that expected in the general population using standardized incidence ratios (SIRs).

Result: RS were identified in 441 (4.9%) of the cohort cases; 384 (87%) of these contained one RS, 42 (9.5%) contained two, nine (2%) contained three, and six (1.5%) contained four or more, with a maximum of 11. RS size information was available in 434 cases. The majority of RS (357/434, 82%) were less than 5 mm in diameter; 61 biopsies (14%) contained 5-9.9 mm RS, and 16(4%) had RS than 10 mm. Table 1 and 2 compare the relative risk of BBD subsets defined by presence, size and number of RS to patient groups lacking RS.

Conclusion: RS imparts no increased breast cancer risk compared to other forms of PDWA (i.e. duct hyperplasia and/or adenosis). Likewise, RS associated with AH also connotes no increased risk above that of AH. Breast cancer risk was not modified significantly by the size or number of RS lesions.

| Table 1: Incident Breast Cancer Relative Risk by Histology and RS Presence |
|-----------------------------|---------|---------|
| Diagnosis | Number | Relative Risk (95% CI) |
| NP | 6048 | 1.07 (0.96, 1.18) |
| PDWA | 2311 | 1.57 (1.37, 1.80) |
| PDWA + RS | 377 | 1.84 (1.33, 2.49) |
| AH | 273 | 4.01 (3.03, 5.21) |
| AH + RS | 64 | 3.33 (1.67, 5.97) |

Relative risks were calculated using Cox Proportional Hazard Regression Analysis. Results are adjusted for age.

| Table 2: Relative Risk By Radial Scar Feature |
|-----------------------------|---------|---------|
| Feature | Number | SIR (95% CI) |
| 1 Scar | 384 | 2.02 (1.48, 2.69) |
| 2+ Scar | 57 | 2.12 (0.85, 4.35) |
| Size < 5 mm | 357 | 1.84 (1.32, 2.51) |
| Size > 5 mm | 77 | 2.50 (1.20, 4.61) |

Relative risks were calculated using Cox Proportional Hazard Regression Analysis. Results are adjusted for age.
COX-2 expression in atypia: Correlation with breast cancer risk.

Lynn C. Hartmann, Wilma L. Lingle, Marlene H. Frost, Shaun D. Maloney, Robert A. Vierkant, V. Shane Pankratz, Thea Tlsty, Amy C. Degnim, Daniel W. Visscher

Background: Women with atypical hyperplasia have a significantly increased risk of a later breast cancer (RR~4.0) and are excellent candidates for chemoprevention strategies. Identification of appropriate molecular targets is a priority. COX-2 is up-regulated in a variety of malignancies by several oncogenic mechanisms. Increased COX-2 expression has been documented in DCIS specimens. We sought to determine COX-2 expression in women with atypia and assess possible correlations with a later breast cancer.

Methods: The Mayo Clinic Benign Breast Disease Cohort includes 9343 women who had an open breast biopsy between 1967 and 1991. For 247 women with atypical hyperplasia, there was formalin-fixed, paraffin-embedded tissue available for assessment of COX-2 expression by immunohistochemistry. Our study pathologist (DWV) scored the COX-2 expression on a scale from 0 (negative) to 3+ (high intensity). We used Cochran-Mantel-Haenszel tests for trend to compare intensity of staining in the samples of women who developed breast cancer (cases) to that of women who did not develop breast cancer (controls).

Results: Forty of the 247 women with atypia have developed breast cancer over a median follow-up of 15 years. The atypia samples displayed a range of COX-2 expression with values of 0 for 28 (11.3%), 1+ for 113 (45.8%), 2+ for 74 (30%), and 3+ staining for 32 (13%). We found significantly higher COX-2 staining intensity in the atypias of those women who went on to develop breast cancer compared to the controls who did not (p=0.04).

Conclusions: Women with atypia are recognized as having a high risk for a later breast cancer. Intense COX-2 expression is associated with a significantly greater likelihood of a subsequent breast cancer in women with atypia and represents one potential molecular target for chemoprevention strategies.

References
Assessment of the Gail model in a cohort of women with atypical hyperplasia.

V. Shane Pankratz, Robert A. Vierkant, Shaun D. Maloney, Marlene H. Frost, Daniel W. Visscher and Lynn C. Hartmann. Mayo Clinic, Rochester, MN

Background: Understanding an individual woman’s risk of developing breast cancer is of high importance if we are to tailor clinical management properly. We sought to evaluate the performance of the Gail model\(^1\) in a cohort of women with atypical hyperplasia, and to determine if other histopathological features might contribute to enhanced risk prediction in this cohort.

Methods: The Mayo Clinic Benign Breast Disease (BBD) Cohort includes 9343 women who had an open breast biopsy between 1967 and 1991.\(^2\) Of these, 336 women had atypical hyperplasia, a group with significantly increased risk of a later breast cancer (RR\(\approx\)4.0). Gail model risk factors, and others, were obtained via survey and medical record review. Lifetime risk (thirty-year probability) of breast cancer was computed for each woman. Logistic regression was used to assess the concordance between the predicted and observed lifetime risk. Proportional hazards regression, with bootstrap model selection, was used to identify a potential risk prediction model for this high-risk group of women.

Results: In this atypia sub-cohort, 64 women experienced a breast cancer with an average follow-up of about 15 years. This number of events was slightly lower than the number predicted by the 30-year Gail model probabilities (rate ratio [95% CI] = 0.94 [0.74 - 1.20]). At the individual level, the concordance between observed breast cancer events and predicted lifetime probabilities of breast cancer was 0.59. This did not reach statistical significance (p=0.13), however, the number of events was low. The model selection process identified one covariate that was associated with breast cancer risk in this sub-cohort: the number of foci of atypia.

Conclusions: Averaging risks across this atypia cohort, the Gail model prediction was on target, but the per-individual concordance between observed and predicted breast cancer was low. Knowledge of the number of foci of atypia provided additional information about breast cancer risk. The development of alternative risk models in this group, and in the entire BBD cohort, are in process.

Epidemiologic Comparison of Disease Incidence Among Populations: The Person-Years Approach.
V.S. Pankratz, R.A. Vierkant, S.D. Maloney, L.C. Hartmann, Mayo Clinic, Rochester, MN

In epidemiological studies it is often of interest to compare disease incidence within a study cohort to that of a reference population. The person-years approach is often used to make and summarize such comparisons. The resulting Standardized Mortality Ratios (SMRs) summarize the degree to which observed cohorts differ from the reference population. While there have been criticisms of this method, there are few alternatives when one wishes to compare study groups with respect to their degree of deviation from population-based expectations. Our study of this topic is motivated by a desire to study the risks of breast cancer in women with a history of a benign breast biopsy relative to a reference population. In this study, follow-up data were obtained from a consecutive series of 9086 women having had a benign breast biopsy. Women with a history of a benign breast biopsy had an SMR of 1.6 (95% CI: 1.5 – 1.7). We present an overview of the person-years method and demonstrate how estimates of per-subject expected events may be used in the place of group-aggregated expected events. We also outline modifications that may alleviate concerns that arise in the use of this approach, motivated by data from the study that motivated our investigations.

This research was supported by the U.S. Army Research and Materiel Command under DAMD17-02-1-0473.
Mammographic breast density is inversely associated with age-related involution.
Karthik Ghosh MD, Lynn C. Hartmann MD, Shaun D. Maloney, Robert A. Vierkant MS, Tia M. Milanese BS, Daniel W. Visscher MD, V. Shane Pankratz PhD., Celine M. Vachon PhD. Departments of General Internal Medicine, Oncology, Biostatistics, Epidemiology, Mayo Medical School, and Anatomical Pathology, Mayo Clinic, Rochester, MN.

**Background:** The breast epithelium is composed of acini that join to form lobules. These lobules undergo age-related involution that has been demonstrated in women even in their 30s. Mammographic breast density is a known risk factor for breast cancer that has also been shown to decrease with age. This study aims to examine the association of age-related lobular involution with mammographic breast density (MBD) in a cohort of women with benign breast disease (BBD).

**Materials and Methods:** Women from the Mayo BBD Cohort who were diagnosed with BBD between 1985 and 1991 and had a mammogram within 6 months of BBD diagnosis were eligible for this study. MBD as a 4-category measure of Bi-RADS density was ascertained from a clinical mammography database that has been maintained since 1985 at the Mayo Clinic. Our breast pathologist (DV), blinded to both the initial diagnosis and clinical outcome, performed pathology review of all the study tissue. Involution in the breast tissue was subjectively assessed as categories of none- 0% involution, partial- 1 – 74% involution, or complete- >75% involution of the terminal duct lobular units. To examine the association between involution and Bi-RADS density, the odds of a low density mammogram (Bi-Rads=1) were estimated for the three categories of involution using logistic regression.

**Results:** A total of 3773 women from the BBD cohort were diagnosed between 1985 and 1991; of these, 2667 (71%) had a Bi-RADS density available within 6 months of BBD diagnosis. Mean age at BBD diagnosis in this sample was 55 years. The distribution of involution and Bi-RADS category of density is shown in the table below.

<table>
<thead>
<tr>
<th>Bi-RADS Breast density</th>
<th>Involution None</th>
<th>Involution Partial</th>
<th>Involution Complete</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56 (15.60%)</td>
<td>325 (19.51%)</td>
<td>174 (27.10%)</td>
<td>555 (20.81%)</td>
</tr>
<tr>
<td>2</td>
<td>31 (8.64%)</td>
<td>221 (13.27%)</td>
<td>126 (19.63%)</td>
<td>378 (14.17%)</td>
</tr>
<tr>
<td>3</td>
<td>54 (15.04%)</td>
<td>412 (24.73%)</td>
<td>176 (27.41%)</td>
<td>642 (24.07%)</td>
</tr>
<tr>
<td>4</td>
<td>218 (60.72%)</td>
<td>708 (42.50%)</td>
<td>166 (25.86%)</td>
<td>1092 (40.94%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>359</strong></td>
<td><strong>1666</strong></td>
<td><strong>642</strong></td>
<td><strong>2667</strong></td>
</tr>
</tbody>
</table>

Age adjusted analyses illustrate an inverse association of involution and Bi-RADS density. Compared to women with no evidence for involution, women with partial involution had 1.1 OR(CI 0.8,1.5) greater odds of low MBD and women with complete involution had 1.4 OR(CI 0.96,2.1) greater odds [p-value test for trend p=0.03].

**Discussion:** Our findings indicate that MBD is inversely associated with involution of breast tissues. Future histological and molecular studies are warranted to understand the process of age-related involution and its association with breast density, in order to improve our understanding of the biology of breast tissues, thereby, of breast cancer.
Age-related lobular involution and reduced risk of breast cancer.

Hartmann LC, Milanese TR, Sellers TA, Frost MH, Pankratz VS, Degnim AC, Visscher DW. Mayo Clinic, Rochester, MN; H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL

**Background:** Lobular involution or regression in the human breast is a natural process associated with aging. To identify tissue-based markers of risk of breast cancer, we studied if the extent of lobular involution was associated with later risk of breast cancer.

**Methods:** We examined the benign breast biopsies of 8,736 women in the Mayo Benign Breast Disease Cohort and classified the lobules in the background breast tissue by degree of involution: none (0%), partial (1-74%), or complete (≥75%). Subsequent breast cancer events and epidemiologic data were obtained from medical records and questionnaires. For relative risks, we compared observed events to those expected, based on the Iowa Surveillance, Epidemiology, and End Results registry.

**Results:** Distribution by degree of involution was: none-1,627 (18.6%); partial-5,197 (59.5%); and complete-1,912 (21.9%). Increased involution correlated with increased age (p<0.0001). Complete involution of lobular units was seen in 19/566 (3.4%) of women <30 at benign biopsy; 53/1037 (5.1%) of those ages 30-39; 142/2446 (5.8%) of those ages 40-49; 455/2109 (21.6%) of those ages 50-59; 724/1600 (45.3%) of those ages 60-69; and 519/978 (53.1%) of those ages 70 and higher. There was a strong, inverse correlation (p<0.0001) between involution and parity. Nulliparous women or women with only one child were more likely to have complete involution of their lobular units. Women with two, three, and four or more children had stepwise decrements in the likelihood of complete involution. The breast tissue from women with a strong family history was less likely to demonstrate involution (p=0.0006).

After a median of 17 years, the relative risk of breast cancer in the cohort overall was 1.40 (95% CI 1.30-1.51). The risk of breast cancer varied significantly by extent of involution, (p<0.001). For those women with no involution, the risk was 1.88 (95% CI 1.59-2.11); with partial involution, 1.47 (1.33-1.61); and with complete involution, 0.91 (0.75-1.10). Moreover, involution of lobular units modified the risk of breast cancer in all subsets, even women with atypia. Women with atypia and complete involution had a risk of 1.49 vs 4.06 with partial involution and 7.79 with no involution (p 0.003). In women with a strong family history of breast cancer and no involution, the RR was 2.77 (95% CI 1.94-3.84), while those with complete involution had a RR of 1.61 (95% CI 0.92-2.61).

**Conclusions:** The degree of age-related lobular involution correlates inversely with breast cancer risk. Aberrant involution may be a biologically important variable in breast cancer biology that can better inform risk prediction at the time of breast biopsy.
Centrosome Amplification is Greatest in Benign Breast Lesions Associated with an Increase in Risk of Cancer.

Wilma Lingle, Vivian Negron, Amy Bruzek, Linda Murphy, Darren Riehle, Robert Vierkant, Shane Pankratz, Daniel Visscher, Lynn Hartmann

Although centrosome amplification is known to be present in invasive breast cancer and ductal carcinoma in situ, it has not been investigated in benign breast lesions. Benign breast disease (BBD) encompasses a spectrum of histologic entities, usually subdivided into non-proliferative lesions, proliferative lesions without atypia, and atypical hyperplasia. A modest increase risk in development of breast cancer in the future has been associated with proliferative lesions, whereas a significant increased risk is associated with atypical hyperplasias. In this pilot study we investigated the status of centrosomes in BBD of various histologies to determine if amplified centrosomes can be detected in the absence of malignancy and invasion, and if any histologic types of BBD have significant levels of centrosome amplification. This study utilizes tissues from patients with BBD who were seen at the Mayo Clinic between 1967 and 1991 and followed up for a median of 15 years. Paraffin embedded BBD sections were stained with hematoxylin and eosin (H&E) for histological assessment. Adjacent sections were immunostained for gamma tubulin as a marker of centrosomes. Images captured using an Apotome-equipped Zeiss Axiovert 200M or Zeiss 510 LSM microscope for z-sectioning. Maximum intensity projection images were analyzed for centrosome size and number. Fibroblast centrosomes, present in each image, were used for normalization. We performed a pilot analysis of 5 non-proliferative lesions, 5 proliferative lesions without atypia, and 42 with atypia. Centrosome amplification was present in 88% of atypical hyperplasia samples, compared to none of the non-proliferative lesions, and only 1 of the proliferative lesions without atypia. Analysis of the complete set of tissues (>40 of each type) will allow us to determine if this difference is significant, and if it is related to development of invasive breast cancer during the patient follow up period.
Assessment of the accuracy of the Gail model in women with atypical hyperplasia.
Judy C. Boughey, Lynn C. Hartmann, Amy C. Degnim, Robert A. Vierkant, Karthik Ghosh, Celine M. Vachon, Shaun D. Maloney, Carol Reynolds, V. Shane Pankratz.

Background: An accurate estimate of an individual woman’s risk of breast cancer is essential for patient counseling and management. Women with atypical hyperplasia (AH) are at an elevated risk of breast cancer. Despite the widespread use of the Gail model, it has not been validated in patients with AH. We evaluate the accuracy of the Gail model in individuals from a well-annotated, well-characterized cohort of women with AH.

Methods: The Mayo Benign Breast Disease (BBD) Cohort is comprised of women aged 18 to 85 who had an open breast biopsy at the Mayo Clinic between 1967 and 1991 with benign pathological findings. Women with atypical ductal or lobular hyperplasia were identified by our study pathologists. Each individual's risk factors for breast cancer were obtained and the Gail model was used to predict five-year, follow-up specific and lifetime (age 90) risks for each woman. For the group level evaluation, the predicted numbers of breast cancers were compared to the observed numbers. For the individual-specific assessment, the concordance statistic was calculated.

Results: Of the 9,376 women in the cohort, 331 women (3.5%) had AH, which make up this study group. Over a mean follow up of 13.7 years, 66 (20%) women have developed breast cancer. The Gail model predicted 31.7 breast cancers in this length of follow up (rate ratio [95% CI] = 2.08 [1.64 – 2.65], p < 0.001). The concordance statistic, which determines the individual-specific agreement between predicted and observed outcomes, suggested that the Gail model did not predict well at the individual level, with a concordance between the individual-specific risk predictions and the observed breast cancer events in the first five years of follow-up of 0.46 (95% CI: 0.27 – 0.64). When evaluated linked to length of follow-up the concordance was 0.58 (95% CI: 0.53 – 0.63).

Conclusion: The Gail model significantly underestimates the risk of breast cancer development in women with atypical hyperplasia. Physicians should be cautious when using the Gail model to counsel individual patients with atypical hyperplasia regarding their risk of breast cancer development.
A novel, tissue-based feature strongly associated with breast cancer risk.

Background: Age-related involution of breast lobules (the anatomic substructure that gives rise to breast cancer), assessed qualitatively, is associated with reduced risk of breast cancer (Milanese et al, JNCI 2006). We hypothesized that a quantitative assessment of lobular involution could be developed to predict breast cancer risk more precisely.

Methods: We performed a nested case control study of 86 cases and 152 controls (238 patients) within the Mayo Clinic Benign Breast Disease Cohort. Hematoxylin and eosin stained slides were scanned into the computer and analyzed using Webslidebrowser software. The 10 largest normal lobules for each patient were analyzed for area measurement and number of acini per lobule. Means were compared for cases and controls, qualitative involution status (none, partial, or complete), histology (nonproliferative, proliferative disease without atypia, or atypical hyperplasia), and family history (none, weak, or strong).

Results: Women who went on to develop breast cancer had a larger lobular area (59,458µ² vs. 49,221µ²; p = 0.0452) and higher number of acini per lobule (21.62 vs. 16.11; p = 0.0006) than women who remained unaffected. Women with no involution had a larger lobular area (102,013µ²) and number of acini per lobule (35.72) than women with partial (56,945µ², 20.85 acini per lobule) or complete involution (27,254µ², 8.72 acini per lobule) (p < 0.0001). The difference between lobular area and number of acini per lobule was not statistically significant when evaluating for effect of histology or family history (p = 0.153 and 0.4770, respectively).

Conclusions: Lobular involution can be quantified and may be useful as a risk predictor for women who have had benign breast biopsies.
Benign breast disease and breast cancer risk in young women.
Ghosh K, Pankratz VS, Reynolds CA, Vierkant RA, Anderson SS, Degnim AC, Visscher DW, Frost MH, Vachon CM, Hartmann LC. Mayo Clinic, Rochester, MN; University of Michigan, Ann Arbor, MI

**Background:** Breast cancer is the leading cause of cancer deaths in younger women (25 to 49 years of age). Young women with breast cancer also have worse overall survival and increased risk of recurrence compared to older women with breast cancer. Innovative approaches to understanding risk factors and tissue characteristics for the younger population can improve understanding of breast cancer etiology and enhance risk-stratification for these women. This study was aimed at examining breast cancer risk factors among young women (<50 years) with BBD.

**Materials and Methods:** Utilizing the Mayo Clinic Surgical and Pathology Indices, women ages 18 to 85 who had benign excisional breast biopsy between January 1, 1967 and December 31, 1991 were identified. The diagnosis of breast cancer served as the study endpoint and was determined using the Mayo medical record and questionnaire information from study participants. The breast pathologist, blinded to the initial diagnosis and clinical outcome, performed pathology review. BBD was classified as non-proliferative disease (NPD), proliferative disease without atypia (PDWA), or atypical hyperplasia (AH). Age-related lobular involution (reduction in number and size of acini per lobule) was classified as none-0%, partial- 1 to74%, or complete- >75% involution. Relative risk (RR) was estimated by comparing the number of observed breast cancers with the number expected, based on breast cancer rates in the Iowa Surveillance, Epidemiology, and End Results registry.

**Results:** Of the 9376 women in the Mayo BBD cohort, 4460 women were aged <50 years at BBD diagnosis and formed the study cohort. The mean age at BBD diagnosis was 39.4 (± 8.3) years. With a median follow-up of 20 years, 326 breast cancer cases were identified. The histologic findings were NPD in 72% of women, PDWA in 26%, and AH in 2%. The relative risk of breast cancer for the overall cohort of young women with BBD was 1.5 (95% CI [1.4, 1.7]). The relative risk among those with AH was 6.9 (95% CI [4.6, 10.1]), compared with a RR of 2.0 (95% CI [1.7, 2.4]) for PDWA, and RR of 1.2 (95% CI [1.0, 1.4]) for NPD. Risk was associated with extent of lobular involution (RR for no involution was 1.7 (95% CI [1.4, 2.1]); partial involution 1.4 (95% CI [1.2, 1.7]); complete involution 0.7 (95% CI [0.3, 1.4]). Family history was available for 83% of the cohort and RR was 2.2 (95% CI [1.7, 2.8]) for women with strong family history and was 1.3 (95% CI [1.1, 1.6]) for women with no family history.

**Discussion:** Young women with BBD are at increased risk of breast cancer. Risk is high in women with atypical hyperplasia, and those with a family history of breast cancer. Lobular involution is associated with reduced breast cancer risk in this population, suggesting a role in modifying breast cancer risk. These findings suggest the need for further research in this population, along with tissue-based studies to examine the processes leading to breast cancer, and enable identification of those women at highest risk.
Estrogen Receptor Expression in Atypical Hyperplasia and Its Association with Type of Atypia and Age.


Background: Estrogen receptor (ER) expression is present in normal breast epithelium and premalignant breast lesions. Prior studies have shown that ER expression increases with age in normal breast epithelium; whereas no age association was seen in atypical hyperplasia and carcinoma in situ.

Design: ER expression was assessed immunohistochemically in archival sections from 246 women with atypical hyperplasia who had an open benign breast biopsy between 1967 and 1991. The ACIS®III (Dako, Carpinteria, CA) was utilized to calculate ER expression (percent staining and staining intensity) in all atypical foci for each woman. Using multivariate linear regression, we examined associations of ER expression with age at biopsy, year of biopsy, indication for biopsy, type of atypia, number of atypical foci, involution status, and family history. Heterogeneity of breast cancer risk across levels of ER expression was also assessed, standardized to a control population (the Iowa SEER registry).

Results: Among the 246 women, 87 (35%) had ADH, 141 (57%) had ALH, and the remaining 18 (7%) had both ADH and ALH. About half (53%) were older than 55 years at diagnosis. About half (53%) were older than 55 years at diagnosis. Forty-nine (20%) developed breast cancer during a median follow up of 14.4 years. Multivariate analysis indicated that type of atypia, year of biopsy, and age at diagnosis were significant predictors of ER percent staining and intensity [P<0.05 (see Table 1)]. ER expression was increased in women with ADH and/or those over the age of 55. The relationship between ER (percent staining and intensity) and breast cancer risk in patients diagnosed with atypia was not significant (P=0.099 and P=0.118, respectively).
**Benign Breast Disease in African-American Women.**
B Sharafeldeen, K Hayek, M Frost, L Hartmann, D Visscher, H Nassar. Wayne State University, Detroit; Mayo Clinic, Rochester; University of Michigan, Ann Arbor; Johns Hopkins, Baltimore

**Background:** Women with benign breast disease (BBD) represent a large and clinically important population. Specific histologic findings in BBD have been shown to be strong indicators of later risk of breast cancer. The major studies of BBD performed to date have been based primarily in Caucasian-American (CA) women. Thus, the prevalence and distribution pattern of BBD in African American (AA) women is not well known.

**Design:** We reviewed archival H and E stained sections of breast needle core and excisional biopsies performed on all AA women in the years 1998 to 2000 at our institution and diagnosed with BBD. BBD was classified, by one pathologist, as nonproliferative (NP), proliferative disease without atypia (PDWA) or atypical hyperplasia including ductal and lobular types (AH), using standard microscopic criteria. We also examined the status of lobular involution in the same biopsies and classified it as none or absent (<1%), partial (1-75%), or complete (75%). We compared lobular involution in our population to that of a cohort of CA women with a diagnosis of BBD within the same age category (<45y; 45-55y and >55y).

**Results:** We identified 520 AA patients with a diagnosis of BBD on breast biopsy. The mean age at diagnosis was 46.4 years (14.7 y). Seventy-five percent were diagnosed with NP, 22% with PDWA, and 3% with AH. Lobular involution increased with age. In women older than 55 years however, the increase in lobular involution appeared to be slower in AA than in CA women (CA had 2.7% none vs. 44% complete and AA had 19% none vs. 31% complete; p<0.001). There was no difference between AA and CA women younger than 55 years in regard to the presence of lobular involution. Lobular involution was similar throughout the different BBD categories in the AA population.

**Conclusions:** In our series of AA women with BBD, the distribution of BBD appears to be similar to other series (including mainly CA women). Our data on lobular involution may indicate that AA undergo lobular involution at a different rate than CA women.
Clinical Analysis of Mucocele-Like Tumors of the Breast: Analysis of a Large Benign Breast Disease Cohort.
DW Visscher, R Vierkant, M Frost, C Reynolds, S Anderson, L Hartmann. University of Michigan, Ann Arbor, MI; Mayo Clinic, Rochester, MN

**Background:** Mucocele-like tumors (MLT) of the breast are unusual lesions characterized by cysts distended with mucin that also dissect/extravasates through the epithelium into surrounding stroma. They are accompanied by variable epithelial proliferation, with an increased frequency of associated atypical ductal hyperplasia (ADH). It is not known whether MLT represent a risk factor for subsequent development of breast carcinoma.

**Design:** Our benign breast disease cohort is comprised of 9376 women who underwent excisional breast biopsy from 1967-1991. Slides from all patients were reviewed retrospectively in a blinded fashion and classified per standard diagnostic criteria by two study pathologists. Mean follow up is 13.7 years. We analyzed subjects with MLT diagnoses for the frequency of proliferative lesions, including ALH/ADH, and for their likelihood of developing breast cancer.

**Results:** The cohort contained 70 MLT (0.75%). Thirty patients (42.9%) were >55yrs of age at time of diagnosis, 24 (34.3%) were 45-54yrs and 16 (22.9%) were <45yrs. MLT were more often associated with proliferative lesions (70% in MLT vs 33% for the cohort overall). ALH/ADH was present in 21.4% MLT lesions, vs 3.4% in the cohort overall (p<0.0001). To date, 6/70 patients with MLT (8.6%) have developed breast carcinoma; this frequency is not significantly different than the BBD cohort overall (p=0.8780).

**Conclusions:** Our findings support previous studies showing a relationship between MLT and atypia. However, beyond the risk associated with atypia itself, we do not observe an additional risk of breast carcinoma associated with the presence of the MLT.
Appendix C: Manuscripts
Benign Breast Disease and the Risk of Breast Cancer

Lyn C. Hartmann, M.D., Thomas A. Sellers, Ph.D., Marilyn H. Frost, Ph.D., Wilma L. Liang, Ph.D., Amy C. Degnim, M.D., KirthikGosh, M.D., Robert A. Walikant, M.A.S., Shain D. Mabrey, B.A., V. Shane Pankratz, Ph.D., David W. Hillman, M.S., Vera J. Suman, Ph.D., Jo Johnson, P.N., Cassandra Blake, M.D., Thea Tlsty, Ph.D., colleague M. Vechon, Ph.D., L. Joseph Molton III, M.D., and Daniel W. Visscher, M.D.

ABSTRACT

BACKGROUND
Benign breast disease is an important risk factor for breast cancer. We studied a large group of women with benign breast disease to obtain reliable estimates of this risk.

METHODS
We identified all women who received a diagnosis of benign breast disease at the Mayo Clinic between 1967 and 1991. Breast cancer events were obtained from medical records and questionnaires. To estimate relative risks, we compared the number of observed breast cancers with the number expected on the basis of the rates of breast cancer in the Iowa Surveillance, Epidemiology, and End Results registry.

RESULTS
We followed 9087 women for a median of 15 years. The histologic findings were nonproliferative lesions in 67 percent of women, proliferative lesions without atypia in 30 percent, and atypical hyperplasia in 4 percent. To date, 707 breast cancers have developed. The relative risk of breast cancer for the cohort was 1.36 (95 percent confidence interval, 1.25 to 1.48), and this increased risk persisted for at least 25 years after biopsy. The relative risk associated with atypia was 4.14 (95 percent confidence interval, 3.26 to 5.41), as compared with a relative risk of 1.66 (95 percent confidence interval, 1.35 to 2.12) for proliferative lesions without atypia and of 1.37 (95 percent confidence interval, 1.13 to 1.59) for nonproliferative lesions. The strength of the family history of breast cancer, available for 4988 women, was a risk factor that was independent of histologic findings. No increased risk was found among women with no family history and nonproliferative findings. In the first 10 years after the initial biopsy, an excess of cancers occurred in the same breast, especially in women with atypia.

CONCLUSIONS
Risk factors for breast cancer after the diagnosis of benign breast disease include the histologic classification of a benign breast lesion and a family history of breast cancer.
Benign breast disease is an important risk factor for later breast cancer. It encompasses a spectrum of histologic entities, usually subdivided into nonproliferative lesions, proliferative lesions without atypia, and atypical hyperplasias with an increased risk of breast cancer associated with proliferative or atypical lesions. The identification of benign breast disease has become more common as the use of mammography has increased, and thus, having accurate risk estimates for women who receive this diagnosis is imperative.

Important questions remain, however, about the degree of risk associated with the common nonproliferative benign entities and the extent to which family history influences the risk of breast cancer in women with proliferative or atypical lesions. Dupont and Page found that women with nonproliferative disease did not have an increased risk of later breast cancer. By contrast, a companion study to the National Surgical Adjuvant Breast and Bowel Project (NSABP) Breast Cancer Prevention Trial (P1) found a relative risk of 1.6 for women who received a diagnosis of a "lower category" of benign breast disease. A limitation of the NSABP study, however, was the lack of central pathological review.

Another major question concerns the possible interplay between atypia and a family history of breast cancer. The Dupont and Page study found that women with atypia and a family history had 1.1 times the risk of those with nonproliferative lesions and no family history. However, two other major studies of benign breast disease did not find a significant interaction between atypia and family history. The duration of increased risk after a finding of benign breast disease or biopsy is also uncertain.

Studies of benign breast disease can also clarify whether there is a continuum of breast alterations that culminates in breast cancer. However, it remains unclear which of the benign entities are actual precursors and which reflect a background of increased risk involving all breast tissue in women. Determining the extent of agreement between the side (right or left) of the benign lesion and the subsequent breast cancer is one means of assessing these issues.

To investigate these questions, we studied 9087 women with benign breast disease for whom we had follow-up data on breast-cancer events. This cohort has been followed for a median of 15 years, and 707 breast cancers have developed, making this, to our knowledge, one of the largest such studies of this kind. We report on the risk of breast cancer according to histologic findings, the age at diagnosis of benign breast disease, and the strength of the family history. We also recorded the side of the cancer (ipsilateral or contralateral) and the time to the diagnosis of cancer.

**Methods**

**Study Population**

We accessed data from the Mayo Clinic Surgical Index and Pathology Index to identify all women 18 to 85 years of age who had undergone surgical excision of a benign breast lesion during the 25-year period from January 1, 1967, through December 31, 1991. For women who had more than one biopsy during this period, we used the first sample. The original list contained 12,132 women, but we excluded 1,047 women for any of the following: a diagnosis of breast cancer or lobular carcinoma in situ, before, or within six months after the biopsy of the benign lesion; mastectomy (unilateral or bilateral) or breast reduction at or before biopsy; or refusal to allow use of their medical records for research. This left 11,085 women. Of these, 2093 (19.5 percent) had no follow-up information after the biopsy. Thus, a total of 10,032 women met our criteria for study entry and had follow-up information. Of these, 945 women had unavailable or unavailable biopsy specimen of the benign lesion. The remaining group of 9087 women constitutes our study cohort. The relative risks of breast cancer (described below) did not differ significantly between the 10,032 women who met our criteria and the 9087 women who made up the study cohort (1.28 and 1.56, respectively).

**Family History and Follow-up**

A questionnaire designed for this study was used to obtain information on family history and other possible risk factors for breast cancer. Thus, our family-history data were obtained at the time of follow-up contact. We categorized family history as none, weak, or strong. The criteria for a strong family history were as follows: at least one first-degree relative with breast cancer before the age of 50 years or two or more relatives with breast cancer, with at least one being a first-degree relative. Any lesser degree of family history of breast cancer was categorized as weak. The questionnaires also asked about breast-cancer occurrences. Follow-up for breast-cancer events was also obtained through the comprehensive (inpatient and outpatient) Mayo medical...
record. Questionnaire information was available for 561 women (61.3 percent). Of the questionnaires, 604 (30.7 percent) were completed by proxy (the next of kin of a deceased patient). As of August 1, 2004, 7,260 (79.9 percent) members of the cohort were still alive. All protocol procedures and patient contact materials were reviewed and approved by the institutional review board of the Mayo Clinic. Submitting the contact materials was considered implied consent.

Histology

Stored hematoxylin-and-eosin-stained sections from each participant were evaluated by a breast pathologist who was unaware of the initial histologic diagnoses and patient outcomes. Biopsy findings were classified according to the criteria of Page et al.25 into the following categories: nonproliferative fibrocystic changes, proliferative fibrocystic changes without atypia, and proliferative fibrocystic changes with atypia: typical duct hyperplasia, atypical lobular hyperplasia, or both (Fig. 1). Biopsy specimens were designated as having proliferative fibrocystic changes if they contained any of the following: ductal hyperplasia (greater than mild), papilloma, radial scar, or adenosis. Cysts, fibroadenoma, or columnar changes were considered nonproliferative unless they also contained one of the lesions denoted above.

Statistical Analysis

The duration of follow-up was calculated as the number of days from biopsy of the benign lesion to the date of the diagnosis of breast cancer, death, or last contact. We estimated relative risks on the basis of standardized incidence ratios (SIRs), dividing the observed numbers of incident breast cancers by population-based expected counts. We calculated these expected counts by apportioning each woman’s follow-up into five-year age and calendar-

Figure 1. Histopathological Appearance of Benign Breast Disease (Hematoxylin and Eosin).

Panel A shows nonproliferative fibrocystic changes; the architecture of the terminal duct lobular unit is distorted by the formation of microcysts, associated with interlobular fibrosis. Panel B shows proliferative hyperplasia without atypia. This is adenosis; a distinctive form of hyperplasia characterized by the proliferation of lobular acini, forming crowded gland-like structures. For comparison, a normal lobule is on the left side. Panel C also shows proliferative hyperplasia without atypia. This is moderate duct hyperplasia, which is characterized by a duct that is partially distended by hyperplastic epithelium within the lumen. Panel D again shows proliferative hyperplasia without atypia, but this is focal duct hyperplasia; the involved duct is greatly expanded by a crowded, rounded, appearing epithelial proliferation. Panel E shows atypical duct hyperplasia; these proliferations are characterized by a combination of architectural complexity with partially formed secondary lumens and mild nuclear pleomorphism in the epithelial cell population. Panel F shows atypical lobular hyperplasia; mononuclear cells fill the lumens of partially distended acini in this terminal duct lobular unit.
period categories, thereby accounting for differences associated with these variables. We used the Iowa Surveillance, Epidemiology, and End Results (SEER) registry as the reference population because of its demographic similarities to the Mayo Clinic population (80 percent of cohort members reside in the upper Midwest). Over 95 percent of our cohort was white, equivalent to that reported in Iowa census data during the study period.13 In the SIR analyses, we considered the time since the original biopsy as a time-dependent variable and all other factors as fixed.

Associations between the risk of breast cancer and histologic findings, the age at diagnosis of benign breast disease, and the strength of the family history of cancer as well as pairwise combinations of these variables, were examined with the use of Cox proportional-hazards regression analysis. The main effects for each categorized variable and the corresponding interaction terms were included in each model, and the statistical significance of each interaction was evaluated with a test of a multiple-degree-of-freedom likelihood-ratio test.

We studied ipsilateral and contralateral breast cancer as a function of the time since biopsy by estimating the relative risk of cancer in the same as compared with the opposite breast for five-year intervals. When calculating the incidence of ipsilateral breast cancer, the ipsilateral breast was considered to be the breast in which the biopsy was performed. The relative risk of contralateral breast cancer was calculated from the incidence of ipsilateral breast cancer.

### Table 1. Characteristics of the Women According to the Histologic Category of Benign Breast Disease

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All Women (N=2007)</th>
<th>Nonproliferative Disease (N=620)</th>
<th>Proliferative Disease without Atypia (N=260)</th>
<th>Atypical Hyperplasia (N=127)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of total</td>
<td>100.0</td>
<td>66.7</td>
<td>29.6</td>
<td>3.7</td>
</tr>
<tr>
<td>Age at biopsy — no. of women (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40 yr</td>
<td>1201 (20.3)</td>
<td>134 (21.6)</td>
<td>247 (27.3)</td>
<td>11 (4.6)</td>
</tr>
<tr>
<td>40–49 yr</td>
<td>2340 (22.7)</td>
<td>414 (25.6)</td>
<td>1124 (40.4)</td>
<td>18 (13.0)</td>
</tr>
<tr>
<td>50–59 yr</td>
<td>2345 (22.6)</td>
<td>1287 (26.1)</td>
<td>1225 (37.9)</td>
<td>13 (7.0)</td>
</tr>
<tr>
<td>60–69 yr</td>
<td>1530 (21.6)</td>
<td>164 (26.1)</td>
<td>1194 (34.9)</td>
<td>11 (4.7)</td>
</tr>
<tr>
<td>≥70 yr</td>
<td>1280 (20.9)</td>
<td>160 (23.7)</td>
<td>1082 (35.5)</td>
<td>11 (3.7)</td>
</tr>
<tr>
<td>Mean age at biopsy — yr</td>
<td>51.4±14.3</td>
<td>46.0±14.4</td>
<td>51.0±13.6</td>
<td>57.2±12.3</td>
</tr>
<tr>
<td>Menopausal status at biopsy — no. of women (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal (&lt;45 yr)</td>
<td>2984 (32.4)</td>
<td>226 (37.2)</td>
<td>1269 (39.0)</td>
<td>86 (15.7)</td>
</tr>
<tr>
<td>Perimenopausal (45–55 yr)</td>
<td>2593 (28.4)</td>
<td>133 (26.6)</td>
<td>1131 (32.3)</td>
<td>101 (19.0)</td>
</tr>
<tr>
<td>Postmenopausal (&gt;55 yr)</td>
<td>3555 (36.1)</td>
<td>222 (36.7)</td>
<td>1118 (33.7)</td>
<td>117 (18.4)</td>
</tr>
<tr>
<td>Family history of breast cancer — no. of women (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>4279 (47.1)</td>
<td>295 (49.9)</td>
<td>1170 (43.1)</td>
<td>139 (41.4)</td>
</tr>
<tr>
<td>Known</td>
<td>1728 (52.9)</td>
<td>102 (51.1)</td>
<td>750 (56.9)</td>
<td>176 (58.6)</td>
</tr>
<tr>
<td>None</td>
<td>2661 (55.5)</td>
<td>175 (56.1)</td>
<td>831 (54.7)</td>
<td>102 (51.8)</td>
</tr>
<tr>
<td>Weak</td>
<td>1374 (24.4)</td>
<td>71 (42.4)</td>
<td>378 (24.9)</td>
<td>41 (20.3)</td>
</tr>
<tr>
<td>Strong</td>
<td>666 (20.1)</td>
<td>50 (19.4)</td>
<td>311 (20.1)</td>
<td>52 (27.0)</td>
</tr>
<tr>
<td>Breast-cancer status as at August 2004 — no. of women (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>6380 (92.2)</td>
<td>552 (93.7)</td>
<td>2425 (90.2)</td>
<td>271 (81.0)</td>
</tr>
<tr>
<td>Positive</td>
<td>707 (7.2)</td>
<td>40 (6.3)</td>
<td>264 (9.8)</td>
<td>56 (19.0)</td>
</tr>
<tr>
<td>Vital status — no. of women (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deceased</td>
<td>1827 (20.1)</td>
<td>132 (19.3)</td>
<td>556 (21.0)</td>
<td>29 (23.5)</td>
</tr>
<tr>
<td>Alive</td>
<td>7260 (79.9)</td>
<td>471 (80.7)</td>
<td>2124 (79.0)</td>
<td>26 (7.5)</td>
</tr>
</tbody>
</table>

* Plus–minus values are means ± SD.
† Menopausal status was categorized according to the age at breast biopsy.
Biopsy. Outcomes were classified as benign or malignant, with malignant outcomes further classified as in situ or invasive. All analyses were performed using SAS (version 9.1) and SPSS (version 15.0).

Table 2. Risk Factors for Breast Cancer after the Diagnosis of Benign Breast Disease.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Women</th>
<th>Person-Years</th>
<th>No. of Observed Events</th>
<th>No. of Expected Events</th>
<th>Relative Risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>9067</td>
<td>144,811</td>
<td>709</td>
<td>432.0</td>
<td>1.56 (1.45-1.68)</td>
</tr>
<tr>
<td><strong>Age at diagnosis of benign breast disease</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30 yr</td>
<td>725</td>
<td>13,593</td>
<td>21</td>
<td>11.5</td>
<td>1.42 (1.33-1.52)</td>
</tr>
<tr>
<td>30-39 yr</td>
<td>1113</td>
<td>20,169</td>
<td>71</td>
<td>35.3</td>
<td>1.43 (1.36-1.50)</td>
</tr>
<tr>
<td>40-49 yr</td>
<td>3474</td>
<td>45,760</td>
<td>212</td>
<td>168.3</td>
<td>1.36 (1.31-1.43)</td>
</tr>
<tr>
<td>50-59 yr</td>
<td>2345</td>
<td>44,140</td>
<td>105</td>
<td>128.2</td>
<td>1.55 (1.49-1.60)</td>
</tr>
<tr>
<td>&gt;60 yr</td>
<td>1630</td>
<td>21,364</td>
<td>142</td>
<td>88.5</td>
<td>1.56 (1.49-1.62)</td>
</tr>
<tr>
<td><strong>Menopausal status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal (age &lt;45 yr)</td>
<td>3948</td>
<td>44,490</td>
<td>100</td>
<td>106.1</td>
<td>1.09 (1.05-1.12)</td>
</tr>
<tr>
<td>Postmenopausal (age 45-55 yr)</td>
<td>2523</td>
<td>45,872</td>
<td>245</td>
<td>113.4</td>
<td>1.36 (1.31-1.41)</td>
</tr>
<tr>
<td>Postmenopausal (age &gt;55 yr)</td>
<td>3516</td>
<td>45,560</td>
<td>209</td>
<td>164.5</td>
<td>1.31 (1.27-1.35)</td>
</tr>
<tr>
<td><strong>Histologic findings</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonproliferative disease</td>
<td>5061</td>
<td>99,109</td>
<td>379</td>
<td>297</td>
<td>1.27 (1.15-1.40)</td>
</tr>
<tr>
<td>Proliferative disease without atypia</td>
<td>2690</td>
<td>41,630</td>
<td>264</td>
<td>140.2</td>
<td>1.88 (1.66-2.12)</td>
</tr>
<tr>
<td>Atypical hyperplasia</td>
<td>335</td>
<td>4,161</td>
<td>64</td>
<td>151</td>
<td>4.24 (2.30-7.87)</td>
</tr>
<tr>
<td><strong>Family history of breast cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>2668</td>
<td>44,974</td>
<td>171</td>
<td>145.4</td>
<td>1.16 (1.01-1.37)</td>
</tr>
<tr>
<td>Weak</td>
<td>1174</td>
<td>17,472</td>
<td>58</td>
<td>65.9</td>
<td>1.34 (1.15-1.55)</td>
</tr>
<tr>
<td>Strong</td>
<td>966</td>
<td>18,087</td>
<td>110</td>
<td>57.0</td>
<td>1.95 (1.62-2.33)</td>
</tr>
</tbody>
</table>

* Numbers of women, person-years, and events may not sum to overall totals because of rounding. 
† The relative risk reflects the observed number of events as compared with the number expected on the basis of Iowa SEER data. All analyses account for the effects of age and calendar period. CI denotes confidence interval. 
‡ Menopausal status was categorized according to the age at first biopsy. 
§ Information on family history was available for 808 of the 907 women.
weekly positive in 1174 (24.4 percent), and strongly positive in 966 (20.1 percent). More women with atypia than without atypia had a strong family history of breast cancer (27.9 percent vs. 19.8 percent, \( P < 0.05 \)). The risk of cancer was highest in the group with atypia; breast cancer developed in 64 of the 336 women (19.0 percent).

**Features of Benign Breast Disease and Subsequent Risk of Breast Cancer**

Patients in the cohort were followed for a median of 13.5 years. A total of 1327 women (28.1 percent) had died and 7260 (79.9 percent) were alive as of August 2004. We have documented 707 breast cancers to date. The median time from the original biopsy to the diagnosis of breast cancer was 10.7 years. Table 2 shows the estimated relative risks of breast cancer associated with the age at the initial biopsy, the strength of the family history, menopausal status, and histologic findings of the biopsy, as compared with expected population-based incidence. The estimated relative risk of breast cancer in the cohort was 1.50 (95 percent confidence interval, 1.45 to 1.60). The risk was inversely associated with the age at biopsy with younger women having a greater risk than older women. The type of benign breast disease identified at biopsy was a major predictor of risk. Atypical hyperplasia had a relative risk of 4.24 (95 percent confidence interval, 3.26 to 5.41); proliferative disease without atypia had a relative risk of 1.88 (95 percent confidence interval, 1.60 to 2.23); and non-proliferative lesions had a relative risk of 1.27 (95 percent confidence interval, 1.15 to 1.40). Family history was an independent risk factor. For women with no known family history of breast cancer, the relative risk was only 1.18 (95 percent confidence interval, 1.01 to 1.37); as compared with 1.43 (95 percent confidence interval, 1.35 to 1.50) for women with weak family history and 1.94 (95 percent confidence interval, 1.58 to 2.32) for those with a strong family history.
Figure 2 shows possible interactions between pairs of the major risk factors of age, histologic findings, and family history. No significant interactions were observed between age and family history or between histologic findings and family history, including atypia and family history. However, there was a significant interaction between age and histologic findings (P=0.05). The risk of breast cancer was 6.39 times the expected risk among women who received a diagnosis of atypia before the age of 45 years; the risk was 9.62 times the expected risk when atypia was diagnosed between the ages of 45 and 55 years, and 5.37 times the expected risk when it was diagnosed after the age of 55 years. An important finding was that for women with non-proliferative disease and no family history or a weak family history, there was no increase in the risk of breast cancer.

**DISCUSSION**

Retrospective and prospective studies have shown a relative risk of breast cancer of 1.3 to 1.6 for women with benign breast disease as compared with women in the general population. The histologic appearance of the benign lesion is a major determinant of risk, yet not all large studies have had access to tissue for re-review. Our investigation was based on a single-institution resource with long-term and complete followup for cancer events. All samples containing the benign lesion were read by a breast pathologist who applied current histologic classifications. More than 70% breast cancers developed in this cohort, giving our study good statistical power. The relative risk of breast cancer for our cohort overall was 1.86 (95% confidence interval, 1.45 to 2.39), and this increased risk persisted for at least 25 years after the initial biopsy.

The histologic appearance of the benign lesion is strongly associated with the risk of breast cancer. For biopsies with non-proliferative findings, the relative risk was 1.27 (95% confidence interval, 1.15 to 1.40), as compared with a relative risk of 1.88 (95% confidence interval, 1.66 to 2.12) for findings of proliferative changes but no atypia, and 2.46 (95% confidence interval, 1.35 to 4.49) for a finding of atypical hyperplasia. When the family history is known, risk profiles can be refined for women with non-proliferative findings and no family history or a weak family history of breast cancer we observed no increased risk. This finding is important, because a sizable proportion...
of women with benign breast disease are in this group (32 percent of our cohort with a known family history status). DePaul and Page made an initial observation in their 1985 report. However, a recent NSABP study found a significantly increased risk of breast cancer among women with lower-category benign breast disease, including nonproliferative disease, in the NSABP P1 trial, which included more than 13,000 women, 3,764 had a breast biopsy with benign findings over a mean follow-up period of 79 months, breast cancer developed in 47 of these women. On the basis of pathology reports from contributing centers, the investigators reported a relative risk of 1.6 among women with lower category findings on breast biopsies as compared with 97 participants who did not undergo a breast biopsy.

In our study, the degree of family history was an independent risk factor. In women with a strong family history of breast cancer, even nonproliferative findings were associated with a risk ratio of 3.62. This subgroup may parallel the high-risk NSABP cohorts. Women with atypia are at a significantly increased risk, but a family history did not significantly modify the atypia-associated risk (Fig. 2). The risk was four times the expected risk among women with atypia and a family history of breast cancer. Regardless of the degree of their family history, among women with atypia without a family history of breast cancer, the risk ratio was 2.95 (95 percent confidence interval, 1.56 to 4.87).

The age at diagnosis of benign breast disease appears to modify the risk related to the histologic appearance of benign breast disease. The presence of atypia in women under 45 years of age is associated with a risk of breast cancer among women over 56 years of age (6.99 and 3.57, respectively), which is not observed in younger women (less than 35 years of age). The age-related risk was not significant.

The Breast Cancer Detection and Demonstration Project showed that the risk of breast cancer among premenopausal women with atypia was elevated by a factor of 2.0 (95 percent confidence interval, 1.0 to 4.0), and the risk of breast cancer was greater among postmenopausal women with atypia. However, in the NSABP study of women with lower categories of benign breast disease, the risk of breast cancer was greatest among postmenopausal women.22

Understanding the risk associated with benign breast disease is important because the increasing use of mammography has increased the frequency of breast biopsies, most of which yield benign findings. In a retrospective study of women undergoing annual mammographic screening, Blamey et al. found that 38 percent of women underwent a biopsy after 10 screening mammograms. The use of hormone therapy may also affect the frequency of breast biopsies. Chlebowski et al., reporting for the Women's Health Initiative investigators, found that relatively short-term therapy with estrogen plus progesterin increased the percentage of women with abnormal mammograms, a major indicator for breast biopsy.20

Regarding the possibility of malignant precursors within benign breast disease, we have information on the side and time to breast cancer for 616 unilateral events. An excess of breast cancers occurred in the same breast during the first years of follow-up, especially in women with atypia (Fig. 4). This finding suggests that precursors to breast cancer exist in benign breast disease. Work in model systems of early steps in mammary carcinogenesis
has identified alterations in key regulatory indicators that can be studied in selected benign breast lesions.24-26.27

In summary, our study shows that histologic features, the age at biopsy, and the degree of family history are major determinants of the risk of breast cancer after the diagnosis of benign breast disease. We found no increased risk among women with nonproliferative lesions, whereas a strong family history was present. No significant interaction between atypia and family history was apparent. The excess risk of cancer in the ipsilateral breast in the first 10 years after the diagnosis of benign breast disease, especially in women with atypia, points to the presence of precursors in some women.

Supported by: Department of Defense Cancer Research Program (DAMD17-94-1-1033), a grant (U24 CA64748-01) from the National Institutes of Health, and the Susan G. Komen Breast Cancer Foundation, the Breast Cancer Research Foundation, and the American Cancer Society.

We are indebted to Dr. Warren and Dr. Port for data collection, and to the nurses of the Breast Research Center for patient follow-up.

REFERENCES

An Analysis of Breast Cancer Risk in Women With Single, Multiple, and Atypical Papilloma

Jason T. Lewis, MD,* Lynn C. Hartmann, MD,† Robert A. Vierkant, MAS,‡ Shaun D. Maloney, BA,§ V. Shane Pankratz, PhD,‡ Teresa M. Allers,† Marlene H. Frost, PhD,‡ and Daniel W. Visscher, MD*

Abstract: Breast papillomas may be single or multiple and associated with atypical ductal or lobular hyperplasias (ADH/ALH). The risk of breast carcinoma development in patients with papillomas, particularly those with multiple or atypical lesions, is incompletely defined. Fibrocystic lesions were histopathologically classified in a benign breast disease cohort of 9155 who underwent biopsy from 1967 to 1991, with papilloma assessment in 9108. Individuals with papillomas (N = 480) were classified into 4 groups: single papilloma (SP, N = 372), single papilloma with ADH or ALH (SP + A, N = 54), multiple (> 5) papillomas (MP, N = 41), and multiple papillomas with ADH or ALH (MP + A, N = 13). Those without papillomas were classified as nonproliferative (NP, N = 6653). Proliferative lesions were classified in a benign breast disease cohort (NP, N = 10232), proliferative without atypia (PDWA, N = 2308), and ADH/ALH (atypical hyperplasia (AH), N = 267). The relative risk of cancer development within our cohort was compared to that expected in the general population using standardized incidence ratios. The relative risk of breast cancer development associated with SP (2.04, 95% confidence interval (CI) 1.43-2.84) was greater than NP (1.28, 95% CI 1.16-1.42) but similar to PDWA (1.90, 95% CI 1.66-2.16). The risk associated with SP + A (5.11, 95% CI 2.64-8.92) was elevated but not substantively different than atypical hyperplasia (4.17, 95% CI 3.10-5.50). Patients with MP are at increased risk compared with PDWA or SP (3.01, 95% CI 1.10-6.55), particularly those with MP + A (7.01, 95% CI 1.91-17.97). There was a marginal increase in breast cancer risk (16%) among patients with proliferative disease if a papilloma was present, but this did not reach statistical significance (P = 0.29). The observed frequency of ipsilateral (vs. contralateral) breast cancer development in papilloma subsets was not significantly different than other patient groups. We conclude that SP imparts a cancer risk similar to conventional proliferative fibrocystic change. The presence of papilloma in, or associated with, atypia does not modify the risk of subsequent breast carcinoma. MP constitutes a proliferative breast disease subset having unique clinical and biologic behavior.

Key Words: intraductal papilloma, atypical hyperplasia, breast cancer

(Pt Am) J Surg Pathol 2006;30:665-672)

Papillomas of the breast are defined by a constellation of pathologic findings including: (1) a discrete intraductal polypoid lesion with (2) an arborizing fibrovascular stroma covered by a layer of myoepithelium, and (3) a second layer of columnar or cuboidal epithelium. They often form palpable nodules, reaching considerable size in some cases, although many are microscopic. Atypical papillomas are often accompanied by significant epithelial hyperplasia and/or periductal sclerosis, resulting in microscopically complex lesions. 

Atypical hyperplasia (AH) may also be present within or adjacent to papilloma. In these so-called atypical papillomas, the histologic distinction from ductal carcinoma in situ may be extremely problematic.

Most early investigators considered intraductal papillomas to be benign lesions without malignant potential or implied risk of developing a subsequent carcinoma. More recent studies have demonstrated that these lesions, like other forms of proliferative breast disease, do increase the risk of developing carcinoma. Some have suggested that papillomas may behave as direct precursor lesions. Neither view, however, has been empirically tested in a sufficiently large cohort of patients with long term follow-up and appropriate population controls. Further, most studies which specifically address pathologic subsets thought to be biologically more aggressive, such as multiple papillomas, or atypical papilloma, consist of relatively small numbers or are enriched by selective inclusion of cases derived from referral consult practices.

We have recently completed pathologic evaluation of a benign breast disease cohort, consisting of all open benign breast biopsies performed at the Mayo Clinic between 1967 and 1991 (N = 9155). All papillary lesions present in these biopsies were routinely defined as a...
component of our microscopic examination. Our objective in this study is to survey the incidence, histologic patterns and relative cancer risk associated with benign papillomas of the breast. We will specifically address the significance of multiple papillomas and papillomas with atypia and whether there is evidence to suggest they are direct precursors.

**MATERIALS AND METHODS**

**Patient Selection**

Patients with benign diagnoses on open excisional biopsy (OEB) of the breast, performed between January 1, 1967 and December 31, 1991, constituted the study population. Searching the Mayo Clinic Surgical Index and Pathology Index identified the cases. The details of the derivation of the study cohort have been published previously. Briefly, subjects were excluded from the study if they met one or more of the following criteria: (1) cancer diagnosis before, at, or within 6 months of the OEB (accounting for possible occult malignancy), (2) unilateral or bilateral mastectomy or reduction before OEB, (3) refusal of research authorization, (4) no follow-up information available, or (5) slides unavailable. Of the 9155 women who met the study criteria, papilloma information for 47 were unavailable. The resulting 9108 patients constituted the study cohort, with a mean follow-up of 16 years.

**Pathology Review**

A pathologist with expertise in breast pathology (D.V.) reviewed the original hematoxylin and eosin (H&E) stained slides of all cases without knowledge of original diagnosis or subsequent outcome. Cases were classified into one of three general categories: nonproliferative (NP) fibrocystic changes, proliferative changes without atypia (PDWA), and AH. NP fibrocystic changes included cyst formation, stromal fibrosis, apocrine metaplasia, and noncomplex fibroadenoma. Proliferative changes without atypia included ductal hyperplasia of usual type, sclerosing adenosis, radial scars/complex sclerosing lesions, and papillomas(s). Atypical ductal or lobular hyperplasia (ADH/ALH) constituted the AH category and the presence or absence of each was documented in each case. The criteria for classification as AH (see below) were applied to epithelial proliferations within or outside of the papilloma.

A papilloma was defined as an intraductal epithelial proliferation of any size that is supported by branching fibrovascular stalks that contain myoepithelium. In many cases they were not the predominant lesions in the biopsy specimen (eg, fluid duct hyperplasia with an incidental, microscopic papilloma). Papillomas were classified as solitary (SP) or multiple (MP), the latter defined as a papillary lesion containing at least 5 papillomas in 2 nonconsecutive tissue blocks. The presence or absence of AH (ADH and/or ALH) was also documented in the papilloma cases. If ADH or ALH was identified within the papilloma or in the surrounding parenchyma, then the case was classified as a single papilloma with atypia (SP + A) or multiple papillomas with atypia (MP + A). For the SP + A cases, the location of the atypia (inside and/or outside the papilloma) was recorded.

**ADH** was defined according to the criteria of Page and others. These lesions exhibited architecturally complex cribriformlike proliferations of monotonous cells that lacked malignant cytologic features and were confined to an area measuring < 2 mm in greatest dimension. Within papillomas, these atypical lesions only partially involved a "basement membrane bound space," with a second nonatypical population of cells composing the remainder. ALH was defined as a proliferation of polygonal, evenly spaced cells with round, monotonous nuclei, and scant cytoplasm. ALH was characterized by partial expansion of acini by atypical cells, often with preservation of luminal spaces, involving less than half of the acini in a lobule.

**Statistical Analysis**

Data were descriptively summarized using frequencies and percentages for categorical variables, and means and standard deviations for continuous variables. We formally compared distributions of certain attributes across papilloma-defined subgroups using z tests and analyses of variance for the continuous variables and χ² tests for categoric variables.

The length of the follow-up for each woman in the study was calculated as the number of days (followed by division by 365.25 to calculate years) from her benign biopsy to the date of breast cancer diagnosis, date of death, or date of last contact. The cumulative incidence of breast cancer by papilloma status was estimated using Kaplan-Meier curves. We compared the observed number of incident breast cancer events in our cohort, stratified by papilloma status, to that expected in the general population using standardized incidence ratios (SIRs). Each individual's person years were apportioned into 5-year age and calendar period categories. Overall category-specific follow-up was then multiplied by the corresponding age-stratified and calendar period-stratified surveillance epidemiology and end results (SEER) incidence rates, and these results were then summed across all categories to calculate the expected number of events. Thus, all risk ratios account for the potentially confounding effects of age and calendar period. The Iowa SEER registry was used as the primary standard population, due to both the proximity of its participants to the Mayo Clinic catchment area and racial/ethnic similarities to our cohort.

As proliferative disease is a complex mixture of many different attributes which may synergistically affect the risk of breast cancer, it is possible that other forms of proliferative change could confound the association of papillomas and breast cancer. Thus, we sought to assess the independent modifying effects of different forms of proliferation using Poisson regression analyses, modeling the individual-specific, log-transformed expected event...
rate as the offset term. This approach facilitates the calculation of SIRs with the added flexibility that generalized linear models provide, such as covariate adjustment and formal assessment of heterogeneity. The Iowa SEER registry was again used to calculate the expected event rates. Analyses were subset to women with proliferative disease. Based on the Poisson models, we calculated relative SIRs (rSIRs), directly comparing ratios of SIRs across levels of each of the proliferation attributes. The accompanying P values assess the heterogeneity of breast cancer SIRs across levels of the attribute. The following types of proliferation were examined: presence of atypia, presence of papillomas, presence of sclerosing adenosis, and presence of radial scars. Two sets of poisson models were fit; one that accounted only for the effects of age and calendar period, and one that accounted additionally and simultaneously for the effects of the other proliferation attributes.

We compared the potentially differential risk of ipsilateral versus contralateral breast cancer within the cohort across papilloma-defined subgroups using a competing risk approach, based on the Poisson distribution. Women with missing biopsy or cancer side information, or with benign breast disease (BBD) or cancer diagnosed bilaterally, were excluded from these analyses. For each subgroup, we compared the incidence rate for ipsilateral cancer to the corresponding rate for contralateral cancer. When calculating incidence for ipsilateral cancer, individuals with contralateral cancer were censored at their date of diagnosis, and vice versa. This approach yields identical person years for each event type, reducing comparisons of incidence to simple comparisons of counts via \(\chi^2\) tests of significance. All statistical tests were 2-sided, and all analyses were carried out using the SAS software system (SAS Institute, Inc, Cary, NC).

**RESULTS**

**Pathologic Findings**

The Mayo benign breast disease cohort has been presented in detail elsewhere.\(^5\) Our current cohort includes 9108 patient biopsies, classified as follows: 6053 (66.5%) NP; 2308 (25.3%) PDWA; and 267 (2.9%) AH. Papilloma was identified in 480 (5.3%) of the biopsies, distributed within the PDWA and AH categories. The majority of papillomas (372, 4.1%) were SP. There were 54 SP + A (0.6%), 41 MP (0.5%), and 13 (0.1%) MP + A. The mean age at biopsy was youngest for the NP group (49.3, SD 14.8), intermediate for the monotypical proliferative groups [PDWA 53.6 (SD 12.1), SP 55.2 (SD 14.5) and MP 53.9 (SD 15.5)], and oldest for the atypical groups [AH 57.3 (SD 11.6), SP + A 59.1 (SD 13.4), and MP + A 65.1 (SD 14.0)].

Because family history is a known risk factor for the development of carcinoma, we compared the frequency of papilloma status with family history to determine if there were any differences among the subsets. Family history was available in 4846 (53%) of the 9108 cases. As Table 1 illustrates, the majority of cases in all subsets did not have a family history of breast cancer. The remainder of the cases varied from a weak to strong family history. \(\chi^2\) tests revealed no differences in distribution of papillomas across levels of family history (\(P = 0.49\)).

Papillomas were accompanied by a complex mixture of proliferative changes. Sclerosing adenosis and usual ductal hyperplasia were both present in at least 50% of cases from each papilloma subgroup (Table 2). Radial scars also occurred at significantly increased frequency (16% SP, 33% SP + A, 34% MP, 31% MP + A) compared to individuals without papillomas (4%, \(P < 0.001\)). Among papilloma cases, radial scars were significantly more common in SP + A, MP, and MP + A compared with SP (\(P < 0.001, \chi^2\) test).

With respect to atypia in the setting of papilloma, most cases (33/51, 65%) contained ADH alone. There were 6 (12%) with ALH and 12 with both ADH and ALH (23%). Examples of atypical papilloma are illustrated in Figures 1 to 4. Of the 45 cases with ADH, atypia was present within the papilloma in 16 (36%), outside of the papilloma in 17 (38%), and present both inside and outside the papilloma in 12 (26%). One SP + A case consisted of 2 biopsies, one contained a solitary papilloma (left breast) and the other ADH (right breast). For purposes of this study, this case was classified as atypia outside of the papilloma.

**Outcome**

Among the overall Mayo cohort, the relative risk of developing carcinoma was: NP 1.3 (95% confidence

<table>
<thead>
<tr>
<th>TABLE 1. Comparison of Family History of Breast Cancer Across the Various Papilloma Subtypes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diagnosis</strong></td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>No papilloma</td>
</tr>
<tr>
<td>SP</td>
</tr>
<tr>
<td>SP + A</td>
</tr>
<tr>
<td>MP</td>
</tr>
<tr>
<td>MP + A</td>
</tr>
</tbody>
</table>

*Family history was missing in 4626 cases.*

© 2006 Lippincott Williams & Wilkins
TABLE 2. Frequency of Proliferative Breast Disease Across the Papilloma Subtypes

<table>
<thead>
<tr>
<th>Ductal Hyperplasia</th>
<th>Sclerosing Adenosis</th>
<th>Radial Scars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes N (%)</td>
<td>Yes N (%)</td>
</tr>
<tr>
<td>No papilloma*</td>
<td>1269 (18)</td>
<td>7055 (82)</td>
</tr>
<tr>
<td>SP+</td>
<td>241 (64)</td>
<td>131 (36)</td>
</tr>
<tr>
<td>MP + A</td>
<td>52 (96)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>MP + A</td>
<td>52 (96)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>MP + A</td>
<td>52 (96)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>MP + A</td>
<td>52 (96)</td>
<td>2 (4)</td>
</tr>
</tbody>
</table>

Ductal hyperplasia includes moderate and florid ductal epithelial hyperplasia.
*This includes NP, proliferative without atypia, and AH cases. Four observations missing values for ductal hyperplasia, 9 for radial scars, and 1 for sclerosing adenosis.
†One observation missing value for sclerosing adenosis and radial scar.

interval (CI), 1.2-1.4], PDWA 1.9 (95% CI 1.7-2.2), and AH 4.4 (95% CI 3.4-5.6). Overall, 724 (8%) of the patients in the cohort have developed breast carcinoma.

Table 3 summarizes the mean age at biopsy, mean interval to development of breast cancer, and risk of carcinoma development among the histopathologic groups with respect to papilloma status. Patients lacking a papilloma(s) who had proliferative disease without atypia had a relative risk of 1.90 (95% CI 1.65-2.16) of developing cancer compared to the control population. Patients with a solitary papilloma without atypia had a risk of 2.04 (95% CI 1.43-2.81), roughly equivalent to other forms of proliferative disease without atypia. Patients with AH and no papilloma(s) had a relative risk of 4.17 (95% CI 3.10-5.50). Individuals with a SP + A had a risk of 5.11 (95% CI 2.64-8.92), slightly greater than those with AH lacking a papillary lesion. The breast cancer risk for multiple papillomas without atypia fell between proliferative disease without atypia and AH (3.01, 95% CI 1.10-6.55). Multiple papilloma cases with atypia had the greatest likelihood of developing cancer, with a relative risk of 7.01 (95% CI 1.91-17.97). A Kaplan-Meier curve depicting cumulative incidence of breast cancer among all histopathologic groups is...
FIGURE 3. At scanning magnification this papilloma is characterized by marked hypercellularity and variable architecture. Higher magnification photomicrograph highlighting confluent growth of epithelium with partially developed cribriform architecture.

FIGURE 4. The architecture is primarily microglandular, but focal complex growth may also be appreciated (arrow). Higher magnification of cribriform area. Lack of uniform involvement and low grade cytology preclude a diagnosis of in situ carcinoma.

presented in Figure 5. The mean interval to cancer development was greater than 5 years in all histologic groups except MP 4.8 (SD 3.2). It was longest in patients with NP 8.7 (SD 7.2). There was no difference in mean interval to cancer between AH and SP + A [6.5 (SD 5.3) vs. 6.2 (SD 4.7), P = 0.87, t test].

As papillomas were most frequently identified in the setting of other proliferative lesions, we attempted to determine the degree to which the apparent breast cancer risk seen in these patients was directly attributable to the papillomas), versus other coexisting forms of proliferation present within the breast. We performed a univariate analysis using Poisson regression models subset to only those women with proliferative changes (ie, NP cases were excluded). After accounting for age and calendar period, women with some form of proliferative disease (eg, AH, radial scar, or sclerosing adenosis) and with a papilloma, had a relative risk of breast cancer roughly 20% higher than those without a papilloma. Thus, within the group of patients with proliferative disease, the presence of a papilloma marginally increased risk. However, this result did not reach statistical significance (P = 0.17). As the univariate model accounted for the excessive risk due to proliferative disease, but it did not adjust for the effects of other individual forms of proliferation, we performed a multivariate analysis adjusting for AH, ductal hyperplasia, sclerosing adenosis, and radial scars. Results were similar to the univariate model: the presence of papillomas increased risk by an additional 16% over those patients without a papilloma. However, this result again failed to reach statistical significance (P = 0.29). We also performed the multivariate analysis subset to proliferative cases without atypia. After adjusting for sclerosing adenosis, radial scars, and duct hyperplasia, the presence of papilloma increased risk by an additional 10% over those patients without a papilloma (P = 0.42).
In the SP+ A patients, risk for breast cancer was not associated with the microscopic location of ADH. Cancers developed in 25% (4/16) of patients with ADH within papilloma compared with 29% (5/17) patients with ADH outside of the papilloma and 17% (2/12) with ADH in both locations. None of the patients with ALH alone (N = 6) has yet developed breast carcinoma.

Table 4 summarizes side of cancer development in relation to the side of the original excisional biopsy. With respect to the overall Mayo cohort, 56% of the patients in the NP, PDWA, and AH groups developed carcinoma in the ipsilateral (same) breast as the biopsy.\textsuperscript{15} Sixty-five percent of the carcinomas in the papilloma group developed in the ipsilateral breast. The likelihood for development of ipsilateral cancer among those with papilloma compared with nonpapilloma groups, was not statistically different ($P = 0.33$, $\chi^2$ test).

**DISCUSSION**

Our study defines the incidence, spectrum of pathology, and breast cancer risk attributable to benign papillomas that were identified in a large cohort of consecutive, nonselected benign biopsies. It is the first to specifically address the cancer risk associated with papillomas, either with or without atypia, using epidemiologically valid comparisons between carefully defined pathologic subsets including nonpapilloma proliferative lesions. The data demonstrate that presence of a single papilloma without atypia conveys an overall breast cancer risk that is similar to or marginally greater than other commonly recognized proliferative fibrocystic lesions. It is unclear, even after multivariate statistical analysis, whether this small difference is due to the more frequent presence of other proliferative lesions in those with papillomas. Second, the presence of ADH/ALH in association with a single papilloma ("atypical papilloma"), does not appreciably modify the risk connotation attributable to atypia overall. Finally, the follow-up data from the cohort imply that patients with multiple papillomas are at a significantly elevated risk for breast cancer, even if atypia is not identified in their biopsy.

The Mayo benign breast disease cohort is derived from the surgical practice at one institution and is not

**TABLE 3. Demographic Characteristics and SIRs of Breast Carcinoma Development for the Mayo Cohort Compared With the Iowa SEER Registry**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Overall N</th>
<th>Age at Biopsy (y) Mean (SD)</th>
<th>Time to Cancer (y) Mean (SD)</th>
<th>Observed Cancers</th>
<th>Expected Cancers</th>
<th>SIR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No papilloma present</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>6639</td>
<td>49.9 (14.8)</td>
<td>8.7 (7.2)</td>
<td>393</td>
<td>298</td>
<td>128 (1.16-1.42)</td>
</tr>
<tr>
<td>Proliferative</td>
<td>296</td>
<td>53.6 (12.1)</td>
<td>7.8 (6.4)</td>
<td>223</td>
<td>122</td>
<td>180 (1.06-2.16)</td>
</tr>
<tr>
<td>AH</td>
<td>267</td>
<td>57.3 (11.6)</td>
<td>6.3 (5.3)</td>
<td>20</td>
<td>0.3</td>
<td>0.01 (3.10-2.26)</td>
</tr>
<tr>
<td>Papilloma present</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP</td>
<td>572</td>
<td>55.2 (14.3)</td>
<td>5.9 (5.0)</td>
<td>37</td>
<td>18</td>
<td>2.04 (1.42-2.81)</td>
</tr>
<tr>
<td>SP + A</td>
<td>54</td>
<td>59.1 (15.4)</td>
<td>6.2 (4.9)</td>
<td>12</td>
<td>2</td>
<td>5.31 (2.04-9.82)</td>
</tr>
<tr>
<td>MP</td>
<td>41</td>
<td>55.9 (15.3)</td>
<td>4.8 (2.2)</td>
<td>6</td>
<td>2</td>
<td>3.01 (1.10-6.52)</td>
</tr>
<tr>
<td>MP + A</td>
<td>13</td>
<td>65.3 (14.0)</td>
<td>5.8 (3.8)</td>
<td>4</td>
<td>1</td>
<td>7.01 (1.91-17.97)</td>
</tr>
</tbody>
</table>

The "Overall" column refers to the total number of cases in each group. Analyses account for the effects of age and calendar period.

**FIGURE 5. Kaplan-Meier curve illustrating cumulative incidence of cancer development among the histologic groups in the Mayo cohort.**

**TABLE 4. Breast Cancer Sidedness Among the Different Diagnostic Groups With Respect to Excisional Biopsy Location**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of Cancers\textsuperscript{a}</th>
<th>Contralateral N (%)</th>
<th>Ipsilateral N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>354</td>
<td>163 (46)</td>
<td>191 (54)</td>
</tr>
<tr>
<td>Proliferative</td>
<td>234</td>
<td>107 (46)</td>
<td>127 (54)</td>
</tr>
<tr>
<td>AH</td>
<td>57</td>
<td>22 (36)</td>
<td>35 (61)</td>
</tr>
<tr>
<td>No papilloma present</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>334</td>
<td>163 (46)</td>
<td>191 (54)</td>
</tr>
<tr>
<td>Proliferative</td>
<td>194</td>
<td>93 (48)</td>
<td>101 (52)</td>
</tr>
<tr>
<td>AH</td>
<td>43</td>
<td>17 (40)</td>
<td>26 (60)</td>
</tr>
<tr>
<td>Papilloma present</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP</td>
<td>54</td>
<td>10 (29)</td>
<td>24 (71)</td>
</tr>
<tr>
<td>SP + A</td>
<td>11</td>
<td>4 (36)</td>
<td>7 (64)</td>
</tr>
<tr>
<td>MP/MP + A</td>
<td>9</td>
<td>5 (36)</td>
<td>4 (44)</td>
</tr>
</tbody>
</table>

MP and MP + A cases were summed because of the small number of events.\textsuperscript{a}Patients with missing side information, unilateral BB, or bilateral cancer have been removed from the analysis.
enriched with extramural pathology consultation materials. Indications for breast biopsy were not different than those employed by comparable institutions during the survey period. To our knowledge, it is the largest benign breast disease cohort that has been subject to standard pathologic review. The size of the cohort and long follow-up allow for robust statistical analysis. The observed proportion of cases and relative risks of carcinoma development associated with NP, proliferative, and atypical lesions is similar to other large surveys of benign breast disease.2,3,6,21

Benign papillomas constitute an important subset of mammary fibrocystic change, collectively accounting for about 5% of proliferative cases overall. As seen in this study, however, papillomas are frequently accompanied by a complex of other proliferative lesions, particularly adenosis and duct hyperplasia. Radial scars are also often present, especially in the SP + A, MP and MP + A subsets (31% to 34%). Finally, papillomas often comprise a background on which atypia develops; 20% of all atypias in our BBD cohort overall were present in cases that also had papillomas. Conversely, about 14% of biopsies with papillomas contained ADH and/or ALH. The observed associations with adenosis and radial scarring are noteworthy in the sense that both are characterized by combined proliferation of epithelial and nonepithelial populations (myoepithelial cells and fibroblasts, respectively). These findings imply that breast tissues harboring papillomas may be characterized by simultaneous activation/proliferation of divergent cell populations. It may be hypothesized that the background of multilinage cell proliferation reflects an especially permissive environment for development of hyperplastic lesions, accounting for the markedly cellular character of this BBD subset, and possibly for the more frequent evolution of atypias.

Although papillomas are often associated with other proliferative or atypical lesions, it is nonetheless true that most benign biopsies with papillomas—about 78%—contained single papillomas and lacked ADH/ALH. Within this subset (ie, single papilloma without atypia) the age at diagnosis and overall breast cancer risk were quite similar to patients with proliferative lesions overall—52.6 versus 55.2 years and 1.90 (95% CI 1.66-2.16) versus 2.04 (95% CI 1.43-2.81), respectively. On the basis of these data we would not advise the risk classification of single, nonatypical papillomas apart from other proliferative fibrocystic lesions.

Some may hypothesize that atypical proliferations developing within papillomas represent biologically distinct, direct precursor lesions. However, the simultaneous presence of papilloma with ADH or ALH (SP + A) was associated with a breast cancer risk (5.11, 95% CI 2.64-8.92) that was similar to, or marginally elevated, relative to other atypias in our cohort (4.17, 95% CI 3.10-5.50). Importantly, in the papilloma cases we failed to identify significant tendency to ipsilateral breast cancer development or short interval to breast cancer diagnosis. Thus, we identify no convincing evidence to suggest that these lesions constitute an homogeneous group of direct cancer precursors. We also attempted to discern whether the location of atypia relative to a papilloma had special significance. Although the number of cases limits definitive interpretation, our data imply that geographic location of ADH relative to a papillary lesion would not necessarily be a clinically useful indicator of breast cancer risk apart from other parameters. However, our data would not support the practice of separately denoting an atypical papilloma as a distinct subset of ADH.

Page et al22 have published a nested case control survey that compares breast cancer risk in 122 patients with papillomas. Their incidence of atypia occurring within or in association with papilloma (17/122, 14%) was similar to our series (54/480, 11%) as was the time interval between biopsy and subsequent breast malignancy. However, the absolute risk of breast cancer after atypical papillomas in the Page et al study was 55% (9/17). In contrast, in our study only 22% of such individuals (12/54) had developed breast cancer at 16 years. They also observed a significantly elevated relative breast cancer risk (2.30 to 3.35 ×) attributable to papilloma that was modified by presence of concurrent atypia (4.40 to 13.10 ×). Because we employed similar diagnostic criteria, we ascribe the differences with our study to their relatively limited number of cases and the study design (ie, case control vs cohort).

Haagensen22 and Murad22 have emphasized the unique clinical behavior of MP, noting from selected series of cases that these patients have significantly elevated breast cancer risk. The incidence and relative cancer risk of MP, however, has not been previously described. Our data show that MP cases constitute a rare subset, accounting for 0.6% of BBD patients. However, depending on the presence of atypical lesions, MP patients have a breast cancer risk that is 3 to 7 times greater than age matched women in the population overall. Thus, our data indicate that MP, even without concurrent atypia, convey a relative risk between proliferative disease overall and AH. On the basis of these findings, we recommend that MP should receive wider recognition as a diagnostic entity and that these patients should be, at a minimum, followed carefully.

REFERENCES
Age-Related Lobular Involution and Risk of Breast Cancer

Tia R. Milanese, Lynn C. Hartmann, Thomas A. Sellers, Marlene H. Frost, Robert A. Verbeek, Shawn D. Maloney, V. Shane Patirakos, Amy C. Degnim, Celine M. Fichon, Carol A. Reynolds, Romayne A. Thompson, L. Joseph Mullen III, Ellen L. Goode, Daniel W. Visscher

Background: As women age, the lobules in their breasts undergo involution or regression. We investigated whether lobular involution in women with benign breast disease was associated with subsequent breast cancer risk. Methods: We examined biopsy specimens of 8736 women in the Mayo Benign Breast Disease Cohort from whom biopsy samples were taken between January 1, 1967, and December 31, 1991. Median follow-up for breast cancer outcomes was 17 years. We classified lobular involution in the background breast tissue as none (0% invovled lobules), partial (1–47%), or complete (>75%). Subsequent breast cancer events and data on other risk factors were obtained from medical records and follow-up questionnaires. To estimate relative risks (RR), standardized incidence ratios were calculated by use of incidence rates from the Iowa Surveillance, Epidemiology, and End Results (SEER) Registry. All statistical tests were two-sided. Results: Distribution of extent of invasion was none among 1617 (18.6%) women, partial among 5197 (59.5%), and complete among 1912 (21.9%). Increased invasion was positively associated with increased age and inversely associated with parity (both P < .001). The relative risk for the entire cohort of 8736 women, compared with the Iowa SEER population, was 1.40 (95% CI = 1.30 to 1.51). Risk of breast cancer was associated with the extent of invasion (for no invasion, RR [i.e., observed versus expected] = 1.88, 95% confidence interval [CI] = 1.59 to 2.21; for partial invasion, RR = 1.47, 95% CI = 1.33 to 1.61; and for complete invasion, RR = 0.91, 95% CI = 0.75 to 1.10; test for heterogeneity P < .001). Lobular involution modified risk in all subsets (e.g., among women with atypia, for no invasion, RR = 7.79, 95% CI = 3.56 to 16.81; for partial invasion, RR = 4.06, 95% CI = 3.03 to 5.33; and for complete invasion, RR = 1.49, 95% CI = 0.41 to 5.82; P = .003).

Conclusions: In this large cohort of women with benign breast disease, lobular involution was associated with reduced risk of breast cancer: Aberrant involution may be a biologically important phenomenon in breast cancer biology. [J Natl Cancer Inst 2006;98:1600–7]
Histology

All slides were reviewed by a breast pathologist (DWV) without knowledge of patient age, cancer outcome, or original histologic diagnosis. Biopsy findings were classified by the most extreme degree of hyperplasia as nonproliferative, proliferative disease without atypia, or atypical hyperplasia, as previously reported (6). Each biopsy specimen was also categorized according to the extent of lobular involution in the background breast tissue. Involved terminal duct lobular units (TDLUs) contain only a few to several small acini that may be distended by cystic change (Fig. 1). Involved lobules also have flattened inconspicuous acinar epithelium with fibrous of specialized intralobular stroma. The degree of involution for each specimen was categorized as none (0% TDLUs involuted), partial (1%–74% TDLUs involuted), or complete (>75% TDLUs involuted). These cut points were set by the pathologists at the initiation of the study to best distinguish the extremes of no involvement from near-complete involution.

In general, viewing five to six lobules was sufficient to assess the extent of involution. One slide from a breast specimen typically contained a dozen or more lobules. There are two exceptions to this statement: 1) when involution was extensive and there are only a few lobular remnants on the slide (which is sufficient to state that complete lobular involution has occurred) and 2) when the entire sample consists of an epithelial hyperplastic lesion, as was the case for 640 (6.8%) of the 9376 women in our original cohort.

Risk Factor Information and Follow-up

To obtain information about family history, reproductive history, and use of hormone replacement therapy, a study-specific questionnaire was sent to patients. 3352 (61%) of the 5476 women or their next of kin returned the questionnaire. Follow-up for breast cancer events was obtained through comprehensive (inpatient and outpatient) Mayo medical records and the questionnaire.

Family history of breast cancer was categorized as strong, weak, or negative. A strong family history was defined as the patient having 1) at least one first-degree relative with breast cancer diagnosed before age 50 years or 2) two relatives with breast cancer at any age, with at least one being a first-degree relative. Patients with family history of breast cancer who did not meet the above criteria were categorized as having a weak family history (6).

Statistical Analysis

Data were summarized descriptively by use of frequencies and percentages. We initially compared the unadjusted distribution of breast cancer risk factors across levels of involution with chi-square tests of statistical significance. Subsequent comparisons were made after accounting for the effects of age by use of multivariate, nominal logistic regression analysis (6). We summarized results from these analyses by use of adjusted percents, carried out by calculating log odds estimates for each 10-year age category (<40, 40–49, 50–59, 60–69, 70–79, or ≥80 years), back-transforming to percent estimates, and then averaging the corresponding percents across all sets of age. This approach was similar to a least-squares means estimate in an analysis of variance setting. Among 243 women with synchronous bilateral biopsy examinations, we assessed the level of agreement across the two readings by use of weighted kappa statistics and their corresponding 95% confidence intervals (CIs).

-84-
The length of follow-up for each woman in the study was calculated as the number of days from her biopsy examination to the date of her breast cancer diagnosis, death, or last contact. We estimated relative risks (RRs) on the basis of standardized incidence ratios by dividing the observed numbers of incident breast cancers by expected numbers of population-based incident breast cancers. Expected values were calculated by apportioning each woman’s person-years of follow-up into 5-year age and calendar-period categories and multiplying these by the corresponding breast cancer incidence rates from the Iowa Surveillance, Epidemiology, and End Results (SEER) Registry. This reference population was chosen because of its demographic similarities to the Mayo Clinic population (80% of cohort members reside in the upper Midwest). Potential heterogeneity in relative risks across levels of involution was assessed by use of Poisson regression analysis, with the log-transformed expected event rate for each individual modeled as the offset term.

In addition to assessing overall breast cancer risk, we also compared rates of ipsilateral to contralateral breast cancer in relation to the side of the benign lesion, both overall and by levels of involution. When calculating incidence for ipsilateral cancer, individuals with contralateral cancers were censored at their date of diagnosis, and vice versa. Women with missing laterality information, bilateral biopsy examination results, or bilateral breast cancer were censored for both events in these analyses. This approach yielded identical numbers of person-years for each type of event. As a result, the length of follow-up was not a factor in the analysis, and the rate comparisons reduced to simple comparisons of the number of events. Thus, we were able to assess whether the relative rate of ipsilateral cancer (compared with contralateral cancer) differed across levels of involution using simple chi-square tests of statistical significance. All statistical tests were two-sided, and all analyses were carried out with the SAS software system (SAS Institute, Inc., Cary, NC).

RESULTS

Extent of Lobular Involution

We characterized the extent of lobular involution in the benign breast biopsies of a cohort of 8736 women with tissue sampled between January 1, 1967, and December 31, 1991, at the Mayo Clinic. The distribution of the patients by level of lobular involution was as follows: no involution among 1627 (18.6%) women, partial involution among 5197 (59.5%) women, and complete involution among 1912 (21.9%) women.

Factors Associated With Involution

As shown in Table 1, the degree of lobular involution increased progressively with age at diagnosis of benign breast disease (P < 0.001). Complete involution of lobular units was observed in only 19 (3.4%) of the 566 women who were younger than 30 years at their benign biopsy; in 53 (5.1%) of the 1037 women aged 30-39 years; in 142 (5.8%) of the 2446 women aged 40-49 years; in 455 (21.6%) of the 2109 women aged 50-59 years; in 724 (43.3%) of the 1600 women aged 60-69 years, and in 519 (53.1%) of the 978 women aged 70 years or older. The gradual nature of the involution process is apparent in that it is already present at least to a partial degree in more than half of the women younger than 40 years and is still ongoing in women older than 70 years.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Extent of lobular involution, No. (%)</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>1627 (18.6) 5197 (59.5) 1912 (21.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Age at biopsy, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-29</td>
<td>108 (54.4) 238 (42.2) 19 (3.4)</td>
<td></td>
</tr>
<tr>
<td>30-39</td>
<td>317 (20.7) 567 (37.4) 57 (3.4)</td>
<td></td>
</tr>
<tr>
<td>40-49</td>
<td>645 (26.3) 1661 (67.9) 142 (5.8)</td>
<td></td>
</tr>
<tr>
<td>50-59</td>
<td>918 (16.3) 1456 (68.1) 455 (21.6)</td>
<td></td>
</tr>
<tr>
<td>60-69</td>
<td>29 (1.8) 847 (28.9) 724 (45.3)</td>
<td></td>
</tr>
<tr>
<td>≥70</td>
<td>12 (1.2) 447 (43.7) 519 (39.1)</td>
<td></td>
</tr>
</tbody>
</table>

*For age at diagnosis of benign breast disease (BBD), percentage values were unadjusted. For all other variables, percentage values were adjusted for age.

†For age at BBD, P values were calculated using chi-square tests of statistical significance. For all other variables, P values were calculated by use of multilogistic regression analyses, accounting for the effects of age. All statistical tests were two-sided.

<table>
<thead>
<tr>
<th>Information on family history of breast cancer</th>
<th>Overall Males Females</th>
<th>Number of children</th>
<th>Number of children</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>131 (17.6) 421 (55.3)</td>
<td>1 3 1</td>
<td>1 3 1</td>
</tr>
<tr>
<td>Strong</td>
<td>75 (17.2) 269 (54.7)</td>
<td>1 2 3</td>
<td>1 2 3</td>
</tr>
<tr>
<td>Parity</td>
<td>324 (22.2) 880 (56.2)</td>
<td>4 4 4</td>
<td>4 4 4</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>357 (22.8) 742 (51.8)</td>
<td>3 4 5</td>
<td>3 4 5</td>
</tr>
<tr>
<td>Parous</td>
<td>235 (26.6) 872 (22.3)</td>
<td>2 4 4</td>
<td>2 4 4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Children breastfed</th>
<th>Overall Males Females</th>
<th>Number of children</th>
<th>Number of children</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>464 (21.6) 1553 (57.4)</td>
<td>2 3 4</td>
<td>2 3 4</td>
</tr>
<tr>
<td>Yes</td>
<td>431 (23.3) 5210 (55.9)</td>
<td>4 4 4</td>
<td>4 4 4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Information on hormone replacement therapy</th>
<th>Overall Males Females</th>
<th>Number of children</th>
<th>Number of children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>481 (22.3) 1330 (57.1)</td>
<td>1 2 1</td>
<td>1 2 1</td>
</tr>
<tr>
<td>Ever</td>
<td>516 (21.8) 1837 (55.4)</td>
<td>2 3 3</td>
<td>2 3 3</td>
</tr>
</tbody>
</table>

| P value† | 0.016 |

The length of follow-up was not a factor in the analysis, and the rate comparisons reduced to simple comparisons of the number of events. Thus, we were able to assess whether the relative rate of ipsilateral cancer (compared with contralateral cancer) differed across levels of involution using simple chi-square tests of statistical significance. All statistical tests were two-sided, and all analyses were carried out with the SAS software system (SAS Institute, Inc., Cary, NC).

We also found a strong, inverse association (P < 0.001) between lobular involution and parity (Table 1). Specifically, the likelihood of complete involution was 27.1% (95% CI = 24.1% to 30.1%) in nulliparous women; 28.0% (95% CI = 24.7% to 31.4%) in women who had one child, 21.5% (95% CI = 19.3% to 23.8%) in women who had two children, 20.4% (95% CI = 17.8% to 23.0%) in women who had three children, and 18.8% (95% CI = 16.1% to 20.0%) in women who had four children or more.

Separating women into categories of ever versus never breastfeeding did not reveal any relationship with extent of lobular involution (P = 0.48). Women who reported having used hormone replacement therapy were slightly less likely to have complete involution (20.3%) than those with no history of hormone replacement therapy use (22.9%) (P = 0.016). Breast tissue from women with a strong family history of breast cancer was less likely than that from women with no or a weak family history of breast cancer to demonstrate lobular involution; i.e., after adjustment for age, women with a strong family history had no involution (24.5%) that those with no or a weak family history had.
Table 2. Association of involvment and other risk factors with breast cancer after the diagnosis of benign breast disease

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of women</th>
<th>No. of person-years</th>
<th>No. of observed</th>
<th>No. of expected events</th>
<th>Relative-risk (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree of involvment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (0%)</td>
<td>1627</td>
<td>32,271</td>
<td>120</td>
<td>78.6</td>
<td>1.88 (1.59 to 2.21)</td>
</tr>
<tr>
<td>Partial (1%–24%)</td>
<td>5197</td>
<td>96,406</td>
<td>440</td>
<td>300.1</td>
<td>1.47 (1.33 to 1.61)</td>
</tr>
<tr>
<td>Complete (25%+)</td>
<td>1912</td>
<td>28,376</td>
<td>106</td>
<td>116.5</td>
<td>0.93 (0.75 to 1.16)</td>
</tr>
<tr>
<td>Histologic type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonproliferative</td>
<td>5736</td>
<td>101,201</td>
<td>355</td>
<td>321.5</td>
<td>1.09 (0.99 to 1.23)</td>
</tr>
<tr>
<td>Proliferative without atypia</td>
<td>2677</td>
<td>45,418</td>
<td>276</td>
<td>158.1</td>
<td>1.75 (1.55 to 1.96)</td>
</tr>
<tr>
<td>Proliferative with atypia</td>
<td>323</td>
<td>4436</td>
<td>65</td>
<td>16.6</td>
<td>3.91 (3.02 to 4.98)</td>
</tr>
<tr>
<td>Age at biopsy, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45</td>
<td>2682</td>
<td>52,055</td>
<td>128</td>
<td>108.4</td>
<td>1.46 (1.24 to 1.70)</td>
</tr>
<tr>
<td>45–55</td>
<td>2259</td>
<td>49,246</td>
<td>254</td>
<td>169.0</td>
<td>1.50 (1.32 to 1.70)</td>
</tr>
<tr>
<td>&gt;55</td>
<td>3495</td>
<td>49,754</td>
<td>284</td>
<td>218.8</td>
<td>1.50 (1.35 to 1.66)</td>
</tr>
<tr>
<td>Family history of breast cancer‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None or weak</td>
<td>4424</td>
<td>81,514</td>
<td>339</td>
<td>269.4</td>
<td>1.23 (1.09 to 1.36)</td>
</tr>
<tr>
<td>Strong</td>
<td>928</td>
<td>18,383</td>
<td>113</td>
<td>79.2</td>
<td>1.93 (1.59 to 2.32)</td>
</tr>
<tr>
<td>Age at birth of first live child, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>711</td>
<td>12,021</td>
<td>71</td>
<td>41.1</td>
<td>1.72 (1.25 to 2.18)</td>
</tr>
<tr>
<td>30</td>
<td>421</td>
<td>77,716</td>
<td>327</td>
<td>257.9</td>
<td>1.27 (1.13 to 1.41)</td>
</tr>
<tr>
<td>&gt;30</td>
<td>388</td>
<td>79,091</td>
<td>30</td>
<td>22.4</td>
<td>1.34 (1.09 to 1.64)</td>
</tr>
</tbody>
</table>

*Number of events expected on the basis of Iowa Surveillance, Epidemiology, and End Results breast cancer incidence data.
*All analyses account for the effects of age and calendar period. CI = confidence interval.

(21.1%), and fewer women with a strong family history had complete involvment (19.3%) than those with no or a weak family history (22.4%). Logistic regression analysis comparing distribution of involvment across levels of family history (P < 0.001).

We also examined the extent of lobular involvment by category of benign breast disease. Among women with nonproliferative disease, 27.2% had complete involvment. However, among women with proliferative disease without or with atypia, only 11.5% had complete lobular involvment (P < 0.001).

Lobular Involvment and Breast Cancer Risk

This cohort of women with benign breast disease was, overall, at increased risk of breast cancer when compared with age-matched women in the general population. Specifically, the relative risk for the entire cohort of 8736 women, compared with the Iowa SEER population, was 1.40 (95% CI = 1.30 to 1.51). In our cohort, degree of involvment was associated inversely with breast cancer risk (Table 2; e.g., for no involvment, RR = 1.88, 95% CI = 1.39 to 2.21; for partial involvment, RR = 1.47, 95% CI = 1.32 to 1.64; and for complete involvment, RR = 0.91, 95% CI = 0.75 to 1.09; test for heterogeneity P = 0.001).

Figure 2 illustrates the observed associations between the extent of involvment and breast cancer risk among strata of age, histology, family history, and parity. Extent of lobular involvment modified age-related breast cancer risk (e.g., for a woman older than 55 years with no involvment, RR = 3.31, 95% CI = 1.90 to 5.08, and for a woman with complete lobular involvment, RR = 0.92, 95% CI = 0.74 to 1.14). The same pattern was observed in all age groups.

Similarly, progressive increases in lobular involvment in background breast tissue were associated with reduced risk of breast cancer among women with benign proliferative disease, even those with atypia (Fig. 2). Among women with atypia, no involvment was more strongly associated with a higher risk of breast cancer (RR = 7.79, 95% CI = 3.36 to 18.78) than complete involvment (RR = 1.49, 95% CI = 0.41 to 5.02) or partial involvment (RR = 4.06, 95% CI = 3.03 to 3.33) (test for heterogeneity, P = 0.003).

Among women with proliferative disease without atypia, no involvment was also associated with a higher risk of breast cancer (RR = 2.94, 95% CI = 2.26 to 3.75) than complete involvment (RR = 1.11, 95% CI = 0.68 to 1.71). The same pattern held true for those with nonproliferative breast disease, i.e., those with no involvment had a higher risk than those with partial or complete involvment.

Lobular involvment modified the influence of family history on risk of breast cancer (Fig. 2). Among women with a strong family history of breast cancer, no involvment was associated with the highest risk of breast cancer (RR = 2.77, 95% CI = 1.59 to 3.84), followed by partial involvment (RR = 1.72, 95% CI = 1.32 to 2.26) and then by complete involvment (RR = 1.61, 95% CI = 0.92 to 2.61). Among women with no or a weak family history of breast cancer: and complete involvment, the risk of breast cancer (RR = 0.59, 95% CI = 0.41 to 0.81) was approximately half of that for the general population, which was based on Iowa SEER data, and approximately fivefold less than the risk of those with strong family history and no involvment (i.e., RR = 2.77, 95% CI = 1.94 to 3.84).

Lobular involvment also modified the risk associated with parity or age at birth of the first live child. Among multiparous women and women whose first live child was born when she was at least 30 years old, no lobular involvment was associated with increased risk of breast cancer (RR = 2.41, 95% CI = 1.25 to 4.61, and RR = 2.74, 95% CI = 1.31 to 5.63, respectively). However, among those two groups of women, when complete lobular involvment had occurred, there was no increase in risk (RR = 1.02, 95% CI = 0.53 to 1.78, and RR = 0.48, 95% CI = 0.10 to 1.40, respectively). Among women whose first live child was born when she was younger than 30 years old, complete lobular involvment was associated with a decreased risk of breast cancer (RR = 0.63, 95% CI = 0.44 to 0.91).

We also asked whether the era in which the biopsy examination was performed—namely, before or after widespread adoption of mammography—affect ed the results. In the first 15 years of the cohort (from 1967 through 1981), 78% of biopsy examinations were done because of a palpable concern (i.e., a palpable

Journal of the National Cancer Institute, Vol. 98, No. 22, November 15, 2006
Lobular Involution: Localized or Field Effect

To address whether or not the degree of involution was relevant only to the area of the biopsy or was representative of the field of breast tissue, we examined 1) whether, for women with bilateral benign biopsy examination results, involution results were concordant and 2) whether the degree of involution at the benign biopsy site was associated with the risk of ipsilateral breast cancer or with both ipsi- and contralateral breast cancers. A subset of 245 women had bilateral biopsy examinations performed at the same time. In 203 (83%) of these women, the same category of involution (no, partial, or complete) was found in the biopsy tissue from both breasts. In 41 (17%), there was a difference of one category between the two breasts. Only one individual had complete involution in the biopsy tissue of one breast and no involution in the contralateral sample. These results indicate a high level of agreement in involution measured across multiple biopsy specimens within a woman (kappa coefficient = 0.72, 95% CI = 0.64 to 0.80; test for agreement beyond that expected by chance P < .001).

We next investigated the extent of involution and the laterality of subsequent breast cancers. In our cohort overall, there is a slight predominance of ipsilateral breast cancers (55.5%) over contralateral breast cancers (44.5%), as reported previously (3); this result is thought to reflect the presence of some direct precursors among these lesions. To determine whether involution at the site of the benign breast disease was relevant to the contralateral breast, we examined the ratio of ipsilateral to contralateral events by degree of involution. With no involution, the ratio was 33.6% ipsilateral to 46.4% contralateral; for partial involution, the ratio was 55.9% to 44.1%; and for complete involution, the ratio was 33.5% to 46.5% (chi-square test for difference in percent ipsilateral across involution status, P = .85). Thus, the relationship between involution extent and breast cancer risk was observed in both the ipsi- and contralateral breast.
breast disease. Our data demonstrate a strong, inverse relation­ship between degree of involution and breast cancer risk. To our knowledge, this is the first study to systematically examine age-related involution in the context of breast cancer risk. Furthermore, greater degrees of involution reduced breast cancer risk even in high-risk subsets defined by age, atypia, reproductive history, or family history. There was a strong direct association between involution and increasing age. There was an inverse association between involution and parity.

As in this study, others have found that older women tend to have fewer lobules or only lobule remnants (4,5). Cowan and Herbert (4) performed a detailed autopsy study of the breast tissue from 102 women, aged 50–104 years, who died without known breast disease. Although considerable individual variability was present, they described a progressive loss of lobules with increasing age. Earlier reports state that age-related involution has already begun in women under the age of 40 years (4,5). Our data confirm that this process is present, at least to a partial degree, in many younger women.

We hypothesize that the degree of involution detected at the benign biopsy site reflects that of the overall field of a woman’s breast tissue. We believe that this hypothesis is reasonable because of our results showing a similar likelihood of contralateral and ipsilateral breast cancers by involution status at the site of the benign breast disease and because of the high concordance an inversion status in women who had bilateral biopsy examinations. However, our study design cannot answer this question definitively. To do so would require examination of the extent of involution throughout all of a woman’s breast tissue.

It is widely appreciated that, as women age, their risk of breast cancer increases. But the rate of increase of breast cancer slows appreciably at approximately age 50 years (10,11), which has been attributed to a reduction in ovarian hormonal production (12). We observed a definite increase in the process of involution at approximately age 50 years (with complete involution present in 5.8% of women aged 40–49 years and in 21.6% of women aged 50–59 years). These data raise the possibility that involution may be contributing to the slowing in the rate of increase of breast cancer among women older than 50 years, as speculated by Henson and Tarone (7).

We examined various factors besides age for their association with degree of involution. We found an inverse association between lobular involution and parity. Others have also reported that the more children a woman has, the more likely she is to have persistence of lobular structures (1,5), which we found was associated with increased risk of breast cancer. Yet, multiparity is generally considered to reduce the risk of breast cancer (13,14). Several factors may explain this apparent contradiction. First, we do not have data on the age at each child’s birth for the women in our cohort. Some epidemiologic work has suggested that full-term pregnancies after 35 years of age are associated with an increased risk of breast cancer (7,13). Thus, data on a woman’s age at each pregnancy and on her age at breast biopsy examination would help to evaluate more definitively the relationships of parity, involution, and breast cancer risk. In addition, our study was limited by the relatively large size of the group of women categorized as having partial involution. More specific, quantitative measures of degree of involution should be explored to determine whether the association between parity status and degree of involution can be defined more precisely. Given the inverse association between complete involution and multiparity and given that both are associated with reduced risk of breast cancer, we hypothesize that the breast cancer risk modification associated with parity is independent of involution status.

There are several biologic mechanisms by which involution or lack thereof could alter a patient’s breast cancer risk. The decrement in epithelial cell number that accompanies involution may decrease breast cancer risk simply because there are fewer epithelial cells to undergo malignant transformation. Another possibility is that aberrant involution may be a marker or phenotype reflecting underlying constitutional susceptibility for breast cancer that is present in the epithelial or stromal compartment or in their relationship with each other. Yet another possibility is that failure to undergo timely or appropriate involution allows prolonged exposure of epithelial cells to intrinsic and/or extrinsic mutagenic stresses (15–20). In this model, the prime targets of such mutagenic processes, such as stem cells or early progenitors, may become quiescent during the process of involution. Experiments to characterize the epithelial and stromal mediators present in tissue with and without involution, in women with and without subsequent cancer, should help to clarify the mechanism of risk reduction.

For our work to date, we divided extent of involution into three categories. We recognized that, although the morphologic patterns of age-related lobular involution have been defined (1,4,5), no histologic standard exists for evaluating the extent of breast involution. In particular, there is no well-characterized method for grading partial degrees of involution. For this reason, we attempted to classify degree of involution with the least amount of subjectivity. Thus, by deciding only whether breast tissue had no lobular involution versus almost complete involution and then by combining the remainder into one category of partial involution, we minimized the subjectivity inherent in judging percent involution.
Our study has several limitations. First, these findings do not necessarily pertain to all women because the cohort included women who had a benign biopsy because of some concern. In addition, women with benign breast disease make up a large population who are understandably concerned about their breast cancer risk (estimated at 1 million US women each year) (21–23). Another limitation lies in our current very broad category of partial involution. This category encompasses a wide range of involution extent (1%–74% of lobules involved). We expect that more-specific gradations would support more refined association studies. Finally, we did not have complete risk factor data for all the women in the cohort, largely because the women with biopsy examinations in the earlier years of the cohort are now elderly or deceased. Fortunately, for purposes of this report, we did not have to depend on the questionnaire for involution status or for cancer outcomes (which were available from our comprehensive Mayo medical record). We had completed questionnaires for 63.8% of the patients with breast cancer and 61.0% of the patients without breast cancer in the cohort.

There are other approaches to the study of involution and breast cancer risk. Henion and Tarone (7) suggested an autopsycase-control series to look at involution as a possible risk factor for breast cancer. Although this approach would provide access to extensive amounts of breast tissue, the availability of clinical risk factor information and of a sufficient number of subjects could be limiting. Women who have breast tissue removed in the course of clinical care are those who have reduction mammoplasty or prophylactic mastectomy. These women are, respectively, those who have breast hypertrophy or a hereditary predisposition to breast cancer. Although involution (or lack thereof) in these women is of considerable interest, their tissue is not necessarily representative of the general population.

The mechanisms controlling age-related involution are of considerable interest. Molecular programs that control postnatal involution in rodents have been studied extensively (24). With postnatal involution, there is dramatic reversal of the developmental changes wrought by pregnancy. Specifically, there is widespread apoptosis of alveolar epithelial cells followed by removal of apoptotic debris and remodeling of the stroma and extracellular matrix (24). These events occur within a matter of days of abrupt weaning and restore the gland to its prepuberty state. In contrast, the molecular orchestration of age-related involution, to our knowledge, has not yet been characterized.

In the past, for women with benign breast biopsy results, the type and extent of epithelial proliferation present in their biopsy has been the principal way to stratify their risk. Results of our study indicate that assessing the status of lobular involution in the biopsied tissue may ultimately add to risk prediction capabilities. It is notable, as shown in Fig. 2, C, that some of the most extreme risk estimates are observed in women whose involution status is unusual for their age—namely, young women with complete involution (RR = 0.43, 95% CI = 0.13 to 1.35) and women older than 55 years with no involution (RR = 3.2, 95% CI = 1.90 to 5.68). It is tempting to speculate that the process of complete involution may be protective and, conversely, that lack of involution identifies higher risk groups. However, confidence intervals were wide around the estimates for these less common categories.

In summary, we have evaluated the extent and effect of age-related lobular involution in a cohort of approximately 9000 women who had a benign breast biopsy examination. We observed a statistically significant reduction in risk of breast cancer among those women whose breast tissue had undergone extensive lobular involution, which was apparently independent of other markers of risk. Among women with benign breast disease, assessment of extent of involution may help to fine-tune current risk prediction approaches. Elucidation of the mechanism of lobular involution may reveal ways to promote the process as a means of risk reduction.

References

NOTES

Supported by a Department of Defense Center of Excellence Grant (FEDDAMD17-02-1-0473-1), a grant (RO1 CA63332) from the National Institutes of Health, and grants from the Susan G. Komen Breast Cancer Foundation (BCTR 99-3152), Anderssen Foundation, Breast Cancer Research Foundation, and Berg Foundation for Breast Cancer Research. The authors take full responsibility for the study design, collection of the data, analysis and interpretation of the data, the decision to submit the manuscript, and the writing of the manuscript.

We are indebted to Joel Womn and Dr Piet de Groen for database development; to William Lingle, Ph.D., and the Biostatistics Core for data processing; to Teresa Allers, Mary Amundson, Mary Campbell, Jannie Johnson, Melanie Kraner, Marge Lepanto, and Lois Penheter for data collection; to Ann Harris and the Survey Research Center for patient follow-up; and to Vicki Shen for help in preparing the manuscript.

Funding to pay the Open Access publication charges for this article was provided by a grant from the Department of Defense.

Manuscript received July 18, 2006; revised August 31, 2006; accepted September 22, 2006.
Lobular Involution: the Physiological Prevention of Breast Cancer

Donald Earl Hensley, Robert E. Tarone, Hala Nsouli

It truly is a remarkable event when traditional pathologic observations lead to new ideas about the prevention of cancer. In this issue of the Journal, Milanesio et al. (1) through a histologic review of breast biopsy specimens, show that the extent of age-related lobular involution is strongly associated with a reduced risk of breast cancer. Breast cancer risk decreased with increasing extent of involution in both high-risk and low-risk subgroups defined by age, epithelial atypia, reproductive history, and family history of breast cancer.

Beginning in the premenopausal period, lobular involution is a physiologic process that occurs over many years whereby the parenchymal elements in the breast progressively atrophy and disappear (2,3). The study reported by Milanesio et al. represents a unique application of the Mayo Benign Breast Disease Cohort to investigate prospectively involution as a risk factor for breast cancer. It is the first study, to our knowledge, to substantiate a hypothesis that is based on pathologic and epidemiologic considerations that delayed involution is a major risk factor for breast cancer (4,5).

As for an explanation of the effect of lobular involution on breast cancer risk, it has been suggested that a reduction in mammary gland tissue that results from involution should lead to a reduction in breast cancer because a progressively smaller amount of epithelial tissue is available for malignant transformation (1,5). The result of involution, therefore, can be considered physiologically analogous to a partial prophylactic mastectomy, with a corresponding reduction in breast cancer risk.

Although a reduction in mammary tissue is a plausible explanation, the underlying issue is one of age or, more precisely, the failure of breast tissue to age normally. The aging process in the breast is under control of various hormones and does not follow the pattern seen in other organs or tissues. Pathologists have long commented on the possibility that persistent atypical lobules might be precursors of invasive breast cancer (6,7). It seems paradoxical that an organ that normally undergoes complete or near complete physiologic atrophy would be a site in which cancer rates steadily increase with age. The continuing increase in breast cancer risk with age is likely associated with the persistence of atypical epithelium beyond the time of normal involution, reflecting an abnormal delay of the aging process in the breast (4,5).

Except for morphologic observations concerning age of onset and progression with age, practically nothing is known about the process of involution. Even less is known about factors that control involution or that delay or accelerate the process. In this context, it is unknown whether the rate of involution is genetically determined and whether known breast cancer risk or environmental factors alter the rate of involution.

Evidence indicates that some risk factors for breast cancer may interfere with the process of involution. In the Mayo study, women with benign proliferative breast disease were substantially less likely to have complete involution than were women with benign nonproliferative disease, and women with a strong family history of breast cancer had slightly less advanced involution than women without such history (1). Late age of menopause, which increases the risk of breast cancer, is likely to result in delayed involution because of persistence of estrogen activity (8). Women whose first full-term pregnancy occurs after age 35 have an elevated risk for breast cancer compared with multiparous women or with women whose first pregnancy was at a much younger age (9,10). After the commencement of involution, late pregnancy with its concomitant increase in the proliferation of the ductal-alveolar epithelium is likely to interrupt the normal process of involution, which typically begins between 30 and 40 years of age. Oophorectomy, which protects against breast cancer (11), leads to the same type of atrophy of breast parenchyma in young women as that seen in older women (12). The reduction in risk may be due to the acceleration of involution induced by oophorectomy.

One of the most striking findings in the study of Milanesio et al. is the degree to which the strong association between extent of involution and breast cancer risk was independent of all known breast cancer risk factors that were investigated (1). This observation suggests that factors unrelated to known risk factors are responsible for the protective effect of involution. For this reason, a greater understanding of the biologic basis for involution will be required to elucidate the mechanisms of the protective effect of lobular involution on breast cancer risk.

The observations reported by the Mayo group may find practical applications for risk prediction (1). It may be useful for pathologists to report the extent of involution in addition to any epithelial changes found in breast biopsy specimens that do not contain cancer. It will be important to determine the extent to which mammographic breast density serves as a surrogate for the extent of involution. By taking extent of involution into account, it should be possible to increase the predictive ability of breast cancer risk models.

Results of the Mayo study provide a new paradigm for breast cancer research and prevention. Age has always seemed the opponent because of the increasing risk of breast cancer with age.
but age may now become an ally. The challenge will be to unravel the natural history of involution and the normal process of aging in the breast. Eventually, involution could become a useful surrogate endpoint for research in breast cancer prevention. A possible approach to prevention may be to develop strategies that achieve complete involution as early as possible afterchildbearing is completed.

REFERENCES


NOTE

The authors would like to acknowledge the constructive comments from Dr William Anderson.
Stratification of Breast Cancer Risk in Women With Atypia: A Mayo Cohort Study

ABSTRACT

Purpose
Atypical hyperplasia is a well-recognized risk factor for breast cancer, conveying an approximately four-fold increased risk. Data regarding long-term absolute risks and factors for risk stratification are needed.

Patients and Methods
Women with atypical hyperplasia in the Mayo Benign Breast Disease Cohort were identified through pathology review. Subsequent breast cancers were identified via medical records and a questionnaire. Relative risks (RRs) were estimated using standardized incidence ratios, comparing the observed number of breast cancers to those expected based on Iowa Surveillance, Epidemiology, and End Results (SEER) data. Age, histologic factors, and family history were evaluated as risk modifiers. Plots of cumulative breast cancer incidence provided estimates of risk over time.

Results
With median follow-up of 13.7 years, 66 breast cancers (19.9%) occurred among 331 women with atypia. RR of breast cancer with atypia was 3.88 (95% CI, 3.00 to 4.94). Marked elevations in risk were seen with multifocal atypia (e.g., three or more foci with calcifications; RR, 10.35; 95% CI, 5.13 to 16.4). RR was higher for younger women (<40; RR, 6.76; 95% CI, 3.24 to 12.4). Risk was similar for atypical ductal and atypical lobular hyperplasia, and family history added no significant risk. Breast cancer risk remained elevated over 20 years, and the cumulative incidence approached 35% at 30 years.

Conclusion
Among women with atypical hyperplasia, multifocal atypia and the presence of histologic calcifications may indicate "very high risk" status (>50% risk at 20 years). A positive family history does not further increase risk in women with atypia.

J Clin Oncol 25:2657-2667. © 2007 by American Society of Clinical Oncology

INTRODUCTION

Atypical hyperplasia is a well-established risk factor for subsequent breast cancer. Multiple studies corroborate an approximately four-fold increased risk of breast cancer in women undergoing surgical biopsy with a finding of atypia.11 Despite good concordance on the estimated relative risk (RR) with atypia, estimates of absolute risk with long-term follow-up are not well established. Reliable breast cancer risk estimates for women with atypia are crucial for risk-benefit analysis and decision making regarding risk-reduction strategies.

The Gail model is currently used to predict a dramatically increased risk for those women who have both atypia and a family history (over that of atypia alone).1 Prior published literature has stated that the risk of breast cancer abates considerably after 10 years after a diagnosis of atypia, whereas more recent evidence indicates otherwise.12 It is also unclear whether breast cancer risk is higher in cases of atypical ductal hyperplasia (ADH) versus atypical lobular hyperplasia (ALH).

Here, we present a comprehensive description of breast cancer risk in women with atypical hyperplasia, based on 331 women with atypia in the Mayo Benign Breast Disease Cohort. Our investigation addresses the effect of family history on atypia risk, the effect of time since biopsy, the influence of ductal versus lobular histology, effects of age at atypia diagnosis, and presence of calcifications on breast cancer risk. In addition, we provide absolute risk estimates over time, and we also present a new histologic feature of atypia—multifocality—that stratifies breast cancer risk among women with atypia.
**Patients and Methods**

**Study Population**

Entry criteria for the study cohort have been previously described. Briefly, this comprises an institutional review board-approved study of women ages 30 to 85 years who had a benign breast biopsy or diagnosis confirmed during 1987 to 1989. The initial cohort included 8,287 women. With additional follow-up data now available for 9,756 women (68.5%) of whom had atypical hyperplasia.

**Follow-Up**

Follow-up for breast cancer events (including both invasive cancer and ductal carcinoma in situ [DCIS]) and risk factor information were obtained for all women with atypia through the Mayo medical record and a study questionnaire. Family history was classified as negative, strong, or weak. The criteria for a strong family history were at least one first-degree relative with breast cancer before the age of 50 years or two or more relatives with breast cancer with at least one being a first-degree relative. Any lesser degree of family history was considered weak.

**Histology**

All available archival hematoxylin and eosin-stained sections were evaluated by our breast pathologists (D.W.W. and C.A.J), without knowledge of the original histologic diagnoses or patient outcomes. The number of slides reviewed per case was variable because of the retrospective nature of the study, with a mean of 3.2 (standard deviation, 3.7). Calculations were recorded for each case when reviewed histologically. A diagnosis of ADH or ALH was based on the criteria of Page et al. ADH was characterized by filling and distortion of invaginated ducts with hyperplasia.

**Characteristics of Patients and Pathologic Specimens**

A total of 381 women with atypia were identified in our cohort between 1987 and 1991. In Table 1, we present the patients’ vital status, breast cancer status, family history, age at biopsy, year of biopsy, indication for biopsy, and histologic features. Women were likely 38 years of age or older and had a breast biopsy performed because of a family history of breast cancer, personal history of breast cancer, and/ or a mammographic abnormality. The overall incidence of breast cancer in women with atypia was 9.4% (95% CI, 8.2 to 10.6) during a 20-year period.

**Statistical Analysis**

Follow-up was defined as the number of days from benign biopsy to date of breast cancer diagnosis, death, or last contact. We estimated RRs with standardized incidence ratios (SIR) and 95% CIs, dividing observed numbers of incident breast cancers by expected counts. We calculated expected counts by applying each individual’s follow-up time since baseline year and calendar period to the 1990 age- and calendar-period–specific population-based incidence rates, thereby accounting for differences in these variables. We used the Iowa Surveillance, Epidemiology, and End Results (SEER) registry as the reference population, because of its proximity to the Mayo Clinic catchment area and social similarity compared with our cohort. We calculated incidence rate ratios for cohort follow-up occurring outside the SEER time frame (1973–2002), such that person-years before 1973 were applied to 1973 to 1979 incidence rates, and person-years subsequent to 2002 were applied to 2001 to 2002 incidence rates. Assuming a two-sided test of hypothesis and a type I error rate of 0.05, we would have 80% power to detect SIRs below 861 if the expected rate was 1.25, as low as 257 if the count is 2,000, as low as 20.8 if the count is 10.3, as low as 10.0 if the incidence is 10.0. Notably, these expected counts reflect the approximate expected numbers of events in our cohort for women with three or more facts of atypia, two facts, one fact, and all subjects combined, respectively.

Recognizing that other biologic mechanisms may modify the association of atypia and breast cancer risk, we formally assessed the potential differential effects of these mechanisms using Poisson regression analyses. This approach allowed us to estimate SIRs with the flexibility that generalized linear models provide, such as covariate adjustment and tests for trend or heterogeneity across subgroups. For all analyses, the log-transformed expected event rate for each individual was modeled as the off-effect term. We displayed observed and expected event rates using cumulative incidence curves and corresponding 95% confidence limits, accounting for the effects of death as competing risk. Expected rates were calculated for each 1-year follow-up interval in a manner similar to that used for determining SIRs. A modified Kaplan-Meier approach was used to calculate expected incidence over these intervals. The expected curve was then smoothed using linear interpolation.

We compared the RR of ipsilateral versus contralateral breast cancer overall and across different clinical characteristics using ratios of corresponding incidence rates. When calculating incidence for ipsilateral cancer, individuals with contralateral cancer were excluded at their diagnosis date and vice versa. Women with missing height or having bilateral biopsies or cancers were excluded for both events. The RRs are equivalent to ratios of observed events, as the approach yields identical person-years for each event type. We used the proportion of the binomial distribution to obtain exact 95% CIs for these RRs. All statistical tests were postulated a priori and were two sided, and all analyses were conducted using the SAS software system (SAS Institute, Cary, NC).

**Results**

The study population included 381 women with atypia and their family members. The mean age at biopsy was 55.1 years (range, 30 to 85 years). The majority of cases were ductal type (67 of 53, 89%), and the remaining six invasive lobular cancers were divided between the ADH and ALH subgroups. Table 2 shows the estimated SIRs for breast cancer associated with various characteristics. The overall group with atypia demonstrates a four-fold RR of breast cancer (RR, 3.88; 95% CI, 3.00 to 4.04) compared with the general population.

**Family History**

We did not see any significant differences in RR seen among the subgroups with a strong family history (RR, 3.96; 95% CI, 1.86 to 8.57) compared with those without a strong family history (RR, 3.00; 95% CI, 1.86 to 4.81).
Table 1. Clinical and Histologic Characteristics Among the 331 Women With Atypical Hyperplasia From the Mammography Breast Disease Cohort Study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Rate</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at biopsy, years</td>
<td>&lt;45</td>
<td>10</td>
</tr>
<tr>
<td>Family history of breast cancer</td>
<td>No</td>
<td>15</td>
</tr>
<tr>
<td>Histologic type</td>
<td>Calcifications</td>
<td>259</td>
</tr>
<tr>
<td>Number of foils of atypia</td>
<td>None</td>
<td>53</td>
</tr>
<tr>
<td>Indication for biopsy</td>
<td>Biopsy within 10 years of BBD</td>
<td>158</td>
</tr>
<tr>
<td>Risk of breast cancer</td>
<td>1967-1971</td>
<td>7.8</td>
</tr>
<tr>
<td>Race</td>
<td>White</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>1.5</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>&lt;45</td>
<td>10</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>&gt;45</td>
<td>9</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Mean age</td>
<td>50</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>SD</td>
<td>10</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>69.5</td>
<td></td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>70.7</td>
<td></td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Males</td>
<td>75</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Females</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>75</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>65</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Female</td>
<td>60</td>
</tr>
<tr>
<td>Age at biopsy, years</td>
<td>Male</td>
<td>70</td>
</tr>
</tbody>
</table>
breast cancer her lifetime risk doubles to 34%. Our data indicate that the Gail model predicts accurately for such women because the increased risk of breast cancer associated with atypia is independent of the effect of family history.

Women in our cohort with atypia and a positive family history of breast cancer had no additional increased risk of breast cancer over that of atypia alone. This finding counters the commonly held view proposed by the Nashville study (ie, that atypia and a positive family history increase breast cancer risk additively). When data from other major studies of benign breast disease are considered along with the Mayo findings, the preponderance of evidence calls into question the result from the Nashville group. In this study, the subgroup of women with atypia and a family history was small (n = 39) with an RR of 8.9 (95% CI, 4.8 to 17.3), compared with 3.5 (95% CI, 1.3 to 5.5) in 193 women with atypia and no family history. In a more recent evaluation of a much larger population in the Breast Cancer Detection and Demonstration Project showed similar frequencies of breast cancer in women with atypia and family history (10% of 2031, 613%) compared with those with atypia alone (51% of 2084, 4.9%). Recent data from the Nurses' Health Study confirm our finding that a family history of breast cancer in a first-degree relative does not further increase risk among women with atypical hyperplasia. To explain these findings, we postulate that atypical hyperplasia is a phenotype reflecting increased risk; this phenotype derives from both inherited risk and lifetime exposure. Thus, the histologic presence of atypia already reflects the increased breast cancer risk inherent in a positive family history.

We have identified a new histologic variable that appears to stratify risk in women with atypical multifocality. The RR of breast cancer increased in a dose-response fashion for women with one, two, and three or more foci of atypia, with a statistically significant trend for increased RR at three or more foci, with atypia and a positive family history (RR = 18% at 25 years). Moreover, in the highest risk subgroup of women with three or more foci and histologic findings, the cumulative incidence exceeded 50% over 25 years. This level of risk approaches that reported for carriers of BRCA mutations. In line with our observation, differential risk based on extent of disease has been established for lobular neoplasia (ie, ALH--lobular carcinoma, A/L and the number of foci of atypia found in core needle biopsy specimens correlates with the likelihood of finding cancer at surgical excision. Some may question whether multifocal atypia may actually represent subtle in situ carcinoma, particularly those of the ADH type. In cases of multifocal ADH, it should be emphasized that individual foci are separate and distinct terminal duct lobular units, none of which measured more than 2 mm. Hence, these examples failed to exhibit the continuous degree of cellular proliferation requisite for a diagnosis of DCIS. We submit that more widespread distribution of atypical foci within breast tissue signals a larger burden of at-risk tissue that has progressed along the continuum toward breast cancer. The data presented in this article provide evidence that the extent of pre-malignant breast change is related to subsequent breast cancer risk. Since this is the first report of the clinical relevance of this histologic finding, we recognize the need for validation and plan to evaluate this factor in a more recent cohort from our institution. Furthermore, we hope that other research groups with large numbers of patients with atypical hyperplasia will also examine the relevance of multifocal atypia in their study sets.

Age at the diagnosis of atypia also emerged as a significant modifier of subsequent breast cancer risk, with a higher RR in younger women. The Nurses Health Study and the Breast Cancer Detection and Demonstration Project have also shown higher risk in younger women with atypia. In our cohort, this increased risk in younger women is not explained by more frequent multifocal disease or a positive family history. Perhaps atypical hyperplasia presents at a young age is the result of previous oncogenic events. Alternatively, breast tissue in younger women may be unusually susceptible to proposed oncogenic estrogen metabolites associated with the premenopausal hormonal environment.

When counseling women with atypical hyperplasia, the length of time at risk is a key element in planning risk-reduction strategies. D'Entremont and Page reported that the greatest risk of breast cancer after...
A diagnosis of atypia lies in the first 10 years, with subsequent RR reduced by half (P < .06). By contrast, the Nurses Health Study found that risk does not decrease over time, with RR slightly higher more than 10 years after biopsy (RR, 3.6) compared with the first 10 years (RR, 3.2). Our data confirm that the RR for breast cancer after a biopsy demonstrating atypia remains significantly elevated for at least 15 years.

Data on long-term absolute risk are more useful than RR estimates when counseling patients. Our study provides estimates of absolute risk for women with atypia and indicates a cumulative incidence of breast cancer with long-term follow-up (10 years) has been reported by other studies. Figure 3 from the study of Dupont and Page shows a cumulative breast cancer incidence of 18% at 20 years and 32% at 25 years in women with atypia. The cumulative incidence is estimated in our cohort were higher: 20% at 20 years and 28% at 25 years. One factor contributing to this difference is our inclusion of DCIS as a recordable breast event, whereas the Nashville study counted only cases of invasive breast cancer. Because DCIS currently receives local treatment (and in some cases, systemic treatment) similar to that for early-stage invasive breast cancer, it is reasonable to include cases of DCIS when estimating risk.

Our data on the latency of subsequent breast cancer do not allow conclusions regarding atypical hyperplasia acting as a precursor lesion, nor is there a suggestion of predisposition for the ipsilateral breast that requires ongoing study. Breast cancers occurring in the first 10 years after atypia diagnosis were significantly more likely to occur in the ipsilateral breast. A recent study of gene expression profiling identified rather similar alterations in gene expression among ADH, DCIS, and invasive cancers found in the same specimen, supporting the role of atypical hyperplasia as a precursor cancer.26 Regarding differences in ipsilateral risk for ductal versus lobular atypia, we found that risk was equal for both breasts after a diagnosis of ADH, which is consistent with the distribution of invasive breast cancers after a diagnosis of lobular carcinoma in situ.24 In contrast, ADH was more likely associated with a later ipsilateral breast cancer, as has been shown for DCIS under🏻 after the diagnosis of biopsy.25

In conclusion, our study provides a comprehensive analysis of breast cancer risk associated with atypical hyperplasia. These findings confirm a four-fold RR of subsequent breast cancer in women with atypical hyperplasia. We estimate that the long-term absolute risk of subsequent breast cancer (in situ or invasive) is higher than previously reported—at least 35% at 25 years, and as high as 50% to 60% in a high-risk subgroup defined by multifocality and calcifications. A positive family history does not correlate significantly increased risk in women with atypia. Improved risk prediction and stratification is now possible to guide risk-reduction counseling for women with atypical hyperplasia.

The authors indicated no potential conflict of interest.


Financial support: Lynn C. Hartman.

Administrative support: Lynn C. Hartman.


References


Breast Cancer Risk in Women With Atypia


Acknowledgment

We thank Joel Werra for database development; Teresa Allen, J. Johnson, Mary Armudisan, Mary Campbell, and Romantia Thompson for data collection; and Ann Hurvitz and the Survey Research Center for patient follow-up.
Breast cancer risk in women with radial scars in benign breast biopsies

Jena C. Berg · Daniel W. Visscher · Robert A. Vierkant · V. Shane Pandolfo · Shawn D. Maloney · Jason T. Lewis · Marlene H. Frost · Karthik Ghosh · Amy C. Degan · Kathleen R. Brandt · Celine M. Vachon · Carol A. Reynolds · Lynn C. Hartmann

Received: 16 April 2007 / Accepted: 22 April 2007 / Published online: 22 May 2007
© Springer Science+Business Media B.V. 2007

Abstract Background: The risk for subsequent breast cancer in women diagnosed with radial scar lesions (RS) on benign breast biopsy remains controversial. We studied the relative risk of radial scar lesions in a large cohort of patients with benign breast disease (BBD).

Methods: Radial scar lesions were identified in a BBD cohort of 9,262 patients biopsied at Mayo Clinic between 1987 and 1991. Radial scar lesions were classified as proliferative disease without atypia (PDWA) unless atypia was present (classified as atypical hyperplasia [AH]). The observed number of breast cancers developing among those with RS was compared to that expected in the general population using standardized incidence ratios (SIRs, mean follow-up interval 17 years).

Results: RS were identified in 439 (4.7%) of the cohort members; 382 (87.0%) contained one RS, 42 (9.6%) contained two, 9 (2.0%) contained three, and 6 (1.4%) contained four or more. The majority of RS (556, 83.4%) were less than 5.0 mm in diameter; 60 (13.5%) were 5.0–9.9 mm; and 16 (3.9%) were 10.0 mm or greater. The relative risk for women with PDWA and RS was 1.88 (95% CI 1.36–2.53), no different from PDWA without RS (relative risk 1.57 (95% CI 1.27–1.91); (P = 0.029). Women with atypical hyperplasia and RS (n = 60) had a relative risk of 2.81 (95% CI 1.29–5.35), while those with RS but without RS had a relative risk of 3.97 (95% CI 2.59–5.19). Conclusions: RS impart no increased breast cancer risk above that of PDWA or AH without RS.

Keywords: Radial scar · Breast cancer

Introduction

Radial scars (RS) are benign breast lesions of uncertain etiology and behavior. Although named as such since 1980 [1], radial scars were previously known by a variety of names, including sclerosing papillary proliferation, non-encapsulated sclerosing lesion, and infiltrating epitheliosis. As these names suggest, radial scars have a characteristic

-100-
The growth pattern in RS can resemble a malignancy. The stellate architecture is difficult to distinguish from invasive carcinomas on mammogram, prompting biopsy of these lesions [3–7]. On histologic examination, the dense stromal fibrosis of RS can distort epithelial structures, mimicking the invasive pattern of carcinomas, especially tubular carcinomas.

The literature regarding the risk of developing subsequent in situ or invasive breast carcinomas following a biopsy diagnosis of radial scar has been mixed. Jacobs et al. examined 99 breast biopsies with RS in a case-control study within the Nurses’ Health Study [8]. Using non-proliferative breast disease as the reference category, they found a relative risk for proliferative disease without atypia (PDWA) and RS of 3.0, and a risk for (or PDWA alone of 1.5 (95% CI, 1.3–3.5 and 1.1–2.1, respectively). This risk was shown to increase with the presence of atypia and RS (RR 5.8 vs. 3.8 for AH alone; 95% CI, 2.7–12.7, and 2.4–5.9, respectively). In both categories, RS acted as an independent risk factor for later breast cancer development, either in situ or invasive carcinomas. This risk increased proportionally with both the number and size of radial scars present. The relative risk for women with PDWA and more than one RS increased to 4.3 (95% CI, 1.7–10.8), and for women with AH and more than one RS, the relative risk increased to 8.4 (95% CI 3.1–22.9). For size determination, the study examined lesions using a cutoff value of 4.0 mm. The relative risk for women with PDWA and RS larger than 4.0 mm increased to 3.5 (95% CI, 1.7–7.3), and for women with AH and RS larger than 4.0 mm, the relative risk increased to 8.8 (95% CI, 3.5–22.0).

Sander et al. examined 880 breast biopsies with RS, 9.2% of the overall Nashville Breast Cohort [9]. They found an overall risk of subsequent invasive breast cancer associated with a diagnosis of RS of 1.82 (95% CI, 1.2–2.7). In contrast to Jacobs et al., the Nashville investigators did not see additive risk with the presence of RS. Specifically, for biopsies containing PDWA and RS, the risk was 2.19 (95% CI, 1.3–3.5), while the risk of PDWA alone was 1.74 (95% CI, 1.2–2.5). The relative risk of biopsies with atypia and RS was 5.39 (95% CI, 2.6–11.0) vs. 4.38 (95% CI, 2.5–7.3) for those with atypia alone. The majority of biopsies had one RS, while 6.7% of biopsies contained more than one RS. This study failed to detect any significant difference in terms of size of radial scar and risk. The authors attribute the risk of RS to the presence of the other epithelial proliferations present, either usual type epithelial proliferative disease or AH, and do not confine an independent risk to RS alone.

The conflicting literature leaves uncertainty over the potential risk associated with a diagnosis of radial scar, yet the increased detection of radial scar lesions by mammographic screening makes accurate risk assessment important for clinical decision making. In this study we sought to clarify the significance of radial scar lesions using the Mayo Benign Breast Disease Cohort.

Methods

Study population

Inclusion and exclusion criteria for the study cohort have been previously described [10]. Briefly, the study population included all women 18–85 years of age who had undergone surgical excision of a benign breast lesion at Mayo Clinic Rochester during the 25-year period from January 1, 1967 through December 31, 1991. The study cohort includes 9,376 women [11]. For 114 breast biopsy samples, the presence or absence of radial scars could not be assessed due to poor slide quality. Thus, the final cohort for this study was 9,262 women. Breast-cancer events were obtained from medical records and questionnaires. Earlier reports of the Mayo Benign Breast Disease Cohort provide detailed descriptions of the characteristics of patients and pathological specimens [10, 11].

Histologic examination

For women who had more than one biopsy during this period, we used the earliest biopsy performed. Stained hematoxylin and eosin stained sections from each participant were evaluated by a breast pathologist (DWW) who was unaware of the initial histologic diagnoses and patient outcomes. Biopsy findings were classified according to the criteria of Page et al. into the following categories: non-proliferative fibrocystic changes, proliferative fibrocystic changes without atypia, and proliferative fibrocystic changes with atypia (atypical ductal hyperplasia, atypical lobular hyperplasia, or both) [12]. Biopsy specimens were designated as having proliferative fibrocystic changes if they contained any of the following: ductal hyperplasia (greater than mild), papilloma, radial scar, or sclerosing adenosis. Cysts, fibroadenomas, or collagenic cell changes were considered non-proliferative unless they also contained one of the lesions denoted above.
Radial scar lesions were systematically identified upon initial histopathologic review of the samples. Radial scars were defined as containing a central fibroelastotic core surrounded by radiating 'arms' of compressed ductal structures with two-cell layers and variable epithelial hyperplasia, and classified as proliferative lesions (Figs. 1–3). Other coexisting non-proliferative (fibroadenoma, cysts) or proliferative lesions (single or multiple papillomas, sclerosing adenosis) were also counted if they appeared anywhere within the same biopsy. If AH was identified within the papilloma or in the surrounding breast parenchyma, then the case was classified as RS with AH (Fig. 4). For RS with AH, the location of the atypia (inside or outside the RS lesion) was recorded. Additional features of size and number of radial scars were also assessed. In cases of multiple RS lesions, the largest size was used for classification purposes.

Statistical analysis

Data were descriptively summarized using frequencies and percents for categorical variables and means and standard deviations for continuous variables. We compared presence of radial scars across levels of categorical variables (including age, indication for biopsy, mammogram era, extent of involution, etc.) using chi-square tests of significance. The duration of follow-up was calculated as the number of days from biopsy of the benign lesion to the date of the diagnosis of breast cancer, death, or the last contact. We estimated relative risks using standardized incidence ratios (SIRs) and corresponding 95% CI, dividing the observed numbers of incident breast cancers by population-based expected counts. We calculated these expected counts by apportioning each woman’s follow-up into 5-year age and calendar period categories, thereby accounting for differences associated with these variables. We used the Iowa Surveillance, Epidemiology, and End Results (SEER) registry as the reference population because of its demographic similarities to the Mayo Clinic population (80% of cohort members reside in the upper Midwest). Over 95% of our cohort was white, equivalent to that reported in Iowa census data during the study period [13]. SIRs were calculated both overall and by subgroups defined by histology and radial scar character-
Table 1. Breast cancer risk by histology and radial scars

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of eligible women (%)</th>
<th>Number with subsequent breast cancer diagnosis</th>
<th>Expected number of breast cancers</th>
<th>Relative risk (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign breast disease (overall)</td>
<td>9,262</td>
<td>721</td>
<td>521.5</td>
<td>1.38 (1.28-1.49)</td>
</tr>
<tr>
<td>Non-proliferative</td>
<td>6,244 (67.4%)</td>
<td>406</td>
<td>344.8</td>
<td>1.16 (1.05-1.26)</td>
</tr>
<tr>
<td>Proliferative disease without atypia (PDWA), total</td>
<td>2,693 (29.1%)</td>
<td>258</td>
<td>159.9</td>
<td>1.61 (1.42-1.82)</td>
</tr>
<tr>
<td>PDWA without RS</td>
<td>2,314 (86%)</td>
<td>215</td>
<td>137.0</td>
<td>1.57 (1.37-1.79)</td>
</tr>
<tr>
<td>PDWA with RS</td>
<td>379 (14.1%)</td>
<td>43</td>
<td>22.9</td>
<td>1.88 (1.36-2.53)</td>
</tr>
<tr>
<td>Atypical hyperplasia (AH) total</td>
<td>325 (35.5%)</td>
<td>63</td>
<td>16.8</td>
<td>3.75 (2.88-4.80)</td>
</tr>
<tr>
<td>AH without RS</td>
<td>265 (81.5%)</td>
<td>54</td>
<td>13.6</td>
<td>3.97 (2.99-5.10)</td>
</tr>
<tr>
<td>AH with RS</td>
<td>60 (18.5%)</td>
<td>9</td>
<td>3.2</td>
<td>2.81 (1.29-5.35)</td>
</tr>
</tbody>
</table>

* Relative risks were calculated using standardized incidence ratios, comparing the observed number of breast cancer events to those expected based on Iowa SEER rates. Expected events account for the effects of age and calendar period.

Results

Pathologic features

Of the 9,262 women in the Mayo Benign Breast Disease Cohort, the benign lesions were classified as non-proliferative disease (NP) in 6,244 (67.4%), proliferative disease without atypia (PDWA) in 2,693 (29.1%), and atypical hyperplasia (AH) in 325 (3.5%) (Table 1). Radial scars were identified in 439 biopsies (4.7%). In these 439 biopsies, 382 (87.0%) contained one radial scar lesion, while 57 (13.0%) contained two or more radial scar lesions.

Of the 2,693 women with PDWA, 379 (14.1%) had at least one RS lesion; 46 (1.7%) had two or more radial scar lesions present. Of the 325 women with AH, 60 (18.5%) had at least one RS lesion; 11 of these (3.4%) had two or more radial scar lesions present.

Size of largest radial scar was available for 432 of the 439 samples. The remaining seven could not be accurately measured due to slide quality. The majority of RS, 356 (82.4%), were less than 5.0 mm in diameter, 60 (13.9%) were 5.0-9.9 mm, and 16 (3.7%) measured greater than 10.0 mm (Table 2). RS was found concurrently with a variety of other proliferative lesions. These included sclerosing adenosis in a majority of cases (362/439, 82.5%), followed in frequency by a single papilloma (77/439, 17.5%), and multiple papillomas 3.9% (17/439). In 63 cases of RS, the

Table 2. Breast cancer risk among women with radial scars, by age and RS features

<table>
<thead>
<tr>
<th>Feature</th>
<th>Number</th>
<th>Number with subsequent breast cancer diagnosis</th>
<th>Expected number of breast cancers</th>
<th>Relative risk (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall radial scars</td>
<td>439</td>
<td>52</td>
<td>26.1</td>
<td>1.99 (1.89-2.61)</td>
</tr>
<tr>
<td>Number of radial scars</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>382</td>
<td>45</td>
<td>22.8</td>
</tr>
<tr>
<td></td>
<td>2 or more</td>
<td>57</td>
<td>7</td>
<td>3.3</td>
</tr>
<tr>
<td>Size of largest scar*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5.0 mm</td>
<td>356</td>
<td>40</td>
<td>21.7</td>
<td>1.84 (1.32-2.51)</td>
</tr>
<tr>
<td>≥5.0 mm</td>
<td>76</td>
<td>9</td>
<td>4.0</td>
<td>2.25 (1.03-4.30)</td>
</tr>
<tr>
<td>Age at biopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>156</td>
<td>17</td>
<td>8.3</td>
<td>2.05 (1.19-3.28)</td>
</tr>
<tr>
<td>≥50</td>
<td>283</td>
<td>33</td>
<td>17.8</td>
<td>1.97 (1.27-2.75)</td>
</tr>
</tbody>
</table>

* Relative risks were calculated using standardized incidence ratios, comparing the observed number of breast cancer events to those expected based on Iowa SEER rates. Expected events account for the effects of age and calendar period.

* Size of largest scar could not be determined for seven of the 439 women with radial scars.
Table 3  Association of radial scars with clinical and histologic variables

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-proliferative disease</th>
<th>Proliferative disease without RS</th>
<th>Proliferative disease with RS</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>6,244 (67.4%)</td>
<td>2,579 (27.8%)</td>
<td>439 (4.8%)</td>
<td></td>
</tr>
<tr>
<td>Age at biopsy:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45</td>
<td>2,302 (76.5%)</td>
<td>827 (20.9%)</td>
<td>78 (2.6%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>45–55</td>
<td>1,657 (62.7%)</td>
<td>832 (31.9%)</td>
<td>152 (5.8%)</td>
<td></td>
</tr>
<tr>
<td>&gt;55</td>
<td>2,287 (63.3%)</td>
<td>1,120 (31.0%)</td>
<td>209 (5.7%)</td>
<td></td>
</tr>
<tr>
<td>Indication for biopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass</td>
<td>3,030 (71.1%)</td>
<td>1,083 (25.4%)</td>
<td>151 (3.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mammographic</td>
<td>1,410 (51.5%)</td>
<td>826 (34.9%)</td>
<td>174 (7.2%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>1,804 (69.7%)</td>
<td>870 (25.9%)</td>
<td>114 (4.4%)</td>
<td></td>
</tr>
<tr>
<td>Time period:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-mammography (1967–1981)</td>
<td>3,219 (72.7%)</td>
<td>1,084 (24.0%)</td>
<td>146 (3.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Post-mammography (1982–1991)</td>
<td>3,025 (62.6%)</td>
<td>1,513 (31.4%)</td>
<td>209 (6.0%)</td>
<td></td>
</tr>
<tr>
<td>Involution:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1,259 (75.6%)</td>
<td>325 (20.9%)</td>
<td>56 (3.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Partial (11–74% TDLU)</td>
<td>2,969 (57.9%)</td>
<td>1,804 (35.2%)</td>
<td>353 (6.9%)</td>
<td></td>
</tr>
<tr>
<td>Complete (75+% TDLU)</td>
<td>1,558 (61.7%)</td>
<td>324 (17.0%)</td>
<td>26 (1.3%)</td>
<td></td>
</tr>
</tbody>
</table>

Values presented as number (percent)

*chi-square test of significance

Additional pathology was solely non-proliferative lesions (fibroadenoma, cysts, or both).

Factors associated with radial scar

We examined the effect of age at biopsy on the likelihood of a RS diagnosis, dividing the cohort into those less than 45, between 45 and 55, and those over 55. As shown in Table 3, women younger than 45 were less likely to have radial scars (2.6%) than those age 45–55 (5.8%), or greater than 55 (5.7%).

Data regarding the indication for biopsy, either a mass or mammographic abnormality, were available for 71.8% of the total cohort. Of the 439 cases containing RS, 151 women (34%) had a palpable lesion, and 174 (40%) had an abnormal mammogram. For 114 (26%), the reason for the biopsy is unknown. For the 2,579 women with proliferative disease but no RS, 1,083 (42%) had a palpable lesion, and 826 (33%) had an abnormal mammogram. The remaining 676 (26%) had no known indication (proportions of palpable versus mammographic indications differ in the RS versus no RS groups—see Table 3).

Mammographic screening is thought to have increased the detection of RS. Incidence rates of RS for the pre- and post-mammographic era were examined using the year 1981 as the division point between the two eras. Women with biopsies during the post-mammography era were more likely to have radial scars than those biopsied during the pre-mammography era (6.0% vs. 3.3%) (Table 3).

Atrophy, or the degree of lobular involution, was previously found to be a risk factor in our benign breast disease (BBD) cohort [11]. This was classified in the background breast parenchyma as none (0%), partial (1–74%), or complete (≥75%) involution. Women with complete involution were less likely to have radial scars (1.3%) than those with no involution (3.5%) or partial involution (6.9%) (Table 3).

To address whether or not atypia is more frequently found in association with RS than with other forms of proliferative disease, we compared the proportion of AH with RS to that of AH with all other proliferative disease. Atypia was found at slightly higher rates in biopsies with radial scar (60/439, 13.7%), than in biopsies with other forms of proliferative breast disease without RS, such as ductal hyperplasia, papilloma or sclerosing adenosis (245/2579, 9.9%). Regarding the type of atypia arising in the setting of RS, 25 (41.7%) contained only atypical duct hyperplasia (ADH), 31 (51.7%) with only atypical lobular hyperplasia (ALH), and 4 (6.6%) with both. Of the 60 cases with any type of atypia, the atypia was present inside the RS in 17 (28.3%), outside the RS lesion in 27 (45.0%), and present both inside and outside the RS in 16 (26.7%).

Breast cancer risk

The overall cohort of women with benign breast disease was at an increased risk of breast cancer development (RR = 1.38, 95% CI 1.28–1.49) compared to the general
population. Out of the 9,262 women in this cohort evaluating radial scar, 721 developed subsequent breast cancer (Table 1). The breast cancer risk in women with radial scars associated with PDWA was 1.88 (95% CI, 1.36–2.53), compared to a relative risk of 1.57 (95% CI, 1.57–1.79) for women with PDWA without radial scar (Table 1). This difference is not statistically significant (test for heterogeneity, \( P = 0.29 \)). Similarly, for women with AH, the presence of radial scar lesions did not significantly modify breast cancer risk; \( RR = 2.81 \) for AH with RS (95% CI, 1.29–5.35) vs. 3.97 for AH without RS (95% CI, 2.99–5.19, test for heterogeneity, \( P = 0.33 \)). RS size and number information were also examined. Women with one radial scar had a relative risk of 1.97 (95% CI, 1.44–2.64) compared to 2.12 (95% CI, 0.85–4.35) for those with two or more radial scars (test for heterogeneity, \( P = 0.86 \)) (Table 2). The size of radial scar lesions also did not modify the risk for subsequent breast cancer, with RR 1.84 for lesions less than 5.0 mm (95% CI, 1.32–1.51) vs. 2.26 for lesions larger than 5.0 mm (95% CI, 1.02–4.30), \( P = 0.38 \). Age at biopsy with RS did not modify the risk for development of subsequent breast cancer, with RR 1.97 for women older than 50 years (95% CI, 1.37–2.73) vs. 2.05 for women younger than 50 years (95% CI, 1.19–3.28, \( P = 0.89 \)).

**Discussion**

Radial scars are a distinctive form of benign, proliferative breast disease for which there are conflicting data regarding the subsequent risk of breast cancer. Our main finding is that RS does not confer increased risk over that of other proliferative lesions, with or without atypia. Moreover, increasing size and number of RS were not found to influence the risk. Although we demonstrated a slightly increased association between RS and AH, the atypia arose more frequently within the background breast parenchyma, and not within the RS itself. Overall, these data argue against a direct premalignant role for RS over and above that of other forms of proliferative disease.

Our findings support the study of Sanders et al. reporting on 880 RS in women from the Nashville Breast Cohort from 1950 to 1986 (Table 4). Their average follow up was 20.4 years. Because the Nashville group did not define RS as a proliferative lesion, 73 biopsies containing non-proliferative changes also contained a RS. In the Nashville study, the risk associated with PDWA containing RS was 2.13 (95% CI, 1.3–3.5) (see Table 4). This study attributed the increased risk associated with RS to the coexistent PDWA, present in the majority (79.2%) of RS. This group detected a modest increase in risk for those women over 49 years of age [9]. In our study we saw no effect on subsequent cancer risk with increasing age at biopsy in women with RS. However, we did detect an increase in the incidence of RS with age.

In contrast to the two cohort studies from Nashville and Mayo, the study by Jacobs et al. found RS to be an independent risk factor for breast cancer (Table 4). This case–control study identified 99 RS in women from the Nurses' Health Study from 1976 to 1992 with an average follow up of 12 years. The relative risk attributed to RS was 3.0 (95% CI, 1.7–5.5) compared to 1.5 for PDWA alone (95% CI, 1.1–2.1). A similar increase was also detected for AH containing RS (RR = 5.8; 95% CI, 2.7–12.7) compared AH alone (RR = 3.8; 95% CI, 2.4–5.9). Additionally, an

<table>
<thead>
<tr>
<th>Table 4 Comparison of studies of risk associated with radial scar</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lesion</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Total cohort</td>
</tr>
<tr>
<td>Number of women with RS</td>
</tr>
<tr>
<td>PDWA, total (n)</td>
</tr>
<tr>
<td>PDWA, no RS (n)</td>
</tr>
<tr>
<td>PDWA, no RS (RR)</td>
</tr>
<tr>
<td>PDWA with RS (n)</td>
</tr>
<tr>
<td>PDWA with RS (RR)</td>
</tr>
<tr>
<td>AH total (n)</td>
</tr>
<tr>
<td>AH no RS (n)</td>
</tr>
<tr>
<td>AH no RS (RR)</td>
</tr>
<tr>
<td>AH with RS (n)</td>
</tr>
<tr>
<td>AH with RS (RR)</td>
</tr>
</tbody>
</table>

* *Reference group for Mayo Benign Breast Disease Cohort = Iowa SEER registry
* **Reference group for Nashville study = women in their cohort with non-proliferative disease and no radial scar
** **Reference group for Nurses Health Study = women in their cohort with non-proliferative disease

© Springer
increase in RS size and number was also found to elevate the risk of subsequent cancer [8].

These differences may be explained, at least in part, by the different study designs: Jacobs et al. used a nested case-control design with four controls selected for each breast cancer diagnosis. The current Mayo study and the Nashville study are retrospective cohort studies. There were 99 biopsies with RS in the Nurses' Health Study compared to 439 (Mayo) and 880 (Nashville) RS lesions. Selection criteria for study participation may also differ between these large studies. As our data span the pre- and post-mammographic era, both types of presentations are represented. We demonstrate an increase in RS since mammographic screening began.

Earlier studies had classified RS as a benign sclerosing lesion lacking premalignant potential [14–17]. In a random examination of both benign breast tissue (n = 34) and cancerous tissue (n = 34), Andersen et al. attributed the presence or absence of RS more to the volume of tissue examined than to any association with carcinoma [15]. In randomized autopsy studies, Nielsen et al. found no difference between cancer-containing and noncancerous breasts in terms of presence or absence of RS [17]. A follow-up study by Nielsen et al. found an increased number of RS in the contralateral breast of women with breast carcinoma; however, no increased incidence of contralateral breast carcinoma was detected [16].

There are several reports of carcinoma arising within a RS [18–22]. These reports include in situ carcinomas, infiltrating ductal and lobular carcinomas [19, 21], as well as metaplastic carcinomas, including adenoid cystic carcinoma [20] and spindle cell metaplastic tumors [18]. Our data do not support a unique relationship between RS and carcinoma; however, because we excluded biopsies containing carcinoma, we cannot directly comment on such isolated case report findings as these. Since most consider atypical hyperplasia to represent a direct precursor of carcinoma, one would expect an increased association between RS and AH if RS was either a precursor or promoter of neoplastic change. While our study did find a slight increased association with AH and RS-containing biopsies, the AH did not preferentially arise within itself. This lack of contiguity with RS and atypia would argue against RS as a direct precursor of subsequent carcinoma.

The etiology of RS lesions remains unclear. Because of their scar-like appearance, they are hypothesized to represent a healing process in breast tissue, with the central relatively acellular core representing a later phase of development [15, 23]. Molecular evidence to characterize the behavior and biologic potential of RS has found similar patterns in both RS and invasive carcinoma for mRNA expression of factors involved in formation of vascularized stroma [24]. Iqbal et al. detected subtle differences in estrogen receptor and Ki67 expression between RS lesions containing epithelial hyperplasia and areas of epithelial hyperplasia outside of the RS lesion, with the RS containing focal clonal areas and slightly diminished estrogen receptor expression [25]. While these findings may suggest that breast tissue containing RS is somehow permissive of proliferation leading to development of carcinoma, clinical studies, including ours, have failed to demonstrate a link with carcinoma [9, 12, 15–17, 26].

In summary, with increased use of mammographic screening, RS has emerged as an important, radiographic mimic of breast cancer. Determining the accurate predictive value of RS diagnosis on breast biopsy has thus become increasingly important. Our data show that RS does not confound increased risk over that of other proliferative lesions, with or without atypia. In our large cohort study, RS emerges as an interesting histopathologic entity, with its greatest clinical significance being the degree to which it generates worrisome mammographic findings.

Acknowledgments Supported by a Department of Defense Center of Excellence Grant (FEDDAM1D1-02-1-0473-1), a grant (RO1 CA46332) from the National Institute of Health, and grants from the Susan G. Komen Breast Cancer Foundation (BCIR-99-3152), Andersen Foundation, and Breast Cancer Research Foundation. We are indebted to Joel Wora and Dr. Pete de Greer for database development; to Wilma Lingle, PhD, and the Biostatistics Core for tissue processing; to Teresa Allen, Mary Campion, Joanne Johnson, Melanie Kamen, Maggie Loprindi, and Betty Anderson for data collection; to Ann Harris and the Survey Research Center for patient follow-up; and to Vicki Shea for help in preparing the manuscript.

References

Association Between Cyclooxygenase-2 Expression in Atypical Hyperplasia and Risk of Breast Cancer

Daniel W. Visscher, V. Shane Pankratz, Marta Santisteban, Carol Reynolds, Ari Ristimäki, Robert A. Vierkant, Wilma L. Lingle, Marlene H. Frost, Lynn C. Hartmann

Background The cyclooxygenase-2 (COX-2) enzyme, which is induced by inflammatory and mitogenic stimuli, plays a protumorigenic role in several human cancers. COX-2 is overexpressed in invasive and in situ breast cancers. Atypical hyperplasia in breast tissue, although benign, is associated with a high risk of breast cancer. We investigated whether COX-2 overexpression in atypical hyperplasia is associated with the risk of subsequent breast cancer.

Methods COX-2 expression was assessed immunohistochemically in archival sections from 235 women with atypia whose biopsy specimens were obtained at the Mayo Clinic from January 1, 1967, through December 31, 1991. COX-2 expression was scored as 0 (negative), 1+ (weak), 2+ (moderate), or 3+ (strong). Risk factor information and follow-up for breast cancer events were obtained via a study questionnaire and the medical records. All statistical tests were two-sided.

Results Forty-one (17%) of the 235 women developed breast cancer during a median follow-up of 15 years. Moderate (category 2+) or strong (category 3+) COX-2 expression was identified in 71 (30%) and 34 (14%) of the 235 samples, respectively. The risk for developing breast cancer, relative to a control population (the Iowa Surveillance, Epidemiology, and End Results registry), increased with increasing COX-2 expression (relative risk [RR] = 2.60, 95% confidence interval [CI] = 1.56 to 4.15, for those with negative or weak COX-2 expression; RR = 3.56, 95% CI = 1.94 to 6.37, for those with moderate expression; and RR = 5.66, 95% CI = 2.69 to 10.75, for those with strong expression; \(P = .07\)). Overexpression of COX-2 was statistically significantly associated with the type of atypia (lobular vs ductal, \(P < .001\)), number of foci of atypia in the biopsy (\(P = .02\)), and older age at time of biopsy (\(>45\) years, \(P = .01\)).

Conclusions COX-2 appears to be a biomarker that further stratifies breast cancer risk among women with atypia and may be a relevant target for chemoprevention strategies.

J Natl Cancer Inst 2008;100:421-427

Women with atypical hyperplasia are at high risk for the development of breast cancer, with a cumulative incidence approaching 25% at 20 years after biopsy examination (1,2). Clinical practice would benefit from the identification of additional predictive features for women with atypia that will enable better risk stratification for these women and from the identification of functional biomarkers that could be modified by chemoprevention strategies.

Cyclooxygenase (COX) enzymes catalyze the synthesis of bioactive prostaglandins from arachidonic acid, which is derived from membrane phospholipids (3). Cyclooxygenase-2 (COX-2), induced in response to various inflammatory and mitogenic stimuli, has been shown to play a protumorigenic role in preclinical models of several tumor systems (4-6). Moreover, it is overexpressed in several human cancers and their precancerous lesions (7). Ristimäki et al. (8) analyzed COX-2 expression by immunohistochemistry in 1576 invasive breast cancer specimens and found moderate to strong expression in 37% of the cancers. In ductal carcinoma in situ, the frequency of COX-2 overexpression appears to be even higher (9,10). Many groups have shown an association between COX-2 expression and an aggressive phenotype in in situ and invasive breast cancer (9-15).

In animal models, COX inhibitors suppress experimental breast cancers (6,7), and several epidemiologic studies in humans have shown that nonsteroidal anti-inflammatory drug (NSAID) use is associated with a reduced incidence of breast cancer (3,16,17).

Affiliations of authors: Department of Surgical Pathology, University of Michigan, Ann Arbor, MI (DNY); Divisions of Biostatistics (VSP, RAV), Medical Oncology (MS, MMF, LCH), Anatomic Pathology (CPO), and Experimental Pathology (WIL), Mayo Clinic, Rochester, MN; Department of Pathology, HUSLAB/University Central Hospital and Genome-Science Biology Research Program, Biomedical Helsinki, University of Helsinki, Helsinki, Finland (AF). Correspondence to: Lynn C. Hartmann, MD, Division of Medical Oncology, Mayo Clinic, 200 1st St Southwest, Rochester, MN 55905 (e-mail: hartmann.lynn@mayo.edu).

See “Funding” and “Notes” following “References.”

DOI: 10.1093/jnci/djn098

© 2006 The Author(s)

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
CONTEXT AND CAVEATS

Prior knowledge
Cyclooxygenase-2 (COX-2) plays a protumorigenic role in several human cancers and is overexpressed in invasive and in situ breast cancers. Atypical hyperplasia in breast tissue, although benign, is associated with a high risk of breast cancer.

Study design
The relationship between the risk of breast cancer and COX-2 expression in archival specimens from women with atypical hyperplasia and a 15-year follow-up was assessed.

Contribution
The risk of developing breast cancer, relative to a control population (the Iowa Surveillance, Epidemiology, and End Results registry), increased with increasing COX-2 expression, in a borderline statistically significant manner, Overexpression of COX-2 was statistically significantly associated with the type of atypia (lobular vs ductal), with number of foci of atypia in biopsy, and with older age at time of biopsy (≥46 years).

Implications
COX-2 may be a biomarker that further stratifies breast cancer risk among women with atypia and may be a relevant target for chemoprevention strategies.

Limitations
Tissue-based biomarker studies, such as this study, are limited by semiquantitative and subjective evaluation of COX-2 status, which is further complicated by the variable nature of immunoreactivity.

Because of the relevance of COX-2 to breast cancer biology, including its presence in preinvasive lesions, we hypothesized that COX-2 expression is increased in atypia and that overexpression is a risk factor for the progression of breast cancer. Accordingly, we studied COX-2 expression in a cohort of women with atypical hyperplasia for whom we have long-term cancer follow-up information.

Participants and Methods

Study Population
Entry criteria for the study cohort have been described previously (1,2). Briefly, this study included all women aged 18–85 years who had been breast biopsy that was surgically excised at the Mayo Clinic from January 1, 1967, through December 31, 1991. The initial cohort included 9087 women (1). With additional follow-up, data for 9376 women were available for this analysis, 331 (3.5%) of whom had atypical hyperplasia (2). Archival paraffin-embedded, formalin-fixed tissue for COX-2 staining was available for 238 of the 331 women.

Risk Factor Information and Follow-up
Follow-up for breast cancer events and risk factor information were obtained for all 238 women with atypia through Mayo medical records and a study questionnaire (1,2). Family history was collected via respondent questionnaires and medical record abstraction and classified as negative, strong, or weak. Criteria for a strong family history were at least one first-degree relative with breast cancer diagnosed before the age of 50 years or two or more relatives with breast cancer, with at least one being a first-degree relative. Any lesser degree of family history was considered to be weak.

All protocol procedures and patient contact materials were reviewed and approved by the Institutional Review Board of the Mayo Clinic. Return and completion of the patient contact materials were considered to be implied consent.

Histology
All archival hematoxylin- and eosin-stained sections were evaluated by the study breast pathologist (DWV), without knowledge of the original histologic diagnosis or patient outcome. A diagnosis of atypical ductal hyperplasia or atypical lobular hyperplasia was based on the criteria of Page et al. (18) and Page and Rogers (19). For each example of atypical hyperplasia, the number of separate foci was defined (2). Multifocal atypia required the identification of atypia in more than one terminal duct lobular unit, as defined by clear separation from another terminal duct lobular unit by nonspecialized interlobular mammary stroma.

Immunostaining
Five-micrometer sections of formalin-fixed, paraffin-embedded samples were deparaffinized with three changes of xylene, rehydrated in an ethanol series (100%, 95%, and 70% ethanol), and rinsed well in running distilled water. Slides were then placed in preheated epitope retrieval buffer (1 mM EDTA, pH 8.0) for 30 minutes, cooled in the buffer for 5 minutes, and rinsed for 5 minutes in running distilled water. An automated slide stainer (AS100 Automarker Plus, DAKO, Carpinteria, CA) was used for all slides at room temperature as follows. Sections were first incubated in a solution of 3% H2O2 in ethanol for 5 minutes to inactivate endogenous peroxidases and then incubated in primary mouse antihuman COX-2, Clone CX-294, monoclonal antibody (1:100 dilution, M1617, DAKO) for 30 minutes. Sections were then rinsed with TBST 10x wash buffer (3006, ultrasofted buffer with Tween 20, DAKO). Sections were then incubated with a peroxidase-labeled polymer conjugated to goat anti-mouse, anti-rabbit immunoglobulins (EnVision Dual Link System-HRP, K4004, DAKO) for 15 minutes. The slides were rinsed with TBST wash buffer, incubated in Nova Red (Vector Laboratories, Burlingame, CA) for 5 minutes, and counterstained with modified Schmorl's hematoxylin for 5 minutes and rinsed for 3 minutes in tap water to set the hematoxylin counterstain. Specimens were dehydrated through a graded ethanol series (70%, 98%, and 100% ethanol), cleared in three changes of xylene, and mounted with a permanent mounting medium. The positive control was colon cancer tissue; the negative control was normal colonic epithelial tissue. All samples were stained simultaneously over a 2-day period.

Evaluation of COX-2 Immunostaining
COX-2 immunostaining was analyzed by the study breast pathologist (DWV), who had no knowledge of patient outcome. The following criteria were established before the reading of the samples (by AR and DWV) (8): 0 = no staining; 1+ = weak, barely perceptible staining in a pancytoplasmic pattern; 2+ = inordinate intensity staining, usually cytoplasmic, with focal plasma membrane distribution; 3+ = strong immunoreactivity with distinct plasma
were combined

Figure 1. Cyclooxygenase-2 staining patterns in atypia. Representative breast atypia samples with corresponding scores were similar, a) Category 0 (no) staining, b) Category 1+ staining, c) Category 2+ staining, and d) Category 3+ staining. Original magnification was ×260. Scale bar = 2.0 mm.

membrane accentuation (Figure 1). Because of the small number of breast cancer events in samples with 0 staining, categories 0 and 1+ were combined for all analyses.

Statistical Analyses

Data were summarized descriptively by use of frequencies and percentages for categorical variables and medians and interquartile ranges (IQRs) for continuous variables. We compared distributions of demographic and clinical attributes from patients with and without available formalin-fixed tissue (for all 331 women with atypia) and across all levels of staining intensity (for the 235 women with COX-2 immunostaining results) by use of χ² tests.

The length of follow-up for each woman in the study was calculated as the number of days from her benign biopsy to the date of her breast cancer diagnosis, death, or last contact. We estimated relative risks (RRs) on the basis of standardized incidence ratios, by dividing the observed numbers of incident breast cancers by population-based expected values. This approach allowed us to compare rates of breast cancer in our cohort with that of the general population rather than an internal referent group, recognizing that all women in our cohort were at some increased risk of breast cancer from their diagnosis of atypical hyperplasia. Expected values were calculated by apportioning each woman's person-years of follow-up into 5-year age and calendar-period categories and multiplying these by the corresponding breast cancer incidence rates from the Iowa Surveillance, Epidemiology, and End Results registry (1). This reference population was chosen because of its demographic similarities to the Mayo Clinic population (80% of cohort members reside in the upper Midwest). Potential heterogeneity in standardized incidence ratios across levels of COX-2 staining was assessed by use of Poisson regression analysis, with the log-transformed expected event rate for each individual modeled as the offset term. We displayed observed event rates by use of cumulative incidence curves, accounting for the effects of death as a competing risk (20). We assessed whether COX-2 staining intensity was associated in a dose-response manner with cumulative incidence by use of tests for trend, which were calculated via Cox proportional hazards regression analysis. We tested for departures from the proportional hazards assumption by use of tests of interaction with follow-up time and found no evidence of nonproportionality. Among breast cancer patients, we compared time to cancer across levels of immunostaining with analysis of variance (ANOVA) methods and homogeneity of breast cancer relative to the original atypia across levels of immunostaining by use of χ² tests. All statistical tests were two-sided, and all analyses were conducted with the SAS (SAS Institute, Inc., Cary, NC) software system.

Results

COX-2 Immunostaining of Atypia Samples

Among the original cohort of 331 women with atypical hyperplasia (2), the distributions of breast cancer status, age at benign biopsy, family history of cancer, and (for breast cancer patients) time to diagnosis were not statistically significantly different between the 235 patients with formalin-fixed tissue available for COX-2 staining and the 96 patients without available tissue (χ² P > .05 for each attribute). Among specimens from the 235 patients with available tissue, 23 (10%) showed no COX-2 staining, 107 (45%) showed category 1+ staining, 71 (30%) showed category 2+ staining, and 34 (14%) showed category 3+ staining (Figure 1). Intensity of staining was statistically significantly associated with type of atypical hyperplasia (lobular vs ductal, P < .001; Table 1). Of the 100 women with atypical ductal hyperplasia only, 77 (or 77%) had either no or weak (categories 0 or 1+) COX-2 staining, 13 (13%) had moderate (category 2+) staining, and 10 (10%) had strong (category 3+) staining.
Table 1. Association of cyclooxygenase-2 staining intensity with demographic and clinical variables, among women diagnosed with atypical hyperplasia

<table>
<thead>
<tr>
<th>Variable</th>
<th>0-1+ (n = 136)</th>
<th>2+ (n = 71)</th>
<th>3+ (n = 34)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at benign biopsy, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40 y</td>
<td>24 (18.0)</td>
<td>6 (20.0)</td>
<td>0 (0.0)</td>
<td>.01</td>
</tr>
<tr>
<td>45-55 y</td>
<td>44 (32.8)</td>
<td>21 (28.6)</td>
<td>10 (13.3)</td>
<td></td>
</tr>
<tr>
<td>&gt;55 y</td>
<td>62 (45.7)</td>
<td>44 (63.0)</td>
<td>24 (19.5)</td>
<td></td>
</tr>
<tr>
<td>Family history of breast cancer†, No. (%)</td>
<td>74 (54.4)</td>
<td>14 (66.7)</td>
<td>12 (35.3)</td>
<td>.02</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weak</td>
<td>22 (63.7)</td>
<td>14 (64.1)</td>
<td>5 (21.7)</td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>26 (67.6)</td>
<td>12 (26.2)</td>
<td>7 (16.6)</td>
<td></td>
</tr>
<tr>
<td>No. of atypical foci, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>80 (61.4)</td>
<td>31 (62.2)</td>
<td>16 (12.2)</td>
<td>.07</td>
</tr>
<tr>
<td>2</td>
<td>23 (47.7)</td>
<td>25 (66.0)</td>
<td>9 (31.0)</td>
<td></td>
</tr>
<tr>
<td>≥3</td>
<td>15 (38.5)</td>
<td>15 (48.5)</td>
<td>9 (23.1)</td>
<td></td>
</tr>
<tr>
<td>Calcifications, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td>.97</td>
</tr>
<tr>
<td>Yes</td>
<td>40 (64.1)</td>
<td>23 (36.1)</td>
<td>11 (14.9)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>50 (35.9)</td>
<td>48 (63.9)</td>
<td>23 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Involvement status, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td>.27</td>
</tr>
<tr>
<td>Partial</td>
<td>104 (68.1)</td>
<td>51 (28.3)</td>
<td>24 (13.4)</td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>14 (38.9)</td>
<td>14 (48.0)</td>
<td>8 (22.2)</td>
<td></td>
</tr>
<tr>
<td>Type of atypical hyperplasia, No. (%)</td>
<td>47 (35.5)</td>
<td>53 (64.4)</td>
<td>22 (18.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ALH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADH</td>
<td>22 (77.0)</td>
<td>13 (13.0)</td>
<td>10 (10.0)</td>
<td></td>
</tr>
<tr>
<td>ALH and ADH</td>
<td>6 (46.2)</td>
<td>5 (38.5)</td>
<td>2 (15.4)</td>
<td></td>
</tr>
<tr>
<td>Indication for biopsy, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td>.14</td>
</tr>
<tr>
<td>Lumbar</td>
<td>46 (60.5)</td>
<td>26 (57.9)</td>
<td>11 (11.6)</td>
<td></td>
</tr>
<tr>
<td>Mammogram</td>
<td>73 (67.8)</td>
<td>35 (62.5)</td>
<td>22 (16.3)</td>
<td></td>
</tr>
<tr>
<td>Vital status, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td>.09</td>
</tr>
<tr>
<td>Deceased</td>
<td>36 (52.1)</td>
<td>19 (26.0)</td>
<td>10 (21.9)</td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>92 (55.0)</td>
<td>52 (32.1)</td>
<td>18 (11.1)</td>
<td></td>
</tr>
<tr>
<td>Year of biopsy, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td>.22</td>
</tr>
<tr>
<td>1967-1971</td>
<td>6 (66.7)</td>
<td>2 (22.2)</td>
<td>1 (11.1)</td>
<td></td>
</tr>
<tr>
<td>1972-1976</td>
<td>12 (48.0)</td>
<td>12 (48.0)</td>
<td>1 (4.0)</td>
<td></td>
</tr>
<tr>
<td>1977-1981</td>
<td>15 (61.7)</td>
<td>7 (26.8)</td>
<td>2 (7.6)</td>
<td></td>
</tr>
<tr>
<td>1982-1989</td>
<td>30 (49.0)</td>
<td>21 (31.5)</td>
<td>13 (19.4)</td>
<td></td>
</tr>
<tr>
<td>1987-1991</td>
<td>64 (61.0)</td>
<td>29 (27.0)</td>
<td>12 (11.4)</td>
<td></td>
</tr>
</tbody>
</table>

* COX-2 = cyclooxygenase-2; ALH = atypical lobular hyperplasia; ADH = atypical ductal hyperplasia. Values expressed as number (percent).
† g test of statistical significance.
‡ Family history was available for 229 of the 235 women. Criteria for a strong family history were at least one first-degree relative with breast cancer diagnosed before the age of 50 years or two or more relatives with breast cancer, with at least one being a first-degree relative. Any lesser degree of family history was considered to be weak.
§ Involvement status was available for 226 of the 235 women. || Indication information was available for 236 of the 236 women.

In contrast, of the 122 women with only atypical lobular hyperplasia, 47 (39%) had no or weak staining, whereas 51 (43%) had moderate staining and 24 (18%) had strong staining (P < .001). Strong immunostaining was also more likely with increasing patient age; no tumor from the 30 women who were younger than 45 years at time of initial biopsy had strong COX-2 staining and only six (20%) had moderate staining. In contrast, among tumors from the 130 patients who were older than 55 years at biopsy, 24 (19%) had strong staining and an additional 44 (34%) had moderate COX-2 staining (P = .01). Finally, the strength of COX-2 immunostaining was associated with increased numbers of atypia foci. Among the 39 patients with three or more foci, nine (23%) showed strong COX-2 staining and 15 (39%) showed moderate staining. In contrast, of the 131 women who had only a single focus of atypia, 16 (12%) had strong COX-2 staining and 31 (34%) had moderate staining (P = .02). The degree of COX-2 immunoreactivity was not associated with family history of breast cancer, calendar year of biopsy, or clinical indication for biopsy (palpable lump vs mammographic abnormality).

COX-2 staining was not limited to atypical foci. Of the 235 subjects in our cohort, 216 (92%) had staining detected in benign lobules, but it was heterogeneous within tissue sections and usually weak or moderate in intensity (category 0, 1+, or 2+ in 202 [94%] of the 216 patients). A total of 179 patients also had staining in (nonatypical) proliferative lesions, such as usual-type duct hyperplasia and adenosis that were present in the tissue sections along with atypia. Most of these lesions were also weakly immunoreactive (category 0 or 1+ in 131 [73%], category 2+ in 40 [24%], and category 3+ in five [3%] of the lesions).
Association of COX-2 Expression With Breast Cancer Risk

Forty-one (17%) of the 235 women with atypia in this study developed breast cancer during a median follow-up of 15 years. Figure 2 illustrates the cumulative incidence of breast cancer as a function of follow-up interval, stratified by category of COX-2 staining intensity. A positive association of borderline statistical significance was observed between COX-2 staining and the subsequent development of breast cancer (test for trend $P = .07$, Cox proportional hazards regression). Risks of developing breast cancer after 15 years of follow-up among women with atypia were as follows: for atypia with no or only weak COX-2 staining, 13% (95% confidence interval [CI] = 6% to 20%); with moderate staining, 19% (95% CI = 7% to 30%); and with strong staining, 25% (95% CI = 6% to 43%). After a follow-up of 20 years, risks of developing breast cancer for atypia with category 0 or 1+, category 2+, or category 3+ staining were 14% (95% CI = 7% to 22%), 24% (95% CI = 10% to 37%), and 31% (95% CI = 8% to 53%), respectively. Poisson regression analyses that compared the observed number of events with the expected number and accounted for age and calendar period also revealed a marginally statistically significant dose-response relationship overall between staining intensity and risk of breast cancer, compared with that of the control population (for category 0 or 1+ COX-2 staining, RR = 2.63, 95% CI = 1.56 to 4.15; for category 2+ staining, RR = 3.56, 95% CI = 1.94 to 6.47; and for category 3+ staining, RR = 5.66, 95% CI = 2.59 to 10.75) (Table 2, test for heterogeneity of relative risks $P = .07$). Results were similar, although slightly attenuated, after further adjustment for number of atypical foci and type of atypia (atypical lobular hyperplasia or atypical ductal hyperplasia) (data not shown).

COX-2 Staining and Cancer Features

Among the 41 women who developed breast cancer during follow-up, 32 had invasive disease; eight had in situ cancer, and one had disease of an unknown type. We compared COX-2 staining intensity (categories 0 and 1+ vs categories 2+ and 3+) in the affected women by cancer type (invasive vs in situ) and found no difference (14 of the 18 women [78%] with category 0 or 1+ staining had invasive disease, as did 18 of the 22 [82%] with category 2+ or 3+ staining, $\chi^2 = P = .75$).

We next examined the time to breast cancer by COX-2 staining intensity. Among the 41 women who developed breast cancer, the median time to breast cancer was 11.6 years (IQR = 7.2-14.5 years). Among the 18 women who developed breast cancer in the category 0 or 1+ COX-2 staining group, the median time to breast cancer was 11.4 years (IQR = 7.9-13.7 years); among the 23 who developed cancer in the category 2+ or 3+ staining group, the median time to breast cancer was 11.8 years (IQR = 5.5-15.0 years) (ANOVA, $P = .87$). Side of breast cancer and side of atypia were known for 34 of the 41 women (14 in the category 0 or 1+ staining group and 20 in the category 2+ or 3+ group). In the category 0 or 1+ group, sideseness was equally distributed (seven ipsilateral and seven contralateral breast cancers). In the category 2+ or 3+ group, there were 13 (65%) ipsilateral and seven (35%) contralateral breast cancers; although seemingly different, these proportions were not statistically significantly different from each other (C$^2 = P = .38$).

Discussion

We studied COX-2 expression in a well-characterized cohort of women with atypical hyperplasia who were followed for breast cancer events for a median of 15 years. Among the atypias from women in this cohort, 44% had moderate or strong COX-2 expression,
and there was a borderline statistically significant increase in risk of late breast cancer associated with increasing levels of COX-2 expression \( (P = .07) \). Atypias with more than one involved focus, which have the highest likelihood of progression to a later breast cancer \( (2) \), were more likely than those with just one involved focus to express COX-2.

There is strong biologic rationale underpinning COX-2 as a relevant biomarker in breast carcinogenesis. COX-2, which is induced by mitogenic and inflammatory stimuli, has many promitogenic downstream effects, including enhanced proliferation, enhanced angiogenesis, resistance to apoptotic cell death, immunosuppression, promotion of invasion, and metastasis \( (9-11,12) \). COX-2 is overexpressed in both invasive breast cancer and ductal carcinoma in situ, and overexpression is associated with aggressive histologic and clinical features \( (8-15) \). COX-2 is also overexpressed in preinvasive lesions in multiple tumor systems, including Barrett esophagus, colorectal adenomas, and cervical intraepithelial neoplasia \( (1,6,7) \).

We identified a relationship between older age at diagnosis of atypia and COX-2 overexpression. Specifically, only 20% of women who were younger than 45 years at diagnosis had atypia with moderate or strong COX-2 expression compared with 41% of women aged 45–55 years and 52.3% of women older than 55 years. Interestingly, these age-dependent differences in COX-2 induction could result in age-related changes in aromatase expression. The enzyme aromatase, encoded by the CYP19 gene, synthesizes estradiol from androgentic precursors \( (7) \). Aromatase is present in breast tissue, and its levels are higher in or near breast cancers \( (21,22) \). It has been shown \( (23,24) \) that prostaglandin \( \mathrm{E}_2 \), which is produced by enzymes downstream of COX-2, stimulates transcription of the CYP19 gene. Thus, increased COX-2 expression and the resultant increased mammary aromatase activity would be expected to increase local estrogen concentrations, in turn further contributing to a promitogenic local environment \( (7) \). This mechanism may be of greater importance in postmenopausal breast cancer, where the synthesis of estrogens is dependent on aromatase in peripheral tissues, especially mammary adipose tissue \( (25) \).

We found that elevated COX-2 expression was more common in atypical lobular hyperplasia than atypical ductal hyperplasia. Perrone et al. \( (26) \) also recently showed that lobular neoplasia expressed COX-2 at high levels. Lobular neoplasia is characterized by loss of adhesion molecules such as E-cadherin, and recent work \( (27-28) \) has shown that COX-2 and prostaglandin \( \mathrm{E}_2 \) induce Snail, a transcriptional regulator that silences the E-cadherin gene CDH1. Our data in atypia suggest that COX-2 may be responsible, at least in part, for the promitogenic loss of adhesion molecules that occurs in lobular neoplasia of the breast.

Many studies \( [\text{for review, see Harris et al.} \, (29)] \) have explored whether administration of NSAIDs, which inhibit COX-2, is associated with the subsequent risk of breast cancer. Recent results from a case-control study \( (17) \) show that users of selective COX-2 inhibitors and nonselective inhibitors, including regular aspirin, ibuprofen, or naproxen, had a reduced risk of subsequently developing breast cancer, whereas users of acetaminophen did not. A previous meta-analysis \( (16) \) found a link between regular use of NSAIDs and reduction in breast cancer risk. These data supplement the extensive literature that supports the use of selective COX-2 inhibitors as chemoprevention agents for other cancers, most notably gastrointestinal malignancies.

This study has several limitations. Tissue-based biomarker studies, such as this study, are necessarily limited by semiquantitative and subjective evaluation of COX-2 status, which is further complicated by the variable and generally weak character of immunoreactivity. More quantitative approaches, such as quantitative reverse transcription–polymerase chain reaction or western blotting, typically require fresh frozen tissue and cannot accurately localize the COX-2 signal in the tissue because of the small size of the atypical foci. The advantages of immunohistochemistry are that we can localize the COX-2 signal to the atypia foci and that we can use archival paraffin-embedded material from patients with adequate follow-up to determine later breast cancer events. One member of our investigative team (AR) has compared various antibodies and approaches in cancer samples in which COX-2 mRNA levels had been measured \( (30,31) \). In general, polyclonal antibodies tended to have relatively weaker staining with higher levels of nonspecific staining than monochonal antibodies. A. Ristimaki, MD, PhD, unpublished data, 2005; thus, we used a monoclonal antibody in this study. An additional challenge is that atypia lesions are small (generally \( <0.2 \) cm in diameter) and focal, thereby rendering our assay vulnerable to sampling artifacts. Hence, the clinical utility of immunohistochemical assessment of COX-2 will require additional research to establish assay consistency and reproducibility. Nevertheless, we were able to study a sizable cohort of women with atypical hyperplasia who had long follow-up time for the subsequent development of breast cancer. The association between COX-2 status and outcome, as well as the observed dose–effect relationship between development of breast cancer and the level of COX-2 staining in atypia, indicates that COX-2 may have utility as a predictive biomarker. Findings in this study support the association between NSAID treatment and risk reduction in breast cancer and also the potential to derive individualized chemoprevention approaches based on patient-specific biomarker assays. For example, in a recent study, Chan et al. \( (32) \) showed that efficacy of aspirin as a chemoprevention strategy for colorectal carcinoma was limited to patients with tumors that express COX-2.

In summary, we found moderate to strong COX-2 expression in 44% of atypical hyperplasia samples from a well-characterized patient cohort. Samples with three or more foci of atypia, which are associated with increased risk of subsequent breast cancer \( (2) \), had stronger COX-2 staining. However, the relationship between COX-2 staining intensity and the risk of subsequent breast cancer was of only borderline statistical significance \( (P = .07) \).

**References**


Funding
Department of Defense (FEDDAMD) 17-02-1-04473; March of Dimes and Bruce Awaram; Regents Foundation for Breast Cancer Research; National Cancer Institute (R01 CA46332).

Notes
We thank Joel Winters for database development; Teresa Allen, Jo Johnson, Mary Campion, Melanie Kasner, and Rosalyn Thompson for data collection, Shawn Maloney and Stephanie Anderson for data analysis, Ann Harris and the Survey Research Center for patient follow-up, Linda Murphy for immunostaining, and Vicki Shea for assistance with manuscript preparation. The authors also full responsibility for all phases of the study, including the design, the collection of the data, the analysis and interpretation of the data, the decision to submit the manuscript for publication, and the preparation of the manuscript.

Manuscript received August 28, 2007; revised January 8, 2008; accepted January 25, 2008.
Lobular involution: localized phenomenon or field effect?

Division of Biostatistics, Mayo Clinic Cancer Center, Rochester, MN, USA.

As women age, the lobules in their breasts undergo involution. We have shown that, in women with benign breast disease, progressive involution assessed near the benign lesion is associated with lower breast cancer risk. However, it is unknown whether the extent of involution is variable or uniform across the entire breast. We compared involution across the four quadrants of both breasts for fifteen women undergoing bilateral prophylactic mastectomy. One pathologist classified involution extent as none (0% involuted lobules), mild (1-24%), moderate (25-74%), or complete (>=75%). We assessed intra-woman concordance using intraclass correlation coefficients (ICCs), kappa coefficients, and pairwise comparisons of agreement. We found strong intra-woman concordance of involution across the eight quadrants of breast tissue (ICC = 0.75, 95% CI 0.59, 0.89). Our study suggests that lobular involution is a homogeneous process, supporting the use of involution measures from a single benign biopsy as a component in breast cancer risk assessment paradigms.

Lobular Involution: Localized Phenomenon or Field Effect?

Robert A. Vierck Jr., M.A.S. 1, Lynn C. Hartmann, M.D. 2, V. Shane Pankratz, Ph.D. 3, Stephanie S. Anderson, B.S. 1, Derek Rallisky, Ph.D. 5, Marlene H. Frost, Ph.D. 5, Celine M. Vachon, Ph.D. 5, Karthik Ghosh, M.D. 5, Tammy J. Distad, B.A. 6, Amy C. Degnim, M.D. 7, Carol A. Reynolds, M.D. 8

1Division of Biostatistics, 2Division of Medical Oncology, 4Division of Epidemiology, 5Division of General Internal Medicine, 6Division of Anatomic Pathology, 7Division of General Surgery, Mayo Clinic Cancer Center, Rochester, MN, 8Department of Biochemistry/Molecular Biology, Mayo Clinic Cancer Center, Jacksonville, FL.

Address correspondence to: Lynne C. Hartmann, M.D., Mayo Clinic, 200 First Street SW, Rochester, MN 55905; Phone (507) 284-3731, Fax (507) 284-1803; Email: hartmann.lynne@mayo.edu

Key Words: breast cancer, lobular involution

ABSTRACT

As women age, the lobules in their breasts undergo involution. We have shown that, in women with benign breast disease, progressive involution assessed near the benign lesion is associated with lower breast cancer risk. However, it is unknown whether the extent of involution is variable or uniform across the entire breast. We compared involution across the four quadrants of both breasts for fifteen women undergoing bilateral prophylactic mastectomy. One pathologist classified involution extent as none (0% involuted lobules), mild (1-24%), moderate (25-74%), or complete (≥75%). We assessed intra-woman concordance using intraclass correlation coefficients (ICCs), kappa coefficients, and pairwise comparisons of agreement. We found strong intra-woman concordance of involution across all eight quadrants of breast tissue (ICC=0.75, 95% CI 0.59,0.89). Our study suggests that lobular involution is a homogeneous process, supporting the use of involution measures from a single benign biopsy as a component in breast cancer risk assessment paradigms.
INTRODUCTION

The epithelium of the human breast is organized into approximately 15-20 major lobes, each comprised of terminal duct lobular units (TDLUs, or lobules) which contain the milk-producing acini. These lobules are the anatomic substructure that gives rise to breast cancer. As a woman ages, her lobules involute, with a resulting reduction in the number and size of acini per lobule (Figure 1). In a recent study, we showed that progressive degrees of lobular involution were associated with lower breast cancer risk in women with pathologically confirmed benign lesions of the breast. That study used a single assessment of involution for each woman based on the normal background lobules at the site of the biopsy. It has been suggested that extent of involution can help predict risk of breast cancer. To do so, the extent of involution in a small tissue sample would need to be representative of the entire field of a woman’s breast tissue. To our knowledge no study has examined the uniformity of involution. Thus, we sought to determine if extent of lobular involution was similar across multiple areas of a woman’s breasts by studying tissue from women undergoing bilateral prophylactic mastectomy.

METHODS

After Institutional Review Board approval, mastectomy specimens from 15 women with no personal history of cancer who had undergone bilateral prophylactic mastectomy at Mayo Clinic between 1998 and 2006 were retrieved from the Tissue Registry. A single section of fibrous breast tissue was sampled from each quadrant of both breasts, for a total of eight samples per individual. For each section, a formalin-fixed, paraffin embedded hematoxylin and eosin slide was prepared for tissue examination. The slides were labeled in a blinded manner, randomly intermixed, and provided to our breast pathologist (CR). Each specimen was categorized by the extent of lobular involution as none (0% involved lobules), mild (1-24%), moderate (25-74%), or complete (≥ 75% involved lobules).

We calculated within-woman concordance of involution extent using intraclass correlation coefficients (ICCs). We first binned all eight values from a woman into a single class, modeling each woman as an experimental unit. Secondary analyses considered the four readings within a breast to be potentially correlated, but assumed readings across breasts were independent. For these analyses, each woman contributed two classes of four measures, effectively modeling each breast as the experimental unit. Initial ICCs pooled the intermediate involution categories of mild and moderate into one “partial involution” category, identical to the measurement in our previous study. Correlations were then re-examined using the four-level categorization of none, mild, moderate and complete.

We also calculated kappa coefficients as a measure of agreement. We used the generalized multiple-rater kappa coefficient to account for the fact that we have more than two measures within each unit, resulting in a conservative, unweighted kappa that does not allow for “partial credit” due to close, but not exact, matches.
To aid in interpretation, we examined and summarized all pairwise comparisons of intra-woman involution values using the three level involution variable defined above (none; partial, pooling mild and moderate; complete). Each woman’s eight measures resulted in 28 pairwise comparisons, or 420 total in our group of 15. We classified each paired comparison as a perfect match (the paired observations agree), partial match (the observations differ by one category), or non-match (the observations differ by two categories). Secondary analyses summarized pairwise measurements only for the four values within a breast, resulting in six breast-specific pairwise comparisons, and thus twelve for each woman and 180 overall.

RESULTS
The mean age at mastectomy for our fifteen women was 53.9 years (range 37-72). Of the 120 assessments of involution, 9 were classified as no involution, 27 as mild, 25 as moderate, and 59 as complete.

Within-woman pairwise comparisons of involution, based on the three level involution variable, are presented in Table 1. Of the 420 total comparisons (28 for each of the 15 women), 341 (81%) were classified as perfect matches, 76 (18%) as partial matches, and 3 (1%) as non-matches. The proportion of perfect and partial matches was similar when confining the pair-wise comparisons to readings within the same breast.

We observed strong correlation among involution measures (Table 1). ICCs for both the four level and three level involution variables were identical (ICC=0.75; 95% CI=0.59,0.89). Correlations were similar when modeling each breast as the experimental unit, indicating that patterns of involution were similar within each breast and across both breasts within a woman.

Kappa statistics also demonstrated strong agreement. Using generally accepted categorizations,[12] the kappa for the three level involution variable fell into the “substantial” agreement category (kappa=0.67, 95% CI 0.59,0.75, Table 1), while the four level involution kappa fell into the “moderate” category (kappa=0.56, 95% CI 0.49,0.62).

DISCUSSION
We found high concordance of lobular involution values across multiple areas of breast tissue in women undergoing bilateral prophylactic mastectomy. Measures of agreement incorporating the eight quadrants across both breasts were similar to those obtained when treating each breast independently, indicating uniformity of effect in the entire field of breast tissue.

We previously reported a strong dose-response association of lobular involution with lower breast cancer risk among women with benign breast disease based on the background tissue surrounding the benign lesion.[7] suggesting that assessing involution extent could improve risk prediction capabilities.[7,8] Our current findings provide evidence that the assessment of involution extent from a single area of the breast is representative of the entire field of breast tissue.
Our study is limited by the large group of specimens falling under the intermediate categories of mild and moderate involution. It is possible that concordance would increase with more objective determinations of involution. We are currently exploring quantitative measures of lobule regression that may better define the involution status of women.[13]

In summary, we observed moderate to high uniformity among measures of age-related lobular involution from multiple areas of breast tissue within a woman. This finding suggests that involution is a consistent physiological process across the field of breast tissue, supporting the use of measures from a single benign biopsy for breast cancer risk prediction.

ACKNOWLEDGEMENTS
We are indebted to Teresa Allara, Romayne Thompson, Joanne Johnson, Melanie Kasner, and Mary Campion for data collection; to Shau Maloney for database design and data management to Tia Milanese for initial study set-up; to Ann Hart for tissue requests and tracking; and to Vicki Shea for help in preparing the manuscript.

Supported by a Department of Defense Center of Excellence Grant (FEDDAMD17-02-1-0473-1), a grant (R01 CA46332) from the National Institutes of Health, and grants from Martha and II. Bruce Atwater Jr. and the Regis Foundation for Breast Cancer Research.

Figure 1 was previously published in the following manuscript: Milanese TK, Hartmann LC, Sellers TA, et al. Age-related lobular involution and risk of breast cancer. *J Natl Cancer Inst* 2006;98:1-8.
REFERENCES

12. Landis JR, Koch GG. (1977) The measurement of observer agreement for categorical data Biometrics; 33:159-174
Table 1. Measures of concordance of involution in 15 women undergoing bilateral prophylactic mastectomy. Eight quadrants assessed per woman (four per breast).

<table>
<thead>
<tr>
<th>Measure</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treating each woman as the experimental unit</strong></td>
<td></td>
</tr>
<tr>
<td>Intraclass Correlation (95% CI)</td>
<td>0.75 (0.50, 0.90)</td>
</tr>
<tr>
<td>Kappa Coefficient (95% CI)</td>
<td>0.67 (0.59, 0.75)</td>
</tr>
<tr>
<td>Pairwise Comparisons, N (%)^1</td>
<td></td>
</tr>
<tr>
<td>Perfect Matches</td>
<td>341 (81)</td>
</tr>
<tr>
<td>Partial Matches</td>
<td>76 (18)</td>
</tr>
<tr>
<td>Non-Matches</td>
<td>3 (1)</td>
</tr>
<tr>
<td><strong>Treating each breast as the experimental unit</strong></td>
<td></td>
</tr>
<tr>
<td>Intraclass Correlation (95% CI)</td>
<td>0.74 (0.60, 0.85)</td>
</tr>
<tr>
<td>Kappa Coefficient (95% CI)</td>
<td>0.65 (0.53, 0.78)</td>
</tr>
<tr>
<td>Pairwise Comparisons, N (%)^1</td>
<td></td>
</tr>
<tr>
<td>Perfect Matches</td>
<td>145 (81)</td>
</tr>
<tr>
<td>Partial Matches</td>
<td>34 (19)</td>
</tr>
<tr>
<td>Non-Matches</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

Results are based on the three-level assessment of involution: none (0% involuted lobules), partial (1–74%), and complete (≥75%). Analyses using a four-level assessment, stratifying the partial involution category into mild (1–24% involuted lobules) and moderate (25–74%), yielded similar results.

1. Pair-Wise comparisons of involution measures within a woman. When treating woman as the experimental unit, the eight involution reads result in 28 pair-wise comparisons per woman, or 420 overall in our group of 15 women. When treating each breast as an experimental unit, the four involution reads result in 6 pair-wise comparisons per breast, or 12 per woman and thus 180 overall.
Figure 1. Histologic features of age-related involution. (A) An example of breast tissue with no lobular involution, with multiple intact terminal duct lobular units, each comprised of multiple acini and specialized stroma (Inset). (B) An example of complete lobular involution with residual terminal duct lobular units, largely depleted of acini (Inset).
Assessment of the Accuracy of the Gail Model in Women With Atypical Hyperplasia

V. Shara Bankins, Lynn C. Harnack, Amy C. DeGrin, Robert A. Vierkamp, Karthik Ghosh, Gilles M. Vichon, Marlene H. Frost, Sharon D. Maloney, Carol Reynolds, and Judy C. Bouffard

ABSTRACT

Purpose
An accurate estimate of a woman's breast cancer risk is essential for optimal patient counseling and management. Women with biopsy-confirmed atypical hyperplasia of the breast (atypia) are at high risk for breast cancer. The Gail model is widely used in these women, but has not been validated in them.

Patients and Methods
Women with atypia were identified from the Mayo Breast Disease (MBD) cohort (1967 to 1991). Their risk factors for breast cancer were obtained, and the Gail model was used to predict 5-year- and follow-up-specific risks for each woman. The predicted and observed numbers of breast cancers were compared, and the concordance between individual risk levels and outcomes was computed.

Results
Of the 9,796 women in the MBD cohort, 321 women had atypia (3.3%). At a mean follow-up of 13.7 years, 68 of 321 (21.2%) patients had developed invasive breast cancer, 1.86 times more than the 34.9 predicted by the Gail model (95% CI, 1.29 to 2.15; P < .001). For individual women, the concordance between predicted and observed outcomes was low, with a concordance statistic of 0.89 (95% CI, 0.44 to 0.95).

Conclusion
The Gail model significantly underestimated the risk of breast cancer in women with atypia. Its failure to discriminate women with atypia into those who did and did not develop breast cancer is limited. Health care professionals should be cautious when using the Gail model to counsel individual patients with atypia.

INTRODUCTION

An accurate estimate of a woman's risk of developing breast cancer is an integral component of patient counseling. It enables physicians to tailor clinical management to the patient's needs and guide patients in the selection of appropriate medical and surgical management. Women with biopsy-confirmed atypical hyperplasia of the breast (atypia) are known to be at high risk for the development of breast cancer. Widespread public awareness of breast disease along with routine use of screening mammograms has led to the increased detection of atypia on breast biopsy. Women with atypia are often counseled to pursue heightened screening and risk reduction strategies such as chemoprevention with tamoxifen or raloxifene. To assist a woman with atypia in making an informed decision, an accurate assessment of her risk is needed.

The original Gail model was developed using data from women who were actively participating in the Breast Cancer Detection and Demonstration Project, a breast cancer screening program. It was updated and validated across a population of women in the National Surgical Adjuvant Breast and Bowel Project P-I study. The updated version of the model (called model 2 in Costantino et al) has been implemented in a variety of formats. It is incorporated in the Breast Cancer Risk Assessment Tool (BCRAT, also referred to in this article as the Gail model), which is available on the National Cancer Institute (NCI) Web site (http://cancer.gov/bcrat) and is viewed 30,000 to 50,000 times per month, suggesting strong demand for this information. The Gail model provides individualized risk estimates of the probability that a woman with specific characteristics will develop invasive breast cancer during the next 5 years, and by age 80 years.
The Gail model incorporates information on risk factors such as age, age at menarche, age at first live birth, number of first-degree relatives with breast cancer, number of prior breast biopsies, and presence of atypical hyperplasia. It is currently the main tool used for breast cancer risk assessment in patients with atypia. Despite its widespread use, the Gail model has not been validated in patients with atypia. Therefore, we evaluated the Gail model in a well-characterized cohort of women with atypia on open breast biopsy.124

### PATIENTS AND METHODS

Details of the study cohort have been previously described. Briefly, the Mayo Breast Biopsy Cohort (BB) cohort comprises 9,376 women age 20-85 years who underwent open breast biopsy at the Mayo Clinic (Rochester, MN) between 1967 and 1991, with benign pathology findings. Women with a history of breast cancer, benign breast disease, or a prior breast biopsy were excluded. Breast biopsies from all women in the cohort were reviewed by our study pathologists (C.E., and David W. Vander, MD) without knowledge of the original histologic diagnosis or patient outcome. A diagnosis of atypia (cystic ductal hyperplasia, atypical ductal hyperplasia, or both) was made in 311 women (3.3%) using the standard criteria and histologic classification of Dupont and Page.125-128

The study was approved by the institutional review board of the Mayo Clinic and all patient contact materials were reviewed and approved.

Each individual’s risk factors for the development of invasive breast cancer were obtained via a study-specific questionnaire and from medical record review. The most current data available for each risk factor were used. Follow-up to date of diagnosis was the time window for each woman in the cohort. The number of breast biopsies was used for each woman in the cohort.

To obtain breast cancer risk estimates, we employed a Framingham program that was modified by NCIC, Gail, J. Burchenal, D. Box, personal communication, February 2007), which contains the code underlying the underlying code for risk estimation in NETS’ DCIS. The standards used in the routine Gail model were used for variables with missing data. Women with unknown age at menarche were assigned an age at menarche of 4 years or age of older. Women with unknown age at first live birth were classified as giving birth before age 25 years. Women with missing family history were classified as having no family history. To improve accuracy of age and follow-up, data, we used the online tool, we randomly selected 100 patients and compared the 5-year and lifetime risk estimates obtained from the code given us to those from the online risk assessment tool. All of these patients were in complete agreement.

The Gail model risk factors of women with atypia were summarized using counts and percentages, as means and standard deviations, both overall and also according to invasive breast cancer status. The cumulative incidence of breast cancer was estimated using methods that corrected for the competing risk of death.121 Cox proportional hazards regression models were used to assess associations between the risk of breast cancer and each of the Gail model risk factors. Hazard ratios (HR) and P-values assessing the associations were obtained.

Gail model predictions were summarized across the study group. Ranges of the predictions were estimated, together with means and standard deviations, for 5-year and follow-up-specific risk estimates. The distributions of the follow-up-specific risk estimates, by invasive breast cancer status, were obtained by computing the proportion of individuals whose risk predictions fell into specified categories. A graph was obtained by plotting these percentages against the center of the risk prediction categories, and thereby interpolating the points. The 5-year predictions and the follow-up-specific probabilities were aggregated to obtain estimates of the number of breast cancer events predicted by the Gail model, both overall and by the categories of the Gail model risk factors. The expected number of breast cancers was compared with the observed diagnoses by using the rate of observed to expected invasive breast cancer. These of significance and 95% CIs were obtained using the Poisson distribution.

### RESULTS

Of the 9,376 patients in the Mayo BB cohort, 531 women (5.6%) had atypia. A mean follow-up of 12.7 years, 58 of the 531 women with atypia (17.2%) developed invasive breast cancer (this cancer, eight in the first 5 years after biopsy). Among the 531 patients with atypia, 75 women (22.7%) died while in active follow-up. Nine of the deaths were among the patients who developed cancer and 66 were among the remainder of the atypia cohort.

In Table 1 we list the Gail model features for the 331 women with atypia. In Figure 1 we show the age-specific cumulative incidence of breast cancer along with population expectations and Gail model predictions for this cohort. Table 1 also lists associations between Gail model risk factors for invasive breast cancer and the risk of invasive breast cancer.

### Aggregate Performance

The Gail model predicted an average 5-year breast cancer risk of 4.2% (standard deviation, 2.7%; range, 0.9% to 18.8%). This equated to a predicted total of 139 breast cancers within 5 years. In this time interval, eight invasive breast cancers were observed. The ratio of observed to predicted exceeded 0.58 (95% CI, 0.29 to 1.19; P = .12).

The Gail model had a calibration value of 1.01 for the predicted cancers. The observed number of invasive breast cancers during the 5-year follow-up period was 10.5% (standard deviation, 6.2%; range, 0.4% to 51.1%). These risk estimates predicted that 34.9 women would experience an invasive breast cancer during this time period. The observed number of events during the observation period was significantly higher than predicted (ratio, 1.66; 95% CI, 1.29 to 2.15; P < .001).

Table 2 summarizes the number of events observed in our cohort and the number of events predicted by the Gail model for each Gail model risk factor. The Gail model underestimated the number of breast cancers, both overall and in the majority of the risk-factor-defined subgroups.
Table 1. Characteristics of the Women With AtRisk According to Whether They Developed Breast Cancer

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All Patients (n = 593)</th>
<th>No Invasive Cancer (n = 279)</th>
<th>Invasive Cancer (n = 64)</th>
<th>Hazard Ratio†</th>
<th>95% CI</th>
<th>p*</th>
</tr>
</thead>
</table>
| Age at menarche             | 10.2                  | 8.8                          | 11.9                     | 0.49†         | 0.43  | 0.56
| Age at first live birth     | 22.9                  | 23.4                         | 22.3                     | 0.69†         | 0.61  | 0.77
| Marital status              | 228                   | 223                          | 235                      | 1.11†         | 1.00  | 1.03
| Family history              | 10.8                  | 10.5                         | 11.2                     | 0.73†         | 0.62  | 0.86
| Education                   | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Employment                  | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Duration of marriage       | 10.2                  | 10.2                         | 10.2                     | 1.00†         | 0.83  | 0.83
| Income                      | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Smoking History             | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Alcohol Consumption        | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Physical Activity           | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Body Mass Index             | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Family History              | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Duration of marriage       | 10.2                  | 10.2                         | 10.2                     | 1.00†         | 0.83  | 0.83
| Income                      | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Smoking History             | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Alcohol Consumption        | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Physical Activity           | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Body Mass Index             | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Family History              | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Duration of marriage       | 10.2                  | 10.2                         | 10.2                     | 1.00†         | 0.83  | 0.83
| Income                      | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Smoking History             | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Alcohol Consumption        | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Physical Activity           | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93
| Body Mass Index             | 20.5                  | 20.5                         | 20.5                     | 0.83†         | 0.73  | 0.93

Abbreviations: † reference.
* p-value and corresponding 95% CI from a Cox proportional hazards regression model.
† p-value is less than 0.05.
‡ p-value is between 0.05 and 0.01.
§ p-value is between 0.01 and 0.001.
¶ p-value is less than 0.001.

Individual-Specific Performance

Figure 2 shows the distributions of the Gall model risk estimates for women who did and did not develop invasive breast cancer. These distributions are shown adjusted to the mean follow-up time of 13.7 years and to the mean age at entry of 50.0 years. This was done to eliminate bias induced by the controls having longer follow-up than the patients, given that follow-up for patients stops at the time of diagnosis of breast cancer, and to account for different risk estimates by age and diagnosis. With a model that perfectly discriminates between groups, the two distributions would not overlap. Here, there is only a slight overlap between the two distributions for the patients and non-cases. The age and follow-up adjusted average risk predictions are slightly lower in the patients (10.0% ± 5.4% vs 10.6% ± 7.3%), although not significantly (P = .46). The concordance between observed and predicted invasive breast cancer events after 5 years, as measured by the c-statistic, was 0.67 (95% CI 0.61-0.72), not significantly different from the value of 0.65 that would be expected by chance (P = .792). When using the risk estimates specific to the length of follow-up, the c-statistic was 0.69 (95% CI 0.64-0.74), not significantly different from the value of 0.65 expected by chance (P = .195).

To determine the degree to which missing data affected estimates of the accuracy of the Gall model predictions, we recomputed the prediction accuracy within the 192 individuals (36%) with complete data. As an additional sensitivity analysis, we also recomputed the prediction accuracy in all participants after imputing the values that would lead to the highest risk prediction. The observed-to-expected ratio of invasive breast cancers in those with complete data, at 1.44 (95% CI 1.04 to 2.00), was somewhat lower than the value of 1.66 observed in all 593 women. However, this still reflected a significant discrepancy between the Gall model predictions and the observed invasive cancers among this subgroup of women with complete data (P = .028). The c-statistic in the complete-data subset was somewhat higher (0.59) than what we observed in the entire cohort (0.50). Even when imputing in a way that leads to the highest possible number of expected cancers, the observed-to-expected ratio was still significantly increased (1.32; 95% CI 1.02 to 1.70, P = .035), whereas the c-statistic (0.59) was similar to what was observed in the complete-data subset.

Discussion

We studied the Gall model in a well-defined cohort of women with at-risk with an average follow-up of nearly 14 years. Measuring the performance of the model, the model slightly overpredicted the number of invasive breast cancers during the first 5 years, but...
Assessment of Gail Model in Atypia

![Graph showing cumulative incidence of invasive breast cancer](image)

**Fig. 1.** Cumulative incidence of invasive breast cancer among women with atypical hyperplasia predicted as a function of age. The red line represents the cumulative incidence, red dots for the competing risk of death, in the atypia cohort. For comparison, two lines representing the Gail-predicted and the baseline predicted rates are included. The blue line reflects the cumulative incidence predicted by the Gail model in this cohort, and the grey line represents the cumulative incidence that serves as the baseline rate for white women in the Gail model calculations.

During the entire 13.7 years of follow-up, the Gail model underpredicted the number of invasive breast cancers during the first 13 years of follow-up. The individual-specific agreement between the Gail model predictions and actual breast cancer outcomes was low. For the first 5 years after biopsy the c-statistic was 0.67 (95% CI, 0.58 to 0.75), no better than chance alone.

During the entire 13.7 years of follow-up, the Gail model predictions were consistent with invasive breast cancer outcomes 50% of the time (95% CI, 44% to 55%), also not significantly better than chance. This finding is lower than assessments of the Gail model in other cohorts, where c-statistics of 0.58 to 0.59 have been reported, although the upper limit of the CI approaches these previously reported values.

This cohort consists of a large collection of women with atypia. However, as we assessed the quality of the risk predictions of the Gail model, there were women for whom complete covariate information was not available (Table 1). When we recomputed the risk estimates in the subset of women with complete data, the estimates were similar. The c-statistic in the complete-data subset indicated performance similar to what was observed in the entire cohort. Even in the situation where the missing data were imputed in such a way to produce the maximum number of predicted breast cancers, the Gail model still predicted a significantly lower number of breast cancer events than were observed. Thus, it seems unlikely that the level of missing data can explain the underestimate of breast cancer risk that is reported here.

Clinical management of women diagnosed with atypia includes quantitative breast cancer risk assessment, comprehensive discussion of risk reduction strategies, and recommendations for future breast cancer screening. The currently available risk reduction options include chemoprevention with agents such as tamoxifen or raloxifene, prophylactic mastectomy, and/or lifestyle modification. Unfortunately, lifestyle modifications,

<table>
<thead>
<tr>
<th>Table 2: Comparison of Observed and Predicted Breast Cancer Events by Gail Model Risk Factors for Invasive Breast Cancer After Diagnosis of Atypia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Overall</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>&lt; 40</td>
</tr>
<tr>
<td>40-69</td>
</tr>
<tr>
<td>&gt; 69</td>
</tr>
<tr>
<td>Age at menarche, years</td>
</tr>
<tr>
<td>&lt; 12</td>
</tr>
<tr>
<td>12-19</td>
</tr>
<tr>
<td>&gt; 19</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Age at first live birth, years</td>
</tr>
<tr>
<td>&lt; 20</td>
</tr>
<tr>
<td>20-24</td>
</tr>
<tr>
<td>25-29</td>
</tr>
<tr>
<td>&gt; 30</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Previous diagnosis with breast cancer</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Number of biopsies</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>&gt; 1</td>
</tr>
</tbody>
</table>

*Compared by applying observed person-years of follow-up to Gail model risk prediction estimates.
such as adoption of a healthy diet, maintenance of a healthy weight, and avoidance of smoking and smoking environments do not seem to provide a substantive reduction in risk of breast cancer. Chemoprevention requires consideration of the risks and benefits of the medications and surgical intervention can be associated with significant morbidity. Gail et al. provide a useful overview regarding the risks and benefits of using tamoxifen, and demonstrate that the use of tamoxifen in women with atypia provides no net benefit. With more reliable assessments of breast cancer risk, it will be possible to provide even better counsel to patients as they consider risk reduction strategies.

Atypia was not included in the initial development of the Gail model due to a lack of pathology assessment for all women in the Breast Cancer Detection and Demonstration Project. Thus, atypia was added to the original model using estimates of the population prevalence of atypia, and the relative risk for breast cancer associated with atypia. This modification was based on a prevalence of atypia of 7.8%, and relative risk for breast cancer of 1.96. However, recent studies evaluating the risk of atypia, based on the more stringent criteria of Dupont and Page, have all reported higher relative risks with atypia (3.6 to 5.3), and lower prevalence (approximately 4%). This may explain the underestimates that we report here.

Another potential explanation for the observed underestimate is that the Gail model is intended as a prospective risk prediction tool, and accounts for death as a competing risk. This result in lower predicted probabilities. In our study, we used the Gail model to predict risk in women for whom outcomes, including death, had already been observed. To assess the degree to which the competing risk of death might have influenced the results of our comparison to the Gail model, we recomputed the Gail model risk probabilities while accounting for death as a competing risk in the most extreme way possible. That is, we considered women who died to still have been at risk until they would have reached age 90 years. Even in this extreme case, the Gail model significantly underestimated the number of breast cancer events (observed-to-expected ratio, 1.44; 95% CI, 1.11 to 1.86; P = .006). However, this approach did result in a higher c-statistic (0.55; 95% CI, 0.49 to 0.60).

The Gail model has been studied in several settings, however, our knowledge, data regarding atypia were not available in these validation studies. In general, the model has fulfilled its original goal—to identify groups of at-risk women suitable for chemoprevention trials. However, the model is increasingly used clinically to predict risk of individual women, and here the Gail model (or others) falls short of the precision required to make treatment recommendations for individual patients. Better performance for population than an individual by these models is explained because the models were derived by averaging information across groups of individuals. When such models are based on large representative groups of patients, this leads to predictions that are well calibrated within an entire group, but do not guarantee accurate predictions for specific individuals within these groups.

To our knowledge, this article is the first report on the Gail model exclusively in women with atypia. It uses data from a large cohort defined by contemporary pathology review, with detailed risk factor information and long-term follow-up. It is limited primarily by the small number of patients that developed breast cancer and uses data from open, rather than overt, biopsy.

The Gail model uses demographic and clinical factors. It is possible that risk assessment could be improved through use of tissue-based risk factors, which should be feasible for all women who undergo a breast biopsy for benign findings (an estimated one million women in the United States alone each year). One of the hypotheses of breast cancer development is the existence of a continuum wherein breast cells undergo successive alterations at a molecular level that lead from normal epithelium, to excess proliferation, to then to atypia, carcinoma in situ, and ultimately invasive carcinoma. If this hypothesis is accurate, then tissue-based and molecular assessments that reflect the current state of the at-risk tissue will likely provide information leading to more accurate risk predictions. For example, in this atypia cohort the risk factors included in the Gail model do not stratify risk (Table 1). Presumably, the risk inherent in these factors (e.g., family history) is already reflected in the tissue type of atypia. We have recently shown that pathologic assessment of number of atypia on biopsy stratifies risk of this patient. We have also shown that the presence of tubular invagination in background breast tissue and cyclooxygenase 2 overexpression further stratifies risk in women with atypia. Addition work in groups of women with measurements of tissue-based biomarkers as well as breast cancer outcomes is likely to provide important information.

In summary, our findings suggest that Gail model risk estimates for our cohort of women with atypia are significantly lower than what was observed with long-term follow-up. At the level of the individual there was low concordance between the Gail model predictions and actual breast cancer events. This study underscores the need for caution when using the Gail model to counsel individual women with atypia regarding their risk of developing invasive breast cancer. Additional research is required to identify highly predictive markers of breast cancer risk, and to incorporate these markers into a more accurate model for use in this high-risk population.

Fig. 2. Distributions of Gail model risk probabilities in women with atypia who develop breast cancer (cases) and those who did not (controls). The plot contains estimates for individualized risk at the end of the available follow-up.

The Gail model was developed as a tool to identify groups of women at high risk for breast cancer, and it has been widely used as a risk assessment tool in clinical settings. However, it is limited by the high rate of false negatives, which can lead to missed cancer diagnoses. These limitations may be addressed by incorporating additional risk factors, such as tissue-based markers, into the model. Our findings suggest that the Gail model may underestimate the risk of breast cancer in women with atypia, and that additional research is needed to improve risk assessment in these patients.
Assessment of Gold Model in Atypia

AUTHORS: DECLARATIONS OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: V. Shane Pankratz, Lynn C. Hartmann, Amy C. Dequin, Kartik Gosh, Robert A. Vierkant, Madeline H. Frot, Sharon D. Maloney
Financial support: Lynn C. Hartmann, Amy C. Dequin, Kartik Gosh, Robert A. Vierkant, Madeline H. Frot, Sharon D. Maloney

REFERENCES

cr 4:315-330, 1995
17. Gail MH, Costantino JP, Bryant J, et al: Weighting the risks and benefits of tamoxifen me-

Acknowledgment

We thank Joel Wunder and Peter de Green, MD, for database development; Sandhya Pruthi, MD, for clinical input; and Teresa Allen, Mary Campion, Joanne Johnson, Melanie Kaiser, Betty Anderson, Romayne Thompson, Ann Harris, and the Survey Research Center for data collection and patient follow-up.

***
A Novel Breast Tissue Feature Strongly Associated with Risk of Breast Cancer.

Kevin P. McKian, M.D.¹, Carol A. Reynolds, M.D.², Daniel W. Vischer, M.D.³, Aziza Nassar, M.D.¹, Derek C. Radisky, Ph.D.⁴, Robert A. Vierkant, M.A.S.⁵, Amy C. Degnim, M.D.⁶, Judy C. Boughhey, M.D.⁷, Karthik Ghosh, M.D.⁵, Stephanie S. Anderson, B.S.⁵, Douglas Minot, B.S.⁵, Jill L. Caudill, B.S.³, Celine M. Vachon, Ph.D.⁷, Marlene H. Frost, Ph.D.¹, V. Shane Pankratz, Ph.D.⁵, Lynn C. Hartmann, M.D.¹

¹Department of Oncology; ²Department of Laboratory Medicine and Pathology; ³Department of Health Sciences Research—Biostatistics; ⁴Department of Surgery; ⁵Department of Health Sciences Research—Epidemiology; ⁶Department of Internal Medicine, Mayo Clinic Cancer Center, Mayo Graduate School of Medical Education, Mayo Clinic College of Medicine, Rochester, MN 55905. ⁷Department of Pathology, University of Michigan, Ann Arbor, MI. ⁸Department of Biochemistry/Molecular Biology, Mayo Clinic Cancer Center, Mayo Graduate School of Medical Education, Mayo Clinic College of Medicine, Jacksonville, FL.

Corresponding author: Lynn C. Hartmann, M.D

Mayo Clinic, 200 First Street SW, Rochester, MN 55905

Phone (507) 284-3731, Fax (507) 284-1803

Email hartmann.lynn@mayo.edu
ABSTRACT
Background: Accurate, individualized risk prediction for breast cancer is lacking. Tissue-based features may help to stratify women into different risk levels. Breast lobules are the anatomic sites of origin of breast cancer. As women age, these lobular structures should regress, which results in reduced breast cancer risk. But this does not occur in all women.

Methods: We have quantified the extent of lobule regression on a benign breast biopsy in 85 breast cancer cases and 142 age-matched controls from the Mayo Benign Breast Disease Cohort, by determining number of acini/lobule and lobular area. We also calculated Gail model 5-year predicted risks for these women.

Results: There is a step-wise increase in breast cancer risk with increasing numbers of acini/lobule (p=0.0004). Adjusting for Gail model score, parity, histology and family history did not attenuate this association. Lobular area was similarly associated with risk. The Gail model estimates were associated with risk of breast cancer (p=0.03). We examined the individual accuracy of these measures using the concordance (c) statistic. The Gail model c statistic was 0.60 (95% CI; 0.50-0.70); the acinar count c statistic was 0.65 (95% CI; 0.54-0.75). Combining acinar count and lobular area, the c statistic was 0.68 (95% CI; 0.58-0.78). Adding the Gail model to these did not improve the c statistic.

Conclusion: Novel, tissue-based features that reflect the status of a woman's normal breast lobules are associated with breast cancer risk. These features may offer a novel strategy for risk prediction.

INTRODUCTION:

The medical community's ability to predict risk of breast cancer for individual women is very limited. In other tissues, optimal cancer risk prediction can occur when the tissue at risk is examined for evidence of premalignant change (e.g. cervix, esophagus, colon). Presumably the field of normal tissue, exposed to an individual's endogenous and exogenous risks, responds with a phenotype (e.g. proliferation, atypical cells) that reflects the increased risk. While breast tissue is not readily available for routine clinical assessment, women with benign breast disease have had breast tissue removed in the course of their care and have an increased risk of a later breast cancer.

Current characterization of benign breast tissue focuses primarily on the degree and type of epithelial hyperplasia, but this focus may overlook other important, easily assessed features. The breast is organized into 15-20 major lobes, each composed of lobules that contain the milk-forming acini. The lobule (or terminal duct lobular unit, TDLU) is the anatomic substructure thought to give rise to breast cancer. Normal aging results in the physiologic regression (or involution) of breast lobules (Figure 1). With regression, there is progressive loss of acini within the lobular units and replacement of specialized intralobular connective tissue with the collagen more typical of the interlobular region (Figure 1). We previously showed that breast cancer risk decreases...
with regression of lobular units, assessed qualitatively as no, partial, or complete involution.\textsuperscript{11}

We hypothesized that a quantitative assessment of involution could be developed as a more precise and physiologic measure of breast cancer risk. Thus, in a nested-case control series within the Mayo Benign Breast Disease Cohort, we have calculated the number of acini within normal lobules and average lobule size. Here we show the risk prediction capabilities of lobule status and compare these results to the current standard, a Gail model assessment of risk performed in the same women.

\textbf{METHODS:}

\textit{Study population}

We performed a nested-case control study within the Mayo Benign Breast Disease Cohort. This cohort includes all women (n=9,376) who had an open breast biopsy, with benign findings, at Mayo Clinic between 1-1-1967 and 12-31-1991.\textsuperscript{10,11} Median follow-up for breast cancer events is 16.9 years.\textsuperscript{11} For cohort members, we assembled risk factor and outcomes data from a study specific questionnaire and the Mayo Clinic medical record. Study pathologists characterized the benign biopsies, including the extent of lobule regression.\textsuperscript{11} All patient contact materials and procedures were reviewed and approved by the Mayo Clinic Institutional Review Board.

For the current study, we selected a random sample of 100 cases who developed breast cancer from the cohort, stratified by five-year categories of year of benign biopsy to represent the entire spectrum of the cohort. We matched two controls to each case based on age and year of benign biopsy. Of these subjects selected, 85 cases and 142 controls had adequate tissue available for assessment.

\textit{Assessment of lobular status}

Extent of lobular regression was previously characterized qualitatively by the study pathologist as none (0\% TDLUs regressed); partial (1-74\% regressed) or complete (\geq75\% regressed).\textsuperscript{11} For the quantitative assessments, one H & E stained slide per subject was scanned into the computer and analyzed using WebSlide Browser software (Bacus Labs product). This software allows the measurement of structural features (lobular area, acini number) as visualized by light microscopy (Figure 2).

The ten largest normal lobules were assessed for each patient by one observer (KPM) without knowledge of case status or previous pathologic assessment. If fewer than ten normal lobules were present, all were assessed. Analysis included (i) counting the number of individual acini per lobular unit and (ii) delineating the circumference of the lobule to measure its area in square microns (Figure 2). We defined countable acini as nuclei forming a distinct circular pattern with or without the presence of a discernible lumen. Distinct lobules were defined by the presence of intersecting stromal tissue. Abnormal lobules, namely those that contained large portions of terminal ducts, atypical lobular or ductal hyperplasia, sclerosing adenosis, large cysts, or proliferative disease without atypia were not included.
Reproducibility

A random sample of 82 slides (25 cases and 57 controls) was read by a second observer (ILC) using the quantitative, manual method described above. A different approach, automated analysis, was performed on another random sample of 95 slides (28 cases and 67 controls) using the Automated Cellular Imaging System (ACIS® II) instrument (Dako, Carpinteria, CA). The ACIS® III automatically scanned the study slides at 4x magnification to obtain an overall image. The images were then visually analyzed (by DM) to determine the 10 largest normal breast lobules (or less if there were not 10 lobules present on the slide). Area measurements were determined by tracing an outline of the lobules using the “free-form” tracing tool. Breast acini were counted within each lobule using the "100x" circle scoring tool. Area measurements and the number of acini were calculated by the instrument and exported from the ACIS® III program to a spreadsheet for statistical analysis.

Gail model calculations

Using age at benign biopsy as the age at risk assessment, the Gail model [NCI Breast Cancer Risk Assessment Tool, (http://cancer.gov/bcrisktool)] was used to predict the five-year risk of breast cancer for each of the women using their risk factor profile. To calculate these estimates, we employed a FORTRAN program provided to us by the NCI (Gail M, Benichou J, Pec D [Information Management Services, Rockville, MD]; personal communication) which we have used previously. This program contains the code that comprises the underlying calculation machinery used in the NCI’s Breast Cancer Risk Assessment Tool (BCRAT). For variables with missing data, we used the standards in the online Gail model. To verify agreement between the code we used and the online tool, we randomly selected 10 subjects from our cohort and compared the 5-year and lifetime risk estimates obtained from the code given us to those from the online risk assessment tool. All of the estimates were in complete agreement.

Statistical analyses:

We studied two measures of involution: the number of acini per lobule and lobule size. Primary analyses used the median of the values obtained across the multiple lobules measured for each woman. Secondary analyses that incorporated the values for all lobules were also performed, using repeated measures approaches, but results were similar to those modeling the medians and thus are not shown.

We compared distributions of number of acini and lobule area across demographic and clinical variables using general linear mixed models, accounting for the matched study design by fitting each case-control set as a random intercept term. Due to data skewness, analyses were run using log-transformed values. The resulting least squares means and 95% confidence intervals were then back-transformed to their original sampling units. We examined correlations between number of acini and lobule area; between these measures and our original three categories of involution (none, partial, complete); and between the quantitative measures obtained by the two manual observers and the ACIS method, using Pearson correlation coefficients, again based on the log-transformed values.
We assessed associations between number of acini, lobule area, and Gail model risk estimates with breast cancer risk using conditional logistic regression analysis. We first modeled each variable as categorical, pooling values into four to six distinct groups. We then assessed dose-response effects by fitting each as a continuous variable in the logistic model. These latter analyses were carried out using log-transformed values for acini and area, as assessments of their functional form revealed sub-optimal model fit using the data in its original scale. We examined univariate associations and models with various combinations of the following variables: the five-year Gail Model risk prediction score, number of live births, family history, and histologic findings. Using the risk estimates from the logistic models, we examined the risk prediction capabilities of these variables using concordance (c) statistics. These statistics can be interpreted as the area under the receiver operating characteristics (ROC) curve, or alternatively as the average sensitivity of the variable across all possible levels of specificity. We used a modified c statistic to account for the matched study design, calculating the number of case-control pairs in each set, as well as the number of “concordant” pairs (those for which the case’s predicted risk exceeded the control’s), then aggregating across all matched sets. 95% confidence intervals (CI) were calculated using 5000 bootstrap samples of case-control sets.

RESULTS:

Patient characteristics

A total of 227 patients were included in the nested case-control study: 85 women who went on to develop breast cancer (cases) and their 142 age-matched controls. The median follow-up for all participants was 16.2 years. The median follow-up for controls was 18.6 years; for cases, 9 years (follow-up ceases after breast cancer diagnosis). The mean age at benign biopsy was 52.1 years. The patient characteristics are provided in Table 1.

Number of acini per lobule (acinar count)

As expected, the average number of acini per lobule was associated with the pathologist’s qualitative category of involution. Namely, women with no involution had a higher mean acinar count [32.0 (95% CI 26.4-38.8)] than women with partial involution [19.7 (95% CI 17.5-22.2)] or complete involution [7.7 (95% CI 5.8-10.3)] (p<0.0001). When comparing the acinar count with histologic category [non-proliferative (NP), proliferative disease without atypia (PDWA), atypical hyperplasia (A II)], the means were not significantly different (18.8 [95% CI: 16.1-22.0], 22.1 [95% CI: 18.6-26.3], and 18.6 [95% CI: 13.2-26.2], respectively) (p=0.309). The acinar count for women with a family history of breast cancer was 22.9 (95% CI: 19.0-27.6) vs. 18.3 (95% CI: 16.4-21.6) for those with no family history (p=0.0683).

When comparing the acinar count for cases vs. controls, women who developed breast cancer had significantly more acini per lobule (24.3) than women who remained unaffected (17.8) (p=0.0008). In Table 2 we show a step-wise increase in risk of breast cancer with increasing numbers of acini/lobule (p=0.0004). This association was similar,
if not slightly stronger, after adjusting for the Gail model five-year risk score (p=0.0001, Table 2). We examined acinar count and breast cancer risk by nonproliferative vs. proliferative histologies and saw a similar dose response association in both groups (data not shown). Further adjustment for other potential confounders including parity and family history did not attenuate the observed association.

Because time from benign biopsy to breast cancer varied from 11 months to 27 years in our cases, we asked if involution measures varied by time to breast cancer. To investigate this, we plotted the ratio of involution in cases to involution in matched controls (on the log scale) as a function of time. We then fit a least squares regression line to this plot. The line had a slight downward trend but always remained above the back-transformed ratio values of 1.0, indicating that the positive association of acinar count with case status was sustained across the entire spectrum of time to cancer and did not vary significantly over time (data not shown).

**Lobule size**

Lobule area was strongly correlated with acinar count (r=0.85, 95% CI: 0.81-0.88). Women who developed breast cancer had a larger lobular area (64.165 µ2) than controls (53.759 µ2) (p=0.065). Logistic regression analyses indicated a step-wise increase in risk of breast cancer with increasing lobule size (p=0.045). Notably, during involution, acini become less cohesive geographically and can drift apart as seen in Figure 1B, resulting in a larger area than might be expected relative to number of acini. While lobule size was associated with breast cancer risk, associations were generally more modest than with number of acini.

**Reproducibility**

We compared the initial quantitative acinar count with those obtained by a second observer and with the automated ACIS readings. There was strong correlation among the three approaches (first and second observer, r=0.91 [95% CI: 0.87-0.94]; first observer compared to ACIS, r=0.78 [95% CI: 0.68-0.84]; second compared to ACIS, r=0.79 [95% CI: 0.68-0.86]).

**Gail model predictions**

The Gail model 5-year estimates were associated with the outcome of breast cancer (p=0.030, Table 2B) for all breast cancer events -- invasive (n=69), in situ (n=13), and three with invasion status unknown. When restricting analyses to invasive cancers only, the Gail model results were very similar (p=0.022).

**Accuracy of risk prediction: Lobule measures vs. Gail model**

We assessed the accuracy of risk prediction, for individual women, for the Gail model and for acinar count and lobular area using the c statistic (Table 3). For the Gail model, it was similar to estimates found in other studies (0.60 [95% CI: 0.50-0.70]). For the Gail model, it was similar to estimates found in other studies (0.60 [95% CI: 0.50-0.70]).14,15 Using acinar count alone, the c statistic was 0.65 (95% CI: 0.54-0.75). Combining acinar count and lobular area increased the c statistic to 0.68 (95% CI: 0.58-0.78). Adding the Gail model to this combined set did not add to predictive accuracy (c statistic, 0.66).
DISCUSSION:

Optimal early detection and prevention strategies for breast cancer require accurate identification of those individuals at significantly increased risk for the disease. Despite our knowledge of many determinants of breast cancer risk, both endogenous and exogenous, our ability to predict risk for individual women remains limited.1,2 Reasoning that a woman’s breast tissue reflects the integration of her exposures to multiple risk-contributing processes, we are working to develop a tissue-based approach to risk prediction for breast cancer. Regression of involuting lobules is a physiologic process that occurs as a woman ages.3,4 Importantly, it is these same structures that give rise to breast cancer.5 Completion of the involuting process, assessed in a qualitative manner, is associated with a significant reduction in breast cancer risk.6 We have now quantified extent of lobular regression for individual women via the number of acini/lobule and lobe size and show a strong association with risk of breast cancer. Importantly, these lobular features, assessed on a single H&E stained slide, identified those women who would later develop breast cancer more precisely than a Gail model prediction. This held true whether or not comparisons were restricted to invasive events. We have also shown reproducibility of these measures, whether obtained manually or in an automated fashion, with correlation coefficients of 0.78–0.91. Of note, these measures appear to be independent of histology, contributing to their risk prediction capabilities.7

Several risk prediction models for breast cancer focus on an individual’s likelihood of carrying a hereditary predisposition to the disease.1,6–22 Outside the hereditary setting, the most widely used tool is the Gail model.12 This model is available on the National Cancer Institute’s website (www.cancer.gov/bcrisktool/) and is viewed approximately 20,000–30,000 times a month,3 demonstrating the strong clinical demand for risk assessment for individual women. While the Gail model has been shown to be well-calibrated in predicting the number of invasive cancers likely to develop in groups of women, its discriminatory accuracy in predicting risk for individual women, as measured by a statistics near or below 0.6, is only slightly better than chance alone. Here we show that a simple physiologic measure of lobular status is more strongly associated with breast cancer risk than the Gail model.

There are several plausible mechanisms by which progressive degrees of lobular involution may reduce breast cancer risk. The most straightforward is that the dramatic reduction in epithelial cell number that occurs with involuting equates to a physiologic ‘‘prophylactic mastectomy.”20 This can be visualized in Figure 3 where age-related lobular involution has essentially removed the TDLUs from the field of breast tissue. Another explanation is that age-related involution invokes some final differentiation-senessece program rendering the remaining cells resistant to carcinogenic influence. It is somewhat counterintuitive that an age-related process like involution is associated with reduced breast cancer risk, when breast cancer risk increases with age. Notably, in studying all women over 55 in our cohort, those who had complete involution had a RR for breast cancer of 0.92 (95% CI: 0.74–1.14) vs. 3.21 (95% CI: 1.90–5.08) for those with no involution.11 This suggests that age-related breast cancer risk may be concentrated in women whose lobules fail to regress normally.
Our study has several limitations. First, these findings do not necessarily pertain to all women because our cohort includes women who had a breast biopsy for some concern. Moreover, the present study is based in a nested case-control study from our larger Mayo Benign Breast Disease Cohort. However, we randomly selected this sample from the entire set of cases and our previous results, based in the entire cohort, showed that involution status assessed qualitatively (none, partial, complete) was strongly associated with breast cancer risk.\textsuperscript{11} Even if our findings are limited to women with benign breast disease, such women number at least one million per year in the United States alone,\textsuperscript{24,26} and they represent a clinically important group, as about 25% of women with breast cancer have had a prior benign biopsy.\textsuperscript{27} A limitation in our comparisons to the Gail model is that our controls were matched to cases on age at benign biopsy. Since age is one of the predictor variables in the Gail model, this matching may limit the risk prediction capabilities of the Gail model. Analysis of only one slide per woman could be a limitation. However, we have looked at uniformity of involution across the field of a woman’s breast tissue in women who had bilateral prophylactic mastectomy and have demonstrated high concordance in involution status across all eight quadrants of their breast tissue.\textsuperscript{28} Importantly, our analyses are based on a modest sample size. Although we found statistically significant associations between acinar count and breast cancer risk, confidence intervals are wide. Further studies are needed to confirm our results.

In summary, we have developed a means to assess degree of regression of normal breast lobules quantitatively. We have shown that higher acinar counts within the lobules, and larger lobule size, are associated with higher risk of breast cancer. These simple physiologic features may offer an alternative strategy for breast cancer risk prediction in women who have had benign breast biopsies.

Acknowledgments:
This work was supported by a Department of Defense Center of Excellence Grant [FEDDAMD17-02-I-0473-1]; Martha and Bruce Atwater; the National Institutes of Health [R01 CA46332]; and the Fred C. and Katherine B. Andersen Foundation.

We are indebted to Teresa Allers, Mary Campion, Joanne Johnson, Melanie Kasner, and Romayne Thompson for data collection; to Emily Barr-Fritcher for help with the reproducibility studies; to Ann Harris and the Survey Research Center for patient follow-up; and to Vicki Shea for assistance with manuscript preparation.

Figure I was previously published in the following manuscript: Milanese TR, Hartmann LC, Sellers TA, et al. Age-related lobular involution and risk of breast cancer. J Natl Cancer Inst 2006; 98:1-8.

Lynn C. Hartman, M.D., had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The authors have no conflicts of interest.
Figure legends
In Figure 1 panel A, there is a field of normal lobules (terminal duct lobular units), each composed of multiple acini. In panel B there has been complete regression (involution) of these lobules leaving small residual structures largely depleted of acini.

In Figure 2 panel A, we subdivide an intact lobule to facilitate counting of individual acini. Panel B demonstrates the delineation of the circumference of the lobule for calculation of lobule area by the computer software.

In Figure 3, whole breast mounts from pre-involutional (A) and post-involutional women. (With kind permission of Springer Science+Business Media. Originally published in "Handbuch der mikroskopischen Anatomie des Menschen." (W. Bargmann, ed.), Vol 3, part 3, Haut und Sinnesorgane, pp. 277-485, 1957. Springer-Verlag, Berlin)
Table I. Clinical and Histologic Characteristics

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Cases (N=85)</th>
<th>Controls (N=142)</th>
<th>Total (N=227)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at Biopsy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>52.5 (10.1)</td>
<td>51.8 (10.1)</td>
<td>52.1 (10.1)</td>
</tr>
<tr>
<td>Histologic Type, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-proliferative</td>
<td>42 (49.4%)</td>
<td>79 (55.6%)</td>
<td>121 (53.3%)</td>
</tr>
<tr>
<td>Proliferative without atypia</td>
<td>31 (36.5%)</td>
<td>56 (39.4%)</td>
<td>87 (38.3%)</td>
</tr>
<tr>
<td>Proliferative with atypia</td>
<td>12 (14.1%)</td>
<td>7 (4.9%)</td>
<td>19 (8.4%)</td>
</tr>
<tr>
<td>Number of live births, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>0 (0%)</td>
<td>1 (0.7%)</td>
<td>1 (0.4%)</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>11 (12.9%)</td>
<td>18 (12.7%)</td>
<td>29 (12.8%)</td>
</tr>
<tr>
<td>1</td>
<td>7 (8.2%)</td>
<td>15 (10.6%)</td>
<td>22 (9.7%)</td>
</tr>
<tr>
<td>2</td>
<td>30 (35.3%)</td>
<td>38 (26.8%)</td>
<td>68 (30%)</td>
</tr>
<tr>
<td>3</td>
<td>26 (30.6%)</td>
<td>29 (20.4%)</td>
<td>55 (24.2%)</td>
</tr>
<tr>
<td>4+</td>
<td>11 (12.9%)</td>
<td>41 (28.9%)</td>
<td>52 (22.9%)</td>
</tr>
<tr>
<td>Family History of Breast Cancer, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>1 (1.2%)</td>
<td>5 (3.5%)</td>
<td>6 (2.6%)</td>
</tr>
<tr>
<td>None</td>
<td>51 (60.0%)</td>
<td>96 (67.6%)</td>
<td>147 (64.8%)</td>
</tr>
<tr>
<td>Weak</td>
<td>16 (18.8%)</td>
<td>22 (15.5%)</td>
<td>38 (16.7%)</td>
</tr>
<tr>
<td>Strong</td>
<td>17 (20.0%)</td>
<td>19 (13.4%)</td>
<td>36 (15.9%)</td>
</tr>
<tr>
<td>A. Number of acini/lobe</td>
<td>Cases</td>
<td>Controls</td>
<td>RR (95% CI)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------</td>
<td>----------</td>
<td>-------------</td>
</tr>
<tr>
<td>0-10</td>
<td>16</td>
<td>37</td>
<td>1.00 (REF)</td>
</tr>
<tr>
<td>11-20</td>
<td>22</td>
<td>41</td>
<td>2.10</td>
</tr>
<tr>
<td>21-30</td>
<td>13</td>
<td>26</td>
<td>3.23</td>
</tr>
<tr>
<td>31-40</td>
<td>8</td>
<td>18</td>
<td>3.23</td>
</tr>
<tr>
<td>41 or more</td>
<td>26</td>
<td>20</td>
<td>11.85</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Gail 5-year risk</th>
<th>Cases</th>
<th>Controls</th>
<th>RR (95% CI)</th>
<th>P-value</th>
<th>RR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-&lt;1</td>
<td>12</td>
<td>33</td>
<td>1.00 (REF)</td>
<td>0.010</td>
<td>1.00 (REF)</td>
<td>0.037</td>
</tr>
<tr>
<td>1-&lt;2</td>
<td>38</td>
<td>72</td>
<td>1.76</td>
<td>(0.64 - 4.87)</td>
<td>1.58</td>
<td>(0.53 - 4.73)</td>
</tr>
<tr>
<td>2-&lt;3</td>
<td>19</td>
<td>38</td>
<td>4.13</td>
<td>(1.28 - 13.32)</td>
<td>3.83</td>
<td>(1.09 - 13.47)</td>
</tr>
<tr>
<td>3+</td>
<td>16</td>
<td>19</td>
<td>3.55</td>
<td>(1.05 - 11.98)</td>
<td>3.51</td>
<td>(0.92 - 13.35)</td>
</tr>
</tbody>
</table>

1. Relative risks and 95% confidence intervals calculated using conditional logistic regression analysis. Analyses account for the matched nature of the data by modeling set ID as a stratification variable. P-values assess the dose-response effects of quantitative involution and the Gail model estimate with risk of breast cancer by modeling each as a continuously distributed predictor variable.
2. Univariate analyses
3. Multivariate analyses, adjusting additionally for the five-year predicted risk of breast cancer based on the Gail model.
4. Multivariate analyses, adjusting additionally for the number of acini/lobe.
Table 3. Assessment of the predictive capability of lobular measures and the Gail model using concordance statistics.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>c statistic (95% CI)</th>
<th>c statistic (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(All cases n=85)</td>
<td>(Invasive only n=69)</td>
</tr>
<tr>
<td>Gail model</td>
<td>0.60 (0.50 - 0.70)</td>
<td>0.59 (0.47 - 0.71)</td>
</tr>
<tr>
<td>Number of acini</td>
<td>0.65 (0.54 - 0.75)</td>
<td>0.69 (0.57 - 0.80)</td>
</tr>
<tr>
<td>Lobular area</td>
<td>0.56 (0.47 - 0.67)</td>
<td>0.59 (0.47 - 0.70)</td>
</tr>
<tr>
<td>Number of acini + area</td>
<td>0.68 (0.58 - 0.78)</td>
<td>0.70 (0.59 - 0.81)</td>
</tr>
<tr>
<td>Number of acini + area + Gail model</td>
<td>0.66 (0.56 - 0.76)</td>
<td>0.69 (0.58 - 0.80)</td>
</tr>
</tbody>
</table>

Statistics calculated using conditional logistic regression analysis, accounting for the matched nature of the data by modeling set ID as a stratification variable.

1 c statistic based on both invasive and in situ events
2 c statistic based on invasive cases only
Figure 1.
(a)

Figure 2
Panel A

Number of Acini per
Panel B

Lobular Area in $\mu^2$
REFERENCES:


