

Prognostic Value of p53 and bcl-2 Expression in Patients Treated with Breast Conservative Therapy

Prognostic value of p53 and bcl-2 expression on treatment outcome in breast cancer patients has been extensively evaluated, but the results were inconclusive. We evaluated the prognostic significance of these molecular markers in patients treated with breast conserving surgery and radiotherapy. One hundred patients whose immunostaining of p53 and bcl-2 expression was available among 125 patients who underwent radiotherapy after breast conserving surgery and axillary lymph node dissection were enrolled into this study. Eighty-seven patients also received adjuvant chemotherapy and/or hormonal therapy. Conventional clinicopathologic variables and treatment-related factors were also considered. The 5-yr loco-regional relapse-free and distant metastasis-free survival rates were 91.7% and 90.9%, respectively. On univariate analysis, age, T stage and the absence of bcl-2 & estrogen receptor (ER) expression were associated with loco-regional relapse-free survival. When incorporating these variables into Cox proportional hazard model, only bcl-2(-)/ER(-) phenotype was an adverse prognostic factor ($P=0.018$). As for the distant metastasis-free survival, age, T stage, and p53 expression were significant on univariate analysis. However, p53 expression was the only prognosticator on multivariate analysis ($P=0.009$). A bcl-2(-)/ER(-) phenotype and p53 expression are useful molecular markers predicting loco-regional relapse-free and distant metastasis-free survival, respectively, in patients treated with breast conserving surgery and radiotherapy.

Key Words : Breast Neoplasms; bcl-2; p53

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INTRODUCTION

Nodal involvement and tumor size are the most important prognostic factors for breast cancer patients at different risk of disease recurrence and/or death (1). Many clinicopathologic variables other than TNM stage have been identified to predict the clinical course of the disease. However, identifying all high-risk patients is still difficult. Beyond the conventional clinicopathologic variables, therefore, novel prognostic factors are needed to be explored to predict the outcome more accurately (2). Molecular markers such as p53 and bcl-2 have been extensively evaluated for their prognostic value since the 1990s (2-5). Although the prognostic value of p53 and bcl-2 expression in breast cancer has been evaluated in a number of studies, the results were controversial. Heterogeneous patient and tumor characteristics might be partly responsible for the discrepancy. Treatments were also various with mastectomy (6, 7) breast conservative therapy

(8-11) or the combination of the two (12-14).

Given these observations, we evaluated the prognostic value of p53 and bcl-2 expression on the treatment outcome, in relatively homogeneous population consisted of early stage breast cancer patients who underwent breast conservative treatment. In addition, the correlation between these markers and triple-negative phenotype was also investigated.

MATERIALS AND METHODS

Between March 2000 and February 2002, 125 patients underwent radiotherapy after breast conserving surgery for breast cancer. Of these, 100 patients whose immunohistochemical staining for p53 and bcl-2 was performed at the time of diagnosis were the cohorts for this retrospective study.

The median age was 44 yr (range; 24-68). Performance status of the patients was good with ECOG grade of 0 for

27 patients and grade of 1 for 73 patients. Histologic subtype was invasive ductal carcinoma in 90 patients (90.0%).

All patients had breast conserving surgery with axillary lymph node dissection. Sixty patients received chemotherapy: cyclophosphamide, methotrexate and 5-fluorouracil in 30 patients; doxorubicin-containing regimen in 27 patients; others in 3 patients. Sixty-two patients received hormonal therapy and 35 of them also received chemotherapy. As regards to radiotherapy, whole breast irradiation was given, up to 50.4 Gy at 1.8 Gy/fraction by using two opposing tangential photon beams. Tumor bed boost was supplemented up to 10 Gy at 2 Gy/fraction.

The clinicopathologic findings according to the expression of p53 and bcl-2 are summarized in Table 1. Median duration of follow-up was 69 months (range; 21-88).

The specimens were immunostained to determine the expression of p53, bcl-2 and c-erbB2 as well as the status of estrogen receptor (ER) and progesterone receptor (PR). Immunostaining was performed in the usual manner, using antibodies to p53 (Dako, Glostrup, Denmark; 1:1,200), bcl-2 (Dako, 1:50), c-erbB2 (Novocastra Laboratories Ltd., New Castle upon Tyne, UK, 1:200), ER (Dako, 1:50) and PR (Dako, 1:50).

The percentage of tumor cells with nuclear staining for p53 was graded semi-quantitatively: 0%, 1-25%, 26-50%,

Table 1. Clinicopathologic variables according to the expression of p53 and bcl-2

Variables	No. of patients		P value	No. of patients		P value
	p53 (+)	p53 (-)		bcl-2 (+)	bcl-2 (-)	
Age						
<40 yr	7	15	0.741*	17	5	0.765*
≥40 yr	22	56		63	15	
Tumor size						
≤2 cm	19	51	0.531*	54	16	0.275 [†]
>2 cm	10	20		26	4	
Nodal involvement						
Negative	17	58	0.015*	58	17	0.248 [†]
Positive	12	13		22	3	
Estrogen receptor						
Negative	12	13	0.015*	11	14	<0.001*
Positive	17	58		69	6	
Progesterone receptor						
Negative	19	37	0.220*	38	18	<0.001 [†]
Positive	10	34		42	2	
c-erbB2						
Negative	20	55	0.373*	62	13	0.248*
Positive	9	16		18	7	
Triple negative						
Yes	10	9	0.011*	8	11	<0.001 [†]
No	19	62		72	9	
bcl-2						
Negative	11	9	0.004*	-	-	-
Positive	18	62				

*chi-square test; [†]Fisher's exact test.

51-75% or >75%. For statistical analysis, p53 expression was considered as negative if ≤25% of tumor cells were stained and as positive if >25% of tumor cells were stained. ER/PR and bcl-2 expression were judged as positive when ≥10% of tumor cells were stained in nucleus and membrane, respectively. C-erbB2 expression was considered as positive if the score was 3, and negative if the score was 0, 1 or 2.

Statistical analysis was done by using SPSS software (release 12.0.1. SPSS Inc., Chicago, IL, USA). Differences in categorical variables between the parameters were compared by using standard chi-square test or Fisher's exact test. The actuarial survival rates were calculated by using the Kaplan-Meier method and statistical significance of differences between the actuarial survival rates was evaluated by the log-rank test. Cox proportional hazard model was used for multivariate analysis. For all statistical tests, *P* values <0.05 were considered significant.

RESULTS

Patients with p53 expression were more likely to have node-positive and ER-negative tumors. In contrast, bcl-2 expression was associated with both ER- and PR-positivity. Expression of p53 was correlated with triple-negative phenotype (*P*=0.011), while bcl-2 negativity was correlated with triple-negative phenotype (*P*<0.001). Inverse relationship was demonstrated between p53 and bcl-2 expression (*P*=0.004, Table 1).

The 5-yr loco-regional relapse-free and distant metastasis-free survival rates were 91.7% and 90.9%, respectively. On univariate analysis, age less than 40 yr and T2 stage were significant prognosticators predicting poor loco-regional relapse-free survival (Table 2). When incorporating these variables into Cox proportional hazard model, however, both lost the statistical significance. Given the association between bcl-2 expression and ER status, we tested interaction of the two variables. Compared with bcl-2(-)/ER(-) phenotype (n=14), other phenotypes (bcl-2[-]/ER[+], bcl-2[+]/ER[-], and bcl-2[+]/ER[+]) achieved higher loco-regional relapse-free survival rate (77.9% vs. 93.9%, *P*=0.033, Fig. 1). On multivariate analysis incorporating age, T stage and the presence of bcl-2(-)/ER(-) phenotype, the bcl-2(-)/ER(-) phenotype was the only adverse prognostic factor for loco-regional relapse-free survival (*P*=0.018). When the use of chemotherapy and hormonal therapy were added in the model, the prognostic significance of the bcl-2(-)/ER(-) phenotype became marginal (*P*=0.064).

For the distant metastasis-free survival, age less than 40 yr, T2 stage and p53 positivity (Fig. 2) were adverse prognostic factors (Table 2). Patients with bcl-2(-)/ER(-) phenotype had an inferior distant metastasis-free survival rate than those with other phenotypes, but the difference was not statistically significant (77.9% vs. 93.0%, *P*=0.153). On multivariate anal-

Table 2. Univariate and multivariate analysis for 5-yr loco-regional relapse-free and distant metastasis-free survival rate

Variables	5-yr LRRFS	P value		5-yr DMFS	P value	
		Uni	Multi		Uni	Multi
Age						
<40 yr	80.6%	0.032	0.081	77.3%	0.033	0.123
≥40 yr	94.7%			94.8%		
Tumor size						
≤2 cm	95.7%	0.046	0.068	97.1%	0.002	0.061
>2 cm	82.6%			76.7%		
Nodal involvement						
Negative	94.7%	0.110	-	94.6%	0.076	0.452
Positive	82.8%			80.0%		
Estrogen receptor						
Negative	93.0%	0.347	-	93.3%	0.323	0.852
Positive	87.6%			83.4%		
Progesterone receptor						
Negative	92.8%	0.650	-	95.5%	0.498	-
Positive	90.7%			87.2%		
p53						
Negative	94.2%	0.181	-	97.1%	<0.001	0.009
Positive	86.2%			75.9%		
bcl-2						
Negative	84.0%	0.140	-	78.7%	0.099	-
Positive	93.5%			93.8%		
c-erbB2						
Negative	90.3%	0.423	-	91.9%	0.359	-
Positive	96.0%			88.0%		
Bcl-2(-)/ER(-)						
Yes	77.9%	0.033	0.018	77.9%	0.153	-
No	93.9%			93.0%		
Triple negative						
Yes	89.2%	0.620	-	78.6%	0.107	-
No	92.3%			93.8%		

LRRFS, loco-regional relapse-free survival; DMFS, distant metastasis-free survival; ER, estrogen receptor.

ysis incorporating aforementioned variables as well as nodal and ER status, which were correlated with p53 expression, only p53 expression was significant ($P=0.009$). The statistical significance of p53 expression remained even after the use of chemotherapy and hormonal therapy were added in the model ($P=0.011$).

In subgroup analysis with triple-negative phenotype ($n=19$), bcl-2(-)/ER(-) phenotype was not associated with loco-regional relapse ($P=0.204$), and p53 expression was not associated with distant metastasis ($P=0.331$).

DISCUSSION

There have been several reports that investigated the prognostic value of p53 or bcl-2 expression on loco-regional failure after breast conservative therapy. Elkhuizen et al. compared 66 patients who experienced local recurrence with 139 matched controls in terms of the expression of several onco-

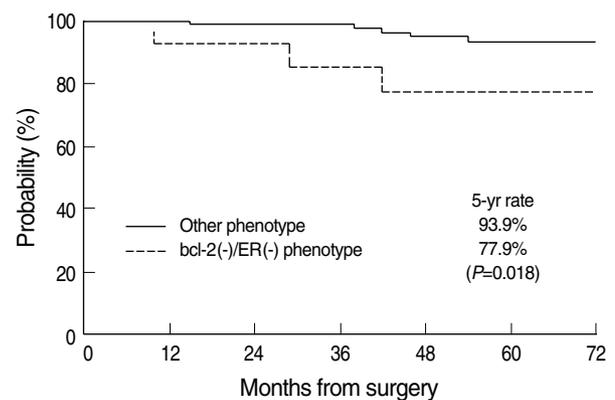


Fig. 1. Loco-regional relapse-free survival curves according to the expression of bcl-2(-)/ER(-) phenotype.

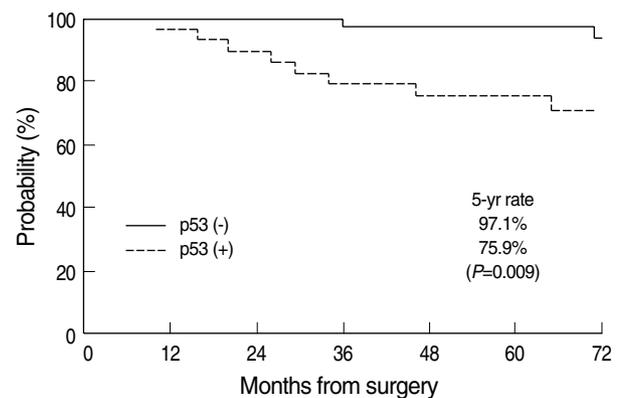


Fig. 2. Distant metastasis-free survival curves according to the expression of p53.

genes along with histological factors. They found that bcl-2 negativity carried a 17 times higher risk of local recurrence in patients >50 yr (10). Noguchi et al. (9) and Turner et al. (15) conducted similar case-control studies and noted the association between local recurrence and p53 positivity. However, a case-control approach has the limitation of inevitable biases in selecting control cases although the process is random. In contrast, other studies which analyzed consecutive patients failed to show any prognostic significance of these markers on local control (8, 11, 16). This might be partly due to the small number of events, that is, local recurrences in early stage breast cancer patients receiving breast conservative therapy.

We also failed to show that any of the two molecular markers was associated with loco-regional relapse-free survival. Instead, the combined variable of bcl-2(-)/ER(-) phenotype was proven to be a significant prognostic factor in univariate and multivariate analyses. Given the association between bcl-2 expression and ER status in a number of studies including ours (11, 17-19), a combined analysis of bcl-2 and ER has already been tried. Callagy et al. showed that the risk of death in bcl-2(-)/ER(-) phenotype is higher than those in other three phenotypes (20). Gasparini et al. demonstrated that the bcl-

2(-)/ER(-) phenotype had a 5.7 times higher risk of overall recurrence compared with bcl-2(+)/ER(-) phenotype on multivariate analysis (12). Likewise, an analysis of several molecular markers in combination provides more accurate prognostic information than that of the single variable, even in the low-risk group like our population.

For the distant metastasis, there are some limited data on the prognostic value of these markers in the breast conservative treatment setting. Aforementioned case-control studies noted a higher risk of distant metastases in patients with p53 expression (9, 15). Again, however, consecutive population-based studies failed to verify the relationship, despite the larger number of patients and events (11, 17). In the current study, p53 positivity was an independent prognostic factor predicting distant metastasis-free survival after adjusting conventional clinicopathologic variables including nodal and ER status and treatment-related factors such as the use of chemotherapy and/or hormonal therapy.

Recently, a number of investigators are seeking for new therapeutic strategy for triple-negative breast cancer, which is associated with inferior distant metastasis-free survival (21-23). Unlike other reports, however, triple negativity was not a significant risk factor for distant metastasis in the present study. However, the correlation between p53 expression and triple-negative phenotype was demonstrated. This finding was also observed by other studies (24, 25). Although the prognostic impact of p53 expression in triple-negative tumors was not established in our study as well as others (25), p53 expression can give therapeutic implication for those patients with triple-negative tumors. Regarding this issue, Bidard et al. showed that the rate of pathologic complete remission was higher in patients with p53-positive tumors than those with p53-negative tumors after neoadjuvant chemotherapy for triple-negative breast cancer (26).

Despite the small number of patients and events, our study demonstrated the prognostic significance of bcl-2 and p53 expression on loco-regional control and distant metastasis, respectively. This is partly because our population is relatively homogeneous, consisted of early stage breast cancer patients treated with breast conserving surgery and radiotherapy. Moreover, accrual period of the patient was relatively short, and therefore our patients were treated in a relatively uniform fashion regarding loco-regional therapy. But, the delivery of systemic therapy was somewhat various as decision to undergo systemic therapy was based on the risk of the individual patient and the subsequent gain with systemic therapy. Further studies with larger population are needed to confirm the results from this study.

Unlike hormonal receptor expression or c-erbB2 expression, there is currently no specific treatment available to target tumors with either p53 expression or bcl-2 negativity. But, breast cancer patients may be stratified into more detailed subtypes by these markers as well as other more relevant molecular markers, which are in the process of development.

Although more diversified patient and tumor specific treatment strategies need to be elucidated from future trials, this approach will eventually benefit patients undergoing treatment for breast cancer.

In conclusion, results from this study show that a bcl-2(-)/ER(-) phenotype and p53 positivity are useful molecular markers predicting loco-regional relapse-free and distant metastasis-free survival, respectively, in patients treated with breast conserving surgery and radiotherapy.

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