Molecular Markers in Male Breast Carcinoma

Daniel Rayson, M.D.
Charles Erlichman, M.D.
Vera J. Suman, Ph.D.
Patrick C. Roche, Ph.D.
Lester E. Wold, M.D.
James N. Ingle, M.D.
John H. Donohue, M.D.

Mayo Clinic Cancer Center, Mayo Clinic, Rochester, Minnesota.

Presented in part at the American Society of Clinical Oncology (ASCO) Annual Meeting, Denver, Colorado, May 17–21, 1997.

Supported in part by the Mayo Clinic Cancer Center Support Grant (CA-15083-24).

The authors would like to thank Steven C. Zeismer for assistance with the immunohistochemistry.

Address for reprints: Charles Erlichman, M.D., Mayo Clinic, 200 First Street, SW, Rochester, MN 55905.

Received October 28, 1997; revision received March 18, 1998; accepted April 6, 1998.

BACKGROUND. In contrast to female breast carcinoma, information regarding the prevalence and prognostic information of new molecular markers is limited in male breast carcinoma. The objective of this study was to assess the degree of expression and prognostic value of estrogen receptors (ER), progesterone receptors (PR), androgen receptors (AR), *bcl*-2, p53, HER-2/*neu*, cyclin D1, and MIB-1 in a cohort of male breast carcinoma patients.

METHODS. A computerized search of the medical index, tumor registry, and tissue registry was used to identify 111 male patients with a diagnosis of primary adenocarcinoma of the breast seen between 1950–1992 at the Mayo Clinic. Of these, 77 patients had adequate tissue specimens available for the immunohistochemical analysis of the markers. Immunoperoxidase staining was performed by an automated avidin-biotin complex method. Progression free (PFS) and overall (OS) survival distributions were estimated using the Kaplan–Meier method. The log rank test was used to determine whether any patient characteristic, tumor feature, or molecular marker was associated significantly with PFS or OS.

RESULTS. The majority of tumor specimens were positive for ER (91%), PR (96%), AR (95%), and *bcl*-2 (94%). Fewer positive specimens were found for cyclin D1 (58%), MIB-1 (38%), HER-2/*neu* (29%), and p53 (21%). The 5-year PFS and 10-year OS for the entire patient cohort was estimated to be 66% (95% confidence interval [CI], 57–77%) and 38% (95% CI, 29–50%), respectively. PFS was decreased significantly for those patients with tumors staining positively for MIB-1 ($P = 0.012$) or negatively for cyclin D1 ($P = 0.009$). OS was not found to differ significantly with respect to these markers.

CONCLUSIONS. The nearly universal expression of hormone receptors in these tumors suggests a central role for endogenous hormones in male breast carcinoma. The high degree of AR expression would suggest that antiandrogen therapy should be explored further. The high frequency of *bcl*-2 positivity may implicate antiapoptotic mechanisms in the carcinogenesis of male breast carcinoma. The finding of decreased PFS in MIB-1 positive tumors supports the role of proliferative activity as a negative prognostic factor in male breast carcinoma. Positive cyclin D1 expression is associated with increased PFS in male breast carcinoma patients, which suggests that interactions among cell cycle regulatory proteins may be important in this disease. ***Cancer* 1998;83:1947–55.**
© 1998 American Cancer Society.

KEYWORDS: male breast carcinoma, molecular markers, hormone receptors, immunohistochemistry.

Breast carcinoma in men is an uncommon disease, accounting for approximately 0.5% of all cases of carcinoma of the breast in the U.S.[1,2] The majority of studies using modern staging techniques and adjuvant therapies have demonstrated similar overall survival (OS) stage for stage to that of breast carcinoma in women.[2–7] However, there are studies that report a decreased disease free survival and OS for males compared with historic female "controls".[8,9].

Prognostic variables are well defined in the female breast carcinoma population and include tumor size, lymph node involvement, histologic type, nuclear grade, and hormonal receptor status.[10,11] There has been a proliferation of new tumor markers in female patients and clinical practice guidelines on their use recently have been published.[12] Although the clinical utility of many of these markers remains under investigation, the study of protooncogenes (e.g., p53 and HER-2/*neu*), cell cycle regulatory proteins (e.g., cyclin D1 and MIB-1), and markers of apoptosis (e.g., *bcl*-2 and *bax*) in female breast carcinoma has led to new insights into the biology of the disease and attempts at utilizing molecular markers as prognostic factors.

The natural history of male breast carcinoma has been described in a number of recent studies,[2–9,13] but data regarding the presence of newer molecular markers and their prognostic significance in males with breast carcinoma are limited in comparison with that in females. Carcinomas of the male breast arise in a different hormonal milieu compared with female breast tumors but appear to behave similarly. The elucidation of new molecular markers in male breast carcinoma may provide insights into the biologic differences between male and female breast carcinoma patients and could aid in clinical management.

The objectives of this study were to describe the tumor characteristics, treatment, and outcome in a cohort of male breast carcinoma patients treated at our institution and to determine the degree of expression and prognostic value of the following molecular markers: estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), p53, *bcl*-2, HER-2/*neu*, MIB-1, and cyclin D1. The majority of these markers were chosen because of the availability of data from female patients and because they represent active areas of investigation in female breast carcinoma.

PATIENTS AND METHODS

Patients

Utilizing a computer search of the medical index, tumor registry, and tissue registry, all male patients with a diagnosis of adenocarcinoma of the breast seen at the Mayo Clinic between the years 1950–1992 were identified. All charts were reviewed and confirmation of the treatment of primary breast carcinoma was obtained for 111 patients. Thirty-four patients did not have pathologic specimens available primarily because they were seen in consultation only and the tumor specimens either were not reviewed or saved at Mayo Clinic. Seventy-seven patients had adequate tissue specimens available for immunohistochemical analysis of the 8 markers and form the basis of this report. In all cases, tissue specimens analyzed were from the initial surgical treatment. No patients received neoadjuvant therapy. Information for the entire cohort of 111 patients regarding risk factors, clinical presentation, primary surgical and adjuvant therapies, tumor recurrence, and subsequent treatment as well as date and cause of death were obtained from the medical record. All therapies not administered at Mayo Clinic were confirmed by review of the clinical notes in the patient's oncology history when they returned for follow-up visits. No patients were contacted. This study was approved by the Institutional Review Board of Mayo Clinic.

Immunohistochemistry

Standard pathologic features of each case including tumor size, nuclear grade, and lymph node status were reviewed and classified according to the TNM system.[14] Immunoperoxidase staining for ER, PR, AR, MIB-1, p53, c-*erb* B-2, *bcl*-2, and cyclin D1 were performed by an automated avidin-biotin complex (ABC) method on a Vantana Techmate 1000 (Vantana, Tucson, AZ). Six-micron paraffin sections were mounted on silanized glass slides, deparaffinized, and rehydrated through graded alcohols to water. Endogenous peroxidase activity was blocked by incubation with 0.6% hydrogen peroxide. Sections were immersed in 10-mM sodium citrate buffer (pH 6.0) subjected to heat antigen retrieval for 30 minutes in a steamer, and then cooled for 5 minutes. Sections then were treated with 10% normal goat serum for 10 minutes to block nonspecific protein binding. Antibody characterization, tissue localization, and positive controls are shown in Table 1. The negative control for each antibody was a nonimmunogenic immunoglobulin (Ig) G antibody.

The mouse monoclonal antibodies to human ER, human PR, MIB-1, p53, c-*erb* B-2, and *bcl*-2 were applied and tissue sections were incubated for 30 minutes at room temperature. The monoclonal antibodies to AR and cyclin D1 were incubated with sections overnight at 4 °C. After brief rinsing, sections were treated with biotinylated antimouse IgG for 30 minutes, rinsed, and then incubated with peroxidase-labeled ABC for 30 minutes. After brief washing, sections were incubated with diaminobenzidine or aminoethylcarbazole (AEC) and hydrogen peroxide for 5 minutes. Detection chemistries were all purchased from BioTek, and used according to the manufacturer's instructions. Sections were lightly counterstained with hematoxylin and then mounted with a coverslip.

The immunostaining was assessed by three reviewers (D.R., L.W., and P.R.) who were blinded with regard to the tumor characteristics and outcomes of the patients. The degree of immunostaining for MIB-1,

TABLE 1
Antibody Characterization, Tissue Localization, and Positive Controls

Antigen	Source	Species	Isotype	Antigen recognized	Cellular localization	Positive control
Estrogen receptor clone ER1D5	Immunotech. Inc. (Westbrook, ME)	Mouse	IgG_1	N-terminal domain of human ER	Nuclear, rarely cytoplasmic	Female breast carcinoma
Progesterone receptor clone 1A6	Vector Laboratories (Burlingame, CA) (Novocastra, Newcastle upon Tyne, UK)	Mouse	IgG_1	Human PR protein	Nuclear	Female breast carcinoma
Androgen receptor clone 2F1Z	Vector Labor (Novocastra, Newcastle upon Tyne, UK)	Mouse	IgG_1	Human AR protein	Nuclear	Prostate carcinoma
MIB-1 clone MIB-1	Immunotech Inc. (Westbrook, ME)	Mouse	IgG_1	Ki-67 nuclear antigen	Nuclear	Tonsil
bcl-2 clone 124	Dako Co. (Carpinteria, CA)	Mouse	IgG_1	Human *bcl*-2 protein	Membrane/cytoplasmic	Tonsil
C-*neu* clone CB11	Vector Laboratories (Novocastra)	Mouse	IgG_{2b}	C-*erb* 2 oncoprotein, internal domain	Membrane	Female breast carcinoma
Cyclin D1 clone P2011F11	Novocastra	Mouse	IgG	Cyclin D1 protein	Nuclear	Female breast carcinoma

Ig: immunoglobulin; ER: estrogen receptor; PR: progesterone receptor; AR: androgen receptor.

bcl-2, cyclin D1, and p53 was assessed jointly by the three reviewers and a consensus was reached in each case. For these markers, specimens with $\geq$ 20% of tumor cells staining for the marker were deemed positive. Immunostains for ER, PR, AR, and HER-2/*neu* were assessed independently by each reviewer. Any tumor cell staining for ER, PR, AR, or HER-2/*neu* was scored positive. The concordance between reviewers ranged from 86% for AR to 97% for ER. In cases in which there was not unanimous agreement, the result recorded by two of the three reviewers was used.

Statistical Analysis

The distributions of OS and progression free survival (PFS) were estimated using the Kaplan–Meier method.[15] For the endpoint of PFS, patients who did not have progression documented in the medical record were censored at the last date their disease status was known. Patients who died of breast carcinoma without documented progression were declared to have progressed on the date of death. The log rank test was used to determine whether any tumor feature, patient characteristic, or molecular marker was significantly associated with PFS or OS.[16] Fisher's exact test was used to assess whether there was a significant pairwise association between positive staining for each marker. Concordance between two tumor markers was said to be observed if both stained positively or both stained negatively.

RESULTS

Patient and Tumor Characteristics

The patient and tumor characteristics for the entire cohort as well as for those patients with adequate tissue are shown in Table 2. All but two of these characteristics, as well as PFS and OS, were not significantly different between the group with tissue for analysis (n = 77) and the group for whom tissue was unavailable (n = 34). The exceptions were stage and lymph node status documentation, in which 24% and 65%, respectively, of patients without tissue samples for analysis had no stage and lymph node status documentation compared with 5% and 32%, respectively, of those with tissue samples for analysis.

The most common surgical therapies were modified radical mastectomy, which was performed in 61 patients (55%), and a radical mastectomy, which was performed in 26 patients (23%). Lumpectomy was an uncommon surgical procedure and was performed in only 2 cases (2%). There was a trend toward less extensive surgery in more recent decades. Seventy-five percent of the 16 patients diagnosed between 1950–1959 underwent radical mastectomy, as did 48% of the 23 patients treated between 1960–1969, and 10% of the 31 patients treated between 1970–1979; none of the 41 patients treated between 1980–1992 underwent this procedure.

The median tumor size was 20 mm among the 86 patients for whom this information was available. Forty-three patients (39%) presented with Stage I disease, 27 (24%) with Stage IIA disease, 22 (20%) with Stage IIB disease, and 7 (6%) with Stage IV disease. Disease stage could not be determined in 12 patients due to incomplete staging information. Among those patients for whom tumor stage could be determined there was no evidence that stage of disease at presentation differed between decades.

The majority of the 104 patients with local or locoregional disease at presentation did not receive adjuvant therapies. Of these patients, information re-

TABLE 2
Patient and Tumor Characteristics

	All patients (n = 111)	Patients with available tissue (n = 77)
Median age (range) (yrs)	64.0 (33–85)	65.5 (33–85)
Family history		
Positive	18 (16%)	13 (17%)
Negative	80 (72%)	55 (71%)
Unknown	13 (12%)	9 (12%)
Surgery		
Biopsy	7 (6%)	3 (4%)
Lumpectomy	2 (2%)	2 (3%)
Simple mastectomy	11 (10%)	7 (9%)
Modified radical mastectomy	61 (55%)	47 (61%)
Radical mastectomy	26 (23%)	17 (22%)
Other	4 (4%)	1 (1%)
Tumor size (median) (range) (mm)	20 (n = 86) (1–50)	20 (n = 67) (1–50)
Stage		
I	43 (39%)	32 (42%)
IIA	27 (24%)	22 (29%)
IIB	22 (20%)	17 (22%)
IV	7 (6%)	2 (3%)
Unknown	12 (11%)	4 (5%)
Adjuvant chemotherapy		
Yes	7 (6%)	6 (8%)
No	82 (74%)	51 (66%)
Unknown	22 (20%)	20 (26%)
Adjuvant hormonal therapy		
Yes	16 (14%)	12 (16%)
No	89 (80%)	61 (79%)
Unknown	6 (5%)	4 (5%)
Adjuvant radiation		
Yes	33 (30%)	21 (27%)
No	72 (65%)	54 (70%)
Unknown	6 (5%)	2 (3%)

garding the use of adjuvant chemotherapy and hormonal therapy was available for 98 and 82 patients, respectively. Only 5 patients (5%) received adjuvant chemotherapy and 13 (16%) received adjuvant hormonal therapy. There was an increasing trend toward the use of adjuvant hormonal therapy in later decades. Adjuvant hormonal therapy was given to 4 of the 27 patients diagnosed in the 1980s and to 6 of the 13 patients diagnosed between 1990–1992.

Adjuvant radiation therapy was administered to 32 of the 104 patients (31%). There was a trend towards decreased use for patients diagnosed between 1980–1992. Seventeen of 32 patients received adjuvant radiation therapy between 1950–1969, as did 6 of 27 patients between 1970–1979, and only 7 of the 39 patients between 1980–1992.

Immunohistochemistry

The results of immunohistochemical analysis of the studied markers are summarized in Table 3. The majority of tumor specimens were positive for ER (91%), PR (96%), AR (95%), and *bcl*-2 (94%). A smaller proportion of tumors with positive staining was found for cyclin D1 (58%), MIB-1 (38%), HER-2/*neu* (29%), and p53 (21%).

TABLE 3
Immunohistochemistry Results

Marker	No.	Positive (%)
ER	76	69 (91%)
PR	76	73 (96%)
AR	74	70 (95%)
HER-2/*neu*	76	22 (29%)
p53	77	16 (21%)
bcl-2	77	72 (94%)
MIB-1	77	29 (38%)
Cyclin D1	73	42 (58%)

ER: estrogen receptor; PR: progesterone receptor; AR: androgen receptor.

Among the 67 tumor specimens analyzed for ER, PR, and *bcl*-2 expression, 57 cases (85%) were concordant for ER and *bcl*-2 and 61 cases (91%) were concordant for PR and *bcl*-2. A strong association was found between positive staining for p53 and cyclin D1 ($P < 0.001$). Other significant associations also were found between ER and MIB-1 ($P = 0.036$), ER and AR ($P = 0.031$), and cyclin D1 and AR ($P = 0.028$).

Progression Free Survival and Overall Survival

At the time of last follow-up, 5 patients were alive and had not progressed, 37 had progressed but were still alive, and 69 had died. Among the 69 patients who had died, 24 had died of breast carcinoma, 35 died of other causes, and 10 died of unknown causes. Among the 42 patients who were alive at last follow-up, the median length of follow-up was 7.8 years (range, 9 days–19.1 years).

The 3-year and 5-year PFS rate for the entire cohort was estimated to be 72% (95% confidence interval [CI], 64–82%) and 66% (95% CI, 57–77%) respectively Fig. 1. The 5-year and 10-year OS rate for the entire cohort was estimated to be 62% (95% CI, 53–72%) and 38% (95% CI, 29–50%) respectively Fig. 2.

Neither PFS or OS was found to be significantly worse for the 64 patients (58% of the entire cohort) for whom lymph node status was known ($P = 0.081$ and $P = 0.792$, respectively). PFS was found to be significantly decreased for those males with tumors staining positively for MIB-1 ($P = 0.012$) Fig. 3, as well as for those with tumors staining negatively for cyclin D1 ($P = 0.009$) Fig. 4. OS was not found to differ significantly with respect to either marker. Due to the high frequency of marker positivity, the association be-

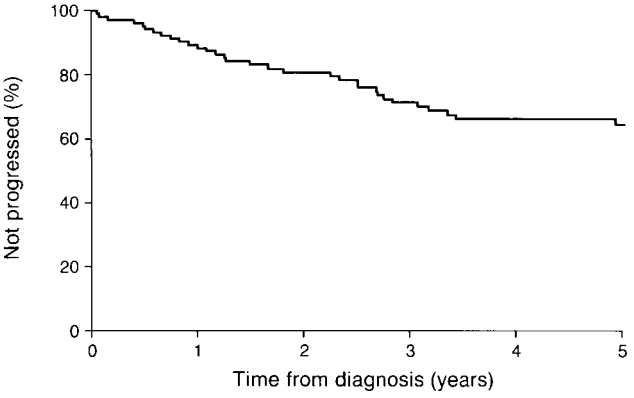

FIGURE 1. Progression free survival. The 3-year and 5-year progression free survival rate was estimated to be 72% (95% confidence interval [CI], 64–82%) and 66% (95% CI, 57–77%), respectively.

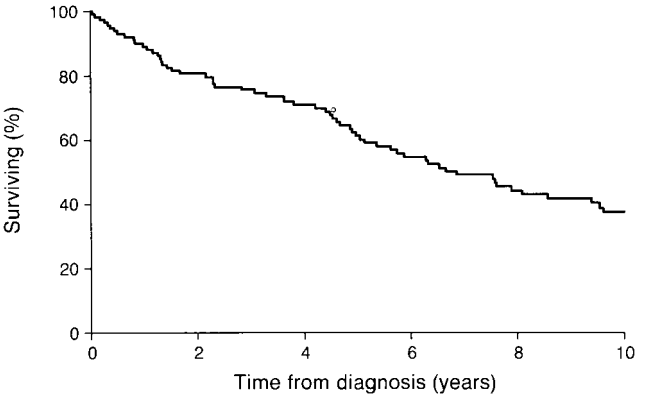

FIGURE 2. Overall survival. The 5-year and 10-year overall survival rate was estimated to be 62% (95% confidence interval [CI], 53–72%) and 38% (95% CI, 29–50%), respectively.

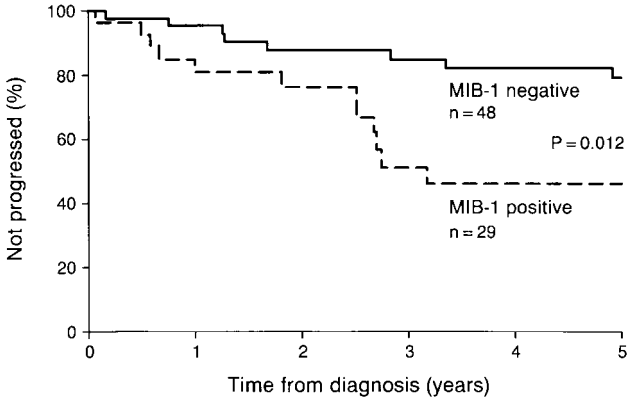

FIGURE 3. Progression free survival and MIB-1. Progression free survival was found to be significantly decreased for those males with tumors staining positively for MIB-1 ($P = 0.012$).

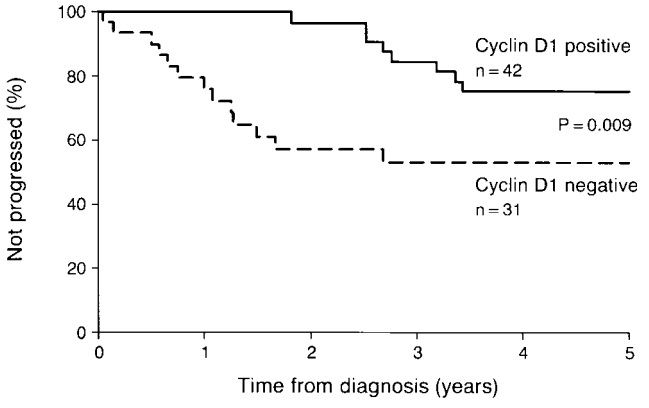

FIGURE 4. Progression free survival and cyclin D1. Progression free survival was found to be significantly decreased for those males with tumors staining negatively for cyclin D1 ($P = 0.009$).

tween ER, PR, AR, and *bcl*-2 and PFS or OS could not be assessed. There was no evidence to suggest that p53 or HER-2/*neu* positivity was associated with PFS or OS.

DISCUSSION

The main results of our study can be summarized as follows: 1) male breast carcinoma demonstrates a high degree of hormone receptor positivity; 2) *bcl*-2 also is highly expressed in these cancers; 3) positive staining for MIB-1 was associated with a significantly decreased PFS ($P = 0.012$); 4) negative staining for cyclin D1 was associated with a significantly decreased PFS ($P = 0.009$); 5) there was a significant association between p53 and cyclin D1 expression ($P < 0.001$); and 6) none of the studied markers were found to be significantly associated with OS.

The presence of ERs in male breast carcinoma tumor specimens was first described by Wittliff in 1974.[17] Subsequent reports have found a higher proportion of ER positive cases in males compared with females. Approximately 64–85.7% of all cases of male breast carcinoma have been found to express ER.[18–22] The prognostic significance of ER remains uncertain in male breast carcinoma. In what to our knowledge is the largest population-based study of hormone receptors in male breast carcinoma performed to date, Stalsberg et al.[19] in a study of 282 men, found a marginally significant trend for patients with ER positive tumors to present with higher stage disease although the influence on disease free and OS was not stated. Data regarding PR are even more limited. Stalsberg et al.[19] found PR to be positive in 76.3% of 139 cases and reported a significant correlation between ER and PR positivity. Our study found ER positivity in 91% of cases and PR positivity in 96% of cases. Although these figures are higher than generally reported, they are consistent with the high degree of expression of hor-

mone receptors that previously has been reported in male breast carcinoma. Stalsberg et al.[19] used a biochemical assay to assess for ER and PR that may be less sensitive than the immunohistochemical analysis utilized in our study, especially for very small lesions and for paraffin embedded tissues.[23] We found no evidence to suggest a significant association exists between age and ER positivity. Thirty-one of the 34 patients age >60 (91%) were ER positive and 38 of the 42 patients age ≥60 years (90%) were ER positive (P = 1.00). This is in agreement with the results from recent studies[18,21] but differ from those of Weber-Chappuis et al.[21], who found that patients age >60 years were more likely to be ER positive (87% ER positive vs. 64% for those patients age <60 years).

Our finding that nearly 95% of tumors in our patient cohort had immunohistochemically detectable ARs is consistent with a previous report in a small group of patients[24] and provides a molecular basis for the response to androgen manipulation that have been reported in cases of metastatic disease. Our results, in conjunction with the reports of Lopez et al.[25] and Doberauer et al.,[26] would suggest that in addition to antiestrogens, antiandrogen therapy should be explored in male breast carcinoma. Due to the high degree of ER, PR, and AR positivity, we were unable to assess whether the presence of these of these receptors increased PFS or OS.

bcl-2 is part of a large family of protooncogenes whose expression serves to block apoptosis.[27] *bcl*-2 expression in female breast carcinoma has been found to be associated with favorable prognostic features including the presence of ER.[28,29] It also has been demonstrated that *bcl*-2 expression is enhanced in ER positive tumors after exposure to tamoxifen[30] and may play a role in predicting the efficacy of adjuvant therapies.[31] *bcl*-2 was expressed in 94% of the cases in our study. Due to the high degree of *bcl*-2 positivity we were unable to assess the prognostic value of this marker. *bcl*-2 expression in the female breast has been associated with hormonal and other growth factors that regulate hyperplastic and involutional changes.[28] The high degree of *bcl*-2 expression in our male breast carcinoma cohort suggests that the male breast may be under similar growth factor control and that resistance to apoptotic cell death may be a factor in the carcinogenesis of this disease.

Mutations in the p53 tumor suppressor gene located on the short arm of chromosome 17 are among the most commonly observed oncogenic abnormalities in human malignancies.[32,33] Immunohistochemical detection of p53 has been found in 0–54% of male breast tumors.[21,22,34] In our series, 21% of the tumors were p53 positive. This result, although within the range previously reported for male breast carcinoma, may be an underestimation when compared with results obtained by polymerase chain reaction techniques.[35,36] The prognostic significance of p53 mutations in male breast carcinoma remains unclear. Anelli et al.[36] found a trend toward a longer disease free survival in a cohort of 34 patients with wild-type p53. Pich et al.[37] found a survival advantage for those cases with p53 negative tumors (median survival of 99 months for p53 negative tumors vs. 33 months for p53 positive tumors). In our study, p53 was not found to be associated with PFS or OS.

The product of the HER-2/*neu* protooncogene is a cell surface growth factor receptor of the tyrosine kinase family, which is related closely to the epidermal growth factor receptor.[38,39] HER-2/*neu* expression occurs in 20–30% of female breast tumors and has been correlated with a number of adverse prognostic factors.[40,41] Immunohistochemically detected HER-2/*neu* expression has been found in 0–95% of male breast carcinoma specimens.[9,21,34,42–44] One study[9] found that HER-2/*neu* positivity was associated with a poorer outcome although this conclusion is limited by the fact that there were only 17 cases studied. Our study found 29% of cases to be HER-2/*neu* positive, an incidence in high agreement with that of female breast carcinoma. In contrast to findings in female breast carcinoma, there was no evidence to suggest a significant association between HER-2/*neu* expression and lymph node or ER status exists nor was HER-2/*neu* expression found to be associated significantly with PFS or OS. This may reflect the small number of patients in our cohort with lymph node positive disease because in female breast carcinoma, HER-2/*neu* provides more prognostic information for lymph node positive compared with lymph node negative patients.[39] Alternatively, other growth factor receptors produced by different protooncogenes may play more important roles than that of by HER-2/*neu*.

The MIB-1 antibody is directed against part of the Ki-67 antigen, a proliferation-associated antigen expressed in all cells that are not in the resting phase (G_0) of the cell cycle, and has been associated with decreased survival in female breast carcinoma.[45] Pich et al.,[46] investigating the role of proliferative activity in male breast carcinoma, studied the immunohistochemical expression of silver-staining nucleolar organizer regions (AgNOR), proliferating cell nuclear antigen, and MIB-1 in 27 cases. Their results suggested that proliferative activity, as assessed by positive staining for AgNORs, may offer significant prognostic information. Our finding of a significantly decreased PFS in MIB-1 positive cases (P = 0.012) supports the con-

cept that proliferative activity is a significant adverse prognostic factor in male breast carcinoma.

Cyclin D1 complexes with cyclin-dependent kinases that bind and phosphorylate the retinoblastoma gene protein (pRB) and is rate limiting for cell entry into S-phase. It acts as a growth factor sensor and depends on mitogenic stimulation for synthesis. Cyclin D1 is overexpressed in a number of human malignancies as a result of gene amplification or translocations involving the D1 locus on chromosome 11q13.[47,48] In female breast carcinoma, approximately 50% of tumors have been found to overexpress cyclin D1[49–51] and cyclin D1 expression has been found to be associated with more aggressive tumor behavior.[52,53] Cyclin D1 overexpression has been assumed to confer a proliferative advantage to tumor cells and thus contribute to a worse prognosis. In a previous study, overexpression of cyclin D1 was found to occur in 41% (18 of 44) of male breast carcinoma specimens.[49] We found that 58% of our cases stained positively for cyclin D1. Contrary to expectation, cyclin D1 negativity was associated with a significantly decreased PFS ($P = 0.009$). This finding is consistent with Gillett et al.[51] who, in a recent study of cyclin D1 expression in 345 female breast carcinoma patients, also found that moderate or strong cyclin D1 staining was associated with a significant disease free survival and OS benefit. One possible explanation for this result may involve the interactions of cyclin D1 with the pRB. It has been demonstrated that mutations or inactivation of pRB leads to down-regulation of cyclin D1.[47,48,54] Tumors with normal pRB may have increased expression of cyclin D1 compared with tumors with mutated pRB. This could account for the improved PFS provided by positive immunostaining for cyclin D1 observed in our study as well as that of Gillett et al.[51] We currently are testing the hypothesis that cyclin D1 may serve as a 'surrogate' marker of pRB in male breast carcinoma and that low levels of expression may indicate the presence of mutated pRB.

The strength of the conclusions from our study is limited by a number of considerations. The small size of our patient cohort precluded a multivariate analysis of the data to assess the independent strength of the observed associations between tumor markers and outcome. Also, due to the small numbers the analysis had limited power to detect small differences among the studied variables. Finally, the use of multiple comparisons increases the possibility of detecting spurious associations between the individual markers themselves and in their relation to PFS and OS. Despite these limitations, a number of interesting avenues of further investigation are suggested by our data.

The nearly universal expression of hormone receptors as well as the significant associations found for ER and MIB-1, ER and AR, and cyclin D1 and AR suggests a central role for endogenous hormones in male breast carcinoma. Based on extrapolation from the female population, tamoxifen is the most commonly used adjuvant hormonal therapy in male patients, although there is no prospective data to confirm efficacy. Further research is needed to clarify the relation between hormone receptor expression and response to hormonal therapies. The role of medical androgen blockade in the treatment of metastatic disease and as adjuvant therapy also remains to be elucidated but is supported by our finding of a high degree of AR expression in male breast carcinoma.

The high frequency of *bcl*-2 expression implicates antiapoptotic mechanisms as playing a role in male breast carcinoma. *bcl*-2 is only one of a large and growing family of regulators of apoptosis that includes molecules with either agonistic or antagonistic activities. The most powerful *bcl*-2 antagonist is bax and there is evidence that the physiologic *bcl*-2/bax balance may play a critical role in apoptotic cell death in female breast carcinoma.[55] We observed a high concordance rate between *bcl*-2 and the expression of ER and PR. It is possible that the action of endogenous hormones may influence the interactions of *bcl*-2 and bax by shifting the balance toward *bcl*-2, thereby causing inhibition of apoptosis and promotion of uncontrolled cellular proliferation.

Due to the rarity of male breast carcinoma and the lack of standardized treatment guidelines, large collaborative studies will need to be performed using strict clinical and laboratory criteria to advance our understanding of this disease as well as to identify the most effective treatment approaches. Although a rare disease, the proliferation of reports from large single institution series describing the natural history and outcomes of male breast carcinoma suggests that prospective clinical trials and collaborative laboratory research potentially are feasible.

REFERENCES

1. Hankey BF, Brinton LA, Kessler LG, Abrams J. Breast. In: Miller BA, Gloeckler Ries LA, Hankey BF, et al., editors. SEER cancer statistics review 1973–1990. Bethesda, (MD): U.S. Dept. of Health and Human Services; 1993 IV.1-IV.19.
2. Jaiyesmi IA, Buzdar AV, Sahin AA, Ross MA. Carcinoma of the male breast. *Ann Intern Med* 1992;117:771–7.
3. Erlichman C, Murphy KC, Elhakim T. Male breast cancer: a 13 year review. *J Clin Oncol* 1984;2:903–9.
4. Yap HY, Tashima CK, Blumenschein GR, Eckles NE. Male breast cancer, a natural history study. *Cancer* 1979;44:748–54.

5. Ribiero GG, Swindell R, Harris M, Banerjee SS, Cramer A. A review of the management of the male breast carcinoma based on an analysis of 420 treated cases. *Breast* 1996;5: 141–6.
6. Cutuli B, Lacroze M, Dilhuydy JM, Velten M, DeLafontan B, Marchal C, et al. Male breast cancer: results of the treatments and prognostic factors in 397 cases. *Eur J Cancer* 1995;31A:1960–4.
7. Willsher PC, Leach IH, Ellis IO, Bourke JB, Blamey RW, Robertson JFR. A comparison outcome of male breast cancer with female breast cancer. *Am J Surg* 1997;173:185–8.
8. Salvadori B, Saccozzi R, Manzari A, Andreola S, Conti RA, Cusumano F, et al. Prognosis of breast cancer in males. *Eur J Cancer* 1994;30A:930–5.
9. Joshi MG, Lee AKC, Loda M, Camus MG, Pedersen C, Heatley GJ, et al. Male breast carcinoma: an evaluation of prognostic factors contributing to a poorer outcome. *Cancer* 1996;77:490–8.
10. Wold LE, Ingle JN, Pisansky TM, Johnson RE, Donohue JE. Prognostic factors for patients with carcinoma of the breast. *Mayo Clin Proc* 1995;70:678–9.
11. McGuire WL, Clark GM. Prognostic factors and treatment decisions in axillary-node-negative breast cancer. *N Engl J Med* 1992;326:1756–61.
12. Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer. *J Clin Oncol* 1996;14:2843–77.
13. Gough DB, Donohue JH, Evans MM, Pernicone PJ, Wold LE, Naessens JM, et al. A 50-year experience of male breast cancer: is outcome changing? *Surg Oncol* 1993;2:325–33.
14. American Joint Committee on Cancer. Breast. In: Beahrs OH, Henson DE, Hutter RVP, et al., editors. Manual for staging of cancer. 4th edition. Philadelphia: J.B. Lipp. 1992: 149.
15. Kaplan E, Meier P. Nonparametric estimation from incomplete observation. *J Am Statistical Assoc* 1958;53:457–81.
16. Peto R, Peto J. Asymptotically efficient rank invariant procedures [with discussion]. *J R Stat Soc A* 1972;135:185–207.
17. Wittliff JL. Specific receptors of the steroid hormones in breast cancer. *Semin Oncol* 1974;1:109–18.
18. Gupta N, Cohen JL, Rosenbaum C, Raam S. Estrogen receptors in male breast cancer. *Cancer* 1974;46:1781–4.
19. Stalsberg H, Thomas DB, Rosenblatt KA, Jiminez LM, McTiernan A, Stemhagen A, et al. Histologic types and hormone receptors in breast cancer in men: a population based study in 282 United States men. *Cancer Causes Control* 1993;4: 143–51.
20. Fox SB, Rogers S, Day CA, Underwood JCE. Oestrogen receptor and epidermal growth factor receptor expression in male breast carcinoma. *J Pathol* 1992;166:13–18.
21. Weber-Chappuis K, Bieri-Burger S, Hurlimann J. Comparison of prognostic markers detected by immunohistochemistry in male and female breast carcinomas. *Eur J Cancer* 1996;32A:1686–92.
22. Bruce DM, Heys SD, Payne S, Miller ID, Eremin O. Male breast cancer: clinico-pathological features, immunocytochemical characteristics and prognosis. *Eur J Surg Oncol* 1996;22:42–6.
23. Roche PC. Immunohistochemical stains for breast cancer. *Mayo Clin Proc* 1994;69:57–8.
24. Sasano H, Kimura M, Shizawa S, Kimura N, Nagura H. Aromatase and steroid receptors in gynecomastia and male breast carcinoma: an immunohistochemical study. *J Clin Endocrinol Metab* 1996;81:3063–7.
25. Lopez M, Natali M, Di Lauro L, Vic P, Pignatti F, Carpano S. Combined treatment with buserelin and cyproterone acetate in metastatic male breast cancer. *Cancer* 1993;72:502–5.
26. Doberauer C, Niederle N, Schmidt CG. Advanced male breast cancer treatment with the LH-RH analogue buserelin alone or in combination with the antiandrogen flutamide. *Cancer* 1988;62:474–8.
27. Yang E, Korsmeyer SJ. Molecular thanatopsis: a discourse on the bcl-2 family and cell death. *Blood* 1996;88:386–401.
28. Leek RD, Kaklamanis L, Pezzella F, Gatter KC, Harris AL. Bcl-2 in normal breast and carcinoma, association with oestrogen receptor-positive, epidermal growth factor receptor-negative tumors and in situ cancer. *Br J Cancer* 1994;69: 135–9.
29. Joensuu H, Pylkkanen L, Toikkanen S. Bcl-2 protein expression and long-term survival in breast cancer. *Am J Pathol* 1994;145:1191–8.
30. Johnston SRD, MacLennan KA, Sacks NPM, Salter J, Smith IE, Dowsett M. Modulation of bcl-2 and Ki-67 expression in oestrogen receptor-positive human breast cancer by tamoxifen. *Eur J Cancer* 1994;30A:1663–9.
31. Gasparini G, Barbareschi M, Doglioni C, Dalla Palma P, Mauri FA, Boracchi P, et al. Expression of bcl-2 protein predicts efficacy of adjuvant treatments in operable node-positive breast cancer. *Clin Cancer Res* 1995;1:189–98.
32. Harris CC, Hollstein M. Clinical implications of the p53 tumor-suppressor gene. *N Engl J Med* 1993;329:1318–27.
33. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. *Science* 1991;253:49–52.
34. Mies C, Mejias A, Nadji M, Morales A. p53 and HER-2 neu are rarely immunohistochemically detectable in male breast cancer. *Mod Pathol* 1994;7:19A.
35. Kovach JS, Hartmann A, Blaszyk H, Cunningham J, Schaid D, Sommer SS. Mutation detection by highly sensitive methods indicates that p53 gene mutations in breast cancer can have important prognostic value. *Proc Natl Acad Sci USA* 1996;93:1093–6.
36. Anelli A, Anelli TFM, Youngson B, Rosen PP, Borgen PI. Mutations of the p53 gene in male breast cancer. *Cancer* 1995;75:2233–8.
37. Pich A, Margaria E, Chiusa L, Ponti R, Geuna M. DNA ploidy and p53 expression correlate with survival and cell proliferative activity in male breast carcinoma. *Hum Pathol* 1996; 27:676–82.
38. Brandt-Rauf PW, Pincus MR, Carney WP. The c-erbB-2 protein in oncogenesis: molecular structure to molecular epidemiology. *Crit Rev Oncog* 1994;5:3134–9.
39. Ravdin PM, Chamness GC. The c-erbB-2 proto-oncogene as a prognostic and predictive marker in breast cancer: a paradigm for the development of other macromolecular markers-a review. *Gene* 1995;159:19–27.
40. Hartmann LC, Ingle JN, Wold LE, Farr GH, Grill JP, Su JQ, et al. Prognostic value of c-erbB2 overexpression in axillary lymph node positive breast cancer. *Cancer* 1994;74:2956–63.
41. Lee AKC, Wiley B, Loda M, Bosari S, Dugan JM, Hamilton W, et al. DNA ploidy, proliferation, and neu-oncogene protein overexpression in breast carcinoma. *Mod Pathol* 1992;5: 61–7.
42. Fox SB, Day CA, Rogers S. Lack of c-erbB-2 oncoprotein expression in male breast carcinoma. *J Clin Pathol* 1991;44: 960–1.
43. Leach IH, Ellis IO, Elston CW. C-erb-B-2 expression in male breast carcinoma. [letter]. *J Clin Pathol* 1992;45:942.

44. Blin N, Kardas I, Welter C, Rys J, Niezabitowski A, Limon J, et al. Expression of the c-erbB2 proto-oncogene in male breast carcinoma: lack of prognostic significance. *Oncology* 1993;50:408–11.
45. Pinder SE, Wencyk P, Sibbering DM, Bell JA, Elston CW, Nicholson R, et al. Assessment of the new proliferation marker MIB-1 in breast carcinoma using image analysis: associations with other prognostic factors and survival. *Br J Cancer* 1995;71:146–9.
46. Pich A, Margaria E, Chiusa L. Proliferative activity is a significant prognostic factor in male breast carcinoma. *Am J Pathol* 1994;145:481–9.
47. Hamel PA, Hanley-Hyde J. G1 cyclins and control of the cell division cycle in normal and transformed cells. *Cancer Invest* 1997;15:143–52.
48. Sherr CJ. Cancer cell cycles. *Science* 1996;274:1672–6.
49. Arber N, Hibshoosh H, Zhang Y, Han Y, Sgambato A, Liu J, et al. Overexpression of cyclin D1 in both male and female breast cancers. *Proc Annu Meet Am Assoc Cancer Res* 1995; 36:227.
50. Bartkova J, Lukas J, Muller H, Lutzhoff D, Strauss M, Barter J. Cyclin D1 protein expression and function in human breast cancer. *Int J Cancer* 1994;57:353–61.
51. Gillett C, Smith P, Gregory W, Richards M, Millis R, Peters G, et al. Cyclin D1 and prognosis in human breast cancer. *Int J Cancer* 1996;69:92–9.
52. Fantl V, Smith R, Brookes S, Dickson C, Peters G. Chromosome 11q13 abnormalities in human breast cancer. *Cancer Surv* 1993;18:77–94.
53. Peters G, Fantl V, Smith R, Brookes S, Dickson C. Chromosome 11q13 markers and D-type cyclins in breast cancer. *Breast Cancer Res Treat* 1995;33:125–35.
54. Muller H, Lukas J, Schneider A, Warthoe P, Bartek J, Eilers M, et al. Cyclin D1 expression is regulated by the retinoblastoma protein. *Proc Natl Acad Sci USA* 1994;91: 2945–9.
55. Binder C, Marx D, Binder L, Schauer A, Hiddemann W. Expression of bax in relation to bcl-2 and other predictive parameters in breast cancer. *Ann Oncol* 1996;7: 129–33.