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Lymph node metastasis and high serum CEA are important prognostic factors in hormone receptor positive and HER2 negative breast cancer

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Abstract. In recent years, treatment options for breast cancer have increased, and prognosis has improved since the 1990s. The present study examined the prognosis for recurrence of breast cancer between 2006 and 2009, in comparison with the results of past treatments, and sought to guide future treatment strategies by elucidating present prognostic factors. A total of 662 patients with breast cancer stage 0-III who underwent surgery at Kitasato University Hospital between January 2006 and March 2009 were included. Cases were classified into four subtypes, based on the presence or absence of hormone receptors and human epidermal growth factor receptor 2 (HER2). Factors associated with recurrence and prognosis were then examined. The 5-year recurrence-free survival (RFS) was 94.9% and the 5-year disease-specific survival (DSS) was 98.4%. Factors related to RFS were pathological lymph node (pN) positive [hazard ratio (HR)=2.85, P=0.001], clinical lymph node (cN) positive (HR=2.28, P<0.01), and hormone receptor negative (HR=1.83, P<0.05). Factors associated with DSS were cN positive (HR=4.55, P<0.01), pN positive (HR=3.40, P<0.05), higher preoperative serum carcinoembryonic antigen (CEA) (HR=3.04, P<0.05), and hormone receptor negative (HR=2.32, P<0.05). In the hormone receptor positive HER2 negative, cN-positive/pN-positive breast cancer group, RFS and DSS were poorer compared with the other groups. In this group, preoperative high CEA level was a poor prognostic factor. The prognosis for hormone receptor positive HER2-negative breast cancer has improved significantly since the 1990s. On the other hand, the prognosis for cN-positive/pN-positive breast cancer was poor. Pre-treatment serum CEA positive cases exhibited a particularly poor prognosis.

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Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in women worldwide, accounting for 23% (1.4 million) of the total new cancer cases and 14% (458,400) of the total cancer deaths in 2008 (1,2).

Breast cancer is a heterogeneous disease and has distinct morphological features and tumor subtypes (3-6). Breast cancer is classified into 4 subtypes: Luminal A (hormone receptor positive, HER2 negative), luminal B (hormone receptor positive, HER2 positive), HER2-enriched (hormone receptor negative, HER2 positive), and triple negative (hormone receptor negative, HER2 negative) by microarray and hierarchical clustering analysis (7-11). It is now classified into 5 subtypes using Ki-67 expression. This classification has been used to formulate guidelines for breast cancer therapy. It was used to determine systemic adjuvant therapies by subtype and risk categories in 2007 per the St. Gallen consensus meeting (12). The 11th St. Gallen (Switzerland) expert consensus meeting on the primary treatment of early breast cancer in March 2009 maintained an emphasis on targeting adjuvant systemic therapies to subgroups as defined by these predictive markers (13,14).

We followed these guidelines and based our breast cancer therapy on expression of hormone receptors and HER2. Little is known about the prognosis of Japanese breast cancer patients treated according to subtype. There are no articles that described long-term prognosis and compared more recent outcomes with those of the 1990s. In this study, we examined prognosis of Japanese breast cancer patients from the 2000s, during which time we followed guidelines for therapy.

Patients and methods

A total of 662 patients with stage 0-III breast cancer underwent surgical resection in the Kitasato University Hospital

between January 2006 and March 2009. We classified them into 4 subtypes: Hormone receptor positive/HER2 negative, hormone receptor positive/HER2 positive, hormone receptor negative/HER2 positive, and hormone receptor negative/HER2 negative known as triple negative. Systemic therapy was consistent with recommendations for each of the biological subtypes. Characteristics of the 662 patients are shown in Table I. Median follow-up period was 77 (2-109) months. TNM classification was used based on the 7th edition of the Union for International Cancer Control (UICC). Clinical lymph node metastasis was considered more than 10 mm minor axis based on computed tomography. The patients with neoadjuvant chemotherapy (NAC) were also classified to pTNM. The positive cut-off value of serum CEA was higher than 5 ng/ml.

We extracted 87 cases with hormone receptor positive/HER2 negative and cN positive/pN positive between April, 2009 and December, 2014 as the validation group. Median follow-up was 51 (5-96) months. The present study was approved by the Ethics Committee of Kitasato University.

Statistical analysis. Recurrence free survival (RFS) and disease specific survival (DSS) were analysed based on clinicopathologic characteristics. RFS and DSS were calculated using the Kaplan-Meier method, and survival differences were assessed using a log-rank test. Variables suggested to be prognostic factors on univariate analysis were subjected to multivariate analysis using a Cox proportional-hazards model. The P-value <0.05 was considered to indicate statistical significance. All statistical analyses were conducted with SAS software package (JMP Pro11, SAS Institute, Cary, NC, USA).

Results

Univariate analysis for RFS and DSS. 5-year RFS of surgically treated breast cancer was 94.9%. Univariate prognostic factors for recurrence were serum value of CEA≧5.0 ng/ml (CEA positive) before cancer therapy (P<0.05), cT2-4 (P<0.001), cN positive (P<0.0001), NAC (P<0.0001), mastectomy (P<0.001), hormone receptor negative (P<0.001), pT2-4 (P<0.01), and pN positive (P<0.0001) (Table II). 5-year DSS was 98.4%. Univariate prognostic factors for DSS were serum value of CEA positive (P<0.01), cT2-4 (P<0.001), cN positive (P<0.0001), hormone receptor negative (P<0.01), pT2-4 (P=0.01), and pN positive (P<0.0001) (Table III).

Multivariate analysis for RFS and DSS. Among the 662 patients, multivariate Cox proportional hazards model identified pN positive (hazards ratio (HR)=2.85, P=0.001), cN positive (HR=2.28, P<0.01), and hormone receptor negative (HR=1.83, P<0.05) as significant independent factors for recurrence (Table II). In the multivariate model for DSS, cN positive (HR=4.55, P<0.01), pN positive (HR=3.40, P<0.05), serum value of CEA positive (HR=3.04, P<0.05) and hormone receptor negative (HR2.32, P<0.05) were identified as independent prognostic factors (Table III).

Kaplan-Meier curve of RFS and DSS by independent prognostic factors based on multivariate analysis. cN status was significantly different in recurrent cases (RFS of cN positive and negative were 70 and 88% respectively, P<0.0001). pN status was significantly different in recurrent cases (RFS of pN positive and negative were 68 and 92% respectively, P<0.0001). RFS of hormone receptor negative breast cancer was worse than that of the hormone receptor positive group (71 and 89% respectively, P<0.001).

cN positive cases had significantly poorer prognoses than the cN negative group (83 and 99% respectively, P<0.0001). DSS of hormone receptor negative breast cancers was 91%, whereas DSS of hormone receptor positive breast cancer was 97% (P<0.01). The pN positive group showed poorer prognoses than the pN negative group (89 and 98% respectively, P<0.0001). DSS of the serum CEA positive group was worse compared with that of the negative group (83 and 97% respectively, P=0.0001).

Intersection of clinical and pathological lymph node metastasis. Fig. 1A shows differences in RFS and DSS based on the intersection of cN and pN factors. The RFS and DSS of the cN positive/pN positive group were 65 and 81%, respectively. This group was significantly worse off than the other groups.

Fig. 1B shows the prognosis of hormone receptor positive/HER2 negative according to intersection of cN and pN. The cN positive/pN positive group showed poorer prognoses than the other groups in hormone receptor positive/HER2 negative types (RFS P<0.0001/DSS P<0.0001). Lymph node metastasis was not associated with recurrence and was not a prognostic factor in the other subtype groups.

Kaplan-Meier curve of RFS and DSS by serum value of CEA before cancer therapy. In hormone receptor positive/HER2 negative breast cancers, RFS and DSS for the serum CEA positive group were significantly worse compared with the CEA negative group (RFS P<0.05, DSS P<0.0001) (Fig. 2A).

In the cN positive/pN positive group, the RFS of CEA positive cases was 52% and for CEA negative cases was 66% (P<0.05). The DSS of CEA positive cases was 43% and CEA negative cases was 90% (P<0.001) (Fig. 2B).

The RFS of CEA positive cases was 50% and that of CEA negative cases was 84% (P<0.05) in the validation group. There was no significant difference in DSS (P=0.26) (Fig. 2C).

Discussion

For the present research, we clarified breast cancer treatment according to hormone receptor expression and HER2 expression. We reported treatment outcomes compared with 1995-1996 patients (15). According to results reported by Nishimiya *et al* (15), recurrence rates for breast cancer in 1995-1996 was 31.2%. Mortality rate was 24.5%. These numbers were 10.4 and 4% respectively in recent years. For hormone receptor positive breast cancers, RFS and DSS were 68.42 and 83.05% respectively in the 1990s. In the 2000s, these numbers were 89 and 97%. These numbers were 60.20 and 64.88% respectively for hormone receptor negative breast cancer. In the 2000s, these numbers were 71 and 91%. For HER2 positive breast cancer, RFS and DSS were 61.17 and 63.16% in the 1990s, and 83 and 94% in the 2000s respectively. For HER2 negative breast cancers, these numbers were 67.40 and 81.26% and 85 and 96% respectively. Prognoses

Table I. Clinicopathologic characteristics of the 662 patients.

Factors	No.	%
Patient	662	100.0
Age (median)	56 (24-93)	
Sex		
Female	657	99.3
Male	5	0.7
Neo adjuvant therapy		
Yes	75	11.3
No	587	88.7
cT[a]		
T0	104	15.7
T1	280	42.3
T2	215	32.5
T3	32	4.8
T4	31	4.7
cN		
N0	530	80.1
N1	91	13.7
N2	32	4.8
N3	9	1.4
cStage		
0	104	15.5
I	255	38.5
II	228	34.7
III	75	11.3
Serum CEA		
5>	607	91.7
5≤	55	8.3
Surgical method		
Lumpectomy	450	68.1
Mastectomy	212	32.0
Lymphadenectomy		
Sentinel lymphnode biopsy	360	54.4
Axillary lymphadenectomy	267	40.3
Not performed	35	5.3
Pathological type		
Ductal carcinoma *in situ*	106	16.0
Lobular carcinoma *in situ*	2	0.3
Invasive ductal carcinoma	500	75.5
Invasive lobular carcinoma	54	8.2
Nuclear grade		
Grade 1	205	31.0
Grade 2	98	14.8
Grade 3	176	26.6
Unknown	183	27.6
pT		
T0	10	1.5
Tis	108	16.3
T1	295	44.6
T2	189	28.6
T3	28	4.2
T4	32	4.8
pN		
pN0	451	68.1
pN1	138	20.8
pN2	39	5.9
pN3	22	3.3
Unknown	12	1.8
pStage		
0	116	17.5
I	225	34.0
II	227	34.3
III	94	14.2
HR (IHC)		
Positive	522	78.9
Negative	140	21.1
HER 2 (IHC and/or FISH)		
Positive	67	10.2
Negative	593	89.6
Unknown	2	0.2
Subtype		
$HR^+/HER2^-$	503	76.0
$HR^+/HER2^+$	19	2.9
$HR^-/HER2^+$	48	7.3
$HR^-/HER2^-$	92	13.8
Postoperative adjuvant therapy		
Yes	597	90.2
No	65	9.8
Recurrence		
Yes	69	10.4
No	593	89.6
Succumbed		
Yes	26	4.0
No	636	96.0

[a]7th edition of the Union for International Cancer Control. IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization; HER2, human epidermal growth factor receptor 2; CEA, carcinoembryonic antigen; HR, hormone receptor.

have improved in all subtypes. In the 2000s, it became clear that prognosis was improving dramatically due to more personalized medical treatment for breast cancer. All in all, it is believed that the overall prognosis of breast cancer has improved with diversification of hormonal therapies and chemotherapy along with the advent of molecular targeted therapeutic agents (16-23). Also in Japan, the prognosis for recurrent breast cancer has improved in the 2000s (24-26).

There is a limitation in this research. Ki-67 subtype was not analyzed yet. Ki-67 is a biomarker used for subtype classification in hormone receptor positive breast cancers. It has been drawing attention as a marker for possibility of recurrence (14,15). Ki-67 cut-off values vary depending on

Table II. Univariate and multivariate analysis for recurrence free survival (RFS).

	RFS					
	Univariate analysis			Multivariate analysis		
Factors	Patient number	RFS (%)	P-value	HR	95% CI	P-value
Age, years			0.87			
>56	322	86				
<56	340	80				
Sex			0.34			
Female	657	84				
Male	5	100				
CEA			<0.05			NS
5>	607	85		1.79	0.86-3.37	
5≤	55	81				
cT			<0.001			NS
T0-1	384	90		1.14	0.41-2.90	
T2-4	278	77				
cN			<0.0001			<0.01
Negative	530	88		2.28	1.26-4.28	
Positive	132	70				
cStage (7th UICC)			<0.0001			
0	102	79				
I	255	91				
II	230	81				
III	75	71				
Neo adjuvant therapy			<0.0001			
No	587	86				
Yes	75	72				
Surgical method			<0.001			
Lumpectomy	450	91				
Mastectomy	212	73				
Pathological type			0.1			
DCIS	106	74				
LCIS	2	100				
IDC	500	86				
ILC	54	45				
Hormone receptor			<0.001			<0.05
Negative	140	71		1.83	1.08-3.01	
Positive	522	89				
HER2 receptor			0.08			
Positive	67	83				
Negative	593	85				
Subtype						
$HR^+/HER2^-$	503	89	0.01			
$HR^+/HER2^+$	19	83				
$HR^-/HER2^+$	48	83				
$HR^-/HER2^-$	92	68				
pT			<0.01			NS
T0-1	413	89		1.02	0.42-2.79	
T2-4	249	77				
pN			<0.0001			0.001
Negative	451	92		2.85	1.52-5.30	
Positive	199	68				

Table II. Continued.

	RFS					
	Univariate analysis			Multivariate analysis		
Factors	Patient number	RFS (%)	P-value	HR	95%CI	P-value
pStage (7th UICC)			<0.0001			
0	116	84				
I	225	90				
II	227	92				
III	94	41				
Adjuvant therapy			0.2			
No	65	94				
Yes	597	83				

HR, hazard ratio; CI, confidence interval; NS, not significant; DCIS, ductal carcinoma *in situ*; LCIS, lobular carcinoma *in situ*; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; HR, hormone recepto; HER2, human epidermal growth factor receptor 2; UICC, Union for International Cancer Contro; CEA, carcinoembryonic antigen.

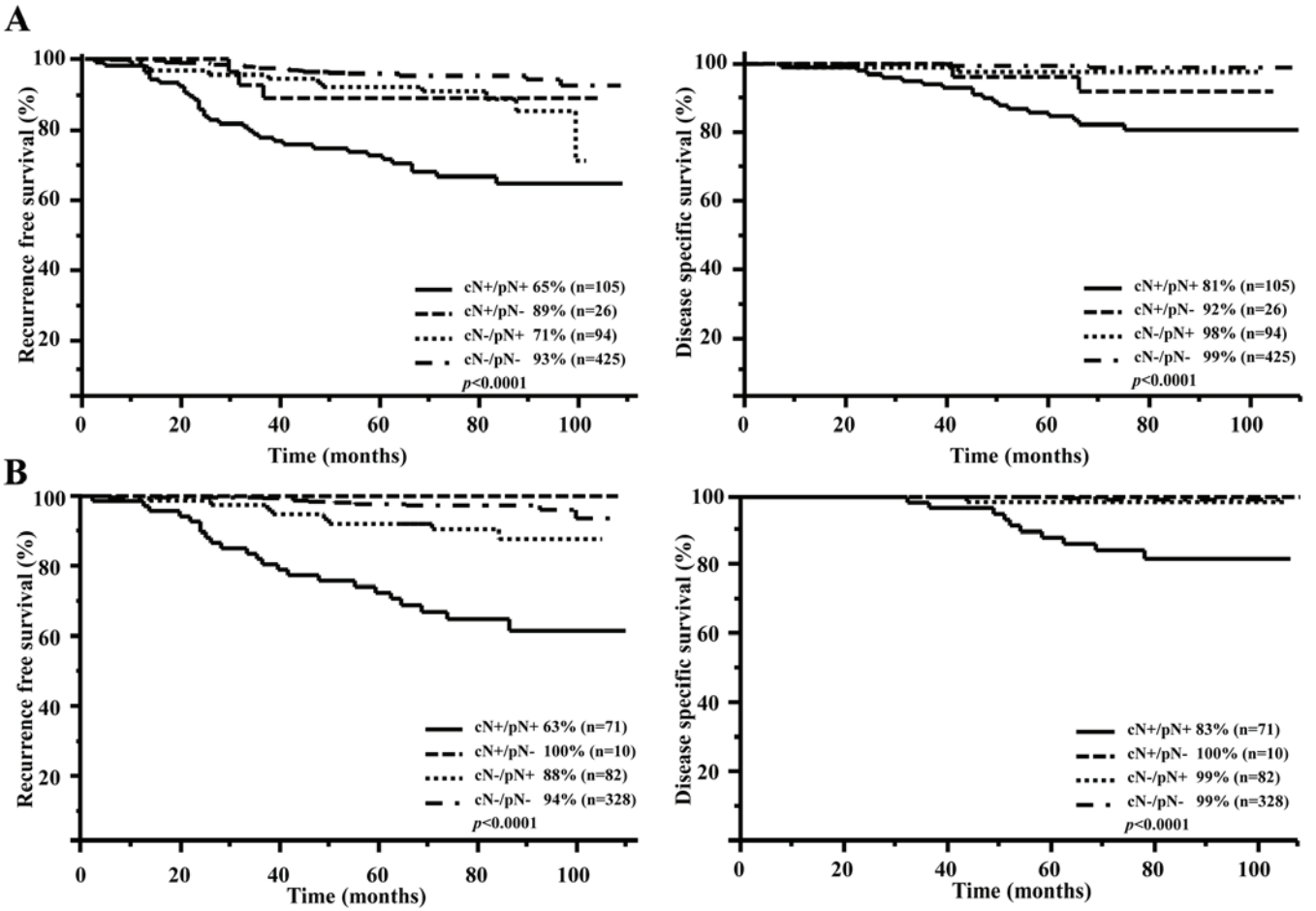

Figure 1. Kaplan-Meier curve of RFS and DSS by cN/pN status. (A) The RFS and DSS of cN positive and pN positive cases were significantly worse than those of the other groups. (B) cN positive/pN positive group showed poorer prognosis than the other groups in hormone receptor positive/HER2 negative type.

lab factors including antibodies, time, staining methods, and methods for scoring. We believe that analysis according to Ki-67 will be necessary in the future.

Within the hormone receptor positive/HER2 negative group, the cN negative group had a good prognosis. In these cases, adjuvant hormone therapy was administered for

Table III. Univariate and multivariate analysis for disease specific survival (DSS).

	DSS					
	Univariate analysis			Multivariate analysis		
Factors	Patient number	DSS (%)	P-value	HR	95% CI	P-value
Age, years			0.66			
>56	322	95				
<56	340	96				
Sex			0.57			
Female	657	96				
Male	5	100				
CEA			<0.01			<0.05
5>	607	97		3.04	1.17-7.03	
5≤	55	82				
cT			<0.001			NS
T0-1	384	98		2.31	0.53-9.45	
T2-4	278	92				
cN			<0.0001			<0.01
Negative	530	99		4.55	1.64-15.18	
Positive	132	83				
cStage (7th UICC)			<0.0001			
0	102	100				
I	255	99				
II	230	93				
III	75	87				
Neo adjuvant therapy			<0.001			
No	587	97				
Yes	75	86				
Surgical method			0.01			
Lumpectomy	450	97				
Mastectomy	212	92				
Pathological type			0.33			
DCIS	106	100				
LCIS	2	100				
IDC	500	95				
ILC	54	96				
Hormone receptor			<0.01			<0.05
Negative	140	91		2.32	1.02-5.17	
Positive	522	97				
HER2 receptor			0.37			
Positive	67	94				
Negative	593	96				
Subtype			<0.05			
HR+/HER2$^-$	503	97				
HR$^+$/HER2$^+$	19	94				
HR-/HER2$^+$	48	93				
HR-/HER2$^-$	92	89				
pT			0.01			NS
T0-1	413	97		1.51	0.38-5.01	
T2-4	249	93				
pN			<0.0001			<0.05
Negative	451	98		3.40	1.08-11.17	
Positive	198	89				

Table III. Continued.

	DSS					
	Univariate analysis			Multivariate analysis		
Factors	Patient number	DSS (%)	P-value	HR	95% CI	P-value
pStage (7th UICC)			<0.0001			
0	116	100				
I	225	97				
II	227	98				
III	94	82				
Adjuvant therapy			0.01			
No	65	93				
Yes	597	96				

HR, hazard ratio; CI, confidence interval; NS, not significant; DCIS, ductal carcinoma *in situ*; LCIS, lobular carcinoma *in situ*; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; HR, hormone recepto; HER2, human epidermal growth factor receptor 2; UICC, Union for International Cancer Control; CEA, carcinoembryonic antigen.

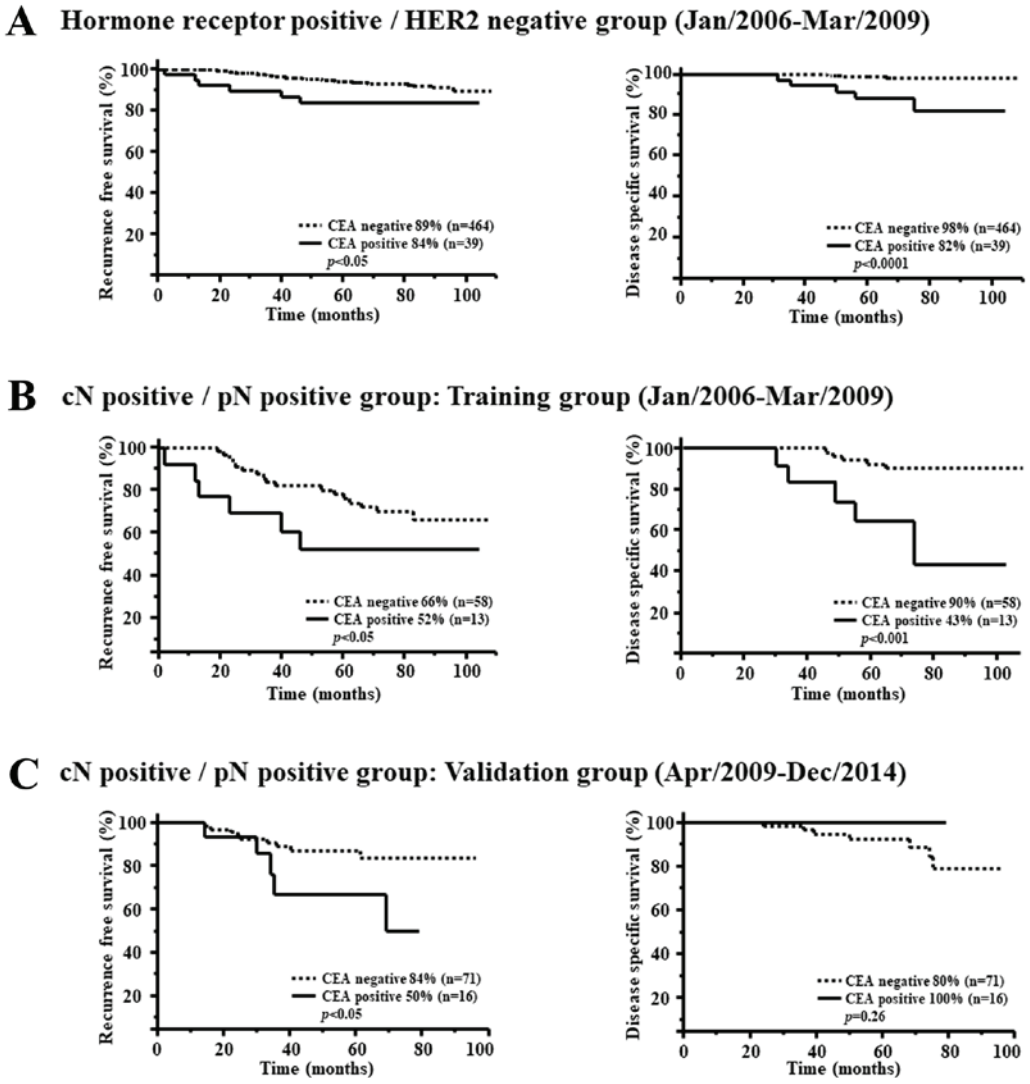

Figure 2. Kaplan-Meier curve of RFS and DSS by serum CEA values before cancer therapy. (A) The RFS and DSS of serum CEA values before cancer therapy positive group were significantly worse compared with the CEA negative group in hormone receptor positive/HER2 negative breast cancers. (B) In the cN positive/pN positive group, the RFS and DSS of CEA positive cases were worse than in the CEA negative group. (C) In the validation group, the RFS of CEA positive cases was worse than in the CEA negative group.

5 years. Recently, studies such as ATLAS (27), attom (28), and MA17R (29) demonstrated the effectiveness of 10 years of hormone therapy. Nevertheless, there is no research determining the particular circumstances under which hormone therapy should be administered. In the present study, in multivariate analysis, factors relating to relapse included cN, pN, and the presence or absence of hormone receptor. Factors relating to prognosis were cN, pN, the presence or absence of hormone receptor, and pretreatment serum CEA values. The analysis of the Intersection of cN and pN was related to poor prognosis. Within the hormone receptor positive/HER2 negative group, the prognosis for the cN positive/pN positive group was particularly poor. Additionally, within the cN positive/pN positive group in the hormone receptor positive/HER2 negative group, there was a poor prognosis for those with high pretreatment CEA levels (Fig. 2B). Serum CEA is a tumor marker used for early detection and monitoring for recurrence of breast cancer, and for evaluating progress of therapy in patients with progressive, recurrent breast cancer in the clinical setting (30-35). Within the 87 cases of the validation group, there was a significant rate of recurrence in those with high serum CEA levels compared with those with low levels of CEA ($P<0.05$) (Fig. 2C). Nevertheless, future surveillance is necessary, since the follow-up period is insufficient. That is to say, in the cN positive/pN positive group, contemporary treatments were inadequate. We believe it is an urgent priority to develop stronger treatment algorithms or to develop new treatments that exceed the current standard. Taking these results into consideration, our facility plans to administer hormone therapy for 10 years to those patients with high serum CEA levels and cN positive/pN positive breast cancer. We will subsequently study the therapeutic effects and side effects such as osteoporosis of prolonged hormone therapy in this population.

Although the number of cases was small, the prognosis was the same in the cN positive/pN negative, and cN negative/pN negative groups. In these 10 cases, pretreatment imaging showed axillary lymph node enlargement of 10 mm or more. Eight out of the 10 cases received neoadjuvant chemotherapy, and these became pN negative (ypN0) after surgery. von Minckwitz *et al* (36) report a good prognosis for patients with ypN0 induced by neoadjuvant chemotherapy. In the hormone receptor positive/HER2 negative/high Ki-67 expression group (Luminal B), the recurrence rate for pathological complete response (pCR) cases was low (36). In the NSABP B18 trial, good prognosis was reported for pCR cases (37,38). There are additional reports of good prognosis in pCR cases with neoadjuvant chemotherapy compared with other cases (39,40). That is to say, aggressive neoadjuvant chemotherapy should be performed for hormone receptor positive/HER2 negative cases that are cN positive and with high levels of Ki-67. It is considered possible to selectively induce good prognosis via neoadjuvant chemotherapy.

The cN positive/pN positive group had a poor overall prognosis. In hormone receptor positive/HER2 negative breast cancer, nodal metastasis was found to be a strong factor relating to the prognosis of recurrence. Furthermore, cases with elevated serum CEA had an especially poor prognosis; therefore the prolongation of hormone therapy is necessary.

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Not applicable.

Conflicts of interest

None.

Funding

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YK and NM performed the statistical analysis and wrote the manuscript. YT, AS, MK, HN, MiW and HK participated in the interpretation of data and the critical review of the manuscript. TS, NS, HT, KY and MaW gave final approval of the version to be published, and made substantial contributions to the conception and design of the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Kitasato University and written informed consents were obtained from the patients for publication of this study.

Patient consent for publication

Written informed consents were obtained from the patients for publication of this study.

Competing interests

The authors declare that they have no competing interests.

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