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Proliferative Activity and Steroid Hormone Receptor Status in Male Breast Carcinoma

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Hormonal factors have been implicated in the development of both female and male breast cancers (MBC). However, MBCs are rare and seem to have different biological behavior than those of females. The aim of this study was to evaluate proliferative activity and to establish an association with steroid hormone receptor concentration and clinicopathological parameters in MBC. Proliferative activity was assessed in 18 MBC by mitotic figure counts and immunohistochemical evaluation of MIB-1 and proliferating cell nuclear antigen (PCNA). Estrogen (ER), progesterone (PR) and androgen (AR) receptors were evaluated in serial section from the same tumor by immunohistochemistry. PCNA (range 17–73%; mean, 51.6%) and MIB-1 (range 18.5–58%; mean 38.4%) were positive correlated with the mitotic rate. High proliferative activity assessed either by mitotic index or MIB-1 expression was associated with more poorly differentiated tumors. Sixty one percent (11/18) of the tumors were ER+ , 72% (13/18) PR+ and 38.5% (5/13) AR+ . Proliferative activity in tumors displaying ER+ /PR+ phenotype showed a tendency to be higher than in ER– /PR– tumors. This difference was statistically significant when MIB-1 expression was used as proliferation marker. An association between AR concentration and age at diagnosis was found; in the AR negative group (8/13) mean age at diagnosis was 54.4 ± 7.3 which was significantly lower than the age of patients with AR+ tumors, 63.2 ± 11.1 (5/13). Results presented here show that decreased androgen action (AR–) within the breast might contribute to an earlier development of MBC. Besides that, the presence of ER and PR in carcinoma cells is considered to provide a growth advantage as shown by the positive association between the phenotype (ER+ /PR+) and high proliferative activity. These results add information for a better understanding of hormonal control of MBC growth and development. © **1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

Breast cancer is an infrequent but serious disease in human males. Knowledge relevant to many aspects of the disease is still limited [1]. Proliferative activity of female breast carcinomas (FBC) is considered to be an important and even independent prognostic factor; in male breast cancer (MBC), on the other hand, information on the cellular kinetics is scant and controversial [2–4].

Hormonal control on growth and development of MBC has been suggested [5]. Estrogens are known to stimulate cell proliferation in both normal and neoplastic breast tissue [6]. This biological effect is exerted when it binds to the estrogen receptor (ER). Epidemiological studies have linked an excess exposure to estrogen with MBC [1, 5]. Besides that, Sasano *et al.* [7] reported an increased aromatase expression in the stromal cells of MBC, suggesting that locally synthesized estrogen may act to promote growth. Estrogen, through ER, may regulate the synthesis of progesterone receptor (PR) and in MBC a positive correlation was found between ER and PR concentration [8]. The role of progesterone on breast tumor cell proliferation is controversial, it may be stimulatory [9] or growth inhibitory [10].

On the other hand, a reduced testicular function and a decreased androgen action within the breast tissue may contribute to the development of MBC [1, 11]. Recent *in vitro* studies suggested that the androgen-induced inhibition of proliferation in

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Table 1. *Clinicopathologic data of eighteen male patients with breast cancer*

Patient No.	Age (yr)	Staging[a]	Histological type[b]	SBR[c]	Receptor status
1	74	T2N1M0	I.D. with cribiform component	I	ER+ /PR+ /AR+
2	50	T2N0M0	invasive ductal (NOS)	I	ER– /PR– /AR–
3	41	T4N1Mx	invasive ductal (NOS)	III	ER+ /PR– /AR–
4	42	T2N0M0	invasive ductal (NOS)	III	ER+ /PR+ /AR+
5	57	T2N0M0	invasive ductal (NOS)	II	ER+ /PR+
6	52	T2N0M0	apocrine carcinoma	I	ER– /PR– /AR–
7	65	T4N1M0	invasive ductal (NOS)	II	ER– /PR– /AR–
8	57	T2N0M0	invasive ductal (NOS)	I	ER– /PR– /AR–
9	48	T2N1M1	invasive tubular carcinoma	II	ER+ /PR+
10	69	T1N0M0	invasive ductal (NOS)	I	ER+ /PR+
11	64	T4N0M0	invasive ductal (NOS)	I	ER+ /PR+
12	71	T4N2M1	I.D. with cribiform component	II	ER+ /PR+
13	55	T4N1Mx	I.D. with comedo areas	II	ER– /PR+ /AR–
14	67	T4N0M0	invasive ductal (NOS)	I	ER– /PR+ /AR+
15	56	T4N1M1	invasive ductal (NOS)	II	ER+ /PR+ /AR–
16	70	T4N1Mx	invasive ductal (NOS)	II	ER– /PR+ /AR+
17	62	T4N0Mx	invasive ductal (NOS)	II	ER+ /PR+ /AR–
18	63	T4N1Mx	invasive ductal (NOS)	II	ER+ /PR+ /AR+

[a]TNM staging: according with American Join Committee on Cancer, 1992.
[b]Histological type: according with WHO histologic classification [41].
[c]Histological grading: according to Le Doussal *et al.* [13] and Elston [14].

MCF7 cells is androgen receptor (AR)-mediated [12]. The correlation between AR concentration and cell proliferation in MBC has not been investigated.

In order to obtain a better understanding of MBC biological behavior we decided to study the proliferative activity and to establish a correlation, if any, with steroid hormone receptor concentration and clinicopathological parameters.

MATERIALS AND METHODS

Patients

Eighteen cases of histologically confirmed MBC were examined retrospectively in this study. Archival paraffin embedded tissues, routinely fixed in 10% formalin, were obtained from different pathology laboratories. Five μm serial sections were mounted on APTES (3-amino propyl triethoxysilane; Sigma, St. Louis, MO)-coated slides, and dried at 37°C during 24 h. Consecutive sections were used for routine hematoxylin-eosin (HE) staining and for immunostaining. Clinical and histopathological features of the patients are summarized in Table 1. Clinical information regarding patient age, primary tumor extent (T), lymph node status (N), distant metastasis (M) for TNM staging, were obtained from the patients' medical records.

Routine pathological examination and mitotic figures

HE stained samples were evaluated by two experienced pathologists to record routine histopathological data using a BH_2 microscope (Olympus optical).

A Scarff–Bloom–Richardson (SBR) system for histological grading was used [13]; the standardized protocol for mitotic figure counts described by Elston [14] was followed. Mitotic counts were started in the most active areas of the neoplasm where the number of mitosis was highest. Once started nine additional fields in the same region were randomly selected. Morphological criteria [15] were applied to the recognition of mitotic figures. Mitotic figure counts were expressed as number per 10 high-power field (MitH) and as mitotic index.

Table 2. *Characteristics of primary antibodies used and MW pre-treatment length*

Antibody	Animal	Working dilution[a]	Source	MW pre-treatment length[b] (min)
ER-LH2 (clone CC4-5)	mouse	1:80	Novocastra, Newcastle, U.K.	15
PR (clone KD68)	rat	1:2 of kit	Abbot, North Chicago, IL, U.S.A.	15
AR (clone 2F12)	mouse	1:20	Novocastra, Newcastle, UK.	15
PCNA (clone PC-10)	mouse	1:400	Novocastra, Newcastle, UK.	10
Ki-67 (clone MIB-1)	mouse	1:100	Amac, Westbrook, Maine, U.S.A.	20

[a]All incubations were done overnight at 4°C.
[b]Defined in Materials and Methods.

Immunostaining

The previously described microwave (MW) pre-treatment technique [16], with minor modifications, was used. In brief, the dewaxed and rehydrated samples were placed in a Coplin jar containing 0.01 M sodium citrate buffer (pH 6.0) for a MW pre-treatment. Samples were heated in a 700 W microwave oven for 3 min at full power (the solution comes to a rapid boil) followed by a variable period (selected for each antigen) at 40% of full power (this power setting adjusts the oven cycles on and off every 12–19 s and the solution is maintained near boiling point). Optimum MW pre-treatment length for each individual antigen is shown in Table 2. Figure 1 shows a validation test on female breast tumors samples with unknown fixation protocol performed to adjust the MW pre-treatment length for MIB-1. After heating, sections were let stand for 20 min in the MW, then rinsed in PBS (pH 7.5) at room temperature for 10 min.

After MW pre-treatment a routine immunohistochemistry protocol was followed [8, 16, 17]. All incubations were done in a moist incubation chamber. The characteristics of the primary antibodies employed in this study as well as working dilutions are summarized in Table 2. Antibody dilutions were adjusted in order to obtain the optimal results determined by the combination of strong immunoreactivity with low background. The specificity of these antibodies has been tested by the suppliers and by us using Western blot assays of tissues containing the proteins under investigation.

The streptavidin–biotin–peroxidase complex method, with diaminobenzidine tetrahydrochloride (Sigma) as chromogen, was used [16, 17]. As second antibodies anti-rat or anti-mouse IgG (whole molecule) (Sigma) biotin conjugated were used (1:20 for ER and AR; 1:80 for PR and MIB-1; and 1:130 for PCNA). Second antibodies were incubated for 30 min at room temperature. The streptavidin–peroxidase (Sigma) complex was used (1:60 for ER and AR; 1:150 for PR, MIB-1 and PCNA) and incubations were done for 30 min at room temperature.

Diaminobenzidine (2 mg/ml)/hydrogen peroxide (0.001%) was used as chromogen substrate. After immunostaining, the slides were slightly counterstained with Mayer's hematoxylin.

Each immunohistochemical run included positive and negative controls. Prostatic hyperplasia was used as control tissue for AR, whereas samples of FBC were used for ER, PR and proliferation markers. In the negative control slides the primary antibody was replaced with normal rat or mouse serum.

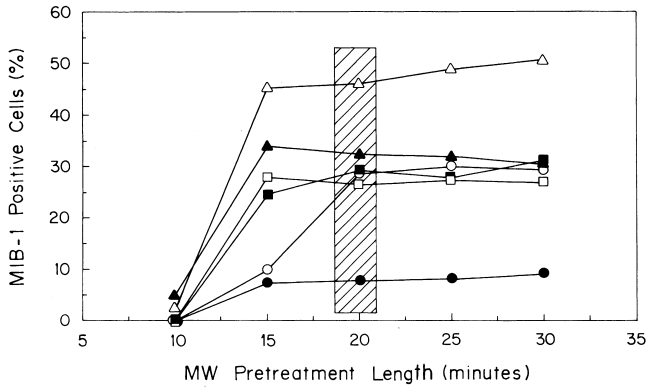

Fig. 1. Effect of MW pre-treatment length on percentage of MIB-1 immunostained cells. Serial sections from six female breast carcinomas received MW pre-treatments of different length for antigen retrieval. MW pre-treatment (3 min at full power + x minutes at 40% of full power, x = 7–27 min). Shaded area shows selected MW pre-treatment length, with less prolonged MW heating the staining was less intense and percentage of immunostained cells was the same or lower, whereas longer heating times did not result in any improved staining intensity or percentage of immunolabelling.

Scoring system

The same scoring system used for ER and PR, previously described in detail [8], was used for AR semi-quantitation. In brief, a score was given to the proportion of cells staining positive: 0% = 0; <1% = 1; 1–10% = 2; 11–30% = 3; 31–66% = 4 and >66% = 5. An intensity score also was given: no staining = 0; weak staining = 1; moderate staining = 2; and strong staining = 3. Intensity and proportion scores were totalled. A tumor with a total score of 0–2 was classified as negative (N). If the score was 3 or 4, the tumor was said to be low-positive (LP), and a score of 5–8 indicated that the tumor was positive (P) [18].

Two different criteria were followed for the proliferating cell nuclear antigen (PCNA) scoring: (1) as we described previously [16] all stained cells were regarded as positive, PCNA(t), (2) only cells with strong nuclear staining were considered to be positive, PCNA(+ + +) [19]. As subjectivity, in establishing intensity cut off values, is likely to affect reproducibility the PCNA(+ + +) quantitation was repeated at a separate sitting one month later by the same observer (MMT) blinded to the previous count. There was an excellent intraobserver correlation (r = 0.89) and the mean of the two counts was considered the PCNA(+ + +) score.

For scoring the Ki-67 staining using MIB-1 antibody, all reactive nuclei were counted as positive (regardless of the staining intensity).

Statistical analyses

The intraobserver correlation for PCNA(+ + +) quantitation was assessed with the Pearson correlation coefficient. For comparing ER and PR positivity within the same patients, the McNemar's test for paired proportions was used [20]. To analyze the interrelationship between proliferation markers, the Spearman rank-correlation coefficient (r_S) and p-

Table 3. Values for proliferation markers in male breast carcinoma

Marker	*n*	Mean ± SD	Median	Range	Units
Mit H	18	12.88 ± 7.49	9.5	3–26	mitosis/10 HPF[a]
PCNA (t)	18	51.64 ± 14.30	52.2	17.3–73.0	% positive cells
PCNA (+ + +)	18	22.33 ± 10.90	20.3	5.4–45.2	% positive cells with strong staining
MIB-1	11	38.40 ± 11.80	38.3	18.5–57.9	% positive cells

[a]HPF: 40 × objective and 10 × eyepiece.

Table 4. Spearman correlations (r_S) of proliferation markers with each other

	Mit H	Mitotic index	PCNA (t)	PCNA (+ + +)
Mitotic index	0.92 (<0.001)[a]	–	–	–
PCNA (t)	0.46 (<0.05)	0.48 (<0.05)	–	–
PCNA (+ + +)	0.45 (<0.05)	0.52 (<0.05)	0.60 (<0.01)	–
MIB-1	0.84 (<0.01)	0.90 (<0.001)	0.24 (NS)	0.51 (<0.05)

Spearman correlation coefficient is given with the *p*-value in parentheses. NS: not significant ($p>0.05$).
[a]*p*-values were calculated according to Siegel [20] (pp. 195–239).

values were calculated [20]. Association between proliferation indices and clinicopathological features was assessed by Kruskall–Wallis and Mann–Whitney *U* tests [20]. The Mann–Whitney *U* test was also applied for comparing the mean age at diagnosis in the AR+ vs AR– groups of patients.

RESULTS

A summary of the clinicopathological characteristics of the patients is presented in Table 1.

Sex steroid hormone receptors

The results of the immunostaining for steroid hormone receptors are summarized in Table 1. Low positive and positive cases were considered positive. Sixty one percent (11/18) of the tumors expressed ER and 72% (13/18) were PR positive. The proportion of ER+ versus PR+ tumors was not significantly different ($p>0.188$) and the status of both receptors was positively correlated (r_S: 0.52; $p < 0.05$). In MBC AR immunostaining was restricted to the nuclei of the epithelial cells, while in prostatic hyperplasia

Table 5. Proliferation indices in MBC according to histological grade, T stage, axillary nodal status and steroid hormone receptors

	Mitotic index				PCNA (+ + +) % positive cells			MIB-1 % positive cells		
	1	2	3		mean ± SD	*n*		mean ± SD	*n*	
All cases	9	5	4		22.3 ± 10.9	18		38.4 ± 11.8	11	
					Histological grade (SBR)					
I	7	0	0	$p = 0.02$[b]	18.6 ± 8.5	7	$p = 0.21$[b]	25.0 ± 5.1	4	$p = 0.027$[b]
II	2	4	3		23.0 ± 12.2	9		45.2 ± 7.6	5	
III	0	1	1		32.1 ± 1.5	2	NS	48.2 ± 1.1	2	
					T stage[a]					
T1–T2	5	2	1	$p>0.05$[c]	21.6 ± 7.0	8	$p>0.05$[c]	29.5 ± 9.9	5	$p = 0.015$[c]
T3–T4	4	3	3	NS	22.9 ± 13.0	10	NS	45.9 ± 7.1	6	
					Lymph node Involvement					
N (–)	7	2	0	$p = 0.01$[c]	21.3 ± 8.2	9	$p>0.05$[c]	31.2 ± 10.3	5	$p = 0.041$[c]
N (+)	2	3	4		23.3 ± 13.0	9	NS	44.4 ± 9.3	6	
					Steroid hormone receptor status					
ER (+)	4	4	3	$p>0.05$[c]	25.9 ± 10.9	11	$p>0.05$[c]	42.4 ± 7.6	6	$p = 0.165$[c]
ER (–)	5	1	1	NS	16.6 ± 8.0	7	NS	33.6 ± 14.0	5	NS
PR (+)	5	5	3	$p>0.05$[c]	23.2 ± 11.0	13	$p>0.05$[c]	43.8 ± 9.3	6	$p = 0.123$[c]
PR (–)	4	0	1	NS	19.9 ± 10.2	5	NS	31.9 ± 11.2	5	NS
AR (+)	2	1	2	$p>0.05$[c]	21.9 ± 12.0	5	$p = 0.177$[c]	45.5 ± 10.8	4	$p>0.05$[c]
AR (–)	5	2	1	NS	18.5 ± 8.0	8	NS	34.3 ± 10.3	7	NS

[a]Primary tumor size and extend according with American Join Committee on Cancer – 1992.
[b]Kruskal–Wallis test was applied.
[c]Mann–Whitney *U* test was applied.

(used as positive control) AR immunostaining was observed in nuclei of both epithelial and stromal cells. AR immunoreactivity was observed in 38.5% (5/13) of MBC.

Proliferation markers

Table 3 shows mean, median and range of values for the proliferation markers studied. The percentage of PCNA and MIB-1 positive cells showed a normal distribution and increased parallel to the mitotic rate. Table 4 shows the correlation of the different proliferation markers values with each other. Mitotic figure counts expressed either as mitotic index or MitH showed high correlation with all proliferation markers. MIB-1 showed a significant positive correlation with PCNA only when strong stained PCNA cells were counted (PCNA(+ + +)). Assuming that PCNA(+ + +) better represent S phase [19] all subsequent analyses, using PCNA expression, were based on PCNA(+ + +) values.

The association of proliferation markers with histological grade, T stage and lymph node involvement is shown in Table 5. High proliferative activity was associated with more poorly differentiated tumors. However, MIB-1 expression better reflexes the stratification of proliferative activity according to histological grade, T stage and lymph node involvement.

Proliferative activity versus ER, PR, and AR status

As is shown in Table 5, statistically significant relationships have not been observed between proliferation indices and ER, PR or AR concentration when each steroid hormone receptor was evaluated independently of each other. However, proliferative activity in tumors displaying ER+ /PR+ phenotype showed a tendency to be higher than in ER– /PR– tumors (Fig. 2). This difference was statistically significant when MIB-1 expression ($p = 0.05$) was used as proliferation marker. A positive association was found between AR concentration and the age at diagnosis; in the AR negative group (8/13) the age at diagnosis was 54.4 ± 7.3, significantly lower than the age (63.2 ± 11.1) of the patients with AR+ tumors ($p = 0.05$). Despite there was no significant correlation between AR and proliferation markers, AR+ tumors showed higher proliferation scores than AR– tumors (Table 5).

DISCUSSION

The results presented in this paper show a close association between ER+ /PR+ phenotype and high proliferative status in MBC. On the other hand, the AR concentration in MBC was correlated with the age at diagnosis; a higher incidence of AR– tumors was found in younger patients. These observations are important to obtain a better knowledge about MBC biological behavior, to analyze differences and similarities between MBC and FBC and may contribute to identify molecular prognostic factors in MBC.

Breast cancer is a heterogeneous disease with a prognosis that varies not only with the extent of the disease, but also with its biologic behavior. Tumor cell proliferation, which is an important biological

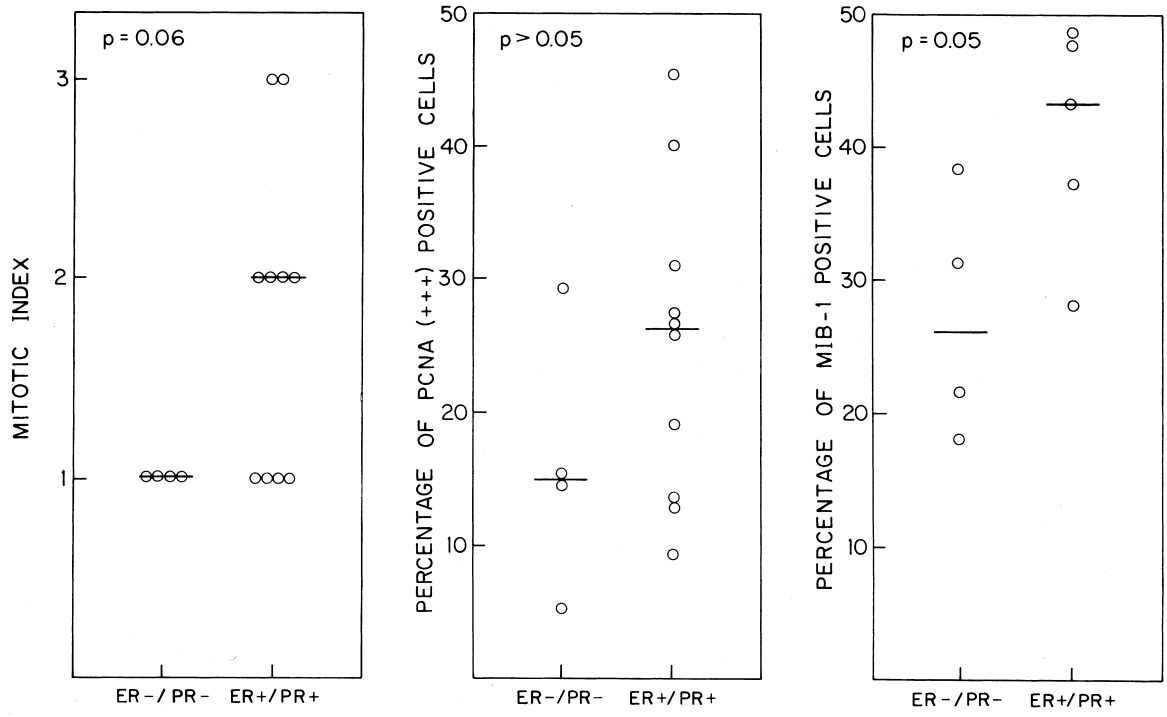

Fig. 2. Proliferative activity evaluated either by mitotic index, PCNA(+ + +) or MIB-1 in ER– /PR– and ER+ / PR+ male breast carcinomas. Bars indicate median.

variable, can be regarded, in FBC, as a prognostic indicator [21, 22]. Several procedures may be used to obtain a reliable measurement of cell proliferation [23, 24]. PCNA and MIB-1 have gained wide acceptance as proliferative markers primarily due to the possibility of being used on routinely processed material, however both markers' expression is seriously affected by fixation and processing procedures. In our experience microwave pre-treatment has proven useful in antigen retrieval giving reliable results when standardized protocols are followed [8, 16]. In this study we have standardized the immunostaining and MW pre-treatment for MIB-1 and AR. As is shown in Fig. 1 the length of MW pre-treatment for MIB-1 is a critical point.

Histological grade, T stage and lymph node involvement are accepted prognostic factors in MBC [1], in the present work a significant positive association between proliferative activity evaluated by MIB-1 expression and these factors was found (see Table 5). These results further support the prognostic value of the cell proliferative activity in MBC that has been previously reported [4]. In our experience MIB-1 better reflexes the stratification of proliferative activity according to histological grade, T and N stages than PCNA. These results could be explained because PCNA is not only a proliferation marker but it is also expressed in cells undergoing DNA repair [25]. Additional information about MBC biological behavior given by quantitative parameters, such as MIB-1 score, might improve the prognostic accuracy of the traditional parameters and the precision with which patients can be selected for systemic adjuvant therapy. The presence of ER and PR in MBC has received considerable attention, but AR has not. A significant difference between breast cancer in males and women is the regularity with which ER and PR are found in MBC [8, 26–28] and its lack of prognostic value. However if a positive association between proliferative activity and ER+ /PR+ phenotype in MBC is established, this might explain the adverse prognosis reported for MBC [26, 29–31]. A better correlation between steroid receptor status and proliferative activity may be hindered either by the complexity of interactions among sex steroid hormones, supported by the critical role of a cross-talk between growth factors, and steroid receptor signalling system [32] or by the small size of our series. In FBC an inverse correlation between ER and PR concentration and cell proliferation has been reported [33–35]; however, some studies support a direct correlation [36, 37].

Besides estrogen and progesterone, androgens have also been implicated in breast cancer development and progression in both males and females. In contrast to ER and PR, considerably less is known about the concentration of AR in MBC. In our present study, 38.5% of tumors expressed AR, a similar percentage as that reported by Everson *et al.* [38], but lower than that found by Sasano *et al.* [7]. The later authors also assessed AR by immunohistochemistry but different results might be explained by differences in the MBC sample studied and/or by the use of different primary antibodies. The mean age at diagnosis of our patients is significantly lower (58 ± 9.7) compared with Sasano's sample (70 ± 11.8). Our results showed that tumors of younger patients present a significant lack of AR expression. Recent *in vitro* studies suggested that the androgen-induced inhibition of proliferation in MCF7, female breast cancer cells line, is AR-mediated [12]. Based on this observation we cannot rule out a role of AR in MBC development, growth and progression. Our results might suggest a possible dual effect of androgen through AR in MBC. A decreased androgen action within the breast tissue may contribute to an earlier development of MBC whereas, once tumor is developed, the presence of AR may contribute to tumor progression (higher proliferative activity in AR+ cases).

In conclusion, the results presented here suggest that decreased androgen action within the breast might contribute to an earlier development of MBC; the study also revealed that tumor phenotype ER+ / PR+ was associated with elevated cell proliferation. Collectively, the present findings are in good agreement with the hormonal control of breast tissue proliferation previously proposed [6, 39, 40]. The positive correlation between MIB-1 expression with histological grade, T stage and lymph node involvement, add information useful to identify molecular prognostic indicators in MBC. It remains to be elucidated to what extend these findings can be related to success or failure of the endocrine therapy in MBC.

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REFERENCES

1. Donegan W. L. and Redlich P. N., Breast cancer in men. *Surgical Clinics of North America* **76** (1996) 343–363.
2. Gattuso P., Reddy V. B., Green L., Castelli M., Haley D. and Herman C. H., Prognostic significance of DNA ploidy in male breast carcinoma: a retrospective analysis of 32 cases. *Cancer* **70** (1992) 777–780.
3. Hatschek T., Wingren S., Carstensen J. and Hultbom R., DNA content and S-phase fraction in male breast carcinomas. *Acta Oncologica* **33** (1994) 609–613.
4. Pich A., Margaria E., Chiusa L., Ponti R. and Geuna M., DNA ploidy and p53 expression correlate with survival and cell proliferative activity in male breast carcinoma. *Human Pathology* **27** (1996) 676–682.
5. Thomas D. B., Male breast cancer. *Epidemiology Reviews* **15** (1993) 220–231.

6. Sunderland M. C. and McGuire W. L., Hormones and breast cancer. *Trends in Endocrinology and Metabolisms* **2** (1991) 72–76.
7. Sasano H., Kimura M., Shizawa S., Kimura N. and Nagura H., Aromatase and steroid receptors in gynecomastia and male breast carcinoma: An immunohistochemical study. *Journal of Clinical Endocrinology and Metabolisms* **81** (1996) 3063–3067.
8. Muñoz de Toro M. and Luque E. H., Lack of relationship between the expression of hsp27 heat shock estrogen receptor-associated protein and estrogen receptor or progesterone receptor status in male breast carcinoma. *Journal of Steroid Biochemistry and Molecular Biology* **60** (1997) 277–284.
9. Kalkhoven E., Kwakkenbos-Isbrücker L., De Laat S. W., Van Der Saag P. T. and Van Der Burg B., Synthetic progestins induce proliferation of breast tumor cell lines via the progesterone or estrogen receptor. *Molecular and Cell Endocrinology* **102** (1994) 45–52.
10. Ballare C., Bravo A. I., Sorin I., Guman N., Schiaffi J. A., Yomha R., Bagnati A., Lema B. and Mordoh J., The expression of progesterone receptors coincides with an arrest of DNA synthesis in human breast cancer. *Cancer* **67** (1991) 1352–1358.
11. Lobaccaro J. M., Lumbroso S., Belon C., Galtier-Dereure F., Bringer J., Lesimple T., Namer M., Cutuli B. F., Pujol H. and Sultan C. H., Androgen receptor gene mutation in male breast cancer. *Human Molecular Genetics* **2** (1993) 1799–1802.
12. Szelei J., Jimenez J., Soto A. M., Luizzi M. F. and Sonnenschein C., Androgen-induced inhibition of proliferation in human breast cancer MCF7 cells transfected with androgen receptor. *Endocrinology* **138** (1997) 1406–1412.
13. Le Doussal V., Tubiana-Hulin M., Friedman S., Hacene K., Spyratos F. and Brunet M., Prognostic value of histologic grade nuclear components of Scarff–Bloom–Richardson (SBR). An improved score modification based on a multivariate analysis of 1262 invasive ductal breast carcinomas. *Cancer* **64** (1989) 1914–1921.
14. Elston C. W., Grading of invasive carcinoma of the breast. In *Diagnostic Histopathology of the Breast*, ed. D. L. Page and T. J. Anderson. Churchill Livingstone, Edinburgh, 1987, pp. 300–311.
15. Baak J. P. A., Mitosis counting in tumors. *Human Pathology* **21** (1990) 683–685.
16. Muñoz de Toro M. and Luque E. H., Effect of microwave pretreatment on proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections. *Journal of Histotechnology* **18** (1995) 11–16.
17. Elias J. M., Margiotta M. and Gaborc B. C., A comparison of the peroxidase–antiperoxidase (PAP), avidin–biotin complex (ABC), and labeled avidin–biotin (LAB) methods for detection of glucagon in paraffin embedded human pancreas. *American Journal of Clinical Pathology* **92** (1989) 62–67.
18. Allred D. C., Clark G. M., Elledge R., Fuqua S. A. W., Brown R. W., Chamness G. C., Osborne C. K. and McGuire W. L., Association of p53 protein expression with tumor cell proliferation rate and clinical outcome in node-negative breast cancer. *Journal of the National Cancer Institute* **85** (1993) 200–206.
19. Tahan S. R., Neuberg D. S., Dieffenbach A. and Yacoub L., Prediction of early relapse and shortened survival in patients with breast cancer by proliferating cell nuclear antigen score. *Cancer* **71** (1993) 3552–3559.
20. Siegel, S., *Nonparametric Statistics for the Behavioral Sciences*. McGraw-Hill, New York, 1956.
21. NIH Consensus Conference, Treatment of early-stage breast cancer. *Journal of the American Medical Association*, **265** (1991) 391–395.
22. McGuire W. L. and Clark G. M., Prognostic factors and treatment decisions in axillary-node-negative breast cancer. *New England Journal of Medicine* **326** (1992) 1756–1761.
23. Woosley J. T., Measuring cell proliferation. *Archives of Pathology and Laboratory Medicine* **115** (1991) 555–557.
24. Keshgegian A. A. and Cnaan A., Proliferation markers in breast carcinoma: Mitotic figures count, S-phase fraction, proliferating cell nuclear antigen, Ki-67 and MIB-1. *American Journal of Clinical Pathology* **104** (1995) 42–49.
25. Dietrich D. R., Toxicological and pathological applications of proliferating cell nuclear antigen (PCNA), a novel endogenous marker for cell proliferation. *Critical Review of Toxicology* **23** (1993) 77–109.
26. Joshi M. G., Lee A. K. C., Loda M., Camus M. G., Pedersen C., Heatley G. J. and Hughes K. S., Male breast carcinoma: An evaluation of prognostic factors contributing to a poorer outcome. *Cancer* **77** (1996) 490–498.
27. Weber-Chappuis K., Bieri-Burger S. and Hurlimann J., Comparison of prognostic markers detected by immunohistochemistry in male and female breast carcinomas. *European Journal of Cancer* **32A** (1996) 1686–1692.
28. Luque E. H. and Muñoz de Toro M., Male breast carcinoma: Prognostic and predictive indicators related with its biological behavior. *Medicina (Bs. As.)* **58** (1998) 95–106.
29. Heller K. S., Rosen P. P. and Schottenfeld D., Male breast cancer: A clinicopathologic study of 97 cases. *Annals of Surgery* **188** (1978) 60–65.
30. Ciatto S., Iossa A. and Bonardi R., Male breast carcinoma: Review of a multicenter series of 150 cases. *Tumori* **76** (1990) 555–558.
31. Salvadori B., Saccozzi R., Manzari A., Andreola S., Conti R. A., Cusumano F. and Grassi M., Prognosis of breast cancer in males: An analysis of 170 cases. *European Journal of Cancer* **30** (1994) 930–935.
32. Dickson R. B. and Lippman M. E., Growth factors in breast cancer. *Endocrine Reviews* **16** (1995) 559–589.
33. Dressler L. G., Seamer L. C., Owens M. A., Clark G. M. and McGuire W. L., DNA flow cytometry and prognostic factors in 1331 frozen breast cancer specimens. *Cancer* **61** (1988) 420–427.
34. Meyer J. S., Koehm S. L., Hughes J. M., Higa E., Wittliff J. L., Lagos J. A. and Manes J. L., Bromodeoxyuridine labeling for S-phase measurement in breast carcinoma. *Cancer* **71** (1993) 3531–3540.
35. Meyer J. S. and Province M. A., S-phase fraction and nuclear size in long term prognosis of patients with breast cancer. *Cancer* **74** (1994) 2287–2299.
36. Fanelli M. A., Vargas-Roig L. M., Gago F. E., Tello O., De Angelis R. L. and Ciocca D. R., Estrogen receptors, progesterone receptors, and cell proliferation in human breast cancer. *Breast Cancer Research and Treatment* **37** (1996) 217–228.
37. Russo J. and Russo I. H., Role of differentiation in the pathogenesis and prevention of breast cancer. *Endocrine-Related Cancer* **4** (1997) 7–21.
38. Everson R. B., Lippman M. E., Thompson E. B., McGuire W. L., Wittliff J. L., De Sombre E. R., Jensen E. V., Shinghakowinta A., Brooks S. C. and Neifeld J. P., Clinical correlations of steroid receptors and male breast cancer. *Cancer Research* **40** (1980) 991–997.
39. Hackenberg R. and Schulz K.-D., Androgen receptor mediated growth control of breast cancer and endometrial cancer modulated by antiandrogen- and androgen-like steroids. *Journal of Steroid Biochemistry and Molecular Biology* **56** (1996) 113–117.
40. Lemieux P. and Fuqua S., The role of the estrogen receptor in tumor progression. *Journal of Steroid Biochemistry and Molecular Biology* **56** (1996) 87–91.
41. Hutter R. V. P., The role of the pathologist in breast cancer management. *Cancer* **66** (1990) 1363–1372.