

# Menopausal MEDICINE

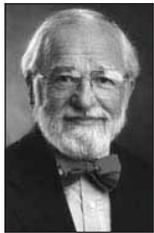
American Society for Reproductive Medicine



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FOR CLINICIANS WHO PROVIDE CARE FOR WOMEN

## The Clinical Implications of Hormone-Induced Breast Density



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Women with a greater mammographic breast density have a higher risk of breast cancer, and about 25% of women on postmenopausal estrogen-progestin therapy have an increase in their breast density. The critical clinical question is whether the short-term increase in density with hormone therapy changes an individual's risk of breast cancer.

### THE RELATIONSHIP BETWEEN BREAST DENSITY AND RISK OF BREAST CANCER

Assessing the impact of breast density on breast cancer screening is complicated by two factors that produce heterogeneous data: (1) Results from programs with biennial screening are less favorable when compared with annual screening and the available data are derived from both, and (2) Results from recent years are better, reflecting improvements in technology. Nevertheless, high breast density on mammography is reported to be associated with a 4- to 6-fold increased risk of breast cancer.<sup>1-4</sup> Increased density impairs the detection of breast masses.<sup>5</sup> A failure

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to detect masses because of high density causes an increase in interval cancers (cancers that present between mammographic screenings, in other words, cancers diagnosed after a negative mammogram).<sup>6</sup> Difficulties in reading high density mammograms also produce false-positive recalls (patients who are recalled for assessment and found not to have cancer). Being recalled for reassessment after an initial mammogram is a cause of significant psychological stress.<sup>7</sup> In addition, at least 25% of the overall cost of mammographic screening in one U.S. program was attributed to investigations of false-positive readings.<sup>8</sup>

These two problems, an increase in interval cancers (a decrease in mammographic sensitivity) and an increase in false-positive recalls (a decrease in mammographic specificity), are consistent with a possible decrease in the detection of cancer. Thus, the concern with dense breasts in postmenopausal women is a reduced quality of mammograms that would decrease the ability to detect early breast cancers. Factors that are associated with greater breast density are nulliparity, older age at first birth, and current use of postmenopausal hormone therapy.<sup>9</sup> Mammographically dense breasts reflect a high proportion of stromal, ductal, and glandu-

### FROM THE EDITOR

David F. Archer, M.D.

Leon Speroff, M.D., reviews the literature relative to hormone therapy and breast density. He points out the inconsistencies in the reports of the association of breast density and breast cancer. The hypothesis that increased breast density delay diagnosis is not substantiated in his review based on the findings of smaller, better differentiated tumors and a decreased overall mortality. His assessment is that the evidence is not conclusive that hormone therapy, by increasing mammographic density, increases the risk of breast cancer diagnosis and results in a poorer clinical outcome.

Peter Conner, M.D., Ph.D., and Bo von Schoultz, M.D., Ph.D., provide their data on the use of Fine Needle Aspiration for evaluating histologic (morphologic) and mitogenic effects in the breast of pre- and postmenopausal women. Their data show a strong correlation between the mitotic activity and mammographic breast density. One of the most interesting findings is that testosterone inhibits the proliferative activity of estrogen in the non human primate breast. There are differences in the morphology and mitotic activity between estrogen and progestin used as hormone therapy and Tibolone. The latter compound, which is not available in the United States, reduces mammographic density and mitotic activity in their studies.

Jennifer Harvey, M.D., introduces the use of the diagnostic methods for assessing breast density by radiography. The relationship between increased breast density and breast cancer is presented, along with the finding that a family history of breast cancer is also associated with an increased risk of breast density. Hormone therapy is associated with an increase in breast density and breast cancer. The latter finding may be due to several factors. First is that density may impede the diagnosis of an early breast lesion, second that the increased breast cancer is due to progestin increasing mitotic activity. At present, there is no substantial evidence that breast density secondary to hormone therapy confers and increased risk of breast cancer development.

Breast density is an important issue for the clinician. The Bi-Rads System to semi-quantitate breast density is used throughout the United States. The relationship of hormone-induced breast density and neoplasia is still unclear. Perhaps the most important aspect of this discussion is the anxiety generated in the patient by a mammogram report indicating further studies are required. False positive rates can be high and are costly. There is no definitive answer to the finding of hormone-induced breast density except that it resolves quickly upon stopping the exogenous hormones.

# Menopausal Medicine

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lar tissue. The likelihood of dense breasts decreases with advancing age and increasing body weight as glandular tissue is replaced by fat.<sup>9</sup> The link with nulliparity supports the contention that a full-term pregnancy early in life produces a change in structure in the breast that persists throughout life and is associated with resistance to proliferation.

## THE IMPACT OF HORMONE THERAPY ON BREAST DENSITY

More current users of hormone therapy have dense breasts than non-users.<sup>9-13</sup> In women younger than age 55, it is difficult to find any differences between hormone users and nonusers.<sup>14</sup> The impact is essentially limited to women older than age 55. The effect of hormone therapy occurs rapidly; thus, duration of use has no effect.<sup>14</sup> In the PEPI three-year randomized trial, almost all increases occurred within the first year, with an increase in breast density observed in 8% of estrogen users and 19% to 24% of estrogen-progestin users, and only 2% in the placebo group, with no differences observed comparing medroxyprogesterone acetate with micronized progesterone.<sup>15</sup> In careful studies, the daily, continuous combined estrogen-progestin regimens have been reported to have a greater effect than sequential regimens, with an increase in density occurring within the first months of treatment and then maintained with no change.<sup>16-20</sup> Therefore, postmenopausal hormone therapy increases breast density mainly in older postmenopausal women; hormone therapy increases breast density in about 10% to 20% of estrogen users and about 20% to 35% of estrogen-progestin users, and the effect occurs within the first months of use and remains stable with no changes with increasing duration of use. An increase in breast density is observed more often in women receiving a daily, continuous combination of estrogen-progestin compared with a sequential regimen.

## THE EFFECT OF HORMONE THERAPY ON MAMMOGRAPHIC SCREENING

Does this hormonal effect on breast density impair mammographic screening? In other words, is there an increase in interval cancers and false-positive recalls in postmenopausal hormone users? In a review of seven studies, there were relatively few

interval cancers in the user groups (from one to 46); nevertheless, six of the seven studies reported decreased mammographic sensitivity in hormone users with increases in interval cancers in users compared with nonusers.<sup>21</sup> Excluding women under age 50, the relative risk for an interval cancer was summarized as 1.7 (CI=1.2-2.4). American, Scottish, and Australian studies have indicated a 5% to 20% decrease in mammographic sensitivity in hormone users who have dense breasts.<sup>22-25</sup> A Finnish study concluded that women with the most dense breasts and using hormones had the highest relative risk of breast cancer, but this conclusion was based on only four cases of cancer in women with dense breasts.<sup>26</sup>

The risk of false-positive recall (mammographic specificity) was investigated in five studies.<sup>22,26-29</sup> The rate of false-positive recall in non-users ranged from 2.1% in the U.K. to as high as 14.7% in an American program; four of the five studies found a slight increase in the risk of false-positive recalls. In a French study, mammographic sensitivity was reduced from 92% to 71% in users because of an incidence of interval cancers that was 3.5 times that of non-users within the first year of the initial exam, and 1.7 times greater during the following two years.<sup>27</sup> Most of the hormone users were on combined estrogen-progestin regimens. The false-positive recall rate was only slightly higher, 3.3% in users and 2.8% in non-users. A prospective study of screening mammograms from Massachusetts General Hospital concluded that recall rates were essentially the same comparing hormone users and non-users and that hormone therapy rarely causes a diagnostic dilemma.<sup>28</sup> However, in New Hampshire, increased breast density and use of hormone therapy independently increased the need for supplemental imaging.<sup>29</sup>

## HORMONE-INDUCED BREAST DENSITY AND THE RISK OF BREAST CANCER

There are several reasons to suspect that the increase in breast density reported with postmenopausal hormone therapy may not be identical to the high breast density associated with an increased risk of breast cancer. Overall, studies have suggested a decrease in mammographic sensitivity with a lesser impact on specificity (false-recall rates). However, the

studies are based on small numbers of interval cancers, and it is uncertain how real or how large this effect is because of the difficulty in controlling for confounding factors (for example, age, age at menopause, and time since menopause). If the effectiveness of breast cancer screening is reduced by postmenopausal hormone therapy, one would expect an adverse impact on breast cancer mortality. Instead, a study that indicated a reduction in mammographic sensitivity also reported smaller, more differentiated (Grade I) tumors among the users compared with the non-users,<sup>20</sup> and most of the studies that have examined the breast cancer mortality rates of women who had used postmenopausal hormone therapy have documented improved survival rates.<sup>30-40</sup> Evidence indicates that hormone users develop smaller, better-differentiated (lower grade) tumors, evidence that is consistent with effects on preexisting tumors and that surveillance/detection bias is not the only explanation for better survival.<sup>41-52</sup> Lower grade tumors are present even when there is no difference in the prevalence of mammography comparing hormone users and non-users or when the data are adjusted for the method of detection.<sup>37,39,47</sup> This is not a totally uniform story in that at least one prospective study concluded that estrogen-progestin users had both lower and higher stage and grade tumors.<sup>53</sup> However, most reports indicate that more tumors in hormone users are detected by screening mammography, and when assessing outcomes in all cancers detected by mammography, hormone users have more ductal in situ tumors, more node-negative cancers, smaller tumors, and less invasive disease and, thus, better survival rates.<sup>54</sup>

In contrast, the Women's Health Initiative (WHI) results in the estrogen-progestin arm indicated an earlier appearance of worse tumors than previously reported in case-control and cohort studies.<sup>55</sup> The WHI pointed out that the results (both the invasive breast cancers and the mammography findings) are consistent with stimulation of growth in established breast cancers (supported by no statistical difference in in situ tumors) but at the same time a delay in diagnosis. This certainly challenges the idea that hormone users have better outcomes because of earlier detection. The WHI suggests that this disagreement could be because of a difference of

mammography use in the observational studies. However, even studies that examine tumor characteristics and outcome in users and non-users who have equally used mammography, lower grade and stage disease with a better outcome is identified in the users.<sup>37,39,54</sup>

Contrary to many reports in the literature, the WHI concluded that their results suggested that invasive breast cancers diagnosed in women who use hormone therapy may have a worse prognosis, basing this conclusion on the differences observed in tumor size and spread of dis-

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*“The older a postmenopausal patient is, the greater the risk of developing an increase in breast density with hormone therapy.”*

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ease. By now, it is well-recognized that the participants in the WHI represent an older postmenopausal population (average age 63 and an average of 18 years since menopause). This older population is more likely to have pre-existing occult tumors that would become detectable quickly after hormonal stimulation. In addition, breast tissue in older postmenopausal women may respond differently to hormone stimulation than breast tissue in women close to their menopause. Is it possible that the WHI results reflect this older population that might have occult tumors that are in fact larger and more prone to respond to hormonal stimulation than tumors in younger women? Other problems with the WHI data include lack of true adjudication of histologic diagnoses that were established by hundreds of pathologists and variations in treatment among the scattered participants (e.g., the introduction of sentinel node assessment midway through the study in

an uncontrolled fashion). Finally, tumors diagnosed in women with increased breast density are more likely to be estrogen receptor-negative, higher grade, and larger,<sup>56</sup> suggesting that the findings in the WHI do not reflect only a difference in hormone use (but a more accurate analysis may be precluded by insufficient numbers).

If breast density were a reflection of estrogen exposure, a strong preponderance of estrogen receptor-positive tumors would be expected in women with increased density. However, women with increased density demonstrated equal increases in risk for both estrogen receptor-positive and estrogen receptor-negative breast cancers, and as noted, another study found a preponderance of estrogen receptor-negative tumors in hormone users with dense breasts.<sup>56,57</sup> This suggests that other factors besides hormone exposure are involved in the relationship between density and breast cancer. For example, there is an association between breast density and family history of breast cancer, indicating an underlying genetic basis for both density and breast cancer.<sup>58</sup> In a case-control study that assessed the relationship between hormone therapy and breast density, leaner women were more likely to increase their breast densities with hormone therapy, but there was no association between the response to hormones and family history, late age at first birth, or history of benign breast disease; the study concluded that recognized risk factors influenced the response to hormone therapy only to a minor degree, suggesting again that unknown genetic factors are involved.<sup>59</sup>

The increase in breast density associated with postmenopausal hormone therapy appears to be a transient, reversible change, a change not consistent with a persistent effect on cellular proliferation. After discontinuing hormone therapy, breast density rapidly decreases so that former users do not display an increase compared to never users.<sup>9,13,60,61</sup> In a retrospective analysis, regression of hormone-induced abnormalities was found to occur within two weeks of cessation of treatment.<sup>61</sup> Similar results were observed in a prospective study observing a reduction in density three weeks after stopping treatment.<sup>62</sup> In a study designed to correlate histologic findings in dense breast tissue, an increase in fibrous stroma and type 1 lobules was observed to be more prevalent in hormone

users, but these changes were also present in non-hormone users, and overall there was no statistically significant difference between histologic features and breast density in women undergoing mastectomy for breast cancer.<sup>63</sup>

## CLINICAL CONCLUSION

There is no doubt that some women develop an increase in breast density with the current use of postmenopausal hormone therapy. But at the present time, the evidence does not provide an answer to the question whether the increase in density with hormone therapy changes an individual's risk of breast cancer. The older a postmenopausal patient is, the greater the risk of developing an increase in breast density with hormone therapy. Therefore, there is a good reason to recommend the discontinuation of hormone therapy for two weeks prior to mammography in women older than age 65 who have dense breasts. In younger women who are recalled for a suspicious or difficult-to-read mammogram, it would be worthwhile to discontinue hormone treatment for two weeks prior to the repeat evaluation.

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## Hormone Therapy (HT) and Breast Response in Postmenopausal Women



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### BACKGROUND

Recently, independent reports have indicated that combined estrogen plus progestogen treatment may increase the risk for breast cancer. On the other hand, data for estrogen alone are less clear.<sup>1</sup> The Million Women Study<sup>2</sup> also suggested an excess risk for estrogen alone and for

tibolone, but these results have been questioned. Observational findings are supported by data from the large randomized prospective Women's Health Initiative (WHI) trial. After a mean follow-up of 5.2 years, there was an increase in the number of breast cancer cases among women treated with a continuous combination of conjugated equine estrogens and medroxyprogesterone acetate.<sup>3</sup> In contrast, no increase in breast cancer was observed during treatment with estrogen alone after a mean follow-up of almost seven years.<sup>4</sup> In clinical practice, many different combinations of estrogen and progestogen are used for hormonal treatment. It is well established that the three major therapeutic regimens, i.e., estrogen only and estrogen in cyclic or continuous combination with progestogen, have markedly different effects in other target organs like the endometrium. Thus far, there have been few studies on the impact of different treatment regimens on the normal breast.

Currently, there is a lack of basic knowledge about the effects of different hormonal treatments on the breast. Such knowledge is vitally important to define therapeutic regimens that are safe and also to identify individual women at increased risk for an adverse response to treatment.

### BREAST PROLIFERATION-A SURROGATE MARKER FOR BREAST CANCER RISK

The basis for risk associated with hormonal therapies may lie in the regulation of cell proliferation. Within populations of cells in vitro and in vivo, a higher rate of cell proliferation may increase the risk of transformation to the neoplastic phenotype. During normal cellular division, approximately one in a million breast cells undergoes spontaneous mutation. Therefore, the risk of developing cancer is highly influenced by the rate of mitosis. Proliferative activity and sensitivity to carcinogenesis seems to be highest in the least differentiated types of lobuli, types 1 and 2, which are present in the immature breast before menarche and in younger nulliparous women.<sup>5</sup> In contrast, parous women have more differentiated breast lobuli which express less proliferative activity and susceptibility to carcinogens.

The hormonal regulation of the breast is substantially different from that of the normal endometrium. In the breast, most cells are quiescent and only a small fraction (1%

to 10%) proliferates, whereas 80% to 100% of glandular endometrial cells proliferate in the follicular phase of the menstrual cycle. During each menstrual cycle, the breast epithelium undergoes proliferation followed by apoptosis. The regulation of the cell cycle in breast cells is complex and the rate of mitosis is controlled by various promoters and inhibitors. The entry of the cell from the quiescent state into the cell cycle is a thoroughly controlled event. When the cell reaches the "restriction point" in late G1, it becomes irreversibly committed to enter the S phase. The passage through the restriction point is regulated by cyclins and inhibitors, e.g., p53. Hormones have been found to influence the signaling systems controlling the cell cycle. Cyclic estrogens stimulate cyclins and also activate oncogenes (e.g., c-myc and c-fos) leading to mitosis and proliferation.

Apart from proliferation, programmed cell death (i.e., apoptosis) is a most important feature in the regulation of growth in most tissues. Normal homeostatic functioning in breast tissue is dependent on epithelial cell turnover and a balance between proliferation and apoptosis. Growth factors and trophic hormones seem to regulate apoptosis and proliferation in a reciprocal manner. Growth factors are critical to maintain cell viability. During the normal menstrual cycle in young healthy women, levels of apoptosis generally peak two to three days after the maximum proliferative activity in the luteal phase.<sup>6</sup> In postmenopausal women, using HT progestogen exposure is of major significance for the balance between proliferation and apoptosis. There are indications that the discontinuation of progestogen is in fact a trigger for initiation of the apoptotic pathway. This is supported by epidemiologic data showing a higher increase in breast cancer risk after continuous combined HRT than treatment with estrogen only.<sup>7</sup>

### MAMMOGRAPHIC BREAST DENSITY

The mammographic pattern of the breast varies depending on the relative amount of fat and connective and glandular tissue. Fat is radiologically lucent and appears dark on the image, whereas stroma and epithelial tissue are more dense and appear white. High amounts of connective and epithelial tissue cause an increased density on mammography.

Mammographic density has been iden-

tified as a strong and independent risk factor for the development of breast cancer. Odds ratios ranging between 2-6 are higher than for many risk factors such as age at menarche, menopause, parity, and body weight.

Mammographic breast density may be of particular importance since it is, in fact, the only known risk factor for breast cancer that is present in the very organ that eventually develops the disease. Density is associated with sex steroid hormones and ovarian function. It also varies with age, parity, height, and body weight as well as menopausal status. Breast density is also related to reproductive status, circulating levels of endogenous sex steroids, peptide hormones, growth factors, and their binding proteins.<sup>8</sup> The increase in mammographic density, when it occurs, seems to occur soon after initiation of HT. The histologic correlation to mammographic density is unclear but is likely to reflect an increased amount of epithelial, connective, and stromal tissue within the breast.

#### **ASSESSMENT OF PROLIFERATION**

There are several methods to measure cell proliferation. The mitotic index is the oldest and cheapest way to assess proliferation and is calculated from the number of mitoses in paraffin-embedded tissues. Immunohistochemistry uses specific antibodies, e.g., the polyclonal MIB-1 antibody against the nuclear Ki67 antigen. This antigen is present throughout the cell cycle in proliferating cells.

The fine needle aspiration biopsy technique (FNA) was developed at the Karolinska hospital more than 30 years ago and is currently established as a diagnostic tool for the evaluation of suspicious lumps in the breast as well as in other locations in the body. Over the years, a high concordance has been shown between the cytological findings at FNA and histopathological analysis in surgical biopsies. Using a 0.6 mm needle attached to a syringe, the procedure is simple, almost painless, and seldom causes hematomas.

FNA biopsies offer a unique opportunity to obtain cell samples from healthy women with normal breasts. It also allows repeated examinations from the same woman during treatment. Needle aspiration can differentiate between solid and cystic lesions and also can be used for investigation of the amount of estrogen and progesterone receptors.

While in young women some 15% of the breast volume consists of epithelial cells, the situation is quite different in the postmenopausal breast. After menopause, and as a consequence of involution, the average amount of epithelium is in the order of 5% or less. Therefore, the use of this technique would be expected to be much more difficult and acquire more skill and experience in the operator performing the biopsies. Our group has adopted this technique for studies of breast cell proliferation in postmenopausal women during prospective randomized trials of various hormonal therapies.<sup>9,10</sup>

#### **HORMONAL THERAPY IN POSTMENOPAUSAL WOMEN**

In clinical practice, a variety of different estrogens and progestogens are used in various doses and combinations. There are suggestions that different hormones, doses, and regimens may have diverging influences on the breast.

Estrogen treatment with various compounds has been used for more than 50 years. There are several types of estrogens with different characteristics and biological activities. Conjugated equine estrogens and estradiol are regarded as moderately potent, whereas the synthetic preparation ethinyl estradiol has high biological potency. Estriol can be characterized as a low potency compound. Estrogen is well established as a mitogen in several target organs, including the breast. High endogenous estrogen concentrations have also been associated with an increased risk of breast cancer in postmenopausal women.<sup>11</sup> Today, conjugated equine estrogens and the native hormone estradiol are the most common substances used for systemic treatment in postmenopausal women, and for many years CEE has been the dominating preparation in the U.S. market.

#### **ADDITION OF PROGESTOGENS**

For many years, it was thought that the breast was hormonally regulated in a similar fashion to the endometrium where estrogen enhanced proliferation and increased the risk of cancer and the addition of a progestogen counteracted this effect. In vitro experiments supported this concept, showing that the addition of a progestogen to breast cells in a estrogenic environment reduces ongoing proliferation. Progestogens, like MPA, are used in high doses for breast cancer treatment. The mechanism explaining this effect is

not clear but may be due to down regulation of estrogen receptors, FSH/LH or through a direct cytotoxic effect. In contrast, in vivo studies of the normal breast have shown that breast epithelial proliferation is highest during the luteal phase of the menstrual cycle under the combined influence of estradiol and progesterone.

Using fine needle aspiration biopsies of the breasts of young premenopausal women, proliferation was found to be increased in the luteal phase and correlated to serum progesterone levels. In young women using combined oral contraceptives, increased proliferation showed a positive correlation to circulating levels of the progestogen levonorgestrel.<sup>12</sup>

In another study, 50 postmenopausal women (mean age 54.9 years) were randomized to receive either estradiol valerate/dienogest or estradiol/norethisterone acetate once daily for six months. FNA biopsies were performed at baseline and after three and six months. Assessment of mammographic density was also performed at baseline and at six months. Here, a three-to four-fold increase in proliferation during treatment with estradiol in combination with either NETA or dienogest was recorded (Figure 1). In the same women, an increase in mammographic density judged both by the Wolfe and percentage scale classification methods was recorded in approximately 50% of cases. A positive association between increase in breast cell proliferation and change in mammographic density was noted.

Data from an experimental in vivo model where surgically postmenopausal macaques were given HT showed that the proliferative response was more pronounced after treatment with conjugated equine estrogens plus medroxyprogesterone acetate than after estrogen alone.<sup>13</sup> Similar findings have been reported in women using HT and undergoing benign breast surgery.<sup>14</sup> The increase in mitotic activity during the luteal phase of the menstrual cycle, as well as the increase in proliferation seen during various types of combined estrogen/progestogen treatments, clearly implies a proliferative action of progestogens. This is also evidence that the breast does not respond to the same proliferative stimuli as the uterus.

The apparently different results between in vitro and in vivo studies may be explained partly by the fact that cultured breast cells are deprived of their natural surrounding milieu of fat and

stroma, thus depriving them of hormonal and paracrine influences. The progestogenic effect on breast tissue is also dependent on whether there is estrogen priming or not.

Altogether, these data suggest that progestogens are mitogenic in the breast. However, the effects surely depend on type, dose, route of administration, and the estrogenic environment. Therefore, different progestogens may have various effects on breast cell proliferation and sex steroid receptor expression.

Several independent epidemiological reports also have indicated that combined estrogen-progestogen HT may carry a risk for breast cancer beyond that of treatment with estrogen alone. While odds ratios (OR) for combined estrogen/progestogen treatment are generally significant, ORs for ERT are around 1.

### ALTERNATIVE REGIMENS

Tibolone is a synthetic compound used for the treatment of climacteric symptoms and the prevention of osteoporosis in postmenopausal women. Tibolone and its metabolites exert weak estrogenic, androgenic, and progestogenic effects. The net effect in different target tissues is the result of various mechanisms that depend on local metabolism, as well as steroid receptor binding and activation.<sup>15</sup>

In vitro data, animal studies, and clinical trials confirm that the effects of tibolone differ from those of estrogens and estrogens plus progestogens. Cellular studies with normal or malignant breast epithelial cells show that tibolone decreases proliferation and increases the rate of apoptosis through modulation of bcl-2, an anti-apoptotic protein. It also reduces the tissue level of biologically active estrogens by inhibiting sulfatase (an enzyme responsible for the conversion of inactive sulphated steroids into active compounds), and by stimulating the synthesis of type II 17 $\beta$ -hydroxysteroid-dehydrogenase and sulfo-transferase (two enzymes that convert estradiol into the weaker estrogen estrone and into inactive sulfated compounds). Animal studies also show that tibolone fails to stimulate the proliferation of normal and malignant breast epithelial cells and tumoral growth of dimethyl-benzan-

thracene (DMBA)-induced tumors inoculated into ovariectomized mice.

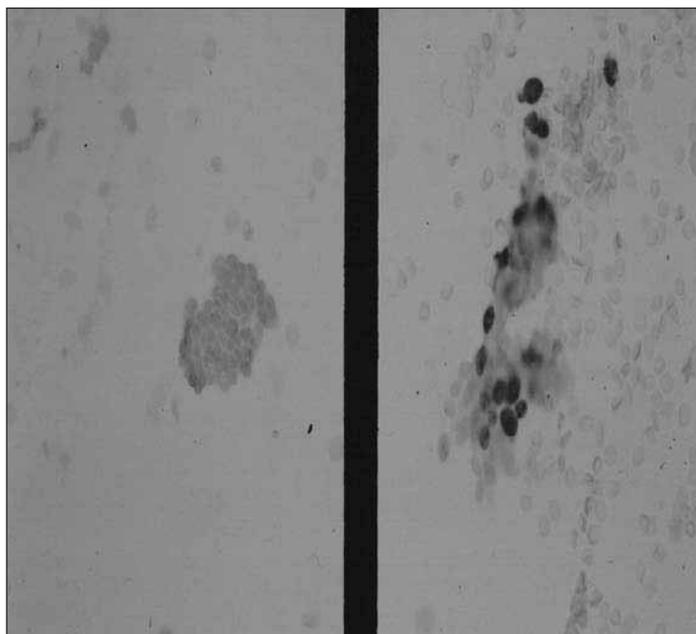
Clinical studies in women indicate that the effects of tibolone on breast tissue are different from those seen with conventional combined estrogen/progestogen treatment. Tibolone has been found not to induce mastodynia and, in contrast to combined therapy, has a minimal impact on mammographic density.

In a prospective, double-blind, placebo controlled study, a total of 166 postmenopausal women were randomized to receive either 2.5 mg tibolone, continuous combined 2 mg estradiol/1 mg norethisterone

almost three-fold after six months ( $p < 0.01$ ), whereas the effect of tibolone did not differ from that of placebo.<sup>10</sup>

In a similar study where 37 women were randomized to treatment with continuous combined CEE plus MPA or tibolone, mammographic density and breast cell proliferation were found to decrease after one year of tibolone treatment. In the continuous combined EP group, there was a stimulation of proliferation and inhibition of apoptosis in parallel to an increase in mammographic breast density.<sup>17</sup>

As compared to continuous combined estrogen/progestogen therapy, the response to tibolone was somewhat more "androgenic" in nature. Estrogen levels were virtually unchanged whereas there was a suppression of SHBG and a clear increase in testosterone levels. In women given continuous combined therapy, the opposite reaction was observed.<sup>10</sup> There are a number of observations to suggest that androgens may counteract the proliferative effects of estrogen and progesterone in the mammary gland. In ovariectomized rhesus monkeys, the addition of a small, physiologic dose of testosterone to standard estrogen therapy treatment almost completely attenuated estrogen induced breast epithelial proliferation. Testosterone was also found to reduce ER $\alpha$ , and the



**Figure 1:** Breast cells from one individual postmenopausal woman before (left) and after (right) six months of continuous combined estrogen/progestogen treatment. Nuclei in proliferating cells stain brown by the Ki-67/MIB-1 antibody.<sup>10</sup>

expression of the mammary epithelial proto-oncogene *myc* which has been linked to tumorigenesis.<sup>18</sup>

acetate, or placebo for six months. The risk of an increase in breast density was significantly less with tibolone than with continuous combined HT. Breast density assessed by either the Wolfe score or percentage classification was increased in approximately 50% of women using conventional HT, whereas only about 5% of women given tibolone had a similar reaction. Also, the percentage of women reporting breast pain as an adverse event during treatment was significantly different between the two groups: 33% among those using continuous combined HT compared to only 3% of women receiving tibolone and 0% when given placebo.<sup>16</sup>

Fine needle aspiration biopsies of the breast were performed in a subgroup of 91 women from this study. During continuous combined treatment, the percentage of MIB-1 positive breast cells was increased

In studies conducted in the primate model, it was also shown that alternative regimens for hormonal therapy may have different effects on proliferation and apoptotic activity within the breast. Ovariectomized cynomolgus macaques were treated with various long-term hormonal combinations using either estrogen alone, in combination with progestogen or tibolone. Breast epithelial proliferation was found to increase significantly following the administration of estrogen (CEE) in combination with progestogen (MPA) compared to treatment with CEE alone, tibolone, or placebo.<sup>19</sup> In the same material, levels of caspase-3 as a marker for apoptosis were found to be significantly lower in the combined treatment group as compared to estrogen

only and controls.<sup>20</sup> A combined effect of increased proliferation and decreased apoptosis could be one possible mechanism to explain the excess breast cancer risk associated with this type of treatment.

### INDIVIDUAL FACTORS

Constitutional and hormonal factors seem to be important predictors of proliferation and mammographic density. Lean, nulliparous women with a shorter duration of menopause have higher hormone levels, whereas older women with low body mass index seem to respond stronger to treatment. Circulating levels of estrogens have a positive association to density and proliferation, and androgens have a negative association.<sup>10</sup>

### CONCLUSION

From a clinical perspective, increased breast cell proliferation and mammographic density during hormonal treatment should be regarded as unwanted and potentially hazardous side effects. However, it is clear that not all women respond in a negative manner to treatment. The individual response to treatment may depend on several factors, e.g., age, BMI, parity, dose and type of regimen, as well as endogenous hormones and growth factors.<sup>8</sup> During continuous combined estrogen/progestogen therapy, a significant increase in breast cell proliferation has been seen to parallel an increase in mammographic breast density. A combined effect of increased proliferation and decreased apoptosis during estrogen/progestogen treatment could be one possible mechanism explaining the excess breast cancer risk reported in clinical and epidemiological studies.

Treatment with tibolone, a compound with estrogenic, progestogenic, and androgenic properties seems to have less impact on breast cell proliferation than conventional continuous combined estrogen/progestogen. Higher androgen and comparably lower levels of circulating estrogens during treatment may add to effects on local steroid metabolism within the breast to explain why tibolone may differ from conventional HT in this respect. Currently, there is rapid development of treatments with selective effects on specific symptoms and target organs. While short-term treatment for symptomatic relief remains uncontroversial, there is much controversy about indications for long-term treatment and prevention. Possible adverse

effects of progestogens have been targeted in recent years, and alternatives for endometrial protection are needed.

*The authors revealed no potential conflicts of interest.*

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## Mammographic Breast Density: The Imaging Perspective



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In 1976, Wolfe described a relative risk of developing breast cancer 33-fold higher for women with dense breast tissue compared to those who were fatty replaced.<sup>1</sup> The association fell out of favor in subsequent years largely due to the variability in applying the Wolfe criteria. However, since the mid-1980s, 12 of 12 studies using quantitative assessment of breast density have shown a moderate to strong statistically significant association between percent breast density and breast cancer risk.<sup>2</sup> Mammographic breast density remains a poorly recognized risk factor for the development of breast cancer despite these numerous studies.

Dense breast tissue on mammography has long been a bane to radiologists due to the lower sensitivity for detecting breast cancers, which has been likened to finding a snowman in a snowstorm. Thus, women at high risk due to greater breast density are also less likely to have their breast cancers detected at mammography. Ancillary screening techniques, such as screening ultrasound or MRI, may therefore be appropriate though sensitivity, specificity, and cost-effectiveness for these methods are not yet established in the screening setting. Ancillary screening should not be substituted for mammography, however, as calcifications, which are frequently the earliest sign of breast can-

cer, are poorly seen on ultrasound and not seen at all on MRI. Digital mammography may be more sensitive for the detection of breast cancer than film-screen mammography due to improved contrast, although no studies have yet demonstrated a benefit.

Women with high mammographic breast density are at 4- to 7-fold increased risk of developing breast cancer compared to women with fatty breasts.<sup>3,4</sup> Breast density is therefore a greater risk factor for developing breast cancer than traditional risk factors such as age at first live birth, age at menarche, and prior benign breast biopsy. Only prior breast cancer, biopsy showing lobular carcinoma in-situ (LCIS) or atypical ductal hyperplasia, and germ line mutations such as BRCA1 or BRCA2 are associated with a greater risk of developing breast cancer. Some studies show a stronger risk for postmenopausal women with dense breast tissue, although others show a stronger risk for premenopausal women. The relationship is monotonic, with increasing density associated with increasing risk. The elevation in risk persists for at least 10 years, though it is unclear how changes in density may affect this risk.

#### METHODS FOR MEASURING BREAST DENSITY

In the United States, mammographic density is classified using the four categories of the Breast Imaging Reporting and Data System (BI-RADS),<sup>5</sup> which are: almost entirely fatty, scattered fibroglandular densities, heterogeneously moderately dense, and extremely dense. The original purpose of including density categories in mammog-

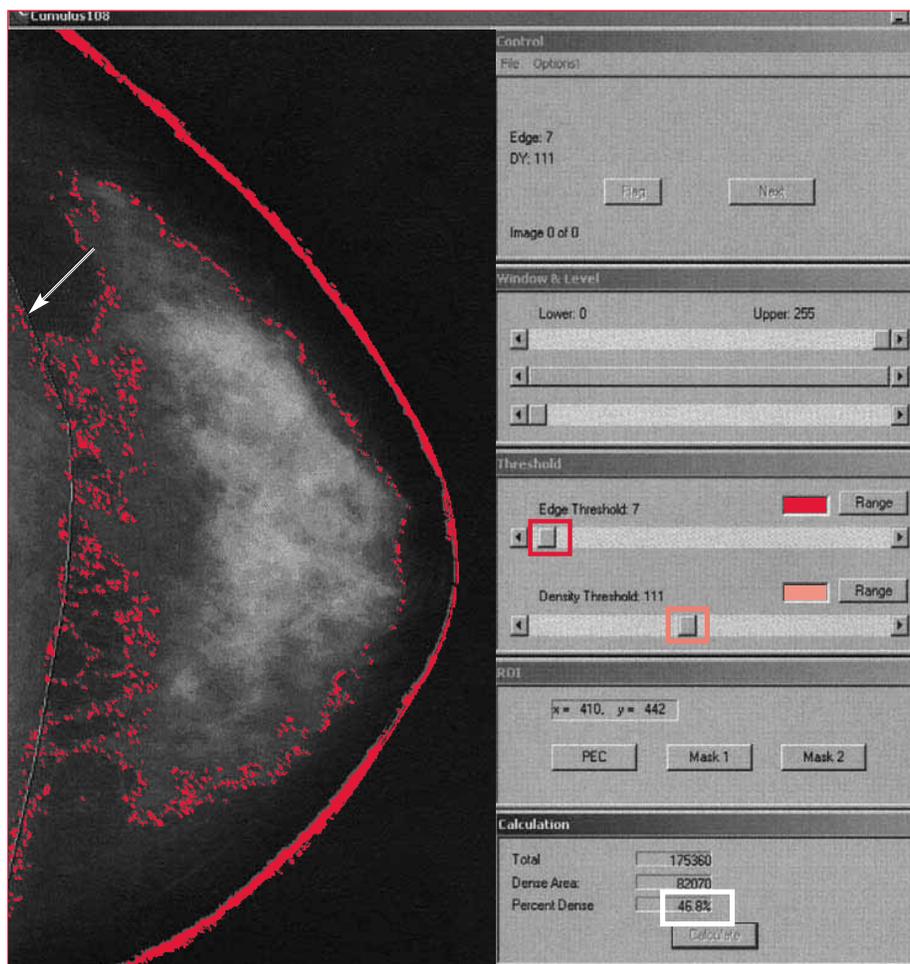
raphy reports was to convey the reduced sensitivity of mammography with increasing breast density. It was not until 2003 that breast density categories were assigned quantitative criteria, defined as <25%, 25% to 49%, 50% to 75%, and >75% dense, respectively. Our work has shown that in practice, the range of percent densities of the BI-RADS categories is considerably lower than these recently issued definitions. The Wolfe criteria are still widely used in Europe and elsewhere. Both the BI-RADS and Wolfe criteria suffer from only fair inter- and intra-reader agreement. An advantage of using BI-RADS density categories, however, is that they are included in nearly every mammography report in the United States in recent years.

Quantitative methods of assessing the percentage of the breast occupied by breast

tissue are considerably more accurate. Manual, and, more recently, computer assisted planimetry methods have been used. Computer-assisted interactive thresholding is a modification of computerized planimetry where the histogram of the mammographic pixel densities is segmented into fatty and dense tissue (Figure 1). This method has much better reproducibility than BI-RADS categories or Wolfe criteria, with kappa values in the range of 0.90.<sup>6</sup> This method is the best validated, though only for film-screen mammography. Use of digital mammograms yields considerably lower percent breast density than film-screen mammography largely due to enhanced visualization of the skin line, which is often not visualized on film mammograms. Percent density correlates better with breast cancer risk than absolute

area of breast tissue,<sup>6</sup> which seems counter-intuitive as one would expect that breast cancer risk would correlate best with the total amount of breast parenchyma. This may be related to the lack of three-dimensional information in assessing breast density with current methods.

A major limitation of current quantitative methods is that the process is quite cumbersome. In order to obtain the percent breast density using computer software methods, an analog mammogram must be digitized, windowed, and converted to bitmap format. The image is opened in the program, where the skin line is defined, pectoral muscle excluded, and the threshold of segmentation defined (Figure 1). While acceptable for studies involving several hundred women, the process soon



**Figure 1.** Interactive thresholding software for calculating percent breast density<sup>6</sup>. The edge of the breast is first selected by sliding the edge threshold bar until the skin line is outlined (red box). The user then selects the appropriate point of segmentation by choosing the point at which the breast tissue is appropriately outlined using the density threshold slider (light red box). The posterior aspect of the breast is defined by manually defining the pectoral muscle (arrow). Percent breast density is calculated by dividing the pixels of breast tissue by the total pixels of breast area (white box). In this case, the breast is 46.8% dense. Note that some areas of breast tissue are denser than others, although this is not accounted for in current models of computerized assessment of breast density.

becomes impractical for studies involving many thousands of women. Quantitative methods are currently used only in the research setting. Some investigators have developed automated programs to measure breast density to improve ease of application and further improve reproducibility.<sup>7</sup>

A second limitation of current quantitative methods is the lack of inclusion of three-dimensional information. Current methods assume that all pixels of breast tissue represent the same amount of breast tissue, meaning that the brightness or pixel depth is ignored. Two women may have the same percent breast density though one may have very low density, wispy breast tissue while the other has a focal area of very dense breast tissue. Methods that are able to include three-dimensional information may therefore better identify women at high risk.

### HORMONAL INFLUENCE ON BREAST DENSITY

Hormonal agents associated with increased breast cancer risk likewise increase breast density, whereas hormonal agents that decrease risk likewise decrease breast density (Figure 2). Seventeen percent to 73% of women will increase their breast density with initiation of menopausal hormone therapy, particularly estrogen with progesterone.<sup>8,9</sup> The breast is rapidly responsive to the initiation and discontinuation of hormones, with the increase in mammographic density occurring largely within the first year<sup>10</sup> and decrease in density occurring in as little as two weeks after withdrawal.<sup>11</sup> Women who experience an increase in breast density associated with initiation of menopausal hormone therapy are also likely to experience breast tenderness.<sup>12</sup>

Increased breast density associated with menopausal hormone therapy use may

mask breast cancer due to decreased sensitivity of mammography with higher breast density. The Women's Health Initiative demonstrated fewer breast cancers detected during the first several years after initiation of therapy in the study group using estrogen with progesterone compared to placebo, followed by a significant increase in breast cancer incidence.<sup>13</sup> The authors hypothesize that this was due to increased complexity of the mammogram. The estrogen-only arm of the Women's Health Initiative study did not show an increased risk of breast cancer compared to placebo.

The little or no association of breast cancer risk with use of estrogen alone in postmenopausal women begs the question of whether estrogen is a culprit in the initiation or promotion of breast cancer. Considerably more work must be done to evaluate the respective role of progestogens in breast metabolism. However, progesterone is known to be a strong potentiator of estrogen action. Thus, menopausal women with extremely low basal progesterone levels may have little response to estrogen use alone, while administration of progesterone may considerably multiply the proliferative effect of estrogen on the breast. This situation may be somewhat similar to pubescent girls where the majority of breast enlargement, due to lobular proliferation, occurs late in puberty when progesterone levels rise.

The method of administration of menopausal hormone therapy may also influence both breast cancer risk and breast density changes. We have shown recently that women using a transdermal preparation of estrogen with a progestogen were less likely to experience an increase in breast density or breast tenderness compared to women using an oral preparation.<sup>14</sup> This may be due to a lower first pass effect of liver metabolism of hormonal compounds.

The Nurse's Health Study showed a moderate positive association between breast cancer risk and serum prolactin levels.<sup>15</sup> Likewise, women using dopamine antagonists such as anti-psychotic medications, which result in increased serum prolactin levels, are at mildly increased risk for developing breast cancer. No studies have shown an association between increased breast density and serum prolactin levels. However, we have presented a case of increased breast density associated with use of domperidone, a dopamine antagonist used for gastrointestinal disorders.<sup>16</sup>

Use of anti-estrogenic compounds such as tamoxifen and raloxifene results in decreased breast density. Tibolone, widely used in Europe for menopausal hormone therapy, has a complex mechanism largely acting as a sulfatase inhibitor. This compound increases breast density in a small percentage of women (2% to 6%). The Million Woman Study showed a small increase in breast cancer risk with the use of tibolone.<sup>17</sup>

### POTENTIAL CAUSES OF THE DENSE MAMMOGRAM

Higher breast density is associated with increased fibrous stroma and an increase in the number of lobules, but not the number of ducts (Harvey et al, unpublished). These changes are likely due to hormonal influence, whether due to overproduction or slower breakdown of estrogen and/or progesterone that result in these proliferative changes. Aromatase activity, which would result in greater conversion of steroids to estrogen, may be higher in dense breasts.

Women who carry the BRCA1/2 gene are known to have greater breast density.<sup>18</sup> These women are at very high risk for developing breast cancer. However, these and other known germ cell mutations account for only about 5% of all breast

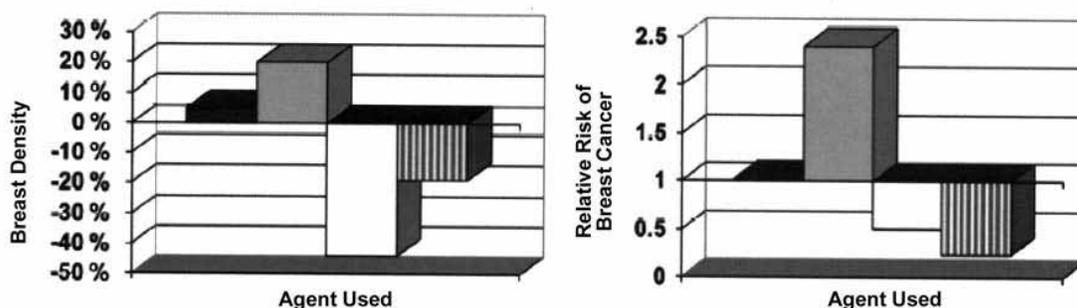


Figure 2. Overall percentage of women with change in density (left) is similar to the relative risk of breast cancer (right) associated with estrogen (black bar), estrogen with progestin (gray bar), tamoxifen (white bar), and raloxifene (striped bar) (with permission, *Radiology*).

cancers (low prevalence, high penetrance genes). Although many women who develop breast cancer have some family history of the disease, they are not known or suspected carriers of the currently known germ cell mutations associated with high breast cancer risk. This makes it likely that there are high prevalence, low penetrance genes present in society that are as yet undetected. These genes may include those involved in estrogen production, such as the CYP19 gene that regulates production of aromatase, or genes that regulate estrogen breakdown. A twin-twin study suggests that breast density is heritable.<sup>19</sup>

While other breast cancer risk factors are difficult, if not impossible, to influence, breast density can be reduced through the use of anti-estrogens such as tamoxifen. A small study from the Netherlands has shown a decrease in breast cancer risk associated with decreasing breast density over a 10-year period, although the confidence intervals were broad.<sup>20</sup> This seems appealing on the surface. However, if women with dense breast tissue are at increased risk due to genetic predisposition, then reducing breast density may not reduce breast cancer risk. Further work is needed to address this issue.

### USING BREAST DENSITY AS A RISK FACTOR

Although breast density is a moderate to strong risk factor for developing breast cancer, no risk assessment models currently include breast density. Breast density could be included in risk models by using the BI-RADS category or the quantitative percent breast density.

An advantage of using the BI-RADS categories in risk assessment models is that these are readily available in mammography reports. However, the BI-RADS categories have only fair reproducibility, which may make validation of the model difficult. An association between BI-RADS categories and breast cancer risk has also only recently been validated in a large population. Vacek et al. have shown that premenopausal and postmenopausal women with BI-RADS extremely dense breast tis-

sue are 4.6 and 3.9 times more likely to develop breast cancer respectively compared to those with BI-RADS almost entirely fatty replaced breasts.<sup>21</sup> The use of BI-RADS density categories could be used as an estimation of breast cancer risk for individual patients keeping in mind that a change in density category may be due to reader variability rather than a true change in percent breast density.

breast density could become a commonly used assessment of breast cancer risk.

Variations in genes (polymorphisms) controlling estrogen production and/or breakdown may be related to breast density. Investigation of this area will be complex since it is likely that more than one gene contributes to breast density and low penetrance of breast cancer due to important polymorphisms in carriers may make identification difficult. Polymorphisms may also involve tissue-specific promoter regions of the gene rather than the transcription portion. Discovery of genetic changes associated with greater breast density will give insight regarding breast cancer initiation and promotion and potentially gene therapy as a preventive strategy.

In summary, high breast density is associated with lower mammographic sensitivity and a moderate to strong risk of breast cancer development. Women with dense breast tissue may wish to consider ancillary screening in addition to mammography, although these techniques need further study before widespread use is recommended. Quantitative measurement of breast density is considerably more reproducible than BI-RADS categories or Wolfe criteria, although it is currently cumbersome to perform. Breast cancer risk assessment models may initially use BI-RADS categories due to com-

mon use but will likely eventually use quantitative breast density derived from digital mammograms. Discovery of the genes that control breast density may lead to insights regarding breast cancer initiation and promotion.

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<b>Breast Density Categories</b>	
<b>Wolfe Categories of Breast Density<sup>1</sup></b>	
<b>N1:</b>	Parenchyma composed primarily of fat with at most small amounts of dysplasia; no ducts visible.
<b>P1:</b>	Parenchyma composed primarily of fat with prominent ducts in the anterior portion of the breast up to one fourth of the breast; may also be a thin band of ducts extending into a quadrant.
<b>P2:</b>	Prominent ductal pattern occupying more than one-fourth of the volume of the breast.
<b>DY:</b>	Severe involvement of dysplasia, often obscures an underlying ductal pattern.
<b>BI-RADS Breast Density Categories<sup>5</sup></b>	
	The breast is almost entirely fat (<25% glandular)
	There are scattered fibroglandular densities (approximately 25% to 50% glandular)
	The breast tissue is heterogeneously dense, which could obscure detection of small masses (approximately 51% to 75% glandular)
	The breast tissue is extremely dense. This may lower the sensitivity of mammography (>75% glandular)

### FUTURE DIRECTIONS

Digital mammography is becoming increasingly more common. Automated measurement of breast density using digital mammography would be ideal since the mammogram is already in electronic format. It is likely that digital mammography vendors will eventually produce software that will provide a measurement of breast density. This would significantly enable use of quantitative breast density in risk assessment models, although models would have to be validated in large populations. Percent breast density by quantitative criteria may prove to be a better predictor of breast cancer risk than serum cholesterol is as a predictor of cardiac event. Therefore, it is not unlikely that quantitative percent

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