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INTRODUCTION:

Hormone replacement therapy (HRT) slows bone loss and improves quality of life for many women, but its use is also associated with a small increased risk of developing breast cancer (1-3). The estrogen plus progesterone arm of the Women's Health Initiative recently closed due to the increased risk of breast cancer without a benefit in prevention of cardiovascular disease or stroke (4). Many women will still choose to use HRT to treat hot flashes and to improve perceived quality of life despite the small increase in breast cancer risk. Currently, it is not possible to predict which women using HRT are at increased risk of developing breast cancer. On the mammogram, HRT is known to slow the normal involution of the breast and causes an increase in mammographic density in 17-73% of women (Figure 1) (5-8). This effect is more common with use of estrogen with progestin compared to estrogen alone(7). Women with increased mammographic density are also known to be at increased risk for developing breast cancer (9). We therefore hypothesize that women who have an increase in mammographic density in response to HRT are at higher risk for developing breast cancer than those women who do not have a change in mammographic appearance in response to HRT.

The purpose of the work funded by this grant is to determine if an increase in mammographic breast density in response to HRT is associated with an increased risk of breast cancer.

The overall goals of the project, as stated in the original application, are to:

- 1) Determine the association between HRT-induced changes in breast density and incident breast cancer
- 2) Quantify the association between initiation and duration of HRT and subsequent change in breast density
- 3) Demonstrate the utility of digital quantitative techniques for determining and reporting breast density

BODY:

As taken from the original Statement of Work, the tasks scheduled to begin and/ or be completed during the project period are as follows:

Task 1. Identify potential cases (months 1 –6).

- List of 1340 women diagnosed with breast cancer at the University of Virginia between 1990 and 1999.
- Update patient listing of women with diagnosis of breast cancer at the University of Virginia (UVA) to include those diagnosed in 2000.
- Include: Postmenopausal by natural menopause or hysterectomy with bilateral oophorectomy, and using estrogen and progestin for at least one year.

- Exclude: Premenopausal or perimenopausal, history of hysterectomy without bilateral oophrectomy, HRT use prior to onset of natural menopause, use of estrogen alone, concurrent use of testosterone, women with implants, diagnosis of cancer prior to the index year.

Task 2. Identify potential controls (months 1 – 6).

- Use mammography database and radiology information system to identify controls
- Controls selected using same inclusion/exclusion criteria as cases.
- Controls will be frequency matched to cases in a 2 control: 1 case ratio by year of diagnosis, age (± 5 years), and time between pre- and post-HRT mammograms (± 6 months).

Task 3. Collect demographic and clinical data (months 7 – 12).

- Use medical records to obtain demographic data
- Collect age, time since menopause, duration of HRT use, parity, age at first childbirth, and height and weight to calculate Body Mass Index (BMI).

Task 4. Locate and select mammograms (months 7 – 12).

- Exclude women with mammograms from other institutions
- Anticipate locating films for approximately 122 cases
- Select pre-HRT mammogram (within one year prior to using HRT)
- Select post-HRT mammogram at least one year after onset of HRT use. Closest date will be used (within 5 years after onset of HRT use).

Task 5. Determine pre-HRT breast density, and the change in breast density with HRT use using digital assessment (months 13 - 18).

- Digitize pre- and post-HRT mammograms.
- Assess breast density of pre- and post-HRT mammograms using digital quantitative analysis to obtain the percentage of the breast occupied by breast tissue.
- Obtain the change in breast density by:

$$\frac{\% \text{ breast occupied by breast tissue}_{\text{post-HRT}}}{\% \text{ breast occupied by breast tissue}_{\text{pre-HRT}}} - \frac{\% \text{ breast occupied by breast tissue}_{\text{pre-HRT}}}{\% \text{ breast occupied by breast tissue}_{\text{pre-HRT}}} = \text{change in density}$$

Task 6. Analyze data and perform statistical analysis (months 19 – 24).

- Summarize patient characteristics for cases and control groups.
- Determine if these data provide evidence that women undergoing HRT who developed breast cancer are more likely to have an increase in mammographic breast density than those who did not develop breast cancer.

- Estimate the odds ratio and construct a 95% confidence interval around the point estimate with and without adjustment for confounding factors.

ACCOMPLISHMENTS:

In regards to Task 1, we have obtained lists of women diagnosed with breast cancer at UVA between 1991-2002 through our pathology department, via searching for both women who underwent mastectomy or lumpectomy during this time period. These lists are being cross-checked through our mammography biopsy database to ensure completeness of data collection. We have reviewed 1248 records to date. Of the reviewed cases, 1154 women have been excluded due to premenopausal or perimenopausal status, having no mammograms prior to cancer diagnosis at UVA, implants or other criteria as listed in Task 1. We identified 87 postmenopausal women using estrogen and progesterone at the time of cancer diagnosis (women using estrogen alone were excluded).

Collection of control subjects took much longer than anticipated (Task 2). We performed manual matching of cases. For each case, we did a search of our mammography database to obtain a list of names of women of age +/- 5 years at the same time as the case (+/- 1 month). The mammography charts were then pulled beginning at the top of the list until two eligible controls subjects were obtained. If no eligible control subjects were obtained, then a new search performed +/- 2 months (protocol calls for +/- 12 months), etc. until 2 control subjects were obtained. This process has been far more time intensive than originally planned. Two cases were diagnosed in 1991 and we were not able to identify controls. However, we have identified 2 control subjects for all of the remaining 85 cases.

Clinical and demographic data have been collected on all cases to date as this has been done at the time of case ascertainment (Task 3).

We have already excluded women that do not have prior mammograms at UVA (Task 4). All mammograms from cases and controls have been digitized (Task 5). The mammograms have been submitted to our biostatisticians to be relabeled (blinding) and randomized.

To better define what compromises a meaningful change in breast density, we performed a study evaluating breast density changes in postmenopausal women on HRT. The manuscript has been submitted to Academic Radiology for publication (see attached original manuscript).

The above results will be used in our study analysis, by defining a significant change in breast density as >3%.

KEY RESEARCH ACCOMPLISHMENTS:

- Reviewed 1248 records, obtaining 85 cases of women using estrogen and progesterone at the time of cancer diagnosis that meet inclusion criteria.

- Women without prior mammograms at UVA have been excluded.
- Clinical data has been obtained on all collected cases to date.
- Identified 2 control subjects for all 85 cases.
- Digitized all mammograms. Currently awaiting randomization.
- Established a scale of clinically meaningful change in breast density for women using HRT by visual and digital assessment techniques.

REPORTABLE OUTCOMES:

Scientific Presentations:

Harvey JA, Williams MB, Petroni G, Bovberg V. Increasing Mammographic Breast Density in Response to Hormone Replacement Therapy and Breast Cancer Risk. The Department of Defense Breast Cancer Research Program Meeting: Era of Hope, September 25-28, 2002, Orlando, Florida. (preliminary data presented)

Harvey JA, Williams MB, Petroni G, Bovberg V. Establishing a Scale of Clinically Meaningful Change in Breast Density in Women using Hormone Replacement Therapy Using Visual and Digital Assessment Techniques. 25th Annual San Antonio Breast Cancer Symposium, December 11-14, 2002, San Antonio, Texas. (preliminary data presented)

Publications:

1. **Harvey JA**, Bovberg V, Smolkin M, Williams MB, Petroni G. Evaluating Hormone Therapy Associated Increases in Breast Density: Comparison Between Reported and Simultaneous Assignment of BI-RADS Categories, Visual Assessment, and Quantitative Analysis. Acad Radiology (submitted).

CONCLUSIONS:

We have identified 85 cases of women using estrogen and progesterone at the time of cancer diagnosis and 170 controls (women using estrogen and progesterone not diagnosed with cancer in or before the matched year). All mammograms have been digitized and are being randomized in preparation for quantitative breast density assessment. In order to perform the density assessment, we developed a scale of meaningful change in breast density for women using HRT, which will aid in our data analysis. This manuscript has been submitted for publication. We anticipate completion of density assessment, data analysis, and manuscript preparation to be completed within

the next six months. Further funding of this project will not be needed to complete the study.

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APPENDICES:

Publications in preparation (final editing not complete!):

1. **Harvey JA**, Bovberg V, Smolkin M, Williams MB, Petroni G. Evaluating Hormone Therapy Associated Increases in Breast Density: Comparison Between Reported and Simultaneous Assignment of BI-RADS Categories, Visual Assessment, and Quantitative Analysis. *Acad Radiology* (submitted).

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Abstract: Rationale and Objectives: Changes in breast density, which are commonly associated with hormone replacement therapy (HRT) use, may imply changes in breast cancer risk. This study explores the ability of different methods to detect hormone replacement therapy (HRT) associated increases in breast density

Materials and Methods: Between 1997 and 2001, 51 postmenopausal women were reported to have HRT associated increases in breast density at our institution. Twenty postmenopausal women not reported to have an increase in density during the same period were selected as controls. Mammograms from date of report and earlier comparison were used. BI-RADS density categories from both dates were obtained from the mammography report. Mammograms were reviewed at separate time points and density changes evaluated by assigning BI-RADS density categories, visual assessment, and computer assisted quantitative analysis.

Results: Mammogram reports were not available for two patients. The remaining 49 women with reported HRT increases in density were included. Reported BI-RADS categories resulted in detection of 57%, simultaneous BI-RADS assignment in 61%, visual assessment in 100%, and quantitative assessment in 94% of women with HRT associated increases in density. Reported BI-RADS category change was the only method that resulted in false positive increases in density for control patients. Minimal HRT associated increases in density were the most difficult to detect, with 90% of these 21 cases not detected by simultaneous BI-RADS category assignment and 3 cases not detected by quantitative methods when defined as an increase of at least 5%.

Conclusion: Visual and quantitative assessment best identified women with HRT associated increases in density, including those with minimal increases. Simultaneous assignment of BI-RADS categories was considerably better than use of reported BI-RADS categories. This information may be helpful in guiding research design of studies evaluating changes in density due the HRT use.

January 21, 2005

Stanley Baum, MD, Editor-in-Chief
Academic Radiology Editorial Office
University of Pennsylvania
3600 Science Center, Suite 370
3600 Market Street
Philadelphia, PA 19104

Dear Dr. Baum,

We humbly submit an original work, "Evaluating Hormone Therapy Associated Increases in Breast Density: Comparison Between Reported and Simultaneous Assignment of BI-RADS Categories, Visual Assessment, and Quantitative Analysis" for consideration of publication in *Academic Radiology*. We look forward to your review of our work.

Thank you for your consideration.

Sincerely,

Jennifer A. Harvey, M.D.
Associate Professor of Radiology
Director, Division of Breast Imaging
University of Virginia

**Evaluating Hormone Therapy Associated Increases in Breast Density:
Comparison Between Reported and Simultaneous Assignment of BI-RADS
Categories, Visual Assessment, and Quantitative Analysis**

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**Evaluating Hormone Therapy Associated Increases in Breast Density:
Comparison Between Reported and Simultaneous Assignment of BI-RADS
Categories, Visual Assessment, and Quantitative Analysis**

Rationale and Objectives: Changes in breast density, which are commonly associated with hormone replacement therapy (HRT) use, may imply changes in breast cancer risk. This study explores the ability of different methods to detect hormone replacement therapy (HRT) associated increases in breast density

Materials and Methods: Between 1997 and 2001, 51 postmenopausal women were reported to have HRT associated increases in breast density at our institution. Twenty postmenopausal women not reported to have an increase in density during the same period were selected as controls. Mammograms from date of report and earlier comparison were used. BI-RADS density categories from both dates were obtained from the mammography report. Mammograms were reviewed at separate time points and density changes evaluated by assigning BI-RADS density categories, visual assessment, and computer assisted quantitative analysis.

Results: Mammogram reports were not available for two patients. The remaining 49 women with reported HRT increases in density were included. Reported BI-RADS categories resulted in detection of 57%, simultaneous BI-RADS assignment in 61%, visual assessment in 100%, and quantitative assessment in 94% of women with HRT associated increases in density. Reported BI-RADS category change was the only method that resulted in false positive increases in

density for control patients. Minimal HRT associated increases in density were the most difficult to detect, with 90% of these 21 cases not detected by simultaneous BI-RADS category assignment and 3 cases not detected by quantitative methods when defined as an increase of at least 5%.

Conclusion: Visual and quantitative assessment best identified women with HRT associated increases in density, including those with minimal increases. Simultaneous assignment of BI-RADS categories was considerably better than use of reported BI-RADS categories. This information may be helpful in guiding research design of studies evaluating changes in density due the HRT use.

Key words: Breast density; parenchymal pattern; Breast cancer risk; hormone replacement therapy; mammography

**Evaluating Hormone Therapy Associated Increases in Breast Density:
Comparison Between Reported and Simultaneous Assignment of BI-RADS
Categories, Visual Assessment, and Quantitative Analysis**

Women with high mammographic breast density are at greater risk for development of breast cancer compared to those with little or no breast density (1). Changes in breast density may therefore alter risk of development of breast cancer. Van Gils et al demonstrated an odds ratio (OR) for development of breast cancer of 5.7 for women that were 5-25% density at baseline that was not changed at 10 years of follow up compared to 1.9 OR for women initially 5-25% density that decreased to <5% density over 10 years (2). However, the confidence intervals were quite broad and overlapped in this small study.

The concept that risk may change with changes in breast density over time is intriguing as hormone replacement therapy (HRT) is known to increase both breast cancer risk (3-6) and breast density (7-11), while tamoxifen and raloxifene both reduce breast cancer risk (12,13) and breast density (14-16). This raises the question of whether women who increase in breast density while using HRT subsequently increase breast cancer risk, while those that do not change in density while using HRT may not increase breast cancer risk. However, differences in technical factors, such as degree of compression, film exposure, and contrast, and acquisition method (film-screen vs. digital mammography) could also affect changes in density. Studying changes in mammographic breast

density over time in large populations of women using HRT would be useful to address this question.

Changes in mammographic breast density can be evaluated in several ways. The most practical means of evaluating large populations for change in breast density is to use reported Breast Imaging Reporting and Data System (BI-RADS) (17) density categories, as this is typically included in standard mammography reports bypassing the need to retrieve mammograms. However, BI-RADS categories have been descriptive in definition until late 2003 when quantitative definitions were issued (17). Not unexpectedly, assignment of BI-RADS density categories has only moderate agreement (kappa statistic 0.43) (18). Change in BI-RADS category may therefore be due to a true change or to variation between readers.

Retrieval of the mammograms allows evaluation of change in density with simultaneous assignment of BI-RADS categories, visual assessment, or quantitative methods. Side-by-side display with simultaneous assignment of BI-RADS categories may reduce changes due to inter-reader variation. However, small changes in density may not be registered a change since BI-RADS categories are relatively broad. Visual assessment would likely be more sensitive to small changes in density than use of BI-RADS categories. Quantitative methods are likely the most reproducible and sensitive, although more time consuming because of the need to digitize images (19). Evaluation of these

techniques may provide guidance for future studies comparing changes in density over time for women using HRT.

In this exploratory study, we evaluate reported BI-RADS categories, simultaneously assigned BI-RADS categories, visual assessment, and quantitative analysis to detect HRT associated increases in breast density over time compared to women not reported to change in density. We then compare the results of these methods to explore strengths and limitations of each.

Materials and Methods:

This retrospective study was given exempt status by our institutional review board. At our institution, observed increases in mammographic breast density secondary to HRT use are noted in the mammogram report. Four attending radiologists with 4 or more years of experience in mammography staffed breast imaging during this time period. Using this information in our computerized database, we identified 51 postmenopausal women during 1997-2001 with increased breast density associated with HRT use (cases). Twenty consecutive postmenopausal women undergoing mammography during a three day period in December 2001 who had at least two mammograms between 1997 and 2000 with no reported change in density during that time period were selected as a control group.

Both the mammogram noting a change in density (index) and a preceding (baseline) mammogram were used. For cases, the preceding mammogram used for comparison according to the mammography report for the index mammogram was used as the baseline. For controls, the most recent mammogram was used in addition to a baseline mammogram that was selected as 1 to 5 years before the matched mammogram depending upon what was available for each patient. An interval of two or more years was preferred to a one year interval as this is our standard when interpreting mammograms at our institution.

Breast density was assessed for index and baseline mammograms using four different methods. All readings were performed in a blinded fashion without knowledge of whether a change in breast density had been reported or not. Readings were performed with at least two weeks between assessments to reduce memory bias.

Reported BI-RADS density categories. BI-RADS breast density categories were recorded from the mammography reports for index and baseline mammograms as: almost entirely fatty, scattered fibroglandular densities, heterogeneously dense, or extremely dense (17).

Simultaneous assignment of BI-RADS density categories. Both index and baseline mammograms were examined simultaneously with the sequence known

to the reader. BI-RADS density categories for both mammograms were assigned by one reader (JAH) with 10 years of experience in breast imaging.

Visual assessment. Mammograms were compared side-by-side with the sequence known to the reader (JAH). Increases in density were categorized as minimal, moderate, marked, or no change.

Quantitative assessment. The mammograms were digitized using a high-resolution Lumisys 75 scanner at 8-bit depth, 2K by 2.8K, optical density range 0.0-3.8, and at 50-micron pitch. Images were reduced in size (12 inch height) in order to view the entire image on the monitor. Only the left craniocaudal views were used to assess quantitative breast density since one view has been shown to be accurate for assessing breast density (20). Computer assisted measurement of percent breast density (20) was performed by one radiologist (JAH) with 3 years of experience in this technique. Index and baseline mammograms were compared side by side on a monitor with the older mammogram on the left and the more recent mammogram on the right. Simultaneous viewing of mammograms has been shown to reduce variability when evaluating changes over time (21). An increase in density was considered to be greater than or equal to 5%.

Statistical analysis

T-tests (for continuous measures) and chi-square tests (for categorical measures) were used to assess differences in study parameters between cases and controls. ANOVA was used to examine differences in quantitative density increase across levels of visual assessment. Kappa statistic and 95% confidence intervals were used to assess rating agreement between reported and simultaneous assigned BI-RADS categories. Spearman rank correlations were used to assess correlation among the four methods. Two-sided $\alpha=0.05$ level tests were used to determine statistical significance.

Results

Mammogram reports were not available for two patients who were excluded from study. The study population includes the remaining 49 cases and 20 controls. The mean age of those with a change in breast density was 59.5 years (range 41 – 77 years), which was similar to those without changes in breast density (mean age 60.3 years, range 49 – 74 years). The time interval from baseline to index mammogram was significantly shorter for those with HRT increases in breast density than control patients (22.8 versus 30.5 months respectively, $p=0.014$).

1. Detection of Increases in Breast Density

The distribution of reported BI-RADS categories, simultaneous assignment of BI-RADS density categories, visual assessment, and quantitative assessment between those with HRT-associated increases in density and those with no reported change is shown in Table 1.

Reported BI-RADS categories. Of the 49 women reported to have HRT-associated increases in breast density, only 28 women (57%) had an increase in reported BI-RADS density category, while 6 of the 20 women (30%) with no change in breast density also increased in reported BI-RADS category (Table 1). Detection of HRT associated increase in broader terms was similar. Twenty-two women (45%) with HRT associated increases in density changed from lower (fatty or scattered densities) to higher (heterogeneously or extremely dense), as did 5 control patients (25%) by reported BI-RADS categories.

Simultaneous assignment of BI-RADS density categories. Thirty of the 49 women (61%) reported to have HRT associated increases in density increased in assigned BI-RADS categories (Table 1). However, unlike use of reported BI-RADS categories, all women in the control group had no change in BI-RADS category when simultaneously assigned.

Visual assessment. All 49 women with reported increases in breast density and all 20 women with no reported increase in density were correctly classified by visual assessment (Table 1). Of interest, 21 women (43%) with HRT associated increases in breast density were identified as having minimal increases by visual assessment, which is similar to the number of women with no change in category by reported or simultaneous assignment of BI-RADS category (Table 1). At the other extreme, 12 women (25%) with HRT associated increases in density had

marked increases by visual assessment, which is considerably larger than the number of women identified as having increases of two categories by using either reported or simultaneous assignment of BI-RADS categories (Table 1).

Quantitative assessment. Forty-six of the 49 women (94%) with reported HRT associated increases in breast density had increases of greater than 5% (Table 1). Women with reported HRT associated increases in breast density had a mean increase in breast density of 19.7% (range 2.0 – 54.8%). All 20 women with no reported increases in density had changes of less than 5% (mean 0.2%, range –4.6 – 4.7%).

II. Assessment among methods

Reported BI-RADS categories. Of women with HRT associated increases in density, the distribution in BI-RADS category was similar whether using reported or simultaneously assigned BI-RADS categories. However, agreement between methods was poor (simple kappa=0.17, 95%CI (–0.07, 0.41); weighted kappa=0.25 (0.03, 0.48)) with perfect agreement occurring in only 25 of 49 (51%) of cases. Correlation with visual assessment was poor as well ($r=0.39$) where 4 of 12 women considered to have marked increases in breast density by visual assessment had no change in reported BI-RADS categories (Tables 2 and 3). Correlation with quantitative assessment shows similar findings (Table 3).

Women with HRT associated increases in breast density (cases) and an increase in reported BI-RADS category had a mean quantitative increase in percent breast density of 21.6%, which was not significantly different than the 17.6% mean increase in breast density seen for cases with no change in reported BI-RADS category ($p=0.33$)(Table 1). Among women not reported to have changes in density (controls), mean breast density change for those with no change in reported BI-RADS category and those with an increase in BI-RADS category was less than 1% each. However, use of reported BI-RADS categories did demonstrate a difference using quantitative assessment between women with HRT associated density change that increased by two reported BI-RADS categories (mean increase 33.2%) compared to women that increased by only one reported BI-RADS category (mean increase 19.0%) ($p=0.03$) (Table 1).

Thus, because of the high false positive rate, use of reported BI-RADS categories did not significantly differentiate between women with HRT associated increases in breast density in women and women with no changes in density. Likewise, an increase in reported BI-RADS category did not identify women with larger HRT associated increases in density compared to those with no change in reported BI-RADS category.

Simultaneous assigned BI-RADS. Simultaneous assignment of BI-RADS categories correlated better ($r=0.86$) with visual assessment than reported BI-RADS categories (Table 3). However, 19 of 21 women (90%) with minimal

increases in breast density by visual assessment had no change in assigned BI-RADS category. These 19 women accounted for 39% of all women with HRT associated increases in density.

Quantitative assessment also correlated ($r=0.78$) better when BI-RADS categories were assigned simultaneously. For women with HRT associated increases in density and no change in simultaneous assignment of BI-RADS categories, increase in density was smaller (mean 9.1%) than for those with an increase in assigned category (mean 26.4%) (Table 1). Likewise, women with HRT associated increases in density and an increase of two assigned BI-RADS categories had a higher mean increase in density (42.3%) compared to those with an increase of only one assigned BI-RADS category (22.8%) (Table 1).

Visual Assessment. Quantitative increases in breast density for no change, and minimal, moderate, marked increases in breast density by visual assessment were significantly different from each other ($p<0.001$, ANOVA) (Table 1) and highly correlated ($r=0.92$) (Table 3). This method best detected small focal increases in density.

Quantitative Assessment. Three cases of HRT associated increases in breast density were not detected by quantitative methods, but were considered minimal increases by visual assessment (Table 1). The reports of these three cases with quantitative increases of less than 5% indicated interval development of focal

asymmetric densities thought due to HRT use. These patients underwent short-term HRT cessation with decrease in size or resolution of the opacities (22).

Discussion

This exploratory study gives insight into the complexities of evaluating HRT associated increases in breast density. The use of reported BI-RADS categories in this study was limited by the high number of false positives. Review of mammograms with use of simultaneous assignment of BI-RADS categories significantly improves results compared to reported BI-RADS categories, but does not detect small increases in density well. Visual assessment outperformed quantitative methods, although the few cases not detected by quantitative methods in this study were new small focal opacities rather than the more common diffuse increase in density and may not be as important when evaluating potential changes in cancer risk. In addition, one would anticipate visual assessment to perform well in this work since cases were selected based on visual assessment at the time of mammography reporting.

Changes in reported BI-RADS categories for evaluating HRT associated increases in breast density would be extremely useful for studying large populations of women. However, it is concerning that our study, though small, showed a lack of significant difference in quantitative change in breast density between women that did and did not increase in reported BI-RADS categories. Using an increase in reported BI-RADS category did not identify women with

larger HRT associated increases in breast density than those with no change in reported BI-RADS category. In our study, women with HRT associated increases in density had a mean increase in breast density of 18% when the reported BI-RADS category was the same, and 19% when the BI-RADS category increased by one category. On the other hand, an increase of two reported BI-RADS categories was associated with a mean increase in breast density of 33%, although this only represented 10% of women with HRT associated increases in density in our study. If reported BI-RADS categories are used to analyze large populations, comparison of increase of two categories to no change or decrease may more accurately reflect risk associations of women with the most obvious HRT associated increases in density, realizing that this represents a small fraction of women with HRT associated increases in density but those with the greatest changes by visual and quantitative measures.

Although use of reported BI-RADS categories clearly has limitations, retrieval of mammograms to perform simultaneous assignment of BI-RADS categories, visual or quantitative assessment may be impractical or impossible in some populations. In these groups, changes in BI-RADS density categories may be the only reasonable method of evaluation. However, although our study has clear limitations, the results may be useful in guiding study planning, performing power analyses to determine study population size, and data analysis when reported BI-RADS categories are to be used to evaluate density changes in large populations. For example, about 5% of women undergoing estrogen replacement

and about 25% of women undergoing estrogen and progesterone therapy will increase in mammographic breast density by visual assessment (10). In our study, only 57% of women with HRT associated increases in density also increased in reported BI-RAD category. Therefore, any examined effect using reported BI-RADS categories to assess HRT associated increases will be considerably diluted by the 30% of women with no HRT associated change that likewise increase in reported BI-RADS category. More women with an increase in reported BI-RADS category will have an increase due to variations in reading rather than an increase in mammographic breast density as seen by visual assessment. The effect will be worse for populations with lower expected HRT-associated increases in density where change in BI-RADS density categories due to inter-reader variability may outweigh HRT-associated increases that may be detected by other methods.

Retrieval of mammograms with simultaneous assignment of BI-RADS categories resulted in improved detection of moderate and marked increases in breast density as detected by visual assessment and quantitative assessment. Women not detected as increased in density with simultaneous assignment of BI-RADS categories largely corresponded to women with minimal increases by visual assessment. In this series, 43% of women with HRT associated increases in density had only minimal increases by visual assessment, and 90% of these did not change in simultaneous assignment of BI-RADS categories. It could be argued however that since the change in breast density is minimal by visual

assessment and percent increase in density is small by quantitative assessment that the change is not clinically important and exclusion is reasonable.

In our study, visual assessment detected slightly more cases of HRT associated increased density than quantitative assessment. All cases with minimal increases in density not detected using simultaneously assigned BI-RADS categories were detected by visual methods, and most were detected using quantitative methods. However, it is not surprising that visual assessment of increases in breast density proved best in our study since cases were identified as those reported to have HRT associated increases in breast density by a radiologist's visual assessment. Thus, the slightly better results in our study of visual assessment over quantitative methods may be due to case selection bias. However, the difference in results between the quantitative and visual assessment methods was very small. In addition, visual assessment was performed by only one reader and results should be validated by evaluating intra-reader and inter-reader variability for this technique. Visual methods are very reproducible in the evaluation of breast density at one point in time (1), so it is likely that this method would also be reproducible in assessing changes over time.

Quantitative methods are very reproducible at assessing breast density at one point in time with agreement of greater than 90% (20). In our study, quantitative methods detected 94% of women with HRT associated increases in density using a criteria of 5% or greater increase. Since the three cases that were not

detected using this method had focal increases of less than 5%, they may not be important in studies of correlation with breast cancer risk.

Quantitative assessment however is cumbersome to perform for large populations as film mammograms must be retrieved, digitized, windowed, and converted to a specific file format. Automated measurement of breast density is being developed (23-26) and will greatly improve the ability to assess breast density changes over time in large populations though these methods will need to be validated and become more widely available. Breast density could then be assessed prospectively in a reproducible fashion on large populations. This is an optimal application of digital mammography as the image is already in digital format negating the need to digitize film-screen mammograms.

A limitation of this study is that cases used were those reported to have an HRT associated change in breast density rather than sequential women undergoing HRT. These cases were likely reported with this finding because the change was thought clinically important, e.g. a focal increase in density that could be mistaken for a potentially malignant process or obvious large change that may result in clinical symptoms of breast tenderness. Thus, it would be useful to repeat this study prospectively evaluating a large number of women on HRT. The type of HRT would be important to evaluate however as the percentage of women with HRT associated increases in density is known to differ by regimen (27). In addition, the time difference between baseline and index mammograms

was significantly longer in our study for women without increases in density. It is unclear why this would occur, although women with HRT associated increases in density may have been obtaining more frequent mammography due to greater symptoms than women that did not change in density since HRT associated increases in breast density is frequently associated with breast pain (9).

Two studies have used BI-RADS categories to assess changes in breast density over time. Rutter et al (28) used reported BI-RADS categories to evaluate changes in breast density in 5212 postmenopausal women in an observational cohort study in Washington State with initiation, discontinuation, and continuing use of HRT. In this study, women with a change from fatty or scattered to heterogeneously or extremely dense were considered to have increased in density, while other changes in BI-RADS categories were not considered a change. Of women that were nonusers of HRT at both exams, 11.6% increased in density by the above criteria. This result is somewhat lower than our study, which found that 25% of controls had a similar change from lower (fatty or scattered densities) to higher (heterogeneous or extremely dense) reported BI-RADS categories. The study by Rutter et al also found that 28% of women increased by the above criteria at the initiation of HRT (28). In our study, only 45% of women with reported HRT associated increases in density had this specific change in BI-RADS categories, indicating that many women with HRT associated increases in density were likely not identified by comparing changes of "lower" to "higher" BI-RADS categories. However, the use of reported BI-

RADS categories in the study by Rutter et al (28) is the only practical method to evaluate a population of this size, realizing that the odds ratio of change will likely be underestimated.

Greendale et al (29) evaluated baseline and mammograms obtained after one year of HRT use from women participating in the Postmenopausal Estrogen/Progestosterone Interventions (PEPI) trial. In this study, mammograms were collected and centrally read with both mammograms read side-by-side with sequence of dates known to the reader. BI-RADS categories were assigned to each mammogram simultaneously. This study showed no density category changes for women on placebo or those using only conjugated equine estrogen (CEE), while 19.4% of women using CEE with continuous progesterone and 23.5% of women using CEE with cyclic progesterone increased in simultaneously assigned BI-RADS categories. These results are similar to our study, finding no density category changes for those on placebo. However, given our finding of only 61% of women with HRT associated increases in density demonstrating an increase of simultaneously assigned BI-RADS categories, the reported percentages of women with increases in density may be underestimated compared to visual or quantitative methods.

Persson et al, used visual methods to assess HRT associated increases in breast density and found that 3% of nonusers, 5% of women using estradiol alone, 10% of women using estrogen with cyclic progesterone, and 28% of

women using continuous progesterone increased in breast density (10). These findings support the results of our study given the small number of false positives in non-users. This study found that 5% of women using estradiol alone had increases in breast density whereas no changes were seen with the Greendale study (29). This may be due to improved detection of small increases in density with visual assessment used in the Persson study.

Greendale et al followed with a study evaluating the mammograms of women participating in the PEPI trial using quantitative assessment (30). This study found a mean increase in breast density of 4.8% with use of estrogen with cyclic progesterone and 3.3% increase with use of estrogen with continuous progesterone. Our study shows a mean increase of 19.7% for women with HRT associated increases in density, but this does not factor in the large percentage of women that use HRT without a change in density. The study also reports that a change of BI-RADS fatty to scattered densities corresponded to a mean increase in breast density of 18.2%, while a change of BI-RADS scattered to heterogeneously dense corresponded to a mean increase in density of 14.4% (30). Our study showed that an increase in prospectively assigned BI-RADS category in women reported with HRT associated increases in breast density had a mean increase of 22.5%. Our higher value may be due to the study population of women with a reported HRT associated increase in breast density. This small difference may be due to selection bias as previously discussed.

Changes in breast density may reflect changes in breast cancer risk. Use of reported BI-RADS breast density categories does not accurately identify women with HRT-associated increases in density. In our study only 57% of women with reported HRT associated increases in breast density increased in reported BI-RADS density category, while 30% of women with no reported change in breast density also increased in BI-RADS density category. Retrieval of mammograms with simultaneous assignment of BI-RADS categories is more accurate but excludes women with minimal HRT associated increases in breast density as detected by visual assessment. Minimal increases in breast density accounted for 43% of the patients with HRT associated increases in density in this study. Visual assessment proved most accurate in detecting HRT associated increases in breast density in our study. However, our study is limited by use of only one reader for this analysis and case selection criteria. Further validation of this method by assessing intra-reader and inter-reader variability would be useful to confirm the high performance of the visual assessment method seen in our study. Quantitative methods are highly reproducible but currently time-consuming. Automated methods of quantifying breast density will eventually allow assessment of breast density to become more practical in large populations.

Visual and quantitative methods in this small exploratory study are more accurate than either reported or simultaneous assignment of BI-RADS categories for detecting HRT associated increases in breast density. Simultaneous assignment

of BI-RADS categories is considerably better than use of reported BI-RADS. Visual and quantitative methods are particularly useful for detecting minimal increases in breast density. These results may be helpful in planning research studies evaluating changes in breast density in women using HRT.

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Table 1. Frequency and mean percent breast density change (by quantitative method) by use of BI-RADS and visual assessment for women reported and not reported to have HRT-associated increases in breast density

	HRT-Associated Increase in Density		Control	
	N (%)	Mean % change in breast density	N (%)	Mean % change in breast density
Overall	49	19.9	20	0.2
Reported BI-RADS				
Decrease in category	0	-	3 (15%)	0.6
No change in category	21 (43%)	17.6	11 (55)	-0.3
Increase in BI-RADS category	28 (57%)	21.6	6 (30%)	0.9
Increase by 1	23 (47%)	19.0	6 (30%)	0.9
Increase by 2	5 (10%)	33.2	0	-
Simultaneous BI-RADS				
Decrease in category	0	-	0	-
No change in category	19 (39%)	9.1	20 (100%)	0.2
Increase in BI-RADS category	30 (61%)	26.4	0	-
Increase by 1	24 (49%)	22.8	0	-
Increase by 2	6 (12%)	42.3	0	-
Visual Assessment				
No Change	0	-	20 (100%)	0.2
Minimal Increase	21 (43%)	8.8	0	-
Moderate Increase	16 (33%)	19.1	0	-
Marked Increase	12 (25%)	40.2	0	-
Quantitative				
No change (<5%)	3 (6%)	3.4	20 (100%)	0.2
Increase (≥ 5%)	46 (94%)	20.9	0	-

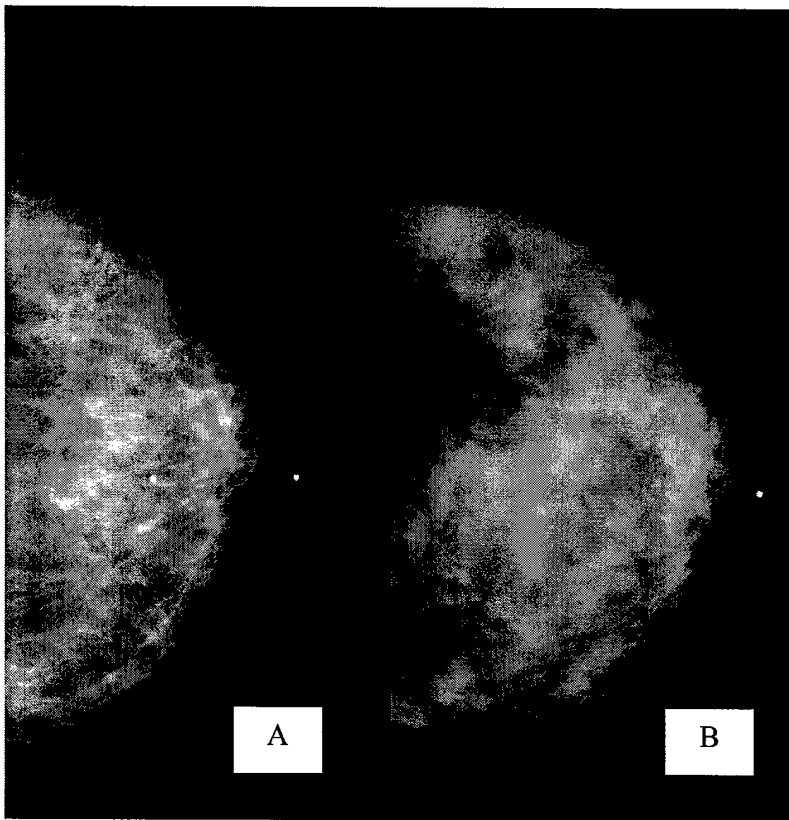
Table 2. Comparison of visual assessment of change in breast density with use of BI-RADS categories in women with HRT associated increases in density compared to women with no reported change in density (control).

	Control	HRT-Associated Increase in Density		
	No Change (n=20)	Minimal (n=21)	Moderate (n=16)	Marked (n=12)
Reported BI-RADS				
Decrease or no change in category	14	12	5	4
Increase of 1 category	6	9	9	5
Increase of 2 categories	0	0	2	3
Simultaneous BI-RADS Assignment				
Decrease or no change in category	20	19	0	0
Increase of 1 category	0	2	15	7
Increase of 2 categories	0	0	1	5

Table 3. Spearman rank correlations among the four assessment methods

	Simultaneous BI-RADS	Visual	Quantitative
	r (p-value)	r (p-value)	r (p-value)
Reported BI-RADS	0.34 (0.016)	0.30 (0.037)	0.27 (0.060)
Simultaneous BI-RADS		0.86 (<0.001)	0.78 (<0.001)
Visual			0.92 (<0.001)

Figure 1. Craniocaudal view of the left breast of a 66-year-old woman at baseline (A) and one year after starting oral estrogen and progesterone (B). BI-RADS categories were reported as heterogeneously moderately dense for both studies. Simultaneous assignment of BI-RADS categories was scattered fibroglandular densities and extremely dense respectively. Visual assessment was marked increase in density. Quantitative assessment was 21% dense at baseline and 76% dense one year later (55% increase in density).



**Evaluating Hormone Therapy Associated Increases in Breast Density:
Comparison Between Reported and Simultaneous Assignment of BI-RADS
Categories, Visual Assessment, and Quantitative Analysis**

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Figure 1A
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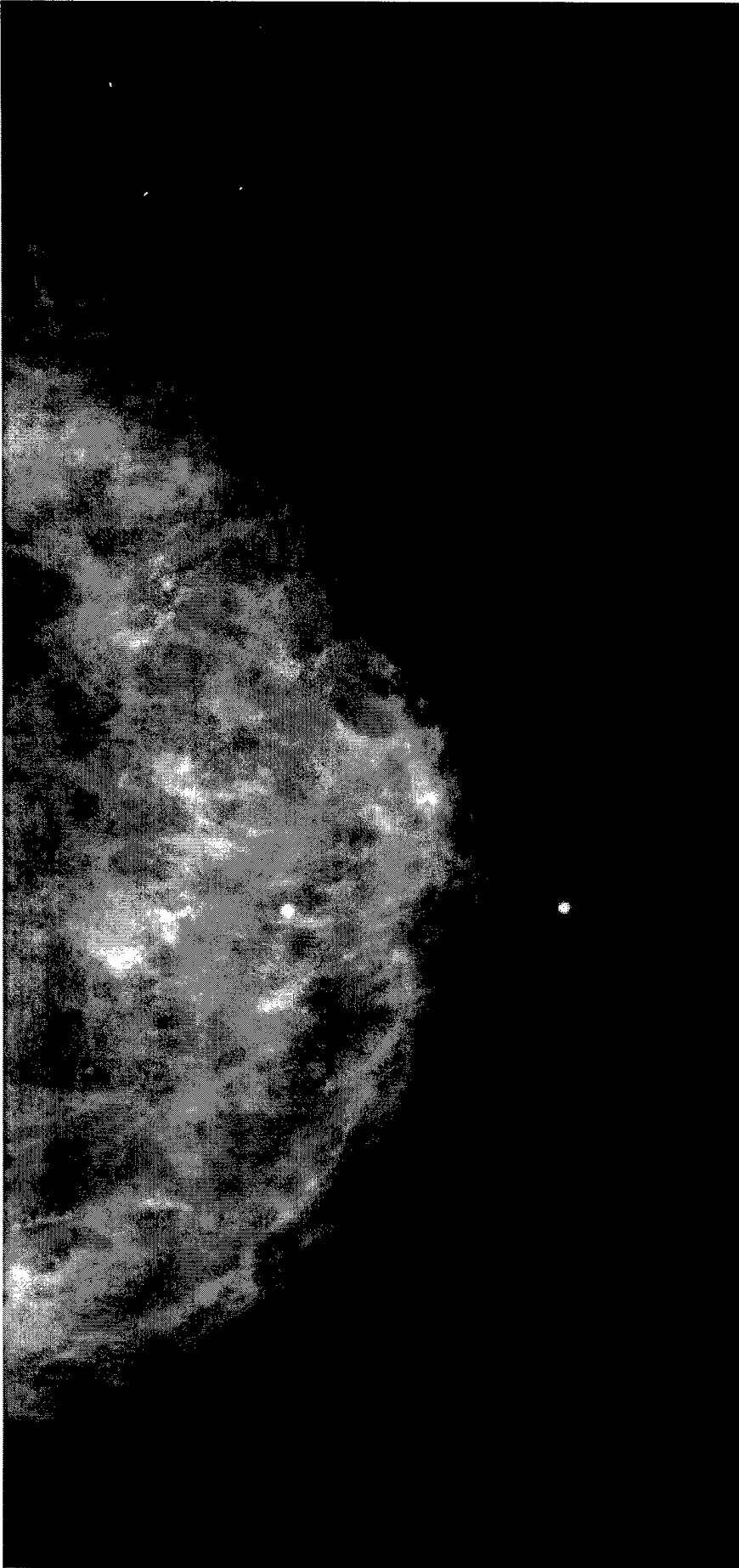


Figure 1B

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