Lactate dehydrogenase, Gleason score and HER-2 overexpression are significant prognostic factors for M1b prostate cancer

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Abstract. It has not been elucidated whether certain types of M1b prostate cancer (M1b PC) are associated with a poor outcome. The present study retrospectively identified predictive factors related to the outcome of M1b PC. The subjects were 104 patients who attended our hospital and received a diagnosis of M1b PC. The observation period ranged from 4 to 122 months (median, 43 months). The parameters investigated were: T classification, N classification, Gleason score (GS), pretreatment prostate-specific antigen (PSA) level, extent of disease (EOD) grade, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), calcium, and hemoglobin (Hb) levels, platelet count, and the status of HER-2 overexpression as determined with a Hercep Test™ Kit using initial needle biopsy specimens for diagnosis. Log-rank test and Cox univariate analysis identified the following factors with statistically significant differences: pretreatment PSA ≥192, N1, GS ≥8, EOD grade 3+4, high LDH, high ALP, low Hb, and HER-2 overexpression. Multivariate Cox proportional hazard analysis identified the factors GS ≥8, high LDH, and HER-2 overexpression with significant differences. The hazard ratio was 5.962, 2.465, and 2.907, respectively, and the probability value was P=0.0218, P=0.0207 and P=0.0090, respectively. When the subjects with GS ≥8, high LDH, and HER-2 over-expression were classified as the high-risk group, the 5-year cause-specific survival rate was 51.2, 29.6, and 20.0%, respectively. The present study showed that M1b PC patients with GS ≥8, high LDH, and HER-2 over-expression have a very poor outcome and thus, should be treated as a high-risk group requiring close follow-up.

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Introduction

Of cancer deaths, in the USA, the incidence of prostate cancer (PC) ranks first in men, while the mortality from PC ranks second after lung cancer. In Europe, about 260,000 people are diagnosed with PC every year (1), and PC accounts for 9% of cancer deaths in men (2). The frequency of PC varies from country to country; it has been reported to be lowest in the Far East, particularly in mainland China and Japan (3). In Japan, however, the frequency in 2015 is expected to increase to about 4.6 times that in 1985 (4), and a recent study reported that PC screening would reduce mortality from PC by 20% (5). PC will thus become an increasingly important disease in men. Patients with PC have only vague symptoms in the early phase of the disease; it is not rare for patients to present with chief complaint of bone pain or neurological symptoms and found to already have PC with bone metastases at the time of diagnosis (6). Most PC is androgen-dependent. Patients with metastatic PC are rarely cured, and most of them are treated by hormone therapy. The majority of such patients, however, progress to castration-resistant prostate cancer (CRPC) within several years. Hormone resistance is considered to be acquired through abnormalities in the androgen receptor as well as a mechanism other than the androgen receptor (7). At present, however, the characteristics of patients who are likely to progress to CRPC have not been clarified, and no effective therapy has been established for CRPC. Docetaxel is the only chemotherapeutic agent reported to improve the outcome (8,9).

Human epidermal growth factor receptor-2 (HER-2 or HER-2/neu) is a proto-oncogene located on chromosome 17q21 and is also a transmembrane tyrosine growth factor. Fundamental research has revealed that HER-2 overexpression induces cancerous transformation in cells, manifesting as greater aggressiveness. Since Slamon *et al* (10) first reported the association between amplification of the HER-2 gene and poor-outcome breast cancer, HER-2 has been considered to be a factor in poor outcome of breast cancer, and HER-2 overexpression breast cancer has been shown to be refractory to hormone therapy (11). Several reports exist on subjects who underwent radical prostatectomy, indicating that those with HER-2 overexpression experienced poor outcome

(12,13). Further *in vivo* study using PC cells discovered that HER-2 regulated activation of the androgen receptor pathway and transcriptional activity (14,15). These finding suggests potential efficacy of treatment with anti-HER-2 agents.

We previously reported that HER-2 was not involved in the regulation of neuroendocrine cell differentiation (16). The present study retrospectively assessed the potential significance of various clinical data (serum biochemical data and pathological findings) and the status of HER-2 protein overexpression, using biopsy specimens obtained at diagnosis, in predicting the outcome of M1b prostate cancer (M1b PC) after hormone therapy.

Materials and methods

Of the 454 patients who had been given a diagnosis of prostate cancer at Aichi Medical University Hospital between January 1998 and December 2006, 104 with M1b confirmed by bone scintigraphy, CT, MRI and with a Karnofsky performance scale of ≥70% were targeted for the present study.

All subjects were treated with hormone therapy. In all subjects, the prostate-specific antigen (PSA) level was confirmed to have decreased to 4.0 ng/ml or less after the initiation of treatment. The last observation was on May 31, 2009. The PSA level was measured using a Tandem-R PSA kit (Hybritech, San Diego, CA, USA). Prostate biopsy (systematic sextant needle biopsy) was performed under transrectal ultrasound guidance with an Aloka SSB-3500 (Aloka Co., Ltd., Tokyo, Japan) using an 18-G biopsy needle (Biopty, C. R. Bard, GA, USA). The clinical stage and the extent of disease (EOD) grade (17) were performed by CT, MRI, and bone scintigraphy. The day of determination of the stage was defined as the first day of observation. Histopathological grading was performed using Gleason score (GS) (18), and the clinical stage was determined based on the International Union Against Cancer classification (19).

The parameters investigated were T classification, N classification, GS, pretreatment PSA, EOD grade, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), calcium (Ca), and hemoglobin (Hb) levels, platelet count (PLT), and the status of HER-2 overexpression as determined by immunohistochemical (IHC) staining with a Hercep Test™ Kit.

The present study was approved by the Institutional Review Board of Aichi Medical University School of Medicine (Approval no. 275). The baseline characteristics of the 104 subjects are shown in Table I.

Immunohistochemical (IHC) procedure and scoring methods. Immunohistochemical staining was performed using the single core that had a highest GS and was occupied largest by a tumor as a result of a systematic sextant needle biopsy. Paraffin sections, 5 μm thick, were stained for expression of the HER-2 protein using a standardized Hercep Test. The primary antibody in this assay is an affinity-purified rabbit polyclonal antiserum raised against an intracellular epitope of the human HER-2 molecule (Specification Sheet: K5205, Dako, USA). Staining was performed on an automated staining apparatus for IHC (Autostainer, Dako) according to the manufacturer's guidelines as follows. Sections were depa-

Table I. Patient characteristics.

Patient no.	104
Age (years)	54-91
Average (SD)	74.2 (±7.4)
Median	74
Serum PSA (ng/ml)	10-100060.0
Average (SD)	920.1 (±1759.3)
Median	268.7
Follow-up period (month)	4-122
Average (SD)	46.9 (±29.1)
Median	43
Treatment	
MAB (%)	93 (89.4)
LH-RH agonist monotherapy (%)	2 (1.9)
Orchiectomies (%)	1 (0.9)
Orchiectomies + anti-androgen (%)	8 (7.7)
Outcome	
Alive (%)	50 (48.0)
Cancer death (%)	45 (43.2)
Other cause death (%)	9 (8.6)
Gleason score	
7 (%)	19 (18.3)
8 (%)	31 (29.8)
9 (%)	48 (46.2)
10 (%)	6 (5.8)
EOD grade	
1 (%)	39 (37.5)
2 (%)	41 (39.4)
3 (%)	15 (14.4)
4 (%)	8 (7.7)
X (%)	1 (0.9)
T classification	
T1 (%)	4 (3.8)
T2 (%)	25 (24.0)
T3 (%)	24 (23.0)
T4 (%)	49 (47.1)
Tx (%)	2 (1.9)
N classification	
N0 (%)	57 (54.8)
N1 (%)	44 (42.3)
Nx (%)	3 (2.8)
HER-2	
Negative (%)	72 (69.2)
Positive (1+) (%)	10 (9.6)
Positive (2+) (%)	14 (13.5)
Positive (3+) (%)	6 (5.8)
Undeterminate (%)	2 (1.9)

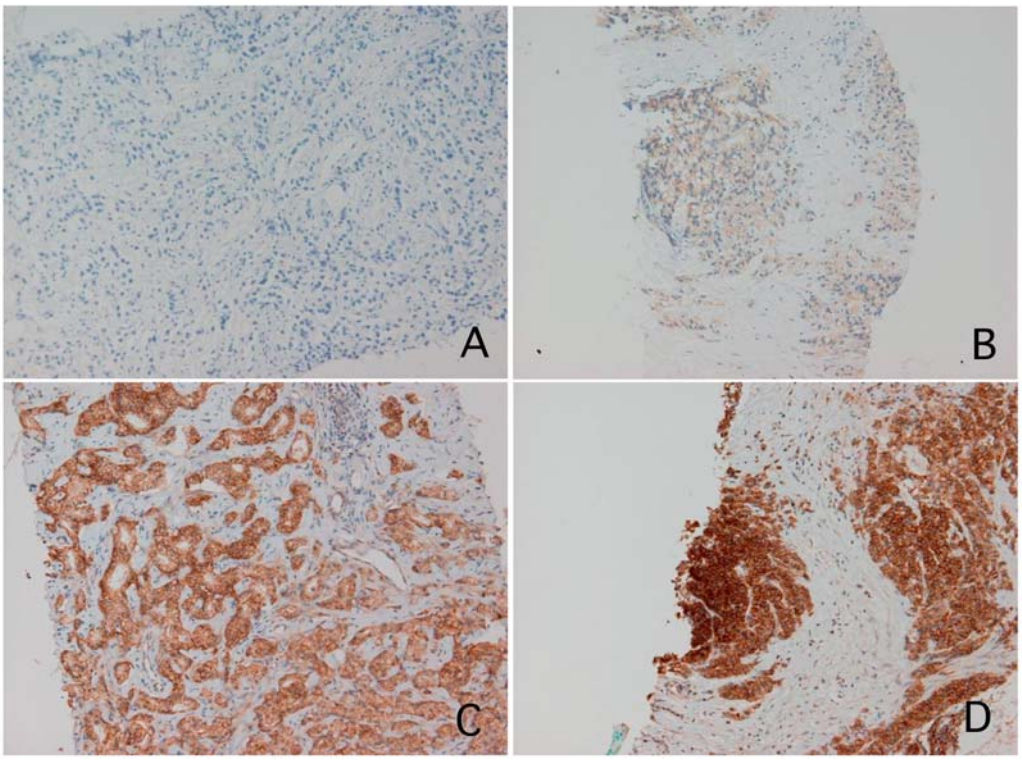

Figure 1. HER-2 expression in four primary tumors detected by immunohistochemistry using the Hercep Test™. (A) No staining or membrane staining observed in <10% of tumor cells (HER-2 score, 0; magnification, x100). (B) Faint or barely perceptible membrane staining detected in >10% of tumor cells: cells are stained only in part of their membrane (HER-2 score, 1+; magnification, x100). (C) Weak to moderate, complete membrane staining observed in >10% of tumor cells (HER-2 score, 2+; magnification, x100). (D) Strong complete membrane staining observed in >10% of tumor cells (HER-2 score, 3+; magnification, x100).

raffinized in xylene and rehydrated through graded ethanols to distilled water. The sections were immersed in Dako epitope retrieval solution (10 mM citrate buffer, pH 6.0) that had been preheated to 95˚C in a water bath and then heat-treated at 95˚C for 40 min. After a 20-min cool-down period at room temperature, the sections were washed with Dako wash buffer, a procedure that followed every subsequent incubation. Endogenous peroxidase was blocked with Dako blocking buffer (0.3% hydrogen peroxide containing 15-mM sodium azide) for 5 min at room temperature. The sections were incubated with the primary polyclonal antibody, an affinity-purified rabbit anti-human HER-2 antibody (1:70) supplied in the kit, for 30 min at room temperature. Bound primary antibody was labeled by incubating the slides with the Dako visualization reagent (horseradish peroxidase-labeled dextran polymer conjugated to affinity-purified goat anti-rabbit immunoglobulins in Tris-HCl) for 30 min. Color development was achieved with 3,3'-diaminobenzidine (DAB) for 10 min. The sections were counterstained with haematoxylin. To confirm validation of the staining run, control cell slides, which were provided in the kit and consisted of three pelleted, formalin-fixed, paraffin-embedded human breast cell lines with known HER-2 positivity (MDA-231, 0; MDA-175, 1+; SK-BR-3, 3+), were also stained simultaneously. In the negative controls, the primary antibody was replaced by normal rabbit serum (Dako negative control reagent) for the HER-2 primary antibody.

Following the Food and Drug Administration Scoring Guidelines for breast carcinomas, the patterns were evaluated using the 0-3+ scales as illustrated in the Hercep Test Kit Scoring Guidelines (0 for no staining at all or membrane staining in ≤10% of the tumor cells; 1+ for only partial, weak staining of the cell membrane of ≥10% of the tumor cells; 2+ for moderate staining of the complete cell membrane in >10% of the tumor cells; 3+ for intense staining of the complete membrane in >10% of the tumor cells).

The results of histological examination and those of staining were interpreted by two pathologists. The scores of 2+ and 3+ were considered HER-2 overexpression and defined as positive, 0 and 1+ defined as negative (Fig. 1).

Statistical analyses. Survival curves were prepared by the Kaplan-Meier method. To identify predictive factors for the outcome of M1b PC, the subjects who had died of causes other than PC were counted as discontinued cases in the calculation of the cause-specific survival rate, and the significance of differences was assessed with the log-rank test. For univariate and multivariate analyses, Cox proportional hazard analysis was employed. All statistical analyses were performed with Statistical Package for Social Sciences (SPSS, Chicago, IL, USA) version 10.0 for Windows. Probability values <0.05 were considered statistically significant.

Results

In the 104 subjects, the 5-year cause-specific survival rate was 56.6%, and the 10-year cause-specific survival rate was 34.9% (Fig. 2A).

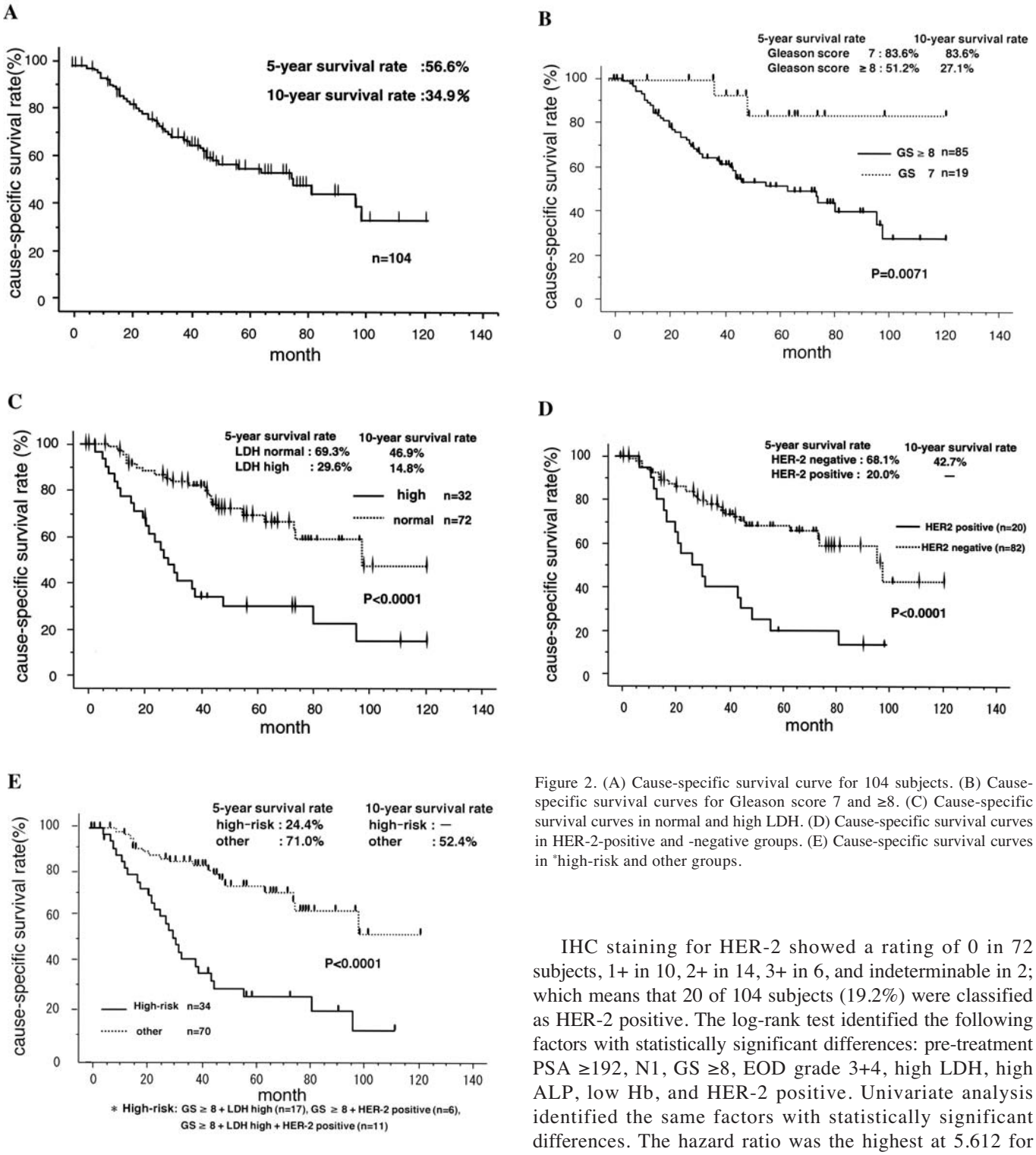

Figure 2. (A) Cause-specific survival curve for 104 subjects. (B) Cause-specific survival curves for Gleason score 7 and ≥8. (C) Cause-specific survival curves in normal and high LDH. (D) Cause-specific survival curves in HER-2-positive and -negative groups. (E) Cause-specific survival curves in *high-risk and other groups.

Each variable was constructed as follows: T classification, T1-3 vs. T4; N classification, N0 vs. N1; GS=7 vs. ≥8 (because there was no subject with GS ≤6); pretreatment PSA level, <192 vs. ≥192 (because a significant difference in survival rate was observed between these groups); EOD grade, 1+2 vs. 3+4; ALP, LDH, and Ca levels, normal values vs. high values (defined as at least 1.15 times higher than the upper limit of normal); PLT and Hb levels, normal values vs. low values (defined as not >0.85 times lower than the lower limit of the normal).

IHC staining for HER-2 showed a rating of 0 in 72 subjects, 1+ in 10, 2+ in 14, 3+ in 6, and indeterminable in 2; which means that 20 of 104 subjects (19.2%) were classified as HER-2 positive. The log-rank test identified the following factors with statistically significant differences: pre-treatment PSA ≥192, N1, GS ≥8, EOD grade 3+4, high LDH, high ALP, low Hb, and HER-2 positive. Univariate analysis identified the same factors with statistically significant differences. The hazard ratio was the highest at 5.612 for GS ≥8 (Table II). Multivariate Cox proportional hazard analysis used the factors which univariate analysis identified as statistically significant, revealed statistically significant differences in the GS ≥8, high LDH, and HER-2 positive, with hazard ratios of 5.962, 2.465, and 2.907, respectively (P=0.0218, P=0.0207, and P=0.0090, respectively) (Table III). When the subjects with GS ≥8, high LDH, and HER-2 positive were classified as the high-risk group, the 5-year cause-specific survival rate was 51.2, 29.6, and 20.0%, respectively (Fig. 2B-D). The 5-year cause-specific survival rate with GS ≥8 + high LDH (n=17), HER-2 positive (n=6), and high LDH + HER-2 positive (n=11) was 24.4%. Outcome was significantly poorer in this group than in the other group,

Table II. Results of the univariate Cox proportional hazard analysis method and log-rank test.

Factors	Univariate Hazard ratio (95% CI[a])	P-value	5-year cause-specific survival rate (%)	10-year cause-specific survival rate (%)	Log-rank test P-value
Pretreatment PSA level (ng/ml)					
<192	1		70.1	45.8	
≥192	1.98 (1.064-3.685)	0.0311	42.3	-	0.0278
T stage					
T1-3	1		60.5	43	
T4	1.285 (0.706-2.338)	0.4123	54.4	-	0.3374
N stage					
N0	1		67.1	50.6	
N1	2.206 (1.207-4.034)	0.0102	41.3	-	0.0083
Gleason score					
7	1		83.6	83.6	
≥8	5.612 (1.358-23.194)	0.0172	51.2	27.1	0.0071
EOD					
1+2	1		60.8	39.3	
3+4	1.978 (1.006-3.889)	0.0479	37.3	-	0.0433
LDH					
Normal	1		69.3	46.9	
High	3.307 (1.835-5.959)	<0.0001	29.6	14.8	<0.0001
ALP					
Normal	1		69.6	65.9	
High	2.903 (1.559-5.405)	0.0008	41.7	13.7	0.0004
Hgb					
Normal	1		72.8	50.4	
Low	2.203 (1.168-4.155)	0.0147	43.4	-	0.0122
PLT					
Normal	1		57.4	38.9	
Low	1.027 (0.519-2.033)	0.9392	52.2	27.9	0.9391
Ca					
Normal	1		57.5	38.1	
High	1.414 (0.595-3.358)	0.4328	51.1	-	0.4293
HER-2					
Negative	1		68.1	42.7	
Positive	3.146 (1.717-5.766)	0.0002	20	-	<0.0001

[a]CI, confidence interval.

which had a 5-year cause-specific survival rate of 71.0% (P<0.0001) (Fig. 2E). Among the subjects with GS=7, the number of subjects with high LDH and HER-2 positive was 3 and 2, respectively.

Discussion

About 80% of patients with M1b PC respond to hormone therapy performed as initial treatment. However, the 5-year survival rate is known to be as low as about 30% in patients with M1b PC in Japan, because more than half of the patients become resistant to hormone therapy within several months to several years (20).

Hormone resistance is considered to be acquired through abnormalities in the androgen receptor as well as a mechanism not mediated by the androgen receptor. Abnormalities in the androgen receptor include: i) androgen receptor amplification (which allows a small amount of androgen to react); ii) androgen receptor gene mutations (which allow anti-androgens, estrogen and corticosteroid to bind to and react with the

Table III. Results of the multivariate Cox proportional hazard analysis method.

Factors	Hazard ratio (95% CI[a])	P-value
T classification (T1-3 vs. T4)	1.136 (0.543-2.374)	0.7351
N classification (N0 vs. N1)	1.199 (0.603-2.384)	0.6035
Gleason score (7 vs. ≥8)	5.962 (1.296-27.414)	0.0218
PSA (<192 vs. ≥192 ng/ml)	1.488 (0.727-3.044)	0.2763
EOD (1+2 vs. 3+4)	1.829 (0.717-4.662)	0.2062
ALP (normal vs. high)	1.657 (0.764-3.591)	0.2009
LDH (normal vs. high)	2.465 (1.148-5.296)	0.0207
Ca (normal vs. high)	0.796 (0.250-2.536)	0.6998
Hgb (normal vs. low)	0.905 (0.395-2.071)	0.8132
PLT (normal vs. low)	0.963 (0.425-2.180)	0.9275
HER-2 (negative vs. positive)	2.907 (1.305-6.477)	0.009

[a]CI, confidence interval.

androgen receptor); iii) abnormalities in co-activators, which potentiate the transcriptional activity of the androgen receptor; and iv) androgen receptor activation caused by abnormal production of growth factors or cytokines. On the other hand, mechanisms not mediated by the androgen receptor include: i) evasion of apoptosis caused by abnormalities in apoptosis-related genes; and ii) the appearance and proliferation of neuroendocrine cells. We also previously reported that neuroendocrine cell differentiation in prostate biopsy specimens is involved in the acquisition of resistance to hormone therapy (21). Debes *et al* (7) suggested that these abnormalities do not occur independently, but are involved in the acquisition of hormone resistance in a complicated manner, but this hypothesis has not yet been verified.

In the present study, the 5-year cause-specific survival rate was 56.6%, and the 10-year cause-specific survival rate was 34.9%. These favorable results may be attributable to the short mean observation period of 47 months. Some patients with M1b survive for a long time, and thus it is sometimes difficult to accurately predict the outcome. With regard to predictive factors for the outcome of M1b PC, some studies recently identified the factors: performance scale, GS, response to endocrine therapy, anemia, and levels of serum albumin, LDH, ALP, and PSA (22,23), while another study showed that EOD grade, interleukin-6 (24), and the status of HER-2 overexpression were useful (16). Still another study reported that serum cholesterol and interleukin-6 levels are involved in cachexia (25). Thus, no consensus has been reached. In the present study, log-rank test and univariate analysis identified the factors: pretreatment PSA ≥192, N1, GS ≥8, EOD grade 3+4, high LDH, high ALP, low Hb, and the status of HER-2 protein overexpression with statistically significant differences.

The presence or absence of lymph node metastasis and GS have been shown to be involved in the outcome of CRPC (26,27), and are widely known to be clinically important indicators. During the present study, multivariate Cox proportional hazard analysis identified the factors GS ≥8, high LDH, and HER-2 overexpression with significant differences, and more than half of such patients died within 2 years. Such patients have a very poor outcome and should be classified as the high-risk group. The present study targeted patients with a Karnofsky performance score ≥70%, and therefore, may have allowed more accurate identification of prognostic factors for M1b PC.

The proto-oncogene HER-2 encodes a protein with a molecular weight of 185 kDa that has a receptor structure penetrating the cell membrane. This protein is a tyrosine-kinase-type receptor on the surface of the cell membrane, and has an amino acid sequence resembling that of the epidermal growth factor receptor. It stimulates cell differentiation and proliferation through the ligand binding to the extracellular domain. Some recent studies indicated that HER-2 stimulates AKT pathway or cyclin D1 and prolongs life or activates growth of PC cells, inhibiting apoptosis (28-30).

With regard to HER-2 expression in patients with PC, Morote *et al* (31) conducted a study on c-erbB-2 expression using biopsy specimens of patients with M1b PC, and found that 64.3% (45/70) of them were classified as having c-erbB-2 overexpression when the observation of staining in 1% or more of the tumor cells was considered positive. Ricciardelli *et al* (32) reported that 70% (37/53) of subjects with localized PC who underwent radical prostatectomy were HER-2 overexpression, and that many of the subjects with advanced stage tumors were HER-2 overexpression and were likely to have distant metastasis. On the other hand, Koeppen *et al* (33) found that of 61 subjects with PC, only 5 had HER-2 overexpression classified as 2+, and none was classified as 3+, while Lara *et al* (34) conducted a phase II trial of trastuzumab plus docetaxel using biopsy specimens of patients with hormone-refractory prostate carcinoma and reported that HER-2 overexpression was seen in 7% (7/100) of the subjects. Our findings were not consistent with the previous findings. The reasons seem to be that the disease stage of the specimens used for comparison varied and that there were differences in the procedure for evaluation of HER-2 overexpression, including the condition of the antigen, type of antibody used, and method of restoration of the antigen.

In the present study, 19.2% of the subjects had HER-2 overexpression. Univariate and multivariate analyses demon-

strated that subjects with HER-2 overexpression had a significantly poorer outcome. This finding is consistent with other reports (12,13,31), and seems to have been attributable to aggressive proliferation of cancer cells because of the action of HER-2 in subjects with HER-2 overexpression. However, further consideration will be required regarding the following: i) the Hercep Test Kit interpreted the result as positive when staining was observed in >10% of tumor cells; and ii) a breast cancer cell line was used as a positive control in this kit. However, it can be said from our results that detection of HER-2 overexpression using the biopsy specimen obtained at diagnosis could be an important factor in consideration of the treatment strategy of M1b PC.

LDH is an intracellular enzyme widely distributed throughout the tissues of the body. The serum LDH level increases when any tissue is injured and LDH is released into the blood. It is generally measured for screening during initial treatment, and fractionation of isozymes is useful for determining the injured organ. The serum LDH level is known to become abnormally high in the presence of diseases including acute myocardial infarction, acute hepatitis, leukemia and malignant lymphoma. The serum LDH level is known to become abnormally high in the presence of testicular tumors, and is used as an indicator of therapeutic effect. However, only limited types of malignant tumor are associated with high values in the early stage. Therefore, serum LDH level is generally used as a predictive factor of outcome or an indicator of therapeutic effect or worsening of symptoms. In patients with M1b PC, few have increased LDH in the early stage. Some studies showed that serum LDH level is a predictive factor for PC with resistance to hormone therapy (35,36), while other studies reported that it is not (23,24). Thus, no consensus has been reached. In the present study, however, patients with high LDH had a very poor outcome. This seems to have been because, in patients with high LDH, cancer cells have great proliferative capacity and thus a shorter cell cycle, which results in increased necrotic cells, and because cancer cells potentiate destruction of normal tissue at sites of metastasis. Therefore, LDH level may be employed as an indicator of tissue destruction in patients with M1b PC.

In conclusion, the present study showed that M1b PC patients with GS ≥8, high LDH, and HER-2 overexpression had a very poor outcome and thus should be treated as a high-risk group requiring close follow-up.

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