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Focus On

What is breast density?

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Abstract Although having increased breast density is one of the strongest risk factors for breast cancer other than increased age and having a BRCA1 or BRCA2 mutation, it is still not clearly understood what that measure of risk is reflecting biologically. It has long been presumed based on indirect evidence that breast density is an indicator of cumulative hormone, particularly estrogen, exposure throughout one's life. However, there is growing evidence that the story may not be that simple. Recent studies suggest that stromal and epithelial proliferation and interaction, and the IGH-axis may all be involved in explaining the breast density and breast cancer risk association. Clearly for breast cancer research to advance it will be necessary to think beyond the presumed association that explains breast density only through estrogen pathways.

Keywords: Breast cancer risk; Breast density; Hormones; Insulin-like growth factor

Introduction

The term breast density has been used to describe the features of the breast tissue as visualized from a mammographic image. On a film-screen mammogram, the stromal and epithelial tissues of the breast appear as shades of gray to white due to the attenuation of the X-rays. In contrast, the fat within the breast is more radiolucent and much darker in appearance on the mammogram. The visual appearance of these different tissues on a mammogram has been classified both qualitatively and quantitatively as a way to describe breast density [1]. The Wolfe parenchymal patterns, Tabar's categories, and the ACR BI-RADS score are similar visual classifications of the appearance of the breast. While each classification system has some unique component and features, they all reduce the variation in breast appearance into four or five categories thought to be associated with a gradient of breast cancer risk. These different categorical classifications tend to be quite highly correlated. Still others have made a visual estimate of the proportion of the breast area on the mammogram that is comprised of the dense stromal and epithelial tissue for a semi-quantitative classification scheme. The intra-rater consistency of both of these ways (categorically and visually estimated) tends to be quite high, but due to the subjective components of the assessment there is greater variation between raters not trained together [2]. In the last decade, more studies have been conducted where the area of the breast that appears dense is measured and the total area of the breast is measured [3–5].

This measurement has been conducted with a planimeter or a computer-assisted technique using digitized copies of the mammogram. There is typically still a subjective component to determine which tissue is considered dense, but this is combined with a more objective assessment of the areas defined as dense. Both the intra- and inter-rater comparisons using measured breast density tend to show very highly correlated measures. An additional question is now raised as to which of the two measures, area of density or the percent of the breast that appears dense (often referred to as percent breast density) is the most

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relevant measure. Since the area of density, sometimes referred to as absolute density, measures the combination of the stromal and epithelial tissue, some argue that this would be the most biologically relevant measure of interest. However studies that have reported both measures (area of density and percent breast density) have consistently found that the percent breast density is associated with a greater gradient in breast cancer risk [1]. Thus, while associations with both measures may be of interest, if the intent is to either measure breast cancer risk or to consider a measure of breast density as a proxy or intermediate marker of breast cancer risk, it appears that the quantitative continuous measure of percent breast density provides the greatest amount of information and can be consistently measured. So while percent breast density is, in some ways a very crude biomarker, it has consistently been associated with up to 4–6-fold increased risk of developing breast cancer [6]. It is the strength of the association between percent breast density and breast cancer risk that compels one to continue to ask 'what is breast density and what is it measuring?'

Some have suggested that breast density is a reflection of the number of epithelial cells at risk [7], however there is growing evidence that it is likely to reflect more than this as the importance of the stromal tissue, and the epithelial and stromal interaction becomes more evident [6]. It has long been presumed that breast density is a reflection of endogenous hormone levels [8]. However, much of this evidence has been indirect in nature and the few studies with direct measures of this association are not consistent with this presumption. Higher breast density has been associated with early age at menarche, late age at first birth, lower parity, being premenopausal, late age at menopause, and alcohol consumption, all factors thought to be associated with an increase in circulating estrogens [9]. Furthermore, there are numerous studies that show that with use of postmenopausal hormones (PMHs) breast density increases.

Exogenous hormones and breast density

Several studies have confirmed a positive association between breast density and exogenous hormones, principally estrogen and progestin in PMH use among women. While oral contraceptives also contain these hormones and may be similarly associated with breast density among premenopausal women, this relationship has not been investigated [10], primarily due to age at mammographic screening and the low prevalence of current oral contraceptive use among women of screening age.

The evidence regarding PMH and breast density was summarized in a comprehensive recent review by Warren [10]. Some of the most detailed evidence of this association emerged from the analyses of the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial, a 3-year, placebo controlled, randomized, double-blind trial of the effects of PMH on cardiac risk factors, and other health outcomes among 875 postmenopausal women at seven clinics throughout the USA [11]. Among 307 women who had not used estrogen, 5 years prior to baseline, Greendale *et al.* evaluated the effects of taking estrogen, estrogen plus one of the three different regimens of progestin, and a placebo on breast density. In the first paper from the dataset, breast density was assessed categorically using the American College of Radiology Breast Imaging Reporting and Data System (BI-RADS) which ranged from (1) entirely fatty to (4) extremely dense. Results showed that breast density increased among 8% of women taking estrogen alone, but increased for 19–24% of users of the estrogen–progestin combinations, and these increases typically occurred within the first year of treatment. Only 2% of women in the placebo group showed an increase in density. Women using the estrogen–progestin combination had a 7–13-fold greater risk of increased density than women using only estrogen, after adjustment for several covariates.

A second study of change in percent density among women in the PEPI trial investigated the effects of the different PMH types on the magnitude of change in breast density at baseline to year 1 [12]. In this study, density was measured as a continuous (percent density) variable using a computer-assisted technique, a method more sensitive to smaller increments in density change. The pattern of results was consistent with those in the first PEPI study of the effects of PMH on breast density. After 12 months of treatment, the mean change in percent density among women using estrogen alone, 1.17%, was not significantly different than baseline density, whereas the mean percent density among women using one of three estrogen–progestin combinations increased from 3% to 5%, and this change from baseline was statistically significant. The mean increases in density across the three combination treatment groups did not differ statistically.

Also several studies have demonstrated that with use of tamoxifen breast density decreases [13,14]. Use of the GNRH agonist drugs that suppress ovarian function have also been related to a reduction in breast density [15]. All of this indirect evidence has supported the assumption that hormones and particularly estrogens determine breast density. But what if these associations were measured directly,

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how do endogenous circulating levels of steroid hormones relate to breast density?

Endogenous hormones and breast density

Boyd *et al.* [16] investigated the association of several hormones considered to be breast mitogens, including growth hormone, prolactin, estradiol, sex hormone binding globulin (SHBG, the primary estradiol binding protein), free estradiol, and progesterone in a sample of 193 premenopausal women and 189 postmenopausal women without current hormone use. Results showed that none of these hormones were associated with breast density in premenopausal women. In postmenopausal women, however, only prolactin and SHBG were positively associated, and free estradiol was negatively associated with both density measures. While the positive relationship demonstrated between prolactin and breast density among postmenopausal women in this study was consistent with the positive relationship between prolactin and breast cancer risk found earlier by Hankinson *et al.* [17], Boyd *et al.* described the patterns of association for SHBG and free estradiol with density as unexpected.

A third PEPI study of 452 postmenopausal women investigated the relationship between change in serum estrone level from baseline to year 1 and change in percent density [18]. Consistent with the other two PEPI studies, there was no association between change in serum estrone levels and percent density from baseline to year 1 among women using only estrogen. Among women using the estrogen–progestin combination treatments, there was a 2.95% increase in percent density per 100 pg/ml increase in serum estrone level, and this increase was primarily attributed to change in the dense area of the breast. While there were differences in the association between serum estrone and percent density across the three combination treatment groups, these differences between the groups were not statistically significant. Ursin *et al.* noted that while the clinical significance of change in breast density is unknown, it may be a marker for those women whose breast cancer risk is increased by PMH use, and that further studies are needed to confirm this hypothesis, and to determine whether PMH dose may be individualized to reduce its harmful effects.

The inconsistent findings of Boyd *et al.* and Ursin *et al.*, regarding the association of circulating levels of estrogen and estrone with breast density among postmenopausal women, may be attributed to the fact that the serum estrone levels among the postmenopausal women in the Ursin *et al.* study may have been manipulated by PMH use in the PEPI trial.

Ziv *et al.* [19] evaluated that if breast density was more associated with ER+ (as would be assumed if working through an estrogen hormonal pathway) or ER− breast cancer in a large study of 44 811 women. The finding that breast density was associated with both ER+ and ER− breast cancer equally provides further support that estrogens alone cannot explain breast density.

Furthermore, the inverse association between breast density and body size is a major inconsistency with the hormone hypotheses. Heavier postmenopausal women will typically have higher circulating endogenous hormone levels [20], yet heavier women have lower breast density. This may in part be due to the high correlation between the measure of percent breast density that has breast size as part of the measure and body size.

Clearly, as researchers we must look beyond the presumed association between estrogen and breast density.

Insulin-like growth factor and breast density

There is accumulating evidence that the growth hormones may influence breast density, and thereby, breast cancer risk. A number of recent studies have found an association between insulin-like growth factor (IGF-I) and breast density [16,21–24], and between IGF-I and breast cancer risk [25–29], IGF-I and its main binding protein, IGFBP-3, are peptide hormones involved in the regulation of cell proliferation, differentiation, and apoptosis [30]. Laboratory studies have shown that IGF-I is mitogenic, whereas IGFBP-3 is antiproliferative and apoptotic [30]. Due to these properties, IGF-I and IGFBP-3 are thought to contribute to the risk of breast cancer associated with mammographic density through the combined effects of mitogenesis and damage to DNA of dividing cells by endogenous mutagens [22]. As the bioactivity of IGF-I in tissue is partially determined by IGFBP-3, their simultaneous effects must be considered when evaluating their role in breast density and carcinogenesis [21]. Circulating concentrations of IGF-I and IGFBP-3 can be measured and have shown wide inter-individual variability. This variability is believed due to differing genetic and environmental factors [31] thus suggesting an important link to understanding the distribution of cancer risk in a population [30].

Byrne *et al.* [21] first reported results showing an association among IGF-I, IGFBP-3, and breast density. They analyzed cross-sectional data from 65 premenopausal and 192 postmenopausal subjects who had served as controls in a previous study of IGF-I, IGFBP-3, and breast cancer risk in the Nurses' Health Study [32]. They compared the percentage of total breast area appearing dense on mammography

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with plasma concentrations of IGF-I, IGFBP-3, and the molar ratio of IGF-I : IGFBP-3 (a measure of the bioavailability of IGF-I). Among premenopausal women, adjusting for covariates (age, IGFBP-3, alcohol intake, laboratory batch, and body mass index (BMI)), IGF-I was positively correlated with density at 0.36 ($P = 0.007$). IGFBP-3 level, adjusted for covariates, was negatively correlated with density at −0.24 ($P = 0.07$). The molar ratio of IGF-I : IGFBP-3 showed the strongest positive association to breast density at 0.39 ($P = 0.004$). In contrast, among postmenopausal women, no associations were found among IGF-I, IGFBP-3, or IGF-I : IGFBP-3 and breast density. These results reflect the pattern found by Hankinson *et al.* [32], who evaluated the association between IGF-I, IGFBP-3 levels, and breast cancer risk, and found that IGF-I was positively associated with breast cancer risk among premenopausal, but not postmenopausal women.

Since Byrne *et al.* reported their findings, four subsequent studies [16,23,24,33] have further investigated the relationship of circulating levels of IGF factors and breast density. Results from three studies [16,23,24] were consistent to those of Byrne *et al.* [21].

Boyd *et al.* [16] evaluated the association of circulating levels of IGF-I and IGFBP-3 with breast density measured as percent mammographic density and area of dense tissue. Several covariates for breast cancer risk were included in their analyses, however, only age and measures of body size influenced the results. Among premenopausal women, results showed that IGF-I was positively associated with percentage of breast density and size of dense area, after adjustment for IGFBP-3, age, and waist, with the adjusted model explaining 54% of the variance in percentage breast density and 35% of the variance in size of dense area. IGFBP-3 was unassociated with either mammographic measure after adjustment for age and waist. This study did not report the association of the molar ratio with breast density. Among postmenopausal women, there were no significant associations for IGF-I, IGFBP-3, and the mammographic measures, after adjustment for age and waist.

Maskarinec *et al.* [23] examined the association of circulating levels of IGF-I and IGFBP-3 with mammographic density in an ethnically diverse sample of 263 healthy premenopausal women. Adjusting for age, BMI, and reproductive factors, they found that IGF-I was positively associated with percent density (0.11), but the relationship was reported as not statistically significant ($P = 0.06$). IGFBP-3 was negatively and significantly associated with percent density at −0.15 ($P = 0.02$), and the IGF-I : IGFBP-3 ratio was positively associated with percent density at 0.13 ($P = 0.03$). Stratification by two levels of BMI showed that the positive relationship between the IGF molar ratio and percent density was confined to those women with a BMI of less than 25 kg/m^2. Maskarinec *et al.* also examined the association of IGF factors with the size of dense and non-dense (fatty) areas in the breast. No associations were found between the IGF factors and the size of dense area, but size of the non-dense area was positively associated with IGFBP-3, and negatively associated with the molar ratio of IGF-I : IGFBP-3, at 0.18 ($P = 0.004$) and −0.15 ($P = 0.02$), respectively. BMI levels, however, did not appear to modify other relationships between IGF-I, IGFBP-3 and breast density measures.

In contrast, a study by Lai *et al.* [33] found no statistically significant associations with circulating level of IGF-I, IGFBP-3, and the molar ratio IGF-I : IGFBP-3 with breast density in the 142 premenopausal or 170 postmenopausal women, after adjusting for age, BMI, and other confounders. The results for women in the premenopausal group are inconsistent with the findings from other published studies, although the findings of no association among postmenopausal women are consistent with Byrne *et al.* and others. Lai *et al.* [33] proposed that local tissue levels of IGF factors, rather than circulating levels, may be a more reliable predictor of premenopausal breast density, and may partially explain their inconsistent findings. Byrne *et al.* [21] offered a similar hypothesis regarding the lack of association of circulating IGF factors and breast density among postmenopausal women in their study. In this regard, a recent study by Gou *et al.* [22] examined the association between tissue levels of IGF factors and breast density. Gou *et al.* hypothesized that levels of growth factors within breast tissue stimulate cell proliferation in the stroma and epithelium that contributes to mammographic density. Using methods of immunohistochemical staining, Guo *et al.* examined the amount of cell nuclei, total collagen, the stromal matrix regulatory protein tissue inhibitor of metalloproteinase-3 (TIMP-3), transforming growth factor-α, and IGF-I in 92 samples of breast tissue from areas surrounding benign lesions. Half of the sample included women with little or no breast density and the other half included women with extensive breast density. Subjects were matched by age at biopsy, and mammograms were taken at the time of biopsy.

Results showed that breast tissue from subjects with extensive breast density contained greater nuclear area, and larger amounts of total collagen, TIMP-3, and IGF-I than the tissue from subjects with little breast density, and these differences were greater in women less than 50 years of age, although menopausal status was not known for these women. Further, the greater stained areas of IGF-I and nuclei

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were associated with density only in women under 50 years of age, whereas in women older than 50 years only the stained area of collagen was associated with density, paralleling the modifying affect of menopausal status on the relationship between circulating IGF-I levels and breast density [16,21,24]. Although the study of Gou *et al.* [22] did not directly compare tissue expression of IGF-I with circulating levels, their findings do not suggest that differences between tissue and circulating levels might explain why the association between IGF-I factors and breast density differ by menopausal status. The results of Guo *et al.* support the hypothesis that stromal and epithelial proliferation is associated with the presence of growth factors in the breast. They note that IGF-I is a known mitogen for breast epithelium, and that several components of the IGF axis have been shown to be dysregulated in breast cancer.

The most recent study of IGF factors and breast density, conducted by Diorio *et al.* [24], examined associations between circulating levels of IGF factors and percent density in the largest sample of women to date; 783 of whom were premenopausal and 791 of whom were postmenopausal. These findings are consistent with those of Byrne *et al.* in both groups of women, and the findings of Maskarinec *et al.* for premenopausal women (the only group in that study); however, the strength of the Diorio *et al.* associations were much weaker across all IGF factors compared to the Byrne *et al.* and Maskarinec *et al.* findings. The strength of the association between IGF-I factors and breast density measures in the Boyd *et al.* study could not be compared with the other four studies because they reported the coefficient of determination for the regression analyses instead of correlation coefficients [24]. Diorio *et al.* found, adjusting for age, BMI, and IGFBP-3, that IGF-I and the molar ratio were positively correlated with percentage breast density at 0.08 ($P = 0.021$) and 0.07 ($P = 0.056$), respectively, in premenopausal women, but not postmenopausal women. Diorio *et al.* also confirmed that IGFBP-3 and breast density were inversely associated in premenopausal women at -0.12 ($P = 0.0005$), and unassociated among postmenopausal women. In addition, Diorio *et al.* also found stronger associations between all three IGF factors and percent density in premenopausal women with BMI less than 25 kg/m^2 compared to women with a BMI greater than 25 kg/m^2. The same pattern was found for height; taller premenopausal women had stronger association of IGF-I and IGFBP-3 levels with percent density than shorter women.

The Diorio *et al.* study was also the first to examine the joint effects of IGF-I and IGFBP-3 circulating levels on breast density. They found that the positive association between IGF-I and percent density in premenopausal women was stronger at low levels of IGFBP-3, while the negative association between IGFBP-3 and percent density was stronger at higher levels of IGF-I, indicating that premenopausal women with high levels of IGF-I and low levels of IGFBP-3 have greater breast density and thus an increased risk of breast cancer.

Conclusions

Taken together, the results of the five studies that have investigated the association between circulating levels of IGF factors and breast density support an association in premenopausal women. Further, the data showed that breast density is associated with local tissue levels of IGF factors bolsters the association. While the first studies [16,21,23,33] reported that the association between IGF-I factors and breast density were provocative, their small sample sizes precluded stratification and effect modification analyses as done in the larger, most recent study [24]. Further studies powered to evaluate that effect modification will allow for greater understanding of the potential biological pathways involved with breast density. Laboratory studies indicating that the IGF axis may play a role in the etiology of breast cancer via its influence on the morphogenesis of breast tissue suggest that breast density may be construed as an intermediate marker of breast cancer risk, at least among premenopausal women. Since premenopausal percent density predicts postmenopausal breast cancer, further study is necessary to determine if premenopausal levels of IGF and related factors predict postmenopausal breast cancer risk, and to what extent the IGF-I : IGFBP-3 ratio and breast density may be independent of breast cancer risk factors. It is also necessary to gain a clearer understanding of the complex pathway among IGF factors, breast density, and breast cancer risk, especially how other endogenous hormones such as estradiol and growth hormone may interact to influence the IGF–breast density association. Studies of the influence of lifestyle factors such as nutrition, physical activity, BMI, alcohol, and tobacco use on the association between IGF factors and breast density may also provide additional insight into underlying biological mechanisms and offer new opportunities for intervention to reduce the breast cancer risk associated with breast density.

What we are learning about breast density, what it is, and what it is related to is suggesting that we may need to broaden our concepts and our way of thinking

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about breast cancer etiology. While estrogens clearly play a role in breast cancer development [34], there appears to be strong evidence that this pathway does not necessarily involve breast density.

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