Breast Density: Biomarker of BC risk

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Brussels
To discuss

- Definition/Biology
- Methods to measure BMD
- BMD and risk of BC
- Factors influencing BMD
- BMD and menopause

**BMD**: Breast Mammographic Density

F. L. BMS 13 03 2010
Definition

**Mammographic Breast Density (MBD)**

- a measure of the extent of radiodense fibroglandular tissue in the breast,
- reflects variation in fat, stromal, and epithelial tissues
- estimates the proportion of fibro glandular tissue relative to fat.
Percentage mammographic density, age, and histological measures.

Histologic markers of mammographic breast density: Core-needle biopsy tissue from healthy volunteers.

- First report of a tissue-based study of BD,
  - sampling mammographically dense and non-dense areas of the breast of healthy women (mean age: 50.1 y; range 40 to 79 y)
- Goal: to examine histologic correlates.
- Quantitative assessment of breast tissue from dense and non-dense areas for epithelium, stroma, and fat.
  - Assessment of the difference in the extent of lobular involution (complete, partial and none) between dense and non-dense areas.

Ghosh K. et al. SABCC Abstract 2008

F. L. BMS 13 03 2010
Histologic markers of mammographic breast density:
Core-needle biopsy tissue from healthy volunteers.

- **Dense tissue**
  - increased stroma and epithelium
  - decreased fat.
- 'no involution': greater in dense tissue compared to non-dense tissue (24% versus 8.8%);
- non-dense tissue: greater proportion of complete involution compared to dense tissue (82.5% versus 35.2%).

Ghosh K. et al. SABCC Abstract 2008
Novel Breast Tissue Feature Strongly Associated With Risk of Breast Cancer

- Quantify the extent of **lobule regression** on a benign breast biopsy
  - 85 patients who developed breast cancer and 142 age-matched controls from the Mayo Benign Breast Disease Cohort,

- **Results**
  - A step-wise increase in breast cancer risk with increasing numbers of acini per lobule ($P = .0004$).
  - Adjusting for Gail model score, parity, histology, and family history did not attenuate this association.

**Definition / Biology**

- Epithelium unlikely to account for the majority of the density
- Stroma plays a major role
  - Fibroblasts, myofibroblasts, endothelial cells, pericytes, macrophages, neutrophils and lymphocytes.
  - Different matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs are synthesized by stromal cells.

Mammographic BD as an intermediate phenotype for breast cancer

- Twin studies: proportion of the breast occupied by density, at a given age, is **highly heritable**,
  - inherited factors explain 63% of the variance.
- **Single-nucleotide polymorphisms (SNPs)** related to MBD:
  - Genetic Polymorphisms Involved in Insulin-like Growth Factor (IGF) Pathway
  - Variants associated with BC risk
  - Genes encoding sex steroid metabolism enzymes and ESRs

**References**

- **Diorio C.** Cancer Epidemiol Biomarkers Prev 2008;17(4).
- **Odefrey F et al.** Cancer Research 2010: 70, 1449
- **Douglas JA** et al. Cancer Epidemiol Biomarkers Prev 2008; 17:3509
To discuss

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# Measurement of mammographic density

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<thead>
<tr>
<th>Qualitative density assessment</th>
<th>Wolfe BIRAD ACR</th>
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<td>Quantitative techniques</td>
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<thead>
<tr>
<th>Bi-Rads</th>
<th>Wolfe</th>
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<tr>
<td>ACR</td>
<td>Wolfe</td>
</tr>
<tr>
<td>BIRADS-1</td>
<td>Predominantly fatty breast (FGD &lt; 5-10%)</td>
</tr>
<tr>
<td>Heterogeneously fatty</td>
<td></td>
</tr>
<tr>
<td>BIRADS-2</td>
<td>Scattered fibroglandular densities (FGD) (FGD &lt; 25%)</td>
</tr>
<tr>
<td>Heterogeneously fatty</td>
<td></td>
</tr>
<tr>
<td>BIRADS-3</td>
<td>Heterogeneously dense</td>
</tr>
<tr>
<td>greater levels of prominence of fibrous tissue</td>
<td></td>
</tr>
<tr>
<td>BIRADS-4</td>
<td>Homogeneously dense</td>
</tr>
<tr>
<td>dense sheets of fibroglandular tissue</td>
<td></td>
</tr>
</tbody>
</table>

F. L. BMS 13 03 2010
fatty radiolucent breast
<10% of dense tissue

Scattered fibroglandular densities
<25% of dense tissue

Heterogeneously dense

>75% of dense tissue
A six-category system for classifying mammographic density.

- (a) 0,
- (b) <10%,
- (c) 10–25%,
- (d) 26–50%,
- (e) 51–75%,
- (f) >75%.

Yaffe Breast Cancer Research 2008
# Measurement of mammographic density

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Calcul de densité

- Calcul en 5 étapes :
  - Image mammographie numérique
  - Segmentation générale
  - Segmentation muscle thoracique
  - Détermination région d'intérêt
  - Détermination signal référence
  - Calcul de densité à partir des valeurs de chaque pixel de l'image

- Résultat dans cette exemple : densité tissus de 73%
Calcul de densité

- Calcul en 5 étapes :
  - Image mammographie numérique
  - Segmentation générale
  - Segmentation muscle thoracique
  - Détermination région d’interet
  - Détermination signal référence
  - Calcul de densité à partir des valeurs de chaque pixel de l’image

- Résultat dans cette exemple : densité tissus de 39%
Agreement between computer-assisted quantitative measurement of mammographic density.

<table>
<thead>
<tr>
<th>Kappa (K) and Weighted Kappa (WK) (95% CI)</th>
<th>Reader 1</th>
<th>Reader 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reader 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K=0.441 (0.270-0.612)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agreement moderate</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Reader 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K=0.568 (0.395-0.741)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate agreement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WK=0.714</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agreement: good</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Reader 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K=0.308 (0.136-0.480)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agreement fair</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WK=0.553</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agreement moderate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K=0.362 (0.186-0.538)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agreement fair</td>
<td></td>
<td></td>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
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</tbody>
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BMD and qualitative measurement by clinicians

Liebens F. et al (abstract) EBCC 2008

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- Factors influencing BMD
- BDM and menopause
Risk of Breast Cancer According to Breast Density in Premenopausal and Postmenopausal Women

Age, mammographic density and the incidence of breast cancer

At all ages, percentage density was greater in those who developed breast cancer. Data from Boyd and coworkers, *N Engl J Med* 2007, **356**:227-236.
Association of dichotomized Wolfe grade, BIRADS, and Tabar classifications with breast cancer risk

- >14,000 cases and 226,000 non cases from 42 studies were included.
  - Associations consistent in studies conducted in the general population
    - highly heterogeneous in symptomatic populations.
  - They were much stronger for percentage density than for Wolfe grade or Breast Imaging Reporting and Data System classification
    - 20% to 30% stronger in studies of incident than of prevalent cancer.
Longitudinal Measurement of Clinical Mammographic Breast Density to Improve Estimation of Breast Cancer Risk

<table>
<thead>
<tr>
<th>Initial density</th>
<th>3 years later</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty</td>
<td>Fatty</td>
<td>1.0</td>
</tr>
<tr>
<td>Fatty</td>
<td>Scattered</td>
<td>1.9</td>
</tr>
<tr>
<td>Fatty</td>
<td>Hetero</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Two breast density measurements separated by an average of 3 years predicted the odds that a women would develop breast cancer more accurately than one measure.

Kerlikowske K et al. JNCI 2007
MBD and the prevalence and incidence of histological types of benign breast disease

Hyperplasia without atypia
RR: 13.85
95% CI 2.65 – 72.49

Atypical hyperplasia and/or carcinoma in situ
RR: 9.23
95% CI 1.66 – 51.48

BDM and recurrence after BC

- **N**: 335 Breast Conserving Surgery for whom a pretreatment mammogram was available
- Retrospective study
- High BDM = >50 % density
- 10-year actuarial risks: 21% (High BMD) vs 5% (Low BMD); [HR], 5.7 [95% CI, 1.6-20; \( P = .006 \)].

Association between Percent Mammographic Density and Subtypes of Breast Cancer? 
Luminal A and Triple-Negative Breast Cancer

- Question: To what extent mammographic density is a predictor for subtypes of BC defined by expression status of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER-2).
- Retrospective case control study
- N: 479 breast cancer patients and 376 control subjects ages 35 to 64 years.
- luminal A BC = ER+ or PR+ plus HER-2- tumors
- BMD is positively associated with both luminal A and triple-negative breast cancer.

Ma H et al; Cancer Epidemiol Biomarkers Prev 2009;18(2):479–85
Mammographic Density and Breast Cancer After Ductal Carcinoma *In Situ*

- N: 504 DCIS (NSABBP B-17)
- Cumulative incidence of any subsequent breast cancers, stratified by percent mammographic density (high >75% vs low <25%)
- RR = 3.2 (1.2-8.5)

BMD: impact on accuracy of screening mammography

87% Sensitivity  63%

97% Specificity  89%

BMD: impact on accuracy of screening mammography

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Breast Density

- Positively correlated with breast cancer
  - **Four to six** times greater risk of breast cancer for women with greater than 60% dense tissue
  - Breast density as a risk factor accounts for as many as 30% of breast cancer cases
- Density was not significantly associated with tumor characteristics
- Can be changed by hormonal, and other interventions
To discuss

- Definition/Biology
- Methods to measure BMD
- BMD and risk of BC
- Factors influencing BMD
- BDM and menopause
Percentile distributions of age-specific, cross-sectional trends in mammographic density, Minnesota Breast Cancer Family cohort, 1990-2003

## Factors influencing BMD

### Increase
- **Ethnicity** (differences consistent with those for BC risk)
- **City life** *(more pronounced in women < 50)*
- **Family history**

### Decrease
- **NSAID** *(continuers use)*
- **Tamoxifan**
- **Age**
- **BMI**
- **Tobacco**

### No change
- **Calcium and Vit D** *(Intake in childhood and young adult)*
- **Serum Vit D** *(in BC survivors)*
- **Dietary patterns**
- **Soy isoflavones**
- **Physical activity**
- **Raloxifan**
- **Letrozole**
- **High dose estrogens** *(childhood and adolescence)*

### References
- McCormick VA 2008; Maskarinec G 2006 review
- Perry NM 2007
- Martin LJ 2010
- Terry MB 2008
- Martin LJ 2009 review
- Kerlikowske K 1996
- Stone J 2009
- **Butler LM 2010**
- Mishra G 2008
- Neuhouser ML 2010
- Takata Y 2007
- Verheus M 2008
- Peters TM 2008
- Pearman LM 2009 review
- Cigler T 2009
- Jordan HL 2010
Factors influencing BMD

- Tamoxifen
- IBIS-1 trial in High-risk women (35 – 70 y)
- After 54 months
  - BMD 28.2% (↓ 13.7%, 95% CI = 12.3% to 15.1%; \( P < .001 \)) in the tamoxifen group and 35.3% (↓ 7.3%, 95% CI = 6.1% to 8.4%; \( P < .001 \)) in the placebo group.
  - Premenopausal (<45y)
    - BMD ↓ 13.4%
  - Menopausal (>55y)
    - BMD ↓ 1.1%

Factors influencing BMD

- **Tamoxifen**
- **High-risk women in IBIS-1 trial**
  - who had at least a 10% reduction in mammographic BD over the first 12 to 18 months of tamoxifen prophylaxis had a 63% reduction in breast cancer risk ($P=0.002$) whereas other women had no benefit ($P=0.89$).
  
- The benefit was even greater for women with precancerous atypical hyperplasia or lobular carcinoma in situ lesions

- If validated, BD changes would be the first biomarker that could be used by clinicians to determine who stands to benefit from continued tamoxifen prophylaxis.

Cuzick J, et al "Change in breast density as a biomarker of breast cancer risk reduction; results from IBIS-1" SABCS 2008; Abstract 61.
To discuss

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- Factors influencing BMD
- BDM and menopause (HRT)
Longitudinal patterns of mammographic density by postmenopausal hormone (PMH) use and age among postmenopausal women, Minnesota Breast Cancer Family cohort, 1990-2003

MBD and HRT

- Prospective cohort study
- N: 329,495 (40-89 y)
- 463,372 screening mammograms from 1996-1998

## Effect of HRT Regimens on MBD

### HRT AND MAMMOGRAPHIC BREAST DENSITY

**Table 3. Distribution of Women According to Changes in Mammographic Densities Between the Two Mammographic Examinations**

<table>
<thead>
<tr>
<th>Exposure Category</th>
<th>Density Reduction</th>
<th>Density Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moderate</td>
<td>Slight</td>
</tr>
<tr>
<td>Unexposed</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>553*</td>
<td>0.7</td>
<td>4</td>
</tr>
<tr>
<td>Exposed</td>
<td>551*</td>
<td>0.5</td>
</tr>
</tbody>
</table>

(1) Estradiol only (orally 2 mg or transcutaneously 50 μg/24 h)
(2) Estradiol (orally 2 mg + cyclically combined progestin)
(3) Estradiol (orally 2 mg + continuously combined progestin)
(4) Weak estrogen (vaginally)

| Estradiol (E2) only orally or transcutaneously | 5% |
| E2 + Progestin cyclically                    | 10%|
| E2 + Progestin continuously                 | 28%|
| Estrogen vaginally                           | 5% |

Conjugated Equine Estrogen in Postmenopausal Women

- Substudy of the Women's Health Initiative Randomized Trial

- **BMD:** mean $\uparrow$ 1.7 % (95% CI, 0.7 to 2.7) versus mean $\downarrow$ 1.2 % (95% CI, –1.8 to –0.5; $P < .001$) in the hormone and placebo groups, respectively.

- These effects were greater in women age 60 to 79 years ($P = .03$ for interaction across age).

Effect of HRT Regimens on MBD

MBD and HRT

- Increased baseline breast density is a risk factor for breast cancer. Boyd NF. *Lancet Oncol* 2005;6:798 (review)

- Combined E + P therapy may cause increased breast density in up to 50% of postmenopausal women, dependent on the regimen (dosage, type of progestogen).

- The average increase in breast density with standard dose is up to 10%.

Influence of patterns of HRT use and MBD on breast cancer detection.

<table>
<thead>
<tr>
<th>Mammographic density</th>
<th>Screen detected, N (%)</th>
<th>Missed interval</th>
<th>True interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>OR (95% CI)*</td>
<td></td>
</tr>
<tr>
<td>Radiologist-determined</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10%</td>
<td>78 (18.2)</td>
<td>9 (11.1) 1.00</td>
<td>24 (8.7)</td>
</tr>
<tr>
<td>10% to &lt;25%</td>
<td>98 (22.8)</td>
<td>11 (13.6) 1.51 (0.52-4.58)</td>
<td>49 (17.7) 1.63 (0.86-3.01)</td>
</tr>
<tr>
<td>25% to &lt;50%</td>
<td>149 (34.7)</td>
<td>31 (38.3) 2.89 (1.03-8.15)</td>
<td>78 (28.2) 1.65 (0.90-3.03)</td>
</tr>
<tr>
<td>50% to &lt;75%</td>
<td>96 (22.4)</td>
<td>28 (34.6) 3.51 (1.19-10.42)</td>
<td>106 (38.3) 3.73 (1.96-7.11)</td>
</tr>
<tr>
<td>≥75%</td>
<td>8 (1.9)</td>
<td>2 (2.5) 4.06 (0.49-33.45)</td>
<td>20 (7.2) 7.76 (2.28-26.48)</td>
</tr>
<tr>
<td>Computer assisted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10%</td>
<td>115 (27.1)</td>
<td>22 (27.5) 1.62 (0.67-3.91)</td>
<td>71 (25.9) 1.44 (0.86-2.42)</td>
</tr>
<tr>
<td>10% to &lt;25%</td>
<td>134 (31.5)</td>
<td>40 (50.0) 2.57 (1.09-6.02)</td>
<td>126 (46.0) 2.45 (1.44-4.17)</td>
</tr>
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<td>25% to &lt;50%</td>
<td>150 (35.3)</td>
<td>40 (6.3) 1.35 (0.35-5.21)</td>
<td>41 (15.0) 4.17 (1.96-8.88)</td>
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<tr>
<td>50% to &lt;75%</td>
<td>26 (6.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥75%</td>
<td></td>
<td>P trend &lt; 0.0001</td>
<td></td>
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<tr>
<td>per 25% increase</td>
<td></td>
<td>1.77 (1.07-2.95)</td>
<td></td>
</tr>
</tbody>
</table>

*ORs adjusted for time since prior screening examination, history of benign breast disease, smoking status, body mass index, family history of breast/ovarian cancer, parity, education, age at menarche, menopausal status, and use of hormone replacement therapy.

Cancer Epidemiol Biomarkers Prev 2006;15(10). October 2006

Chiarelli AM et al. 2006
Effect of HRT short disruption on MBD

60y; E2 continuously

Before HRT

After 1 year

After 2 weeks of disruption
Models for Phase II Chemoprevention Trials for Women at High Risk of BC

Tissue Based Biomarkers

Morphology
Proliferation

RPFNA
Random periareolar fine needle aspiration

DL
Ductal lavage

NAF
Nipple aspiration fluid

Mammographic Breast Density

RANDOMIZATION

Study Agent

Repeat Biomarkers

6-12 months

Placebo

Adapted from Fabian C. Endocrine related Cancer 2005
To conclude

- Given the large population attributable risk of MBD, the routine measurement of MBD should be given more consideration.
- This marker has great potential to be used for research into the etiology and prevention of breast cancer.
• **ABSTRACT:** Associations between Polymorphisms in Glucuronidation and Sulfation Enzymes and Mammographic Breast Density in Premenopausal Women in the United States [Cancer Epidemiology, Biomarkers and Prevention; Subscribe]

• Objective: Sex hormones are metabolized to less active compounds via (a) glucuronidation catalyzed by UDP-glucuronosyltransferases (UGT) and (b) sulfation catalyzed by sulfotransferases (SULT). Functional UGT and SULT polymorphisms can affect clearance of sex hormones, thereby influencing exposure in hormone-sensitive tissues, such as the breast. We assessed relationships between functional polymorphisms in the UGT and SULT genes and breast density in premenopausal women.

• Methods: One hundred seventy-five women ages 40 to 45 years, who had a screening mammogram taken within the previous year, provided a genomic DNA sample. Mammograms were digitized to obtain breast density measures. Using generalized linear regression, we assessed associations between percent breast density and polymorphisms in the UGT1A and UGT2B families, SULT1A1, and SULT1E1.

• Results: Women with the SULT1A1(H213/H213) genotype had 16% lower percent breast density compared with women with the SULT1A1(R213/R213) genotype after controlling for ethnicity (P = 0.001). Breast density was 5% lower among women carrying at least one copy of the UGT1A1(TA7)-UGT1A3(R11)-UGT1A3(A47) haplotype compared with the UGT1A1(TA6)-UGT1A3(W11R)-UGT1A3(V47A) haplotype (P = 0.07). No associations were observed between polymorphisms in the UGT2B family or SULT1E1 and breast density.

• Conclusion: Polymorphisms in SULT1A1 and the UGT1A locus may influence percent breast density in premenopausal women.
Biological Hypotheses

- Stromal and epithelial cells
  - Influenced by stimuli to cell proliferation (mitogenesis).
  - Genetic damage to both cells (mutagenesis) could initiate carcinogenesis

Stromal fibroblasts
- collagen,
- pre-adiopocytes

Martin and Boyd *Breast Cancer Research* 2008 10:201
There is a need to place HRT in perspective.\textsuperscript{1,2,3} The RR of breast cancer using ccHRT for >5 years is similar to 2 drinks of alcohol per day.\textsuperscript{4}

![Various risk factors influencing Breast Cancer Risk](image)


• **ABSTRACT:** The relation of leptin and adiponectin with breast density among premenopausal women [European Journal of Cancer Prevention; Subscribe; Sample]

• The adipocytokine leptin may increase breast cancer risk, while adiponectin may be protective. We examined the association of the two circulating markers with mammographic density, a strong predictor of breast cancer risk. For 183 premenopausal participants of a nutritional trial, mammograms performed at baseline, year 1 and year 2 were assessed for density using a computer-assisted method. Serum samples obtained at the same time were analyzed for leptin and adiponectin by enzyme-linked immunosorbent assay. We applied mixed models to incorporate the repeated measurements while adjusting for confounders including body mass index (BMI). At baseline, the mean age of the participants was 42.6±2.9 years; 40% were of Asian ancestry. Leptin was lower and adiponectin higher in normal weight than overweight women. Neither marker was related to absolute breast density. The significant inverse association of leptin with percent density disappeared when BMI was added to the model. After stratification by weight, percent density decreased with higher leptin levels in normal weight women, whereas it increased among overweight participants. After adjustment for BMI, the positive association between percent density and adiponectin was greatly reduced and no longer significant. These results do not support a strong association of leptin or adiponectin with breast cancer risk as assessed by mammographic density. In contrast, the findings suggest the possibility that the inverse association of BMI with breast cancer risk in premenopausal women is mediated by adipocytokines.
Endogenous Hormone Levels, Mammographic Density, and Subsequent Risk of Breast Cancer in Postmenopausal Women

Rulla M. Tamimi, Celia Byrne, Graham A. Colditz, Susan E. Hankinson

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Correspondence to: Rulla M. Tamimi, ScD, Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, 181 Longwood Ave, Boston, MA 02115 (e-mail: rulla.tamimi@channing.harvard.edu).

Background: Mammographic density and circulating sex hormones are two well-confirmed predictors of breast cancer risk. Whether mammographic density reflects levels of endogenous sex hormones is unclear. We examined whether these predictors are independently associated with breast cancer risk in a prospective study.

Methods: We conducted a nested case–control study within the Nurses' Health Study cohort of 253 case subjects with breast cancer and 520 control subjects. All participants were postmenopausal women who were not using postmenopausal hormones at the time of both blood collection and mammography. Plasma levels of estradiol, free estradiol, testosterone, and free testosterone were evaluated. Mammographic density was assessed by use of computer-assisted analysis of mammograms. Logistic regression models that were adjusted for matching variables and potential confounders were used to calculate relative risks (RRs) and 95% confidence intervals (CIs). All statistical tests were two-sided.

Results: Levels of circulating sex steroids and mammographic density were both statistically significantly and independently associated with breast cancer risk. The relative risk of breast cancer associated with mammographic density (RR for highest quartile compared with lowest quartile = 3.8, 95% CI = 2.2 to 6.6; \( P_{\text{trend}} < .001 \)) changed little when the analysis was adjusted for circulating estradiol (RR = 3.9, 95% CI = 2.2 to 6.9; \( P_{\text{trend}} < .001 \)) or circulating testosterone (RR = 4.1, 95% CI = 2.3 to 7.2; \( P_{\text{trend}} < .001 \)). Circulating levels of estradiol (RR = 2.4, 95% CI = 1.4 to 4.0) and of testosterone (RR = 2.0, 95% CI = 1.2 to 3.1) were both associated with breast cancer risk, before and after adjustment for mammographic density. In a joint analysis of mammographic density and plasma testosterone, the risk of breast cancer was highest in the highest tertiles of both relative to the lowest tertiles of both (RR = 6.0, 95% CI = 2.6 to 14.0). A similar pattern was observed in the joint analysis of estradiol and mammographic density (RR = 4.1, 95% CI = 1.7 to 9.8).

Conclusions: Circulating sex steroid levels and mammographic density appear strongly and independently associated with the risk of breast cancer in postmenopausal women.

CONTEXT AND CAVEATS

Prior knowledge

Mammographic density and circulating sex hormones are two confirmed predictors of breast cancer risk, but it is unclear whether the risk associated with mammographic density is primarily related to levels of endogenous sex hormones. Study design A nested case–control study within the Nurses' Health Study cohort with 253 case subjects with breast cancer and 520 control subjects who were postmenopausal at the time of blood collection and mammographic examination. Contribution Among postmenopausal women, levels of circulating sex steroids and mammographic density were both statistically significantly and independently associated with breast cancer risk. Implications The mechanism by which mammographic density increases the risk of breast cancer among postmenopausal women is independent of the levels of circulating sex steroid hormones. Limitations Circulating levels of steroid hormones were used as a proxy for the more biologically relevant measure of the levels of steroid hormones in breast tissues.

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Epidemiology

Premenopausal Mammographic Density in Relation to Cyclic Variations in Endogenous Sex Hormone Levels, Prolactin, and Insulin-like Growth Factors

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Key Words: breast cancer • mammographic density • steroid hormones and receptors • growth factors and receptors • prolactin

Mammographic density is strongly associated with breast cancer risk, and endogenous hormones, which are risk factors for breast cancer, may be involved in the mechanism. This cross-sectional study of 494 premenopausal women is the first to account for cyclic variations in estrogen levels, by measuring urinary estrone glucuronide (E1G) in the periovulatory and luteal phases of the menstrual cycle, and to assess the role of androgens. Computer-assisted density readings were obtained from digitized mammograms. Mean ovulatory E1G level and daily E1G load were both positively associated with percent density before adjustment for body mass index (BMI), with women in the top fourth having 10.2% (95% CI: 2.9%, 18.1%) and 8.9% (1.7%, 16.7%), respectively, higher density than those in the bottom fourth (P trend before/after BMI adjustment = 0.006/0.11 and 0.01/0.13, respectively). Neither the peak nor luteal E1G levels were predictive of density after adjustment for E1G levels at other points in the cycle. The plasma androgens testosterone, androstenedione, and dehydroepiandrosterone sulfate were negatively associated with density. In mutually adjusted analyses, density was positively associated with insulin-like growth factor (IGF)-I and negatively with IGF-II (P trend = 0.006 for both) but not with IGF binding protein-3. There was also weak evidence of a positive association of prolactin with density. The study supports the hypothesis that endogenous hormones affect density in premenopausal women; in particular, it shows a positive association between estrogen levels and density and suggests that the mean level throughout the cycle is the most biologically relevant measure. Most of these hormone-density associations were attenuated with further adjustment for BMI. [Cancer Res 2009;69(16):6490–99]
Endogenous Sex Hormone Levels and Mammographic Density among Postmenopausal Women

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Abstract
Background: Mammographic density is one of the strongest predictors of breast cancer risk. The mechanism by which breast density increases breast cancer risk is unclear although it has been hypothesized that breast density reflects cumulative exposure to estrogens.

Methods: To evaluate this hypothesis, we conducted a cross-sectional study among 520 postmenopausal women in the Nurses' Health Study that examined the relation between circulating sex hormones and mammographic density. Women were postmenopausal and not taking exogenous hormones at the time of blood collection and mammogram. Percent breast density was measured from digitized mammograms using a computer-assisted method. Circulating estrone, estradiol, androstenedione, testosterone, DHEA, DHEA sulfate, sex hormone–binding globulin, progesterone, and prolactin were measured in plasma.

Results: In contrast to the prior hypothesis, circulating estrogens were inversely related to percent mammographic density. The mean percent mammographic density was 25.6% among women in the lowest quartile of circulating estradiol compared with 14.4% among women in the highest quartile (Spearman correlation ($r = -0.22, P < 0.0001$)). Circulating estrogens alone explained 1% to 5% of the variation of mammographic density. Body mass index was positively associated with circulating estradiol levels ($r = 0.45, P < 0.0001$) and inversely related to percent mammographic density ($r = -0.51, P < 0.0001$). After adjustment for body mass index, there was no association between estradiol and breast density ($r = 0.01, P = 0.81$). Likewise, there was no relation between the other sex hormones measured or prolactin and mammographic density after adjustment for body mass index.

Conclusion: These findings indicate that in postmenopausal women, mammographic density is independent of circulating sex hormone levels.