Aromatase Activity and Estradiol in Human Breast Cancer: Its Relationship to Estradiol and Epidermal Growth Factor Receptors and to Tumor-Node-Metastasis Staging

By Pascual Bolufer, Enrique Ricart, Ana Lluch, Carlos Vazquez, Antonio Rodriguez, Amparo Ruiz, Francisco Llopis, Javier Garcia-Cande, and Rafael Romero

Purpose: The present report attempts to clarify whether there is a relationship between aromatase activity (ARAC) and estradiol (E_2), hormonal receptors, E_2 receptor (ER), and epidermal growth factor receptor (EGFR), as well as with tumor stage and histopathology in human breast cancers.

Materials and Methods: We studied 225 breast carcinomas, 67 of which were premenopausal and 158 postmenopausal. In each sample, ARAC, EGFR, ER, and E_2 were quantified. ARAC was quantified by Thompson and Siiterii's method, EGFR was quantified with a two-point assay method using radioactive iodine (^{125}I)-EGF as ligand, and ER was measured by the Scatchard method using ^{3}H-E_2. E_2 was quantified by radioimmunoassay in the diethylether tumor extract.

Results: ARAC was found in 64% of the cancers studied. There is a strong direct association between ARAC and tumor size in postmenopausal patients ($P = .001$). In the postmenopausal group, the proportion of ARAC-positive (ARAC+) tumors is significantly higher among ER-positive (ER+) than ER-negative (ER−) ones ($P < .001$). ER+ tumors also have significantly higher levels of E_2 than do ER− ones ($P < .0001$); similarly, ARAC+ tumors have significantly higher levels of E_2 than do ARAC− ones ($P < .0001$). There is a significant multiple linear correlation between the log of the levels of ARAC, ER, and EGFR and the log of tumor E_2 ($P < .0001$). The correlation coefficients obtained show that ARAC and ER have a positive effect on tumor E_2.

Conclusion: The results obtained suggest the importance of tumor ARAC in the tumoral levels of E_2 and reinforce the possible biologic significance of tumor ARAC, especially in postmenopausal breast carcinoma patients.

J Clin Oncol 10:438-446. © *1992 by American Society of Clinical Oncology.*

THE MAIN SOURCE of estrogens in postmenopausal women are the C19 androgenic adrenal steroids, androstenedione (A) and testosterone (T), which by the action of the aromatase system are converted into estrone (E_1) and estradiol (E_2), respectively.[1-5] Due to their aromatizing capacity, normal mammary tissue[4,6] and some breast cancers[1,4,7,8] are able to synthesize their own estrogens, which in turn prompt the growth of the hormone-dependent breast cancers.[4,6]

Aromatase activity (ARAC) has been found in 60% to 70% of breast cancers[1,4,7,8] and is greater in breast cancers than in normal breast tissue[7] or even mammary fat.[8] However, and contrary to brain ARAC for which specific biologic significance related to sexual differentiation has been described,[6,10] little is known about the significance of ARAC in breast cancers. In one study, tumoral ARAC is related to the response to aminoglutethimide,[11] and in other studies, an association between ARAC and E_2 receptor (ER) status was found.[12] However, in the majority of the studies in this field, no relation has been found between ARAC and ER or progesterone receptors (PRs),[1,4,5,7,8] or between ARAC and tumoral tumor-node-metastasis (TNM) staging.[1,8]

Moreover, the origin of tumoral E_2 has not been completely elucidated. It has been suggested[3] that mammary sulfatase might play a more important role than ARAC in the production of E_1 through conversion of E_1 sulfate to E_1, with further transformation to E_2 by the action of 17β-hydroxysteroid dehydrogenase. However, some recent studies performed in vivo support the relevance of tumor ARAC in the tumoral synthesis of estrogens.[11] Higher E_2 concentrations have been found in breast cancer than in normal breast tissue or in plasma[7,13]; nevertheless, only a relationship between E_2 and ER status was found.[7]

The introduction of aromatase inhibitors in breast cancer treatment makes clarification of the possible significance of tumor ARAC and E_2 essential. The inconsistency of the results of the different studies on this subject may be attributable to the small number of cases included. For this reason, the present study quantified ARAC and tumor E_2 in 225 primary breast cancers to ascertain whether there is a relationship between ARAC and E_2, hormonal receptors, ER, and epidermal growth factor receptor (EGFR), as well as tumor stage and histopathology.

From the Department of Clinical Pathology, Hospital La Fé, Valencia; Department of Clinical Oncology, University of Valencia, Valencia; Instituto Valenciano de Oncologia, Valencia; Department of Surgery, Hospital of Sagunto, Valencia; and Department of Statistics, Politechnic University, Valencia, Spain.

Submitted June 3, 1991; accepted October 18, 1991.

Address reprint requests to Pascual Bolufer, MD, Laboratorio de Hormonas, C Maternal, Hospital La Fé, Avd Campanar 21, 46009 Valencia, Spain.

© 1992 by American Society of Clinical Oncology.

0732-183X/92/1003-0018$3.00/0

MATERIALS AND METHODS

The study included 225 breast carcinomas from consecutive patients. Sixty-seven carcinomas were from premenopausal women with a median age of 40 years (range, 24 to 49 years) and 158 from postmenopausal women with a median age of 62 years (range, 42 to 87 years). Four patients older than 45 years having menstrual disorders were included in the postmenopausal group.

Tumor samples, all of which should have a minimum weight of 400 mg, were obtained at the time of mastectomy or tumorectomy and were stored in liquid nitrogen until analyzed. In each sample, ARAC, tumoral E_2, cytosolic ER (cER), nuclear ER (nER), and EGFR were quantified. The tissue sections were stained with hematoxylin-eosin, and in 140 tumors the degree of dedifferentiation was assessed by the Bloom-Richardson (BR) index.[14,15] The tumors were grouped into three grades: grade I comprised scores 3 to 5; grade II, 6 to 7; and grade III, 8 to 9.

One hundred eighty-eight cancers were classified as ductal carcinoma, 27 as lobular carcinoma, and 10 as medular, inflammatory, and mucinous carcinoma. Almost 50% of the tumors (111 of 225) were T_2, with the rest distributed among the other sizes (Tables 1 and 2). Eighty-four patients had nodal involvement at the time of the mastectomy, and 16 patients had distant metastases (Tables 1 and 2).

Reagents

Reagents included tritium-labeled A, 1β-^{3}H(N)-androst-4-ene-3, 17-diona (^{3}H-A), 27.4 Ci/mmol and 99% of purity (Du Pont de Nemours, Germany); cold A, androst-4-eno-3,17-diona (A; Sigma, St Louis, MO); β-nicotinamide-adenine-dinucleotide phosphate (NADPH; Sigma); tritium-labeled estradiol, 2,4,6,7-^{3}H17β-estradiol (^{3}H-E_2), 91 Ci/mmol (Amersham, Buckinghamshire, United Kingdom); tritiated water (^3H_2O), 5 mCi/mL (Amersham); iodine-labeled EGF (^{125}I-EGF); 164 to 178 μCi/μg (Du Pont de Nemours); and cold EGF (Biomedical Technologies, Ltd, Stoughton, MA).

Table 1. ARAC and Qualitative Parameters in Premenopausal Breast Cancer Patients

	ARAC−	ARAC+	Total	$\chi^2(df)/P$
BR index				3.17 (2)/.20
I	8	6	14	
II	10	8	18	
III	1	5	6	
T				1.80 (3)/.61
1	8	5	13	
2	14	20	34	
3	6	5	11	
4	4	5	9	
N				0.66 (1)/.42 (Yates)
0	16	13	29	
+	16	22	38	
M				0.01 (1)/.91 (Yates)
0	29	33	62	
1	3	2	5	
ER status				0.002 (1)/.96 (Yates)
−	24	25	49	
+	8	10	18	
EGFR status				0.24 (1)/.61 (Yates)
−	22	27	49	
+	10	8	18	

Table 2. ARAC and Qualitative Parameters in Postmenopausal Breast Cancer Patients

	ARAC−	ARAC+	Total	$\chi^2(df)/P$
BR index				0.98 (2)/.61
I	10	21	31	
II	13	38	51	
III	4	16	20	
T				12.52 (3)/.0005
1	8	8	16	
2	30	47	77	
3	3	18	21	
4	7	37	44	
N				1.89 (1)/.16 (Yates)
0	21	34	55	
+	27	76	103	
M				1.57 (1)/.21 (Yates)
0	47	100	147	
1	1	10	11	
ER status				12.44 (1)/.00041 (Yates)
−	35	45	80	
+	13	65	78	
EGFR status				2.11 (1)/.14 (Yates)
−	40	78	118	
+	8	32	40	

Assay of ARAC in Breast Cancer

Quantification of the ARAC was done by Thompson and Siiterii's method,[16] which consists of radiometric quantification of ^3H_2O yield in the process of aromatization of ^{3}H-A to E_1.

Between 250 and 300 mg of tissue was taken and homogenized to complete powder using a Mikro-Dismembrator (Braun, Melsungen, Germany). The tissue powder was resuspended in 0.5 mL of assay buffer (0.066 mol/L phosphate, 9.82 mmol/L nicotinamide, 10.56 mmol/L $MgCl_2$, 1.12 mmol/L edetic acid (EDTA), 15.38 mmol/L NaN_3 and 1/1,000 Triton-X-100, and 0.125 mmol/L dithiothreitol, pH 7.3) per 200 mg tissue and left in vortex for 1 hour at 4°C. Aliquots of 100 μL and 400 μL of the homogenate were taken and kept frozen at −20°C for total protein (TP)[17] and E_2 determinations, respectively.

In a glass tube, 1 μCi ^{3}H-A and 0.69 μmol/L of cold A, both in ethanolic solutions, were combined and dried under a stream of N_2. The extract was dissolved in 200 μL of tissue homogenate or replaced with assay buffer in the blank tube. Next, 10 μL of aqueous solution of 0.64 nmol/L NADPH was added and incubated for 1 hour at 37°C. The reaction was stopped by placing the tubes in an ice bath. Then 200 μL of charcoal-dextran suspension (5 g charcoal and 0.5 g dextran T-70 per 100 mL water) was added, and the tubes were centrifuged at 4,000 rpm for 15 minutes to remove the supernatant containing the ^3H_2O. The supernatant was seeded in the charcoal-sephadex chromatographic columns (glass columns of 6 × 15 cm; filled with 5 cm charcoal at the top and 5 cm sephadex 25 to 50 at the bottom) eluted with 6 mL of distilled water, and collected in 12 aliquots of 500 μL each. According to the results obtained after counting the radioactivity of eluted ^3H_2O, a tumor was considered as aromatase-positive (ARAC+) when the sum of counts-per-minute of the 12 aliquots was at least two times the counts-per-minute obtained for the reagent blank. Final results, corrected by the recoveries obtained by checking the columns with ^3H_2O, were expressed as femtomoles of E_1 per milligram of TP per hour.

The method was validated by quantifying ARAC in different tissues and by checking the reduction of ARAC in ARAC+ breast cancers in the presence of the aromatase inhibitor, aminoglutethimide (AG). The inhibitory capacities of the imidazol-derivative CGS16949A,[18] AG, 4-hydroxy androstendione, and dihydrotestosterone in human placental homogenates were also tested.

Tumor E_2 Quantification

Duplicate 200-μL samples of tissue homogenate were extracted with 2 mL of freshly distilled diethylether. E_2 was quantified in these extracts using a radioimmunoassay kit for plasmatic E_2 (^{125}I-estradiol direct Radioimmunoassay Kit, Baxter Dade, Düdingen, Switzerland). The antibody used is highly specific, having negligible crossreactivities with other related steroids. All the samples were analyzed in duplicate, and the concentrations of most of the samples lay between the third and fourth point of the standard curve corresponding to 147 and 367 pmol/L, respectively. Final results were expressed in picomoles of E_2 per gram of TP tissue.

Sample Preparation for EGFR and ER

The same sample homogenate was used for ER and EGFR determination. A minimum of 150 mg of tissue was homogenized in HEPES-EDTA-dithiothreitol buffer (20 mmol/L HEPES, 1.5 mmol/L EDTA, 0.125 mmol/L dithiothreitol; pH 7.4), 1 mL for each 50 mg of tissue. After the tissue was homogenized, it was centrifuged at 5,000g for 15 minutes at 4°C to yield a cytosolic supernatant and a nuclear pellet. The nuclear pellet was kept for quantification of nER, but the supernatant was centrifuged at 25,000g for 30 minutes at 4°C. The supernatant was kept for quantification of cER, and the sedimented plasma membranes were resuspended in one half the original volume with assay buffer (1 mmol/L HEPES, 5 mmol/L NaCl, pH 7.4, containing 1 g bovine serum albumin [BSA] per liter). A small aliquot of cytosolic fraction was centrifuged separately to quantify plasma membrane TP.

EGFR Quantification

The EGFR assay consisted of incubating duplicate 100-μL samples of plasma membranes plus 100 μL ^{125}I-EGF (40,000 cpm, ≈ 200 to 300 fmol/mL) and 200 μL of assay buffer[19] to evaluate the total binding; or with 200 μL of a solution of EGF in assay buffer containing 8,333 fmol of cold EGF to evaluate the nonspecific binding. The tubes were incubated at room temperature for 2 hours and then centrifuged at 20,000g for 10 minutes at 4°C. The specific binding was obtained by subtracting the mean counts-per-minute of nonspecific binding from that of total binding. Results were expressed in terms of femtomoles of ^{125}I-EGF bound to plasma membranes per milligram of plasma membrane TP.

Homogenates of placental tissue (rich in EGFR) were used in each assay as positive controls.

An EGFR concentration greater than 0.5 fmol/mL homogenate, with the nonspecific binding lower than 70%, was used as the criterion of positivity (EGFR+).[19]

ER Quantification

The ER was quantified by the method reported by Leake et al.[20] Fifty microliters of ^{3}H-E_2 with or without 100 × diethylstilbestrol (DES) in seven increasing concentrations in the 10^{-10} to 10^{-8} mol/L range was added to 150 μL of cytosolic and nuclear fractions. All the tubes were incubated at 4°C for 18 hours. The ^{3}H-E_2–receptor complex was separated from the free ^{3}H-E_2 using the dextran-coated charcoal method for the cytosolic fraction and by filtering for the nuclear fraction.

Results were expressed in femtomoles of cER per milligram of TP for the cytoplasmic fraction and femtomoles of nER per milligram of DNA for the nuclear pellet. The DNA was determined following a modification of the method reported by Burton.[21,22]

A tumor was considered ER-positive (ER+) when ER was simultaneously present in the cytosolic (cER+) and nuclear fractions (nER+) and was considered ER-negative (ER−) when it was not present in either cellular fraction (−/−) or was present in only one of them (+/− or −/+).

The results obtained with the ER method were validated through participation in an independent quality control program (European Organization for Research and Treatment of Cancer [EORTC] Receptor Study Group Quality Control) and by verifying that the results obtained with the control materials were within the limits of mean ± 2SD of the participants using equivalent methods.

Statistics

To compare the results of two or more groups, the Kruskal-Wallis (KW) test was used. The proportions were compared with the χ^2 test using Yates's correction for 1 degree of freedom (*df*). In multiple comparisons with *P* equal to .05 as the level of statistical significance, the *P* value was corrected for six comparisons (comparison within premenopausal or postmenopausal groups) and for 12 comparisons (both groups), resulting in *P* values of .01 and .005, respectively, as limits of statistical significance.

Multiple regression analysis was used to evaluate the specific effect of quantitative variables ARAC, ER, and EGFR together with the qualitative variables of tumoral T, N, and M on tumor E_2.[23] In this analysis, ER is the sum of cER and nER for the ER+ tumors; for ER− tumors, it is nil. In the analysis, the transformations log of E_2 and log of quantitative variables plus 1 were used to normalize the distributions of these variables, which showed a clear skew. To test the formal contribution of the variables of T, N, and M to the model, the analysis of variance (ANOVA) test for deletion of variables was applied; to check the difference in the significance pattern of premenopausal and postmenopausal patients, multiple regression analysis in groups was performed.[23]

RESULTS

ARAC was identified in 145 of the 225 (64%) breast cancers studied. The postmenopausal group tended to have a higher proportion of ARAC+ tumors (70% *v* 50%) and higher median levels of ARAC than the premenopausal group, although the differences did not reach statistical significance (Fig 1).

To check the qualitative or quantitative associations of ARAC with BR index, TNM staging, and ER and EGFR status, the χ^2 test or the nonparametric KW ANOVA, respectively, were used.

In the premenopausal group, no relationship between the presence of ARAC and T was demonstrated (χ^2 = 1.8, not significant [NS]; Table 1), but a statistically significant direct quantitative association of the two

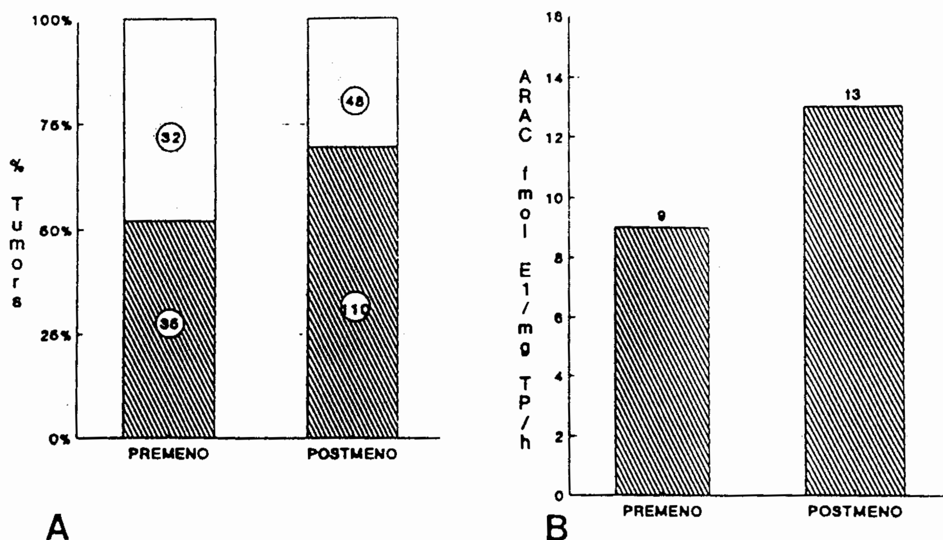

Fig 1. ARAC and menstrual status. Presence of ARAC (A); the figures inside the circles represent the number of tumors. ARAC+ (▨) v ARAC− (□): χ^2 = 5.46, P = .019. Quantity of ARAC (B); the figures at the top of the bars represent the median values: KW test = 6.21, P = .012.

parameters was found (KW = 10.11, P = .01; Table 3, Fig 2). In contrast, in the postmenopausal group there is a statistically significant increase in the proportion of ARAC+ tumors with T (χ^2 = 12.52, P < .001; Table 2, Fig 2); however, no quantitative relationship between the two parameters was found (KW = 3.22, NS; Table 3).

No qualitative or quantitative relationship between tumor ARAC and N involvement was found in the

Table 3. ARAC of ARAC+ Breast Cancers: Relationship With BR Index, Tumor Characteristics, and Hormonal Receptors

	Premenopausal Group		Postmenopausal Group	
	Median fmol E_1/mg TP (range)	No. of Samples	Median fmol E_1/mg TP (range)	No. of Samples
BR index				
I	9.7 (5-22.5)	6	13.0 (5.2-25.2)	21
II	11.8 (5.6-99.6)	8	12.3 (5.0-56.7)	38
III	12.3 (6.5-22.7)	5	11.3 (5.0-22.1)	15
KW; P	0.48; .78		1.69; .42	
T				
1	6.9 (5.1-8.1)	5	7.3 (5-17.4)	9
2	9.1 (5-14.3)	20	13.3 (5-92.9)	47
3	18.0 (7.5-22.5)	5	11.5 (8.1-24.0)	17
4	19.7 (5-99.7)	5	12.9 (5-31.8)	37
KW; P	10.11; .01		3.22; .35	
N				
0	7.9 (5-21.2)	13	12.9 (5.3-56.7)	34
+	10.7 (5-99.7)	22	11.7 (5-92.9)	76
KW; P	0.94; .33		0.01; .90	
M				
0	8.9 (5-99.7)	33	12.0 (5-56.7)	107
1	22.6 (22.5-22.7)	2	18.9 (5-92.9)	10
KW; P	4.85; .027		6.64; .0091	
ER status				
−	8.1 (5-99.6)	25	13.9 (5-92.9)	44
+	11.4 (5-22.7)	10	11.4 (5-56.7)	66
KW; P	2.8; .08		2.31; .13	
EGFR status				
−	9.1 (5-99.6)	27	11.6 (5-92.9)	79
+	9.4 (5.2-22.7)	8	13.8 (5-25.3)	31
KW; P	0.0034; .95		1.27; .258	

Abbreviation: KW, the nonparametric ANOVA of Kruskall-Wallis test.

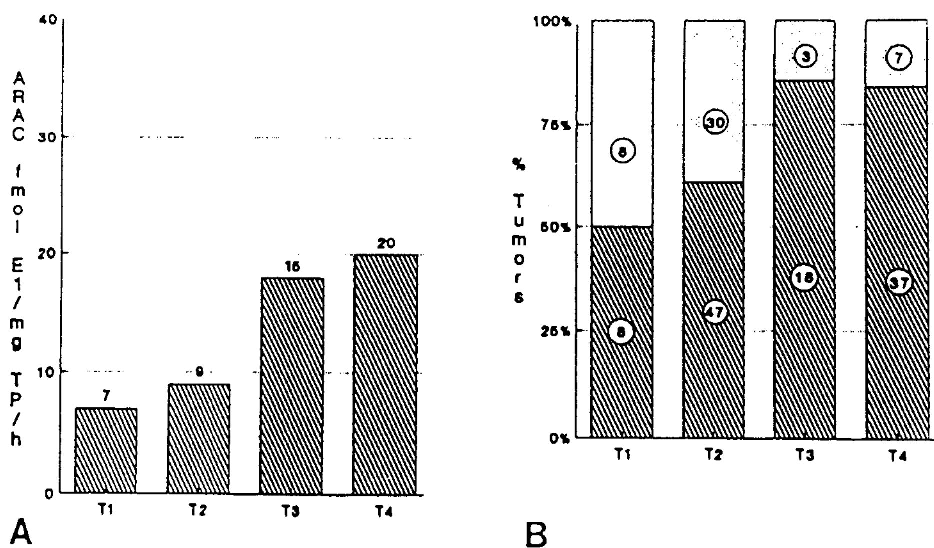

Fig 2. ARAC and tumor size (T). Quantity of ARAC and T in premenopausal group (A); the figures at the top of the bars represent median values: KW test = 10.11, P = .017. Presence of ARAC (ARAC+, ▧; ARAC−, ☐) and T in the postmenopausal group (B); the figures inside the circles represent the number of tumors: χ^2 = 12.52, P = .0005.

groups studied (Tables 1 to 3), nor is there any relationship between ARAC and distal M in the premenopausal (χ^2 = 0.01, NS; Table 1) or postmenopausal group (χ^2 = 1.57, NS; Table 2). However, a trend toward higher median values of ARAC in tumors with distal M was found in both groups (Table 3, Fig 3).

The presence of ARAC+ tumors is strongly associated with ER+ status in the postmenopausal group (χ^2 = 12.44, P < .001; Table 2), with 83% of ARAC+ tumors in ER+ cancers versus 56% in the ER− tumors. However, this relationship disappears in the premenopausal group (Table 1, Fig 4). No quantitative association between ARAC and ER status has been found in any of the groups studied (Table 3).

No relationship, either qualitative or quantitative, was found between ARAC and EGFR in the two groups studied (Tables 1 to 3), nor was there any association of tumor ARAC with the BR index of histologic differentiation (Tables 1 to 3).

The quantitative associations of the tumor E_2 with BR index, TNM staging, ARAC, and ER and EGFR status were assessed using the nonparametric KW ANOVA.

Tumor E_2 did not show significant changes with the menstrual status (KW = 1.28, NS); for this reason, tumor E_2 was studied in the whole group, regardless of menstrual status of the patients.

No relationship between tumor E_2 and TNM staging was found (Table 4). However, a significantly strong and

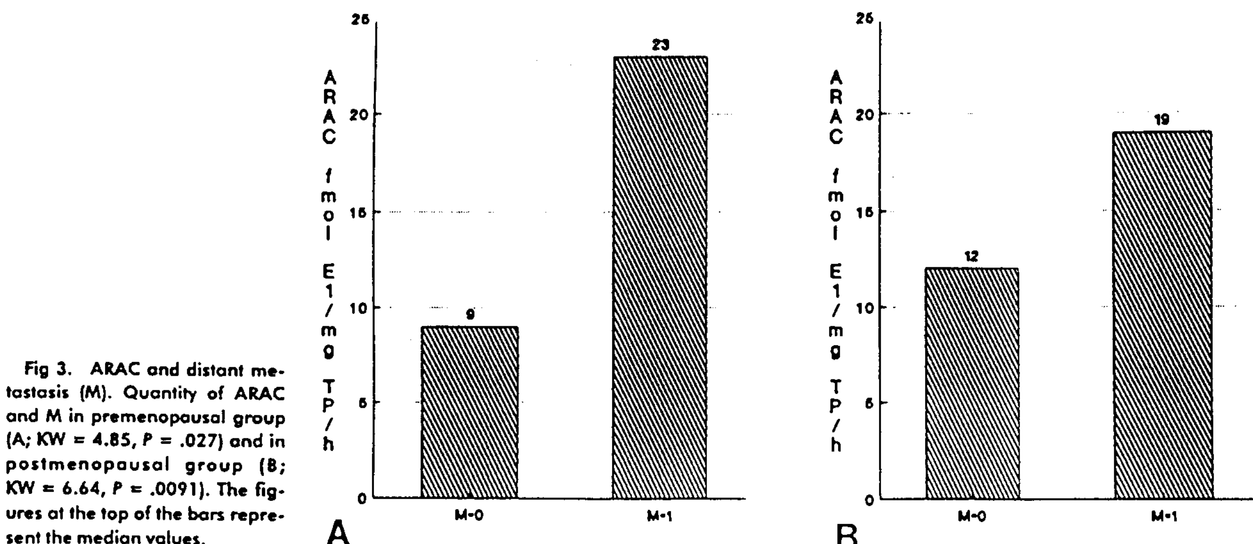

Fig 3. ARAC and distant metastasis (M). Quantity of ARAC and M in premenopausal group (A; KW = 4.85, P = .027) and in postmenopausal group (B; KW = 6.64, P = .0091). The figures at the top of the bars represent the median values.

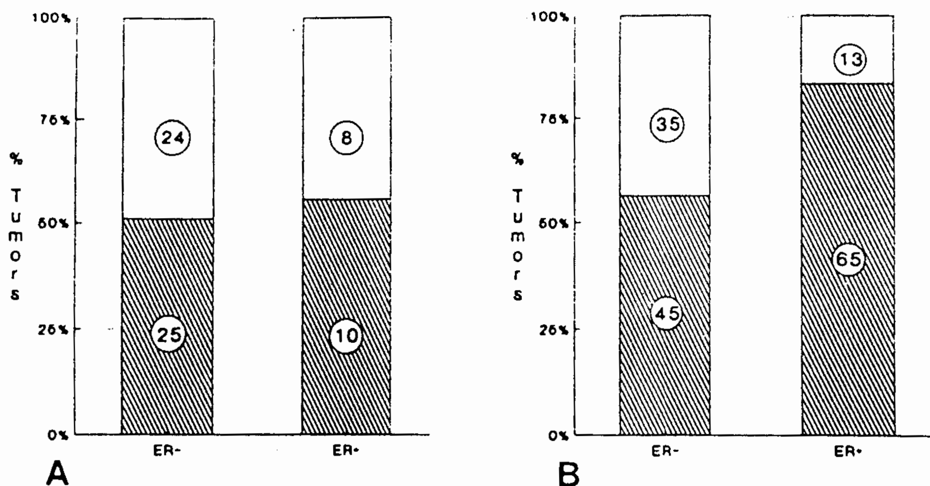

Fig 4. Presence of ARAC (ARAC+, ▨; ARAC−, □) and ER status in premenopausal (A; χ^2 = 0.002, NS) and postmenopausal (B; χ^2 = 12.44, P = .0004) patients. The values inside the circles represent the total number of tumors.

direct association of tumor E_2 with the presence of ARAC (KW = 42.93; P < .0001; Table 4, Fig 5)—even stronger than that found for ER status (KW = 30.7, P < .0001; Table 4)—was observed. Thus, the ER+ or ARAC+ tumors have median values of E_2 that were almost threefold those of their corresponding counterparts.

To evaluate the contribution of TNM stage and the quantitative variables ARAC, ER, and EGFR to tumor E_2 content, the multiple linear correlation analysis was used.

A statistically significant correlation coefficient of the log E_2 versus the log of quantitative variables, ARAC, ER, and EGFR plus 1, together with tumor TNM stage was found (R^2 = .56, F = 15.7, P < .0001; Table 5). However, only the partial regression coefficients of the quantitative variables were statistically significant, and those of the tumor characteristics T, N, and M lacked statistical relevance (Table 5). Moreover, the removal of the variables T, N, and M from the regression did not modify the multiple correlation coefficient (F[3,218] for deletion of variables = 0.04079, NS). The increments in

Table 4. Breast Cancer E_2: Relationship With BR Index, Tumor Characteristics, ARAC, and Hormonal Receptors

	Median pmol E_2/g TP (range)	No. of Samples	KW	P
BR index			0.55	.75
I	27.8 (1.4-91.7)	45		
II	23.4 (0.4-261.6)	69		
III	22.3 (0.4-70.1)	26		
T			2.86	.41
1	13.5 (1.5-60.9)	29		
2	22.3 (0.4-261.6)	111		
3	24.9 (0.4-218.4)	32		
4	23.8 (2.2-141.6)	53		
N			2.91	.08
0	20.5 (1.4-261.6)	84		
+	23.1 (0.4-218.4)	141		
M			2.02	.154
0	22.3 (0.4-261.6)	209		
1	37.4 (1.8-73.4)	16		
ARAC presence			42.93	<.0001
−	11.7 (0.4-218.4)	80		
+	30.1 (0.4-261.6)	145		
ER status			30.7	<.0001
−	12.8 (0.4-218.4)	129		
+	31.2 (3.6-261.6)	96		
EGFR status			3.9841	.04
−	24.2 (0.4-261.6)	167		
+	4.1 (0.4-112.6)	58		

Abbreviation: KW, the nonparametric ANOVA of the Kruskall-Wallis test.

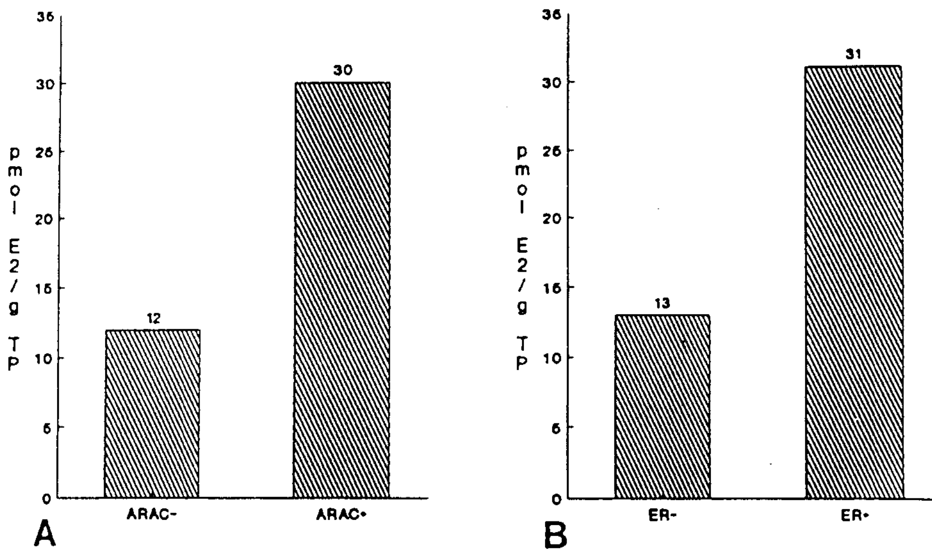

Fig 5. Tumor E_2 and ARAC (A; KW = 42.93, $P < .0001$) or ER status (B; KW = 30.7, $P < .0001$). Values at the top of the bars represent the median values of E_2.

ARAC and ER have a positive effect on intratumor E_2, whereas the increments in EGFR have a negative influence (Table 5). In the postmenopausal group, the multiple correlation coefficient obtained with ARAC, ER, and EGFR is much stronger ($R^2 = .655$; Table 5) than the one obtained for premenopausal patients ($R^2 = .36$; Table 5). The patterns of difference, however, lack statistical significance (F[4,217] regression analysis in groups = 2.104, NS).

DISCUSSION

The 64% of ARAC+ breast tumors found in this study lies between the percentages found in similar studies.[4,8,24] The trend toward higher proportions of ARAC+ tumors and higher amounts of ARAC found in postmenopausal patients lends support to the studies that point to an increase in ARAC after menopause.[25,26] However, other studies on human breast cancer did not reach this conclusion,[4,5,7,24] probably because of a scarcity of statistics.

In this study, a strong positive association between the presence of tumor ARAC and T was observed in postmenopausal patients. However, this relationship could not be demonstrated in the premenopausal group, even though a quantitative association between the amount of tumor ARAC and T was found. Our results are in agreement with those of an experimental study in the rat prostate gland, in which a similar ARAC and T relationship was found,[2] but differ from the findings of an earlier study that showed that ARAC tended to decrease in tumor with T stage greater than 2.[8] The lack of relationship between E_2 and T found in our study makes it difficult to understand how aromatase could mediate the effects on tumor growth and suggests that ARAC may affect tumor growth by ways other than estrogen synthesis or that the increase in ARAC related

Table 5. Multiple Regression Analysis of Qualitative and Quantitative Parameters Versus Log E_2

	Premenopausal + Postmenopausal Group			
	All Parameters PRC (*P*)	T,N,M Deletion PRC (*P*)	Premenopausal Group PRC (*P*)	Postmenopausal Group PRC (*P*)
Constant	0.942 (.0000)	0.945 (.0000)	1.056 (.0000)	0.831 (.0000)
Log (ARAC + 1)	.360 (.0000)	.358 (.0000)	.283 (.0051)	.423 (.0000)
Log (ER + 1)	.077 (.0002)	.077 (.0002)	.098 (.0663)	.069 (.0016)
Log (EGFR + 1)	−.089 (.0131)	−.089 (.0109)	.015 (.8470)	−.133 (.0005)
T	−.020 (.7471)	—	—	—
N	.071 (.5645)	—	—	—
M	−.001 (.9964)	—	—	—
R^2 (F; *P*)	.56 (18.4; .000)	.56 (36.6; .000)	.36 (4.3; .008)	.66 (40.4; .000)

NOTE. In the analysis, ARAC, ER, and EGFR are quantitative variables and T, N, and M are qualitative variables.
Abbreviation: PRC, partial regression coefficient.

to T is not a causative relation; rather, it is a consequence.

The finding of higher ARAC in breast cancers with distant M is not verified by other studies,[1] but it may explain the better response achieved with AG than with tamoxifen in patients with bone metastases.[27]

As in earlier studies,[5] no relation between ARAC and the degree of histopathologic differentiation was identified in this study.

We have found a strong association between the presence of ARAC and ER+ tumors in postmenopausal patients, a finding confirmed in a previous study.[12] However, most studies found no such association.[1,4,5,7,8,11] This may be attributable to the combined effects of the scarcity of cases and the masking effect of the premenopausal group, which lacks this association.

The lack of significant variations in tumor E_2 with menstrual status might be attributed to the increase in the quantity and greater proportion of tumor ARAC observed in postmenopausal patients. This in turn may explain the greater intratumor synthesis of E_2, which could compensate for the synthesis of E_2 of ovarian origin.

The relationship between intratumor E_2 and ER status has been reported in an earlier study,[7] but the present study shows an even stronger positive association between intratumor E_2 and the presence or quantity of ARAC. This strong association of intratumor E_2 with ARAC lends support to the hypothesis that ARAC plays an important role in the intratumor synthesis of E_2, suggested by some experimental in vivo studies.[13]

The positive effect of the increase in ARAC or ER on the intratumor E_2 might be explained as a consequence of the larger aromatization or greater capacity for binding of E_2 to ER, respectively. It is more difficult to explain the negative effect of EGFR on tumor E_2, which may be an expression of tumor dedifferentiation or may be related to unknown factors associated with EGFR.

The strong positive association between ARAC and T, its relation with distal M, and the strong relationship between intratumor E_2 and ARAC, ER, and EGFR (especially in postmenopausal patients) found in this study indicate that tumor ARAC has biologic relevance. However, firm evidence of this conclusion requires demonstration that tumors with higher ARAC synthesize a higher level of E_2. Points that require further study include the relationship between ARAC and T.

ACKNOWLEDGMENT

We are grateful to Esperanza Afan de Ribera and Enrique Lerma for their expert technical assistance in the determinations of ER and EGFR.

REFERENCES

1. Bezwoda WR, Mansoor N, Dunsey R, et al: Aromatization of androstendione by human breast cancer tissue: Correlation with receptor activity and possible biologic significance. Oncology 44:30-33, 1987

2. Marts SA, Padilla GM, Petrow V: Aromatase activity in microsomes from rat ventral prostate and Dunning R3327H. J Steroid Biochem 26:25-29, 1987

3. Santen RJ: Aromatase inhibitors for treatment of breast cancer: Current concepts and new perspectives. Breast Cancer Res Treat 7:23-36, 1986 (suppl)

4. Mallet NT, Santner SJ, Feil PD, et al: Biological significance of aromatase activity in human breast tumors. J Clin Endocrinol Metab 57:1125-1128, 1983

5. Lipton A, Santen RJ, Santner SJ, et al: Correlation of aromatase activity with histological differentiation of breast cancer. A morphometric analysis. Breast Cancer Res Treat 12:31-35, 1988

6. Roselli CHE, Ellinwood WE, Resko JA: Regulation of brain aromatase activity in rats. Endocrinology 11:192-199, 1983

7. Vermeulen A, Deslypere JP, Paridades R, et al: Aromatase, 17B-hydroxysteroid dehydrogenase and intratissular sex hormone concentrations in cancerous and normal glandular breast tissue in postmenopausal women. Eur J Clin Oncol 22:515-525, 1986

8. Lipton A, Santner SJ, Santen RJ, et al: Aromatase activity in primary and metastatic human breast cancer. Cancer 59:779-782, 1987

9. O'Neill JA, Elton R, Miller WR: Aromatase activity in adipose tissue from breast quadrants: A link with tumour site. Br Med J 296:741-743, 1988

10. Connolloy PB, Roselli CE, Resko JA: Aromatase activity in adult guinea pig brain is androgen dependent. Biol Reprod 43:698-703, 1990

11. Bezwoda WR, Mansoor N, Dansey R: Correlation of breast tumour aromatase activity and response to aromatase inhibition with aminogluthetimide. Oncology 44:345-349, 1987

12. Miller WR, Hawkins RA, Forrest APM: Significance of aromatase activity in human breast cancer. Cancer Res 42:3365-3368, 1982 (suppl)

13. Lonning PE, Dowsett M, Powles TJ: Postmenopausal estrogen synthesis and metabolism: Alterations caused by aromatase inhibitors used for the treatment of breast cancer. J Steroid Biochem 35:355-366, 1990

14. Bloom HJG, Richardson WW: Histologic grading and prognosis in breast cancer. Br J Cancer 11:359-377, 1957

15. Contesso G, Mooriesse H, Friedman S, et al: The importance of histologic grade in long-term prognosis of breast cancer: A study of 1,010 patients, uniformly treated at the Institut Gustave-Roussy. J Clin Oncol 9:1378-1386, 1987

16. Thompson DB, Siiteri PK: Utilization of oxygen and reduced nicotinamide adenine dinucleotide phosphate by human placental microsomes during aromatization of androstendione. J Biol Chem 249:5364-5367, 1974

17. Bradford MM: A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-254, 1976

18. Mason JI, Murray BA, Olcott M, et al: Imidazole antimycotics: Inhibitors of steroid aromatase. Biochem Pharmacol 34:1087-1092, 1985

19. Bolufer P, Miralles F, Rodriguez A, et al: Epidermal growth factor receptor in human breast cancer: Correlation with cytosolic and nuclear ER receptors and with biological and histological characteristics. Eur J Cancer 26:283-290, 1990

20. Leake RE, Laing L, Smith DC: The role of estradiol-nuclear receptor measurement in the management of human breast cancer, in King RJB (ed): Steroid Receptor in Human Breast Cancer Tumors: Methodological and Clinical Aspects. Cardiff, United Kingdom, Alpha Omega Press, 1978, pp 75-86

21. Burton K: A study of the conditions and mechanism of diphenylamine reactions for colorimetric estimation of desoxyribonucleic acid. Biochem J 62:315-322, 1956

22. Katzelenbogen BS, Leake RE: Distribution of estrogen-induced protein between endometrial and myometrial fractions of immature and mature rat uterus. J Endocrinol 63:439-449, 1974

23. Armitage P: Multiple regression and multivariate analysis, in Armitage P (ed): Statistical Methods in Medical Research. Oxford, UK, Blackwell Scientific, 1977, pp 302-348

24. Miller WR, Forrest ADM: Oestradiol synthesis from C19 steroids by human breast cancers. Br J Cancer 33:116-118, 1976

25. Brodie AMH, Dowsett M, Coombes RCH: Aromatase inhibitors as new endocrine therapy for breast cancer, in Osborne CK (ed): Endocrine Therapies in Breast Cancer. Boston, MA, Kluwer Academic, 1988, pp 51-65

26. Lueprasitsakul P, Longcope C: Aromatase activity of human adipose tissue stromal cells: Effects of thyroid hormones and progestogens. Proc Soc Exp Biol Med 194:337-341, 1990

27. Bhatnagar AS, Nadjafi CH, Steiner R: Aromatase inhibitors in cancer treatment, in Stall SA (ed): Endocrine Management of Cancer. 2. Contemporary Therapy. Basel, Germany, Karger, 1988, pp 30-42