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Relationship of Age and Menopausal Status to Estrogen Receptor Content in Primary Carcinoma of the Breast

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The cytosolic estrogen receptor (CER) content of 1037 primary breast carcinomas was evaluated by sucrose density gradient analysis. Tumor specimens from premenopausal patients had significantly lower levels of CER (14.6 ± 1.5 (mean ± SEM) 8S binding fmole/mg protein) compared with carcinomas from postmenopausal patients (57.5 ± 3.9 fmole/mg protein; $p < 0.001$). The proportion of specimens with CER levels above threshold values of 3, 7, or 10 fmoles/mg protein were significantly higher for postmenopausal patients (72%, 63%, 59%, respectively) than for premenopausal patients (56%, 42%, 36%, $p < 0.001$). When compared within half-decades, no statistically significant differences between premenopausal and postmenopausal patients were observed for mean, median, or rank sums of CER levels ($p > 0.3$). When patients were compared by half-decades, both mean and ranked sums of CER levels were significantly different ($p < 0.001$). The proportion of specimens that demonstrated CER levels above a threshold value of 10 fmole/mg protein increased sequentially from a low of 13/51 (26%) for patients <35 years to a high of 60/81 (74%) for patients >75 years.

PRIOR TO ROUTINE estrogen receptor (CER) analysis of breast carcinoma specimens, the clinical parameters of age, menopausal status, and length of disease-free interval from mastectomy were used to select therapy in patients with metastatic breast carcinoma.[1] In particular, elderly patients with breast carcinoma when compared with younger patients were more often responsive to hormonal manipulation and had improved survival.[1-3] Since the initial report by Jensen et al.[4] that the measurement of CER content in metastatic breast carcinoma tissue was useful in predicting response to endocrine therapy, this determination has been widely used to select therapy.[5] It has also been reported[6] that patients with primary tumors that were CER-rich experienced improved survival compared with patients whose carcinomas are CER-poor.

Compared with premenopausal patients, postmenopausal patients have quantitatively higher levels of CER and a greater proportion of their tumors are CER positive.[5,7-10] With the exception of large series reported by Elwood,[10] most investigators[5,7-9] have interpreted their data to suggest a significant correlation between CER levels and menopausal status rather than between CER levels and age. The present study evaluates the interrelationship between age, menopausal status, and cytosolic estrogen receptor content of primary tumors in patients with carcinoma of the breast.

Material and Methods

Patient Population

One thousand and thirty-seven primary mammary carcinomas were obtained from Duke University Med-

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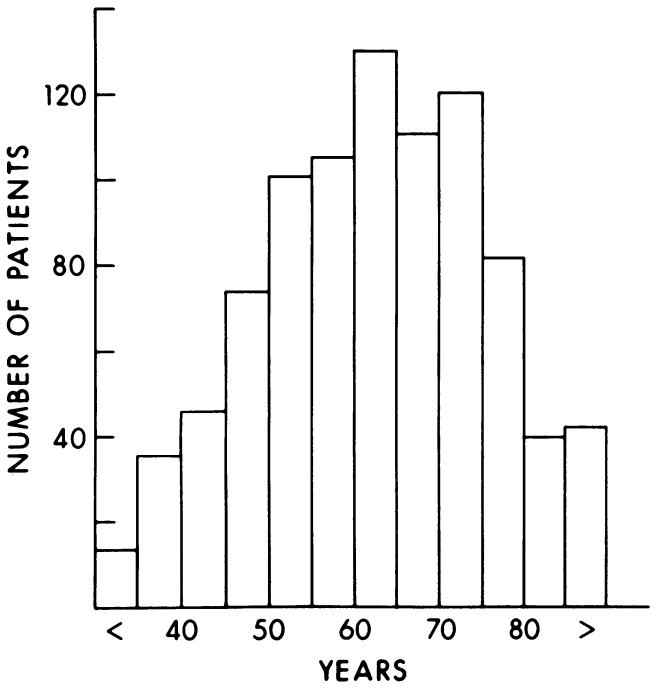

FIG. 1. Age distribution of study patients with primary carcinoma of the breast. A total of 1037 patients ranged from 23 to 97 years with a median age of 58 years.

ical Center and cooperating community hospitals in North Carolina. Menopausal status was determined either from direct interview data in a protocol obtained by a trained observer (437 patients) or from review of protocol data submitted by the referring physician. Patients were considered to be premenopausal if they had had a menstrual period within the previous six months. Patients were considered postmenopausal if their last menstrual period was more than six months prior to mastectomy or if they were surgically castrate at least six months prior to mastectomy. Patients were designated as indeterminant if menstrual histories could not accurately classify menopausal status.

Estrogen Receptor Analyses

The estrogen receptor content of each tumor was assessed by sucrose density gradient analyses (SDGA) as previously described.[11] All tissues were washed in buffer (0.1 M EDTA, 0.1 M thioglycerol, 0.05 M TRIS, 0.05 M HEPES, pH 7.4 at 4 C) immediately after excision. Cryostat-prepared sections were made of each tissue specimen to confirm the presence of carcinoma. The quantity of estrogen receptor present in each specimen was determined from the amount of diethylstilbestrol-inhibitable 8S binding species. Indistinct binding in the 8S region was not considered in the quantitation of CER; 4S binding was not included for the purpose of this study, since 8S binding species demonstrate higher specificity and predictive value for response to hormonal manipulation in patients with metastatic breast carcinoma.[12,13]

Statistical Methods

Data was stored and analyzed using the CLINFO (Bolk, Beranek and Newman, Inc., Boston, MA) and TORO (Duke Comprehensive Cancer Center) data analysis systems.

Two group comparisons were made using the Mann Whitney U test.

The general linear model was used for multivariable analysis of covariates related to CER. CER values were log transformed because of their large range of variation and marked skew toward higher values. Exploratory data analysis revealed better linearity of the relationship between age after log transformation of CER, further supporting its desirability.

Results

Patient Population

One thousand and thirty-seven patients with primary breast carcinoma were included in this study. Median age of the population was 58 years (range 23 to 97 years) (Fig. 1). There were 265 premenopausal patients, 603 postmenopausal patients, and 169 patients in whom menopausal status was indeterminant.

Relationship of Receptor Levels to Menopausal Status

Post menopausal patients had significantly higher CER levels than premenopausal patients ($p < 10^{-6}$, Mann Whitney U test). In linear regression analysis, 6.7% of the total variance in CER was explained by variation in menstrual status. The cumulative distribution of CER by menstrual status demonstrated a higher percentage of premenopausal patients with unmeasurable CER values, as well as a lower incidence of tumors with values above any particular threshold value in the premenopausal group (Fig. 2).

Relationship of Receptor Levels to Age

Examination of the scatter plot of log CER values against age revealed steadily increasing CER values with age from the third decade into the tenth decade. CER was measurable above the threshold of the SDG assay in a progressively higher percentage in proceeding from youngest to oldest patients with the disease. When patients with unmeasurable CER values were excluded, there was still a significant relationship between increas-

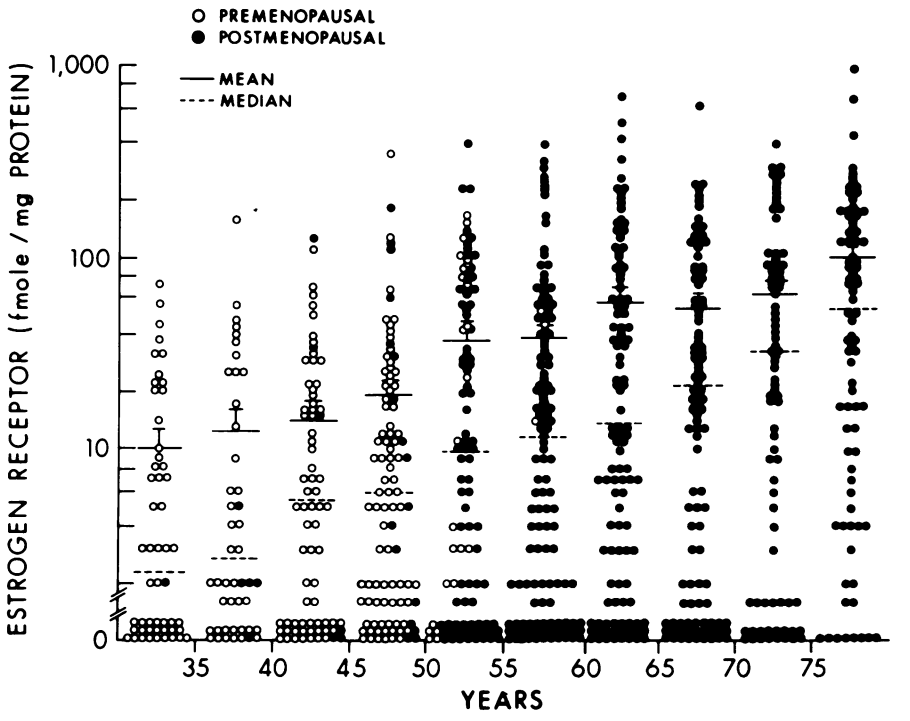

FIG. 2. Age distribution of estrogen receptor values. The distribution of ER values was categorized in 5-year increments. Estrogen receptor values represent 8S binding (SDGA). Premenopausal patients—open circles; postmenopausal patients—solid circles; mean—solid horizontal bars; SEM—solid vertical bars; median value—dashed horizontal bar.

ing age and CER level. Furthermore, the strength of the relationship as judged by correlation coefficient was unchanged when patients with unmeasurable levels are excluded. Thus, the increasing CER with age is not simply due to an increase in the number of patients with measurable levels but a true upward modulation of CER values. In regression analysis, 10.7% of the total variance in log CER was explained by age variation. The cummulative frequency distribution (CFD) for half-decades graphically represents the nature of this shift to higher CER levels in older patients (Fig. 3). Tumors from patients <35 years had quantitatively lower receptor values: 40% had no demonstrable CER, 70% had CER values less 10 fmole/mg protein and none were observed with CER values exceeding 100 fmole/mg protein. This was in contrast to the distribution of CER values in tumors of patients >75 years: less than 10% had no demonstrable CER; over half had CER values above 50 fmole/mg protein and CER values exceeding 100 fmole/mg protein were observed in 29/81 (35.8%). Patients 45 to 49.9 years had fewer specimens without demonstrable CER (23%) compared with patients <35 years, but the distribution of CER values above the median was similar to the younger patients—the two CFD curves are closely approximated. Patients 60 to 64.9 and 45 to 49.9 age group had similar numbers of specimens without CER. The distribution of CER values demonstrated a considerable number of tumors with higher quantities of estrogen receptor; *i.e.*, the CFD is shifted to the right and displaced downward (Fig. 3).

Simultaneous Analysis of Receptor Levels, Menopausal Status and Age

Examination of the scatter plot of CER *vs.* age reveals no discernable discontinuity in the perimenopausal pe-

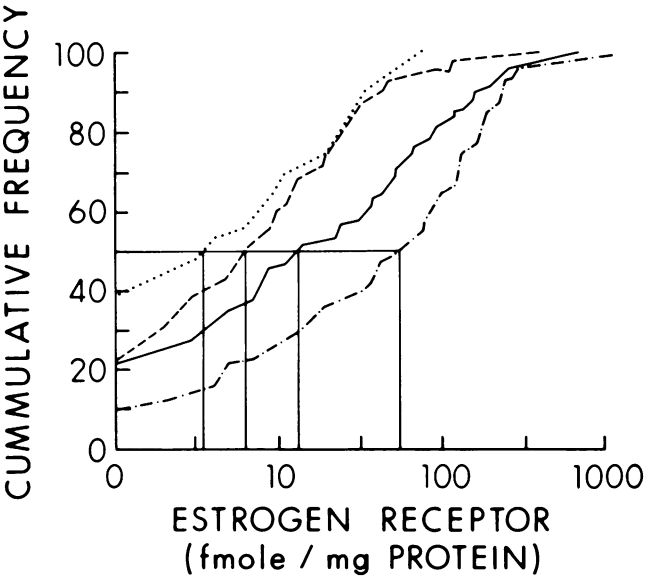

FIG. 3. Commulative frequency distribution of estrogen receptor values. The cummulative frequency distribution of ER values is shown for four groups of patients. The perpendicular lines drawn from the 50% line represent median values. Patients >35 years—dotted line; patients 45 to 49.9 years—dashed line, patients 60 to 64.9 years—solid line; patients <75 years—dashed and dotted line.

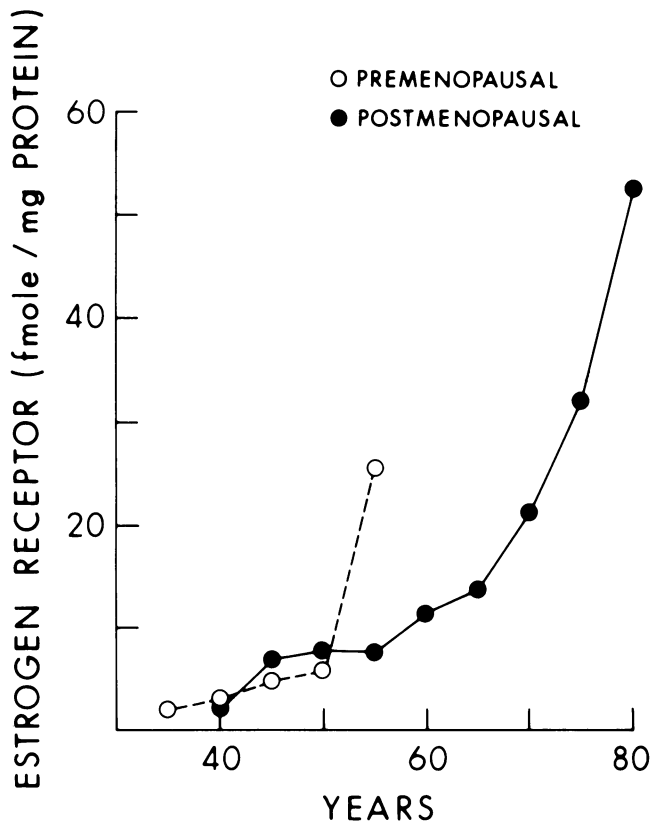

FIG. 4. Median values for premenopausal and postmenopausal patients. The median values for premenopausal and postmenopausal patients was plotted for each five-year increment. Premenopausal patients—open circles, dashed line; postmenopausal patients—closed circles, dotted line.

riod. CER values were not significantly different between premenopausal and postmenopausal patients within any half-decade group (Figs. 4, 5), although the median CER for menstruating patients aged 50 to 55 tended to be higher than for postmenopausal patients aged 50 to 55. In a multiple regression analysis, age provided significant additional information once menopausal status was accounted for. However, once age was accounted for, menopausal status provided no additional information.

Discussion

The analyses of the data from these 1037 patients with primary carcinoma of the breast indicate that CER increases with age from the third through the tenth decade. Knowledge of menstrual status does not further clarify the variation in CER once age-specific variation is adjusted for, yet age adds substantially to the explanation of CER variation even after menstrual status has been taken into account. Most previous studies[5,7-9] have emphasized menopausal status, suggesting that premenopausal patients have a lower incidence of CER positive tumors as well as quantitatively lower tumor levels of estrogen receptor compared with postmenopausal patients. Martin et al.[9] found that although mean CER levels increased for each decade, significant differences were only noted between premenopausal and postmenopausal patients. Allegra et al.[8] were also unable to define a direct correlation between CER levels and age. These studies did not evaluate the relationship between CER levels and menopausal status within the same age range. In addition, the sample size of older patients may have been insufficient to permit a statistically valid assessment of this relationship.

In a recent report, Elwood et al.[10] described a positive correlation between age and CER levels, noting that this relationship could explain the association of menopausal status and CER levels observed by others. Our data generally confirm this report although we note a monotonic increase in the proportion of CER positive tumors without a detectable peak at 45 to 49 years and without a decrease in patients older than 75 years. This difference may be related to the use of the SDG analysis while others have used the somewhat less specific multiconcentration titration assay.[10] The quantitation of CER using the 8S binding species obtained from SDG appears to be more predictive of response to hormonal therapy than the DCC assay.[12,13]

The reason for the CER content increase with patient age may be related to a number of factors. Some studies have shown a negative correlation between serum estrogen levels and CER content of the breast carcinoma.[14,15] Since postmenopausal women have lower

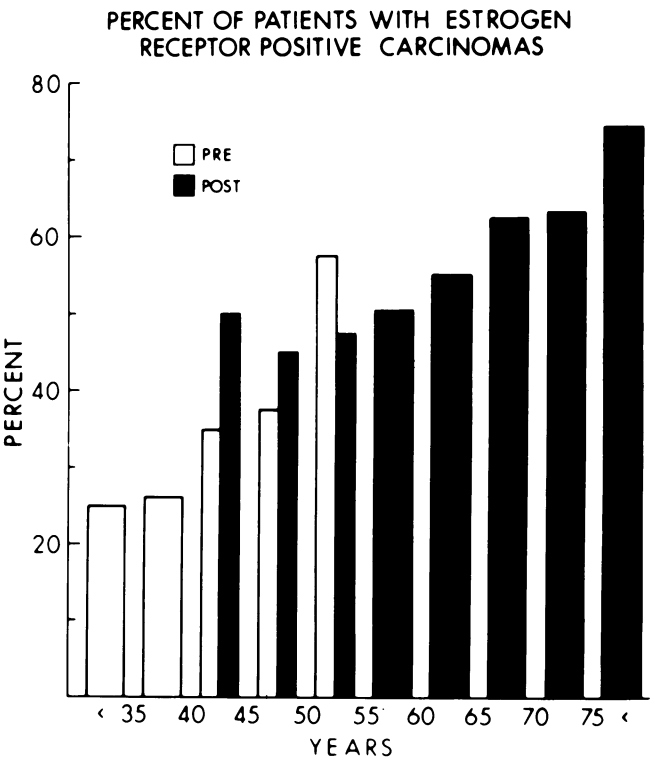

FIG. 5. Percentage of patients with estrogen receptor values <10 fmole/mg protein are shown for each five-year increment. Premenopausal patients—open bars; postmenopausal patients—solid bars.

circulating estrogen levels,[16] the higher CER levels observed in tumors from these patients have been suggested to be the result of an increase in unoccupied cytosolic receptor rather than an increase in total cytosol receptor.[14] Saez et al.[17] have postulated that the cyclic levels of serum progesterone in premenopausal patients limit CER synthesis. This later hypothesis is supported by the menstrual cycle variations of CER observed in normal human endometrium[18] and recently shown in normal human breast.[19] In both the breast and endometrium, preluteal CER values were significantly higher than CER levels during the luteal phase when plasma progesterone is high. Thus, the higher CER levels observed in postmenopausal patients may be related to chronic unopposed estrogen stimulation, to a decrease in the progesterone down regulation of CER or a combination of these factors. Pituitary-ovarian function is also highly dependent upon age.[16] Younger patients, with cyclic ovarian activity, have tumors with the lowest CER content. Patients in their early 40s and through early 50s have an increased incidence of anovulatory cycles with more frequent failure of progesterone secretion. Elderly patients, in whom cyclic ovarian function has ceased but who may have noncyclic levels of adrenal and ovarian androgen that are converted peripherally to estrogens,[20] have carcinomas with the highest CER levels. It is possible that age and menstrual status may show correlation with CER levels as a reflection of the hormonal millieu in which the breast carcinoma developed.

The integrity of receptor regulation of breast carcinoma growth is reflected in the tumor's biologic behavior.[21] The improved survival and response rate to hormonal therapy observed in elderly patients with breast carcinoma[1-3] suggests that these patients would be more likely to have an intact receptor control mechanism, compared with younger patients. The fraction of the total variability in CER which is explained by age is only about 10%. The remaining 90% probably relates in large measure to variable expressivity of the individual tumor type in the amount of estrogen receptor per cell or proportion of CER rich cells. The variation of CER by age may be an important clue to the biologic characteristics associated with malignant transformation but does not obviate the need for determination of CER in individual patients for purposes of treatment planning and prognostic assessment.

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