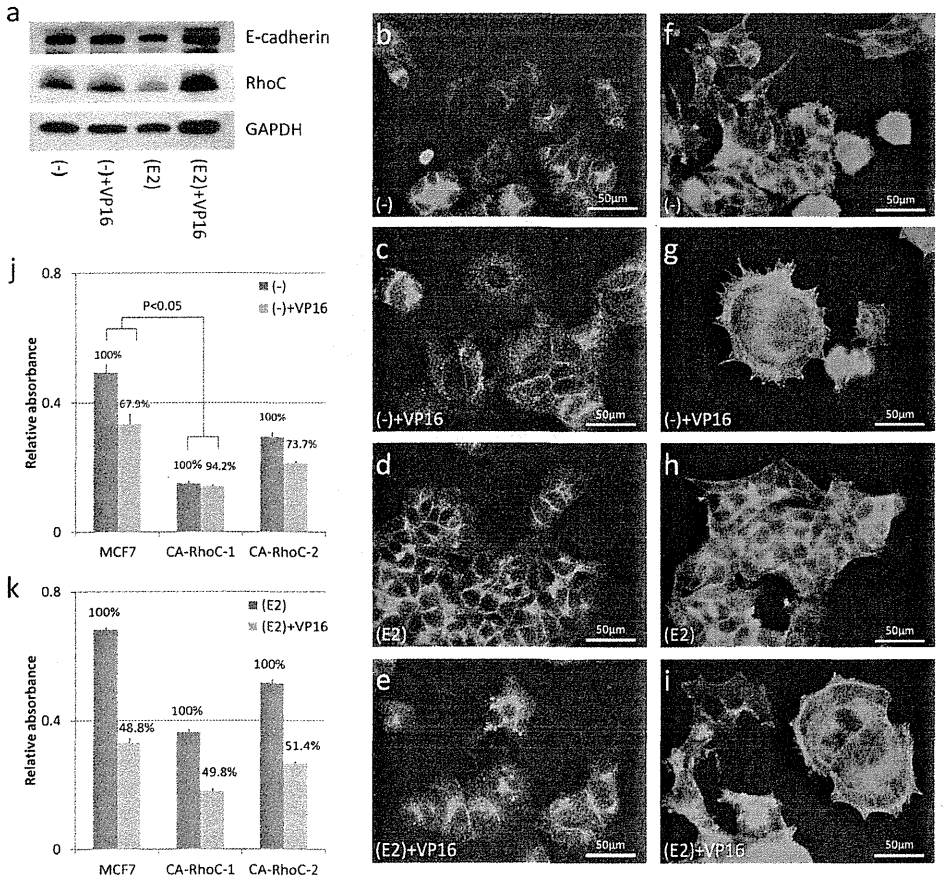


**Fig. 5** Effects of etoposide treatment in MCF-7 cells. Western blots (**a**). MCF-7 cells ($1.2\times10^6$/dish) were plated in a phenol-red-free RPMI 1640 medium supplemented with 2 % FBS treated with dextran-coated charcoal (dcc) in the absence of E2 and insulin. The cells were further cultured for 5 days in a phenol-red-free RPMI 1640 medium containing 2 % dcc-treated FBS in the absence or presence of 100 nM E2 and/or 10 μM of etoposide, with medium changes every other days. Cell extracts were prepared, and Western blots were similarly performed using anti-RhoC and anti-E-cadherin antibodies. The *bottom panel* shows the loading controls of GAPDH. Immunocytochemistry of E-cadherin (**b**, **c**, **d**, **e**) and phalloidin staining (**f**, **g**, **h**, **i**). MCF-7 cells were similarly plated and culture onto a 4-well chamber slide. After culture for 5 days, cells were fixed with 4 % paraformaldehyde. The chamber slides were either stained with rhodamine-labeled phalloidin or reacted with the anti-E-cadherin antibody and then with an FITC-conjugated secondary antibody. The slide was observed with a fluorescence microsocpe. MTT assay (**j**, **k**). Parental (MCF) and transfectant (CA-RhoC-1, -2) MCF-7 cells ($6\times10^3$/well) were plated onto a 96-well plate in a phenol-red-free RPMI 1640 medium, supplemented with 2 % dcc-treated FBS. The next day, the cells were either left untreated or cultured with 10 μM etoposide (VP16) in the absence (−) or presence of 100 nM E2 (E2). The medium was changed every other day. MTT assays were performed after incubation for 4 more days. Percentages compared to the values of untreated cells are also shown. The other trial gave a similar result

of apoptosis is minimal under this dose of etoposide treatment. Taken in sum, RhoC may not directly contribute to chemotherapy resistance in MCF-7 cells.

## Discussion

As long as we know, this is the first report to compare various EMT-related molecules in human breast cancer specimens of the same patient before and after chemotherapy. Through this comparison, we demonstrated a reduction of E-cadherin and an upregulation of fibronectin in clinical breast specimens resected after chemotherapy. In earlier studies, BMI1 and Snail and Twist homologs were found to take part in the EMT-mediated therapy resistance of tumor cells [23–28]. Unexpectedly, no evidence of SNAIL1, SNAIL2, TWIST1, or BMI1 upregulation was observed in the post-chemotherapy specimens examined in the present study. Interestingly, significantly more upregulation of RhoC was found in the specimens resected after chemotherapy versus the specimens biopsied before. Human breast cancer MCF-7 cells stably transfected with the CA-RhoC plasmid exhibited an EMT-like phenotype characterizing by reduced membranous E-cadherin expression and disorganization of actin fibers. In addition, RhoC was also upregulated in the similar EMT-like

process under etoposide treatment in MCF-7 cells. Taken in sum, RhoC may be a key regulator in the EMT-like process in human breast cancer during chemotherapy. Notably, an earlier study showed that RhoA silencing reverts the resistance to doxorubicin in human colon cancer [29]. In another study, RhoC overexpression was found to be independently predictive of poor response to doxorubicin-based chemotherapy [22]. These findings tempt us to speculate that RhoC is involved in cell survival during chemotherapy in clinical breast cancer. The present study, however, failed to demonstrate significant chemotherapy resistance in CA-RhoC-expressing MCF-7 cells in MTT assay. It is likely that the involvement of the EMT-core regulators or BMI1 might be required for chemotherapy resistance through EMT. To explore this issue, we are now under investigation of cell survival signaling activated by EMT in clinical colon cancer specimens and cultured colon cancer cell lines.

In conclusion, the present study showed that RhoC is more upregulated in breast cancer specimens after chemotherapy than before. Further, CA-RhoC-expressing MCF-7 cells had the potential to reduce the membranous expression of E-cadherin. RhoC may be an attractive molecule to prevent the EMT process.

**Acknowledgments** We are grateful to A. Fukushima, S. Itoh, M. Tamura, J. Kosegawa, M. Yamamoto, K. Honmou, S. Sato, M. Kikuchi, M. Hoshino, and Y. Haga at Jichi Medical University Hospital for their technical assistance. This study was supported in part by Grant-in-Aid of Young Scientists (B) of the Ministry of Education, Culture, Sports, Science and Technology (KAKENHI 26860244). This study was also supported in part by the Jichi Medical University Young Investigator Award.

**Conflict of Interest** The authors declare no conflicts of interest.

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Breast Cancer Res Treat (2014) 147:103–112
DOI 10.1007/s10549-014-3090-8

CLINICAL TRIAL

# Treatment outcomes and prognostic factors for patients with brain metastases from breast cancer of each subtype: a multicenter retrospective analysis

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Received: 9 May 2014 / Accepted: 29 July 2014 / Published online: 9 August 2014


**Abstract** To define prognostic factors for breast cancer patients with brain metastases, compare their clinical courses and prognoses according to breast cancer subtypes, and analyze the causes of death in such patients. We retrospectively analyzed 1,466 patients diagnosed with brain metastases between April 1, 2001 and December 31, 2012, from 24 institutions of the Japan Clinical Oncology Group. Overall, 1,256 patients with brain metastases were included. The median overall survival (OS) was 8.7 months (95 % confidence interval [CI] 7.8–9.6 months). Univariate and multivariate analyses revealed that patients diagnosed with brain metastasis within 6 months of metastatic breast cancer diagnoses, asymptomatic brain disease, or HER2-positive/estrogen receptor-positive tumors had increased OS. Median OS after the development of brain metastases was 9.3 months (95 % CI 7.2–11.3) for the luminal type, 16.5 months (95 % CI 11.9–21.1) for the luminal-HER2 type, 11.5 months (95 % CI 9.1–13.8) for the HER2 type, and 4.9 months (95 % CI 3.9–5.9) for the triple-negative type. Luminal-HER2 type patients had significantly longer OS than patients with the luminal type (hazard ratio [HR] = 1.50, $P < 0.0001$) and triple-negative type (HR = 1.97, $P < 0.0001$); no significant differences were noted compared to HER2-type patients (HR = 1.19, $P = 0.117$). The prognosis and clinical course of patients with brain metastasis from breast cancer before and after developing brain metastases vary according to subtype. Focusing on the subtypes of breast cancer can optimize the

**Electronic supplementary material** The online version of this article (doi:10.1007/s10549-014-3090-8) contains supplementary material, which is available to authorized users.

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prevention, early detection, and improved treatment of brain metastases.

**Keywords** Breast cancer · Brain metastases · Retrospective analysis · Subtype

## Introduction

Brain metastasis—an increasingly problematic issue in the management of breast cancer—is associated with impaired quality of life because of progressive neurological impairments [1]. Although brain metastases are less common than bone or visceral metastases in patients with breast cancer, they are associated with considerably poorer prognosis and are less responsive to systemic therapies than other metastases. Recently, a trend of increased incidence of brain metastases has been noted [2, 3]. The reasons for this increased incidence include the more frequent use of sensitive detection methods such as contrast-enhanced magnetic resonance imaging (MRI), increased awareness among patients and clinicians, and improvements in systemic therapies that prolong survival [4–7]. Another possible reason is that adjuvant and systemic therapy with a drug having low penetrance through the blood–brain barrier (BBB), such as trastuzumab, may—while decreasing the risk of distant metastases in general and prolonging overall survival—lead to an increased risk of brain metastases in breast cancer patients [8, 9]. A meta-analysis revealed adjuvant trastuzumab therapy to be associated with a significantly increased risk of the central nervous system (CNS) being the site of first recurrence in HER2-positive breast cancer patients [9]. However, the HERA trial showed no increased risk of CNS relapses with adjuvant trastuzumab therapy [7]. In patients with metastatic HER2-positive breast cancer receiving front-line trastuzumab-based therapeutic regimens, a high incidence of CNS metastases—ranging from 28 to 43 %—was reported [10]; these were considerably higher than those reported historically. Brufsky et al. reported CNS metastases in 377 (37.3 %) of 1,012 patients with confirmed HER2-positive tumors [4].

CNS metastases generally tend to occur late in the course of metastatic breast cancer (MBC) and are associated with 1- and 2-year survival rates of only 20 % and <2 %, respectively [11, 12], with most patients dying of systemic disease progression [13]. The median survival time for patients with breast cancer with untreated brain metastasis is 4 weeks; this can be increased up to 4–6 months with whole-brain radiotherapy and stereotactic radiosurgery or up to 16 months if solitary metastases can be removed surgically [14, 15]. Dawood et al. reported that patients with brain metastases have different survival times depending on HER2 status [6], and anti-HER2 treatment after brain metastases has been associated with a survival benefit [6, 16–18].

Because an intact BBB prevents most chemotherapeutic agents from entering the CNS, chemotherapy is not used routinely to treat CNS metastases [4]. Although surgery can be an effective treatment option for patients with a limited CNS disease burden [19], steroids, and radiotherapy remain the mainstay of the treatment of CNS metastases [20, 21]. However, in patients with fewer lesions, stereotactic radiosurgery is associated with longer survival [20].

The classification of breast cancer has been evolving continuously; it now encompasses a group of heterogeneous, genomically defined disease subsets [22]. Breast cancer subtypes are known to affect the clinical course and prognosis of not only primary breast cancer [22–24] but also of MBC [25]. Sperduto et al. demonstrated that the tumor subtype is an important prognostic factor for survival in patients with breast cancer brain metastases [26, 27]. With regard to the clinical course of patients with brain metastases from breast cancer, limited information is available for specific breast cancer subtypes. Therefore, our study aimed to define the prognostic factors in breast cancer patients with brain metastases and estimate their prognostic impact based on estrogen receptor (ER) and

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HER2 status. Further, we also analyzed the causes of death in breast cancer patients with brain metastases using a large multicenter retrospective dataset.

## Methods

### Participants

We retrospectively identified patients diagnosed with brain metastasis as the first recurrence of breast cancer during follow-up as well as those who developed brain metastases during systemic treatment for metastatic disease. Patients diagnosed with brain metastases between April 1, 2001 and December 31, 2012, at each participating institution were included in this analysis. The presence of brain metastases was defined on the basis of appropriate imaging and/or histological findings. This study was planned by the breast division of the Japan Clinical Oncology Group that includes 34 clinical institutions in Japan. For this study, 24 institutions provided datasets of patients with brain metastases without identifying the individuals. The study was coordinated by the Tokai University School of Medicine. It was approved by the institutional review board of the Tokai University School of Medicine, which waived the need for written informed consent because of the retrospective nature of the study.

We collected data for 1,466 patients diagnosed with brain metastases from breast cancer. Of the 1,466 patients with brain metastases, 210 were excluded because (i) they were not diagnosed in the inclusion period (April 2001–December 2012) of this study ($n = 166$) or (ii) no survival data were available in this dataset ($n = 44$). Thus, 1,256 patients were evaluated in this analysis.

### Staging and pathology review

Metastatic brain disease was confirmed by histopathological analysis if specimens were available. Primary tumors were histologically classified using the World Health Organization criteria [28]. A patient was considered to have HER2-positive disease if the primary tumor or a metastatic tumor had a score of 3+ on HER2 immunohistochemical analysis or if fluorescence in situ hybridization revealed amplification of the *HER2* gene. A patient was considered to have hormone receptor (HR)-positive disease if at least 1 % of the tumor cells stained positive for ER or progesterone receptor on immunohistochemical analysis. Histological grading was assessed using the Nottingham grading system [29].

### Definition of breast cancer subtypes

Based on immunohistochemical staining findings, we defined breast cancer subtypes as follows: "luminal type," ER-positive and HER2-negative tumors; "luminal-HER2 type," ER-positive and HER2-positive tumors; "HER2 type," ER-negative and HER2-positive tumors; and "triple-negative type," ER-negative and HER2-negative tumors.

### Definition of death due to brain metastases

According to information from patient charts, we classified the causes of death in patients with brain metastases as follows: (i) "Certainly/Definitely related" if the cause of death was related to the brain metastases, for example, altered consciousness or coma, (ii) "Probably related" for patients who discontinued systemic treatment because of brain metastases, (iii) "Possibly related" for patients who changed systemic treatment because of brain metastases to a less toxic treatment, and (iv) "Not related" if the cause of death was systemic disease without any symptoms of brain metastases.

### Statistical methods

Median values and standard deviations were used to summarize age at diagnosis. Frequencies and proportions were used to present categorical clinical characteristics. Brain metastasis-free survival (BMFS) was defined as the time interval from the diagnosis of primary breast cancer until the diagnosis of brain metastasis or the last follow-up date, whichever occurred first. Data on patients who remained alive without progression of brain metastases at the last follow-up were censored in the BMFS analyses. Overall survival (OS) was defined as the length of time from diagnosis of brain metastases to death or to the last follow-up date if patients were alive at the last follow-up. Data on patients who were alive at the last follow-up were censored in the OS analyses. BMFS and OS were estimated by the Kaplan–Meier product-limit method. Kaplan–Meier curves were used to present BMFS and OS for patients in each group. Univariate and multivariate Cox proportional hazards regression models were used to assess the effects of treatment and other predictive factors. The analyses were performed using SPSS ver. 21 (SPSS Inc., Chicago, IL, USA). Two-sided $P$ values of $<0.05$ were considered statistically significant.

## Results

### Clinical characteristics

Overall, 1,256 patients diagnosed with brain metastasis from breast cancer were included in this study (Table 1). The median follow-up time was 50.6 months, and a total of 1,072 (86 %) patients were died. The median OS was

**Table 1** Patient characteristics

| | Total ($n$ = 1,256) | | Luminal ($n$ = 343) | | Luminal-HER2 ($n$ = 162) | | HER2 ($n$ = 270) | | Triple negative ($n$ = 337) | |
|---|---|---|---|---|---|---|---|---|---|---|
| ER | | | | | | | | | | |
| Positive | 554 | 44.1 % | 343 | 100 % | 162 | 100.0 % | 0 | 0.0 % | 0 | 0.0 % |
| Negative | 645 | 51.4 % | 0 | 0.0 % | 0 | 0.0 % | 270 | 100 % | 337 | 100 % |
| Unknown | 57 | 4.6 % | 0 | 0.0 % | 0 | 0.0 % | 0 | 0.0 % | 0 | 0.0 % |
| HER2 | | | | | | | | | | |
| Positive | 437 | 34.8 % | 0 | 0.0 % | 162 | 100.0 % | 270 | 100 % | 0 | 0.0 % |
| Negative | 688 | 54.8 % | 343 | 100 % | 0 | 0.0 % | 0 | 0.0 % | 337 | 100 % |
| Unknown | 131 | 10.4 % | 0 | 0.0 % | 0 | 0.0 % | 0 | 0.0 % | 0 | 0.0 % |
| Symptoms | | | | | | | | | | |
| Symptomatic | 932 | 74.2 % | 256 | 74.6 % | 121 | 74.7 % | 204 | 75.6 % | 252 | 74.8 % |
| Asymptomatic | 262 | 20.9 % | 72 | 21.0 % | 36 | 22.2 % | 53 | 19.6 % | 76 | 22.6 % |
| Unknown | 62 | 4.9 % | 15 | 4.4 % | 5 | 3.1 % | 13 | 4.8 % | 9 | 2.7 % |
| Number of brain metastases | | | | | | | | | | |
| 3 or less | 492 | 39.2 % | 133 | 38.8 % | 72 | 44.4 % | 118 | 43.7 % | 128 | 38.0 % |
| more than 3 | 663 | 52.8 % | 186 | 54.2 % | 84 | 51.9 % | 133 | 49.3 % | 192 | 57.0 % |
| Unknown | 101 | 8.0 % | 24 | 7.0 % | 6 | 3.7 % | 19 | 7.0 % | 17 | 5.0 % |
| Treatment for brain metastases | | | | | | | | | | |
| Operation | 186 | 14.8 % | 46 | 13.4 % | 19 | 11.7 % | 53 | 19.6 % | 47 | 13.9 % |
| STI | 291 | 23.2 % | 75 | 21.9 % | 52 | 32.1 % | 66 | 24.4 % | 67 | 19.9 % |
| WBI | 611 | 48.6 % | 168 | 49.0 % | 74 | 45.7 % | 132 | 48.9 % | 176 | 52.2 % |
| Others | 2 | 0.2 % | 0 | 0.0 % | 0 | 0.0 % | 0 | 0.0 % | 2 | 0.6 % |
| No treatment | 33 | 2.6 % | 12 | 3.5 % | 3 | 1.9 % | 2 | 0.7 % | 12 | 3.6 % |
| Unknown | 133 | 10.6 % | 42 | 12.2 % | 14 | 8.6 % | 17 | 6.3 % | 33 | 9.8 % |
| Time from relapse to developing brain metastases | | | | | | | | | | |
| Less than 6 months | 408 | 32.4 % | 86 | 25.1 % | 45 | 27.8 % | 85 | 31.5 % | 153 | 45.4 % |
| More than 6 months | 777 | 61.8 % | 238 | 69.4 % | 105 | 64.8 % | 170 | 63.0 % | 169 | 50.1 % |
| Unknown | 71 | 5.6 % | 19 | 5.5 % | 12 | 7.4 % | 15 | 5.6 % | 15 | 4.5 % |
| Histological grade | | | | | | | | | | |
| G1 | 81 | 6.4 % | 23 | 6.7 % | 15 | 9.3 % | 14 | 5.2 % | 22 | 6.5 % |
| G2 | 185 | 14.7% | 56 | 16.3 % | 40 | 24.7 % | 41 | 15.2 % | 37 | 11.0 % |
| G3 | 388 | 30.9% | 75 | 21.9 % | 34 | 21.0 % | 103 | 38.1 % | 149 | 44.2 % |
| Unknown | 602 | 47.9 % | 189 | 55.1 % | 73 | 45.1 % | 112 | 41.5 % | 129 | 38.3 % |

*STI* stereotactic radiotherapy, *WBI* whole-brain radiotherapy

**Table 2** Cox model for univariate and multivariate analyses

| | Univariate analysis | | | | Multivariate analysis | | | |
|---|---|---|---|---|---|---|---|---|
| | HR | 95.0 % CI | | $P$ | HR | 95.0 % CI | | $P$ |
| Time from relapse to developing brain metastases (more than 6 months/less than 6 months) | 0.810 | 0.710 | 0.920 | 0.002 | 0.819 | 0.708 | 0.947 | 0.007 |
| Symptoms of brain metastases (symptomatic/asymptomatic) | 0.810 | 0.690 | 0.940 | 0.006 | 0.850 | 0.720 | 1.004 | <0.0001 |
| Number of brain metastases (multiple/less than 3) | 0.590 | 0.520 | 0.670 | <0.0001 | 0.587 | 0.511 | 0.676 | 0.056 |
| HER2 (positive/negative) | 1.540 | 1.350 | 1.760 | <0.0001 | 1.666 | 1.441 | 1.925 | <0.0001 |
| ER (positive/negative) | 1.200 | 1.060 | 1.350 | 0.004 | 1.347 | 1.172 | 1.549 | <0.0001 |
| Histological grade (G1 and 2/G3) | 0.894 | 0.744 | 1.075 | 0.235 | | | | |

*HR* hazard ratio

8.7 months (95 % confidence interval [CI] 7.8–9.6 months). Of the 1,256 patients with brain metastases, 554 (44.1 %) patients had ER-positive primary tumors, while 645 (51.4 %) patients had ER-negative tumors. Further, 437 (34.8 %) patients had HER2-positive primary tumors and 688 (54.8 %) patients had HER2-negative tumors. Of the patients with brain metastases, 74.2 % showed symptoms of CNS involvement, and 20.9 % had no such symptoms. In

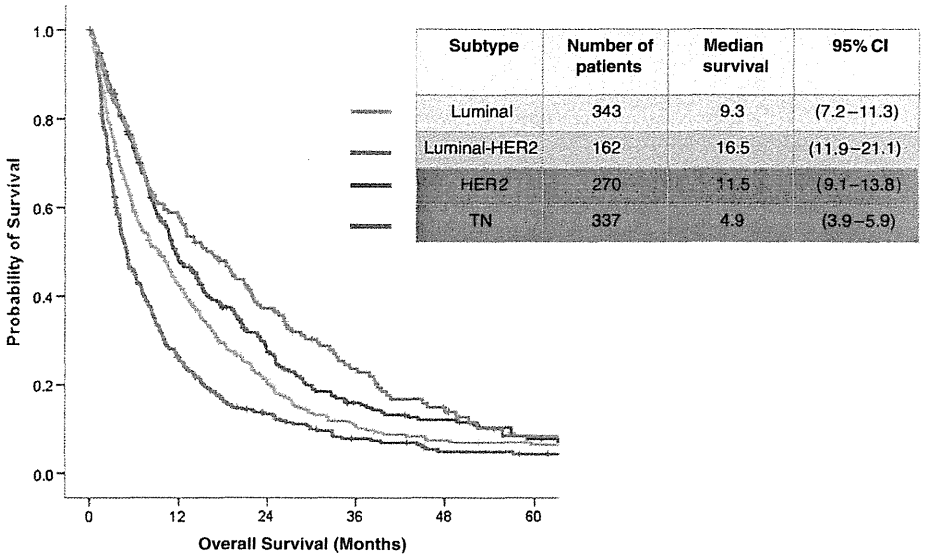



**Fig. 1** Overall survival after developing brain metastases according to breast cancer subtype. *CI* confidence interval

this dataset, the first treatment for brain metastases was surgery in 14.8 % patients, stereotactic radiotherapy in 23.2 %, and whole-brain radiation in 48.6 % patients.

## Prognostic factors

Table 2 shows the results of univariate and multivariate analyses of OS for various clinical factors among all the patients. The results revealed that patients diagnosed with brain metastases within 6 months from the diagnosis of other metastases, asymptomatic brain disease, or HER2/ER-positive tumors had longer OS than those diagnosed with brain metastases after 6 months from the diagnosis of other metastases, symptomatic brain disease, or HER2/ER-negative tumors. In multivariate analysis, patients diagnosed with <3 brain metastases did not show longer OS than patients with multiple brain metastases.

## Breast cancer subtypes and treatment outcomes

The clinical characteristics and Kaplan–Meier OS curves according to breast cancer subtype are shown in Table 1 and Fig. 1. The median survival for patients with brain metastases was 9.3 months (95 % CI 7.2–11.3) for the luminal type, 16.5 months (95 % CI 11.9–21.1) for the luminal-HER2 type, 11.5 months (95 % CI 9.1–13.8) for the HER2 type, and 4.9 months (95 % CI 3.9–5.9) for the triple-negative type. Patients with luminal-HER2-type tumors showed longer OS than patients with luminal-type tumors (hazard ratio (HR) = 0.66, $P < 0.0001$) and triple-negative-type tumors (HR = 0.50, $P < 0.0001$) but not as compared to patients with HER2-type tumors (HR = 0.84, $P = 0.117$). Patients with triple-negative-type tumors had poorer OS than patients with luminal-type tumors (HR = 1.37, $P < 0.0001$), luminal-HER2-type tumors (HR = 1.97, $P < 0.0001$), and HER2-type tumors (HR = 1.75, $P < 0.0001$) (Supplementary Table 1).

We next analyzed the time from the diagnosis of primary breast cancer to the development of brain metastases according to subtype (Fig. 2a). Patients with luminal-type tumors showed longer BMFS than patients with luminal-HER2-type tumors (HR = 0.61, $P < 0.0001$), HER2-type tumors (HR = 0.44, $P < 0.0001$), and triple-negative-type tumors (HR = 0.45, $P < 0.0001$). Among patients with HER2-positive tumors, the luminal-HER2 type was associated with longer BMFS than the HER2 type (HR = 0.69, $P < 0.0001$) (Supplementary Table 2).

Subsequently, we analyzed the time from the diagnosis of MBC to the development of brain metastases according to subtype (Fig. 2b). Brain metastases were diagnosed as the first metastatic lesion in 15 % of luminal-type patients, 16 % of luminal-HER2-type patients, 15 % of HER2-type patients, and 20 % of triple-negative-type patients (Fig. 3). The duration from MBC diagnosis until brain metastasis was longer in patients with luminal-type tumors than that in patients with luminal-HER2-type tumors (HR = 0.80, $P = 0.03$), HER2-type tumors (HR = 0.54, $P < 0.0001$), and triple-negative-type tumors (HR = 0.50, $P < 0.0001$). Further, among patients with HER2-positive tumors, the luminal-HER2 type was associated with a longer time from MBC diagnosis until brain metastasis as compared to the HER2 type (HR = 0.67, $P < 0.0001$) (Supplementary Table 3).

The lines of treatment at the time of progression of the first brain metastasis are shown in Fig. 3. The line of treatment takes into consideration the systemic treatment

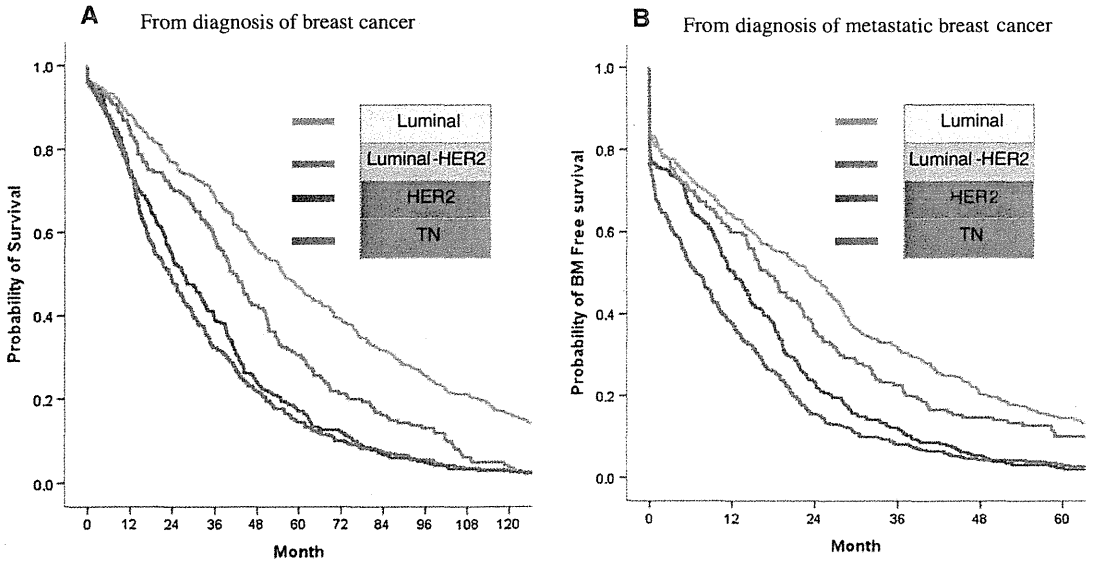



**Fig. 2** Time until brain metastasis according to tumor subtype. **a** Brain metastasis-free survival from the time of diagnosis of primary breast cancer to that of brain metastases according to breast cancer subtype. **b** Brain metastasis-free survival from the time of diagnosis of metastatic breast cancer to that of brain metastases according to breast cancer subtype. *TN* triple negative

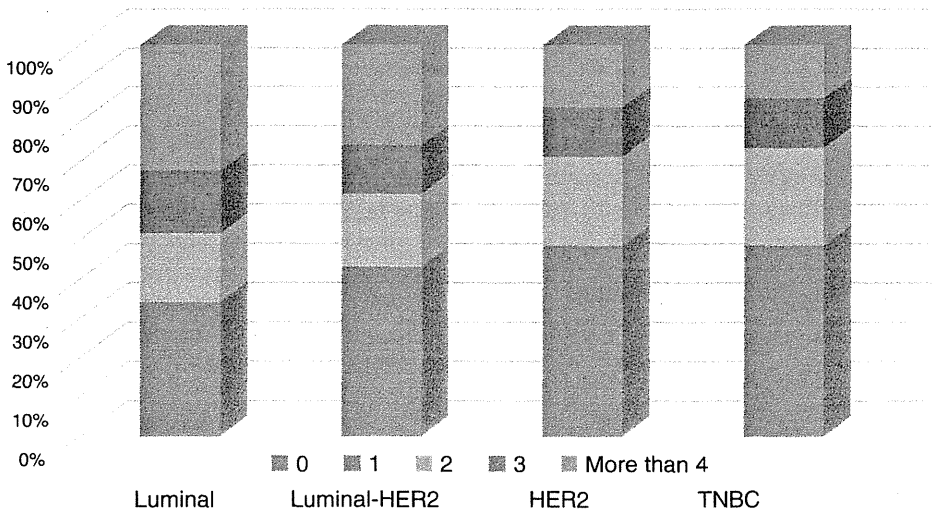
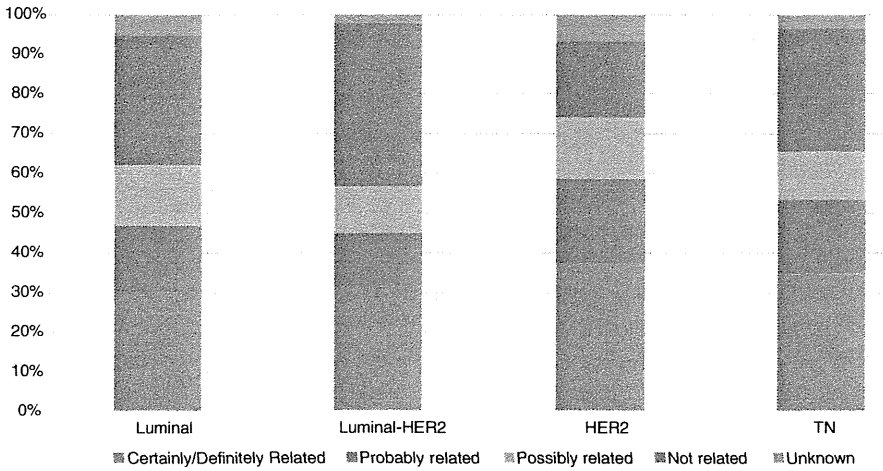



**Fig. 3** Lines of treatment at the time of progression of the first brain metastasis. The line of treatment takes into consideration the systemic treatment that patients received until any brain metastasis-related progressive disease events

that patients received until any brain metastasis-related progressive disease events. Of the 302 patients with luminal-type tumors who developed brain metastases during treatment for MBC, 58 (19 %) patients were receiving first-line therapy for MBC at the time of brain metastasis development; 52 (17 %), second-line therapy; 49 (16 %), third-line therapy; and 97 (32 %), subsequent lines of therapy (Fig. 3). Among the 220 patients with HER2-type MBC, 74 (33 %) patients were receiving first-line therapy for MBC at the time; 50 (22 %), second-line therapy; 28 (12 %), third-line therapy; and 35 (15 %), subsequent lines of therapy (Fig. 3). Of the 302 patients with triple-negative-type MBC, 85 (28 %) patients were receiving first-line therapy for MBC at the time; 75 (24 %), second-line therapy; 39 (12 %), third-line therapy; and 41 (13 %), subsequent lines of therapy (Fig. 3). Patients with luminal-type and luminal-HER2-type tumors tended to develop brain metastases later during treatment as compared to those with the other subtypes.

## Cause of death in patients with brain metastases

Figure 4 shows the causes of death in patients with brain metastases. In our dataset, more than 50 % of patients (695/1,256) with brain metastases died directly due to brain metastases. Among the 220 HER2 patients, the causes of




| Subtype | Certainly/Definitely related | Probably related | Possibly related | Not related | Unknown | Total |
|---|---|---|---|---|---|---|
| Luminal | 91 | 49 | 46 | 97 | 16 | 299 |
| Luminal-HER2 | 40 | 17 | 15 | 52 | 3 | 127 |
| HER2 | 82 | 47 | 34 | 42 | 15 | 220 |
| TN | 107 | 57 | 38 | 95 | 11 | 308 |

**Fig. 4** Causes of death in patients with brain metastases, with the number of patients for each cause of death. *TN* triple negative

death in relation to brain metastases were Certainly/Definitely related in 82 (37 %) patients, Probably related in 47 (21 %), and Possibly related in 34 (15 %); in only 42 (19 %) patients, the cause of death was not related to brain metastases. However, among the 127 patients with luminal-HER2-type tumors, the cause of death in 52 (40 %) patients was not related to brain metastases. These data suggested that the major cause of death in patients with HER2-type tumors was brain metastasis, which was more frequent than in patients with luminal-HER2-type tumors.

Treatment after the diagnosis of brain metastases

Of the 1,256 patients with brain metastases, 186 (14.8 %) underwent surgery as part of the treatment for brain metastases; 291 (23.2 %) received stereotactic radiotherapy, and 611 (48.6 %) underwent whole-brain radiotherapy as first-line treatment for brain metastases; and 33 (2.6 %) patients did not receive any of these treatments. Patients treated with surgery and stereotactic radiotherapy for brain metastases (median survival: 16.3 months) showed longer OS than those treated by whole-brain radiotherapy (median survival: 7.2 months) as first-line treatment (HR = 0.52; 95 % CI 0.45–0.59; $P < 0.0001$).

## Discussion

To the best of our knowledge, this is the largest study documenting treatment and patient outcomes in breast cancer patients with brain metastases. We found that patients with brain metastases in each subtype showed different prognoses, time from diagnosis of primary breast cancer to developing brain metastases, time from diagnosis of MBC to developing brain metastases, and causes of death. The favorable prognostic factors for survival in patients with brain metastases were as follows: diagnosis of brain metastases within 6 months of MBC diagnosis, asymptomatic brain disease, and HER2/ER-positive tumors.

Previous studies have shown that the median OS for patients with brain metastases from breast cancer following whole-brain radiotherapy and stereotactic radiosurgery is 4–6 months [14, 15]. Sperduto et al. demonstrated that various tumor subtypes are associated with different time intervals from primary diagnosis to the development of brain metastases as well as with varying survival after brain metastasis [26, 27]. Our data revealed a similar median OS (8.7 months); further, patients with various subtypes showed different median OS, i.e., 9.3 months for the luminal type, 16.5 months for the luminal-HER2 type, 11.5 months for the HER2 type, and 4.9 months for the triple-negative type. Patients with ER-positive tumors, such as luminal and luminal-HER2-type tumors, developed brain metastases later in the natural course of MBC than patients with ER-negative tumors. Moreover, patients with HER2-positive tumors, such as luminal-HER2- and HER2-type tumors, had longer OS than patients with HER2-negative tumors after developing brain metastases.

Potential prognostic factors for OS after developing brain metastases were similar to those previously noted for breast cancer patients, including HER2-positive status, Karnofsky Performance Scale score, tumor subtype, age, and early detection of brain metastases [4, 6, 26, 27, 30]. Our data showed that brain metastases diagnosed within

6 months of MBC diagnosis and asymptomatic brain disease were good prognostic factors for survival in these patients. Further, patients with HER2-positive disease had clearly longer OS than patients with HER2-negative disease; as a previous study demonstrated, trastuzumab controlled systemic metastases and prolonged survival [16–18] but did not control brain metastases. Screening for brain metastases by imaging studies is not routinely performed. A prospective trial evaluating screening for brain metastases showed that patients with symptomatic brain metastases had similar survival as patients with occult brain metastases [31]. However, our data revealed that the early detection of brain metastases was associated with longer OS as compared to symptomatic brain metastases. We consider that this discrepancy exists because the previous study was published 10 years ago, when systemic treatments (particularly anti-HER2 treatment), sensitive detection methods (such as contrast-enhanced MRI), and CNS-specific therapies (e.g., stereotactic radiotherapy and surgical resection for smaller, solitary lesions) were not yet developed. Our data demonstrated that ER-negative patients had shorter survival after developing brain metastases as well as a shorter time until the development of brain metastases from diagnosed MBC. Randomized trials using imaging studies might, therefore, be necessary in ER-negative patients with MBC who are at high risk for brain metastases.

CNS metastases tend to occur late in the course of MBC and are associated with 1- and 2-year survival rates of only 20 and <2 %, respectively [11, 12], with most patients dying of systemic disease progression [13]. However, our findings demonstrated that more than 50 % of patients died due to the symptoms of brain metastases. These results indicate that controlling the symptoms of brain metastases would improve OS because CNS-specific therapies are administered early in the course of treatment (e.g., stereotactic radiotherapy or surgical resection for smaller, solitary lesions). Further, such improved OS after the development of brain metastases may lead to the improved OS of MBC patients.

Our findings demonstrated that over 50 % of our patients died due to the symptoms of brain metastases. These results indicate that controlling the symptoms of brain metastases would improve OS since CNS-specific therapies are administered early in the course of treatment. Further, such improved OS after the development of brain metastases may lead to the improved OS of MBC patients.

Our study has certain limitations. First, as a retrospective evaluation of data collected from a dataset, this study suffers from biases associated with any retrospective study, such as an inherent selection bias. Second, tissue processing before immunohistochemical analyses was conducted in different laboratories, which may have led to variations in the results. Third, our study included patients who received different treatments for brain metastases and differing systemic treatment in multiple institutions, which may have affected the overall outcomes.

## Conclusion

Despite the inherent limitations associated with a retrospective study, our results are important for several reasons. Using the largest dataset of patients with brain metastases from multiple institutions, we evaluated patient outcomes and prognostic factors in breast cancer patients with brain metastases. Different subtypes showed different prognoses and clinical courses before and after developing brain metastases. Favorable prognostic factors for survival in patients with brain metastases included the early detection of brain metastases, asymptomatic brain disease, and HER2/ER-positive status. Future research should focus on each separate subtype of breast cancer in order to optimize the prevention, early detection, and improved treatment of patients with brain metastases.

**Acknowledgments** We would like to thank the following individuals for providing us with data on patients with brain metastases: Yasuyuki Sato, Nagoya Medical Center; Yoshimasa Kosaka, Kitasato University School of Medicine; Tsutomu Iwasa, Kinki University School of Medicine; Hiroyasu Yamashiro, Kure Medical Center/Chugoku Cancer Center; Tadahiko Shien; Okayama University Hospital; Kazuya Miyoshi, NHO Fukuyama Medical Center; Anan Keisei, Kitakyushu Municipal Medical Center, Department of Surgery; Hitoshi Arioka, Yokohama Rosai Hospital; and Maeda Shigeto, National Nagasaki Medical Center. We would also like to thank Editage for providing editorial assistance. This study was supported by the National Cancer Center Research and Development Fund (23-A-17) and Tokai University School of Medicine Research Aid, 2013.

**Disclosure** There are no conflicts of interest to declare.

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Breast Cancer Res Treat (2014) 145:143–153
DOI 10.1007/s10549-014-2907-9

CLINICAL TRIAL

# Survival of HER2-positive primary breast cancer patients treated by neoadjuvant chemotherapy plus trastuzumab: a multicenter retrospective observational study (JBCRG-C03 study)

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Received: 3 March 2014 / Accepted: 4 March 2014 / Published online: 30 March 2014


**Abstract** We investigated the disease-free survival (DFS) of HER2-positive primary breast cancer patients treated with neoadjuvant chemotherapy plus trastuzumab, as well as predictive factors for DFS and pathologic response. Data from 829 female patients treated between 2001 and 2010 were collected from 38 institutions in Japan. Predictive factors were evaluated using multivariate analyses. The 3-year DFS rate was 87 % [95 % confidence interval (CI) 85–90]. The pathologic complete response (pCR: ypT0/is + ypN0) rate was 51 %. The pCR rate was higher in the ER/PgR-negative patients than in the ER/PgR-positive patients (64 vs. 36 %, $P < 0.001$). Patients with pCR showed a higher DFS rate than patients without pCR (93 vs. 82 %, $P < 0.001$). Multivariate analysis revealed three independent predictors for poorer DFS: advanced nodal stage [hazard ratio (HR) 2.63, 95 % CI 1.36–5.21, $P = 0.004$ for cN2–3 vs. cN0], histological/nuclear grade 3 (HR 1.81, 95 % CI 1.15–2.91, $P = 0.011$), and non-pCR (HR 1.98, 95 % CI 1.22–3.24, $P = 0.005$). In the ER/PgR-negative dataset, non-pCR (HR 2.63, 95 % CI 1.43–4.90, $P = 0.002$) and clinical tumor stage (HR 2.20, 95 % CI 1.16–4.20, $P = 0.017$ for cT3–4 vs. cT1–2) were independent predictors for DFS, and in the ER/PgR-positive dataset, histological grade of 3 (HR 3.09, 95 % CI 1.48–6.62, $P = 0.003$), clinical nodal stage (HR 4.26, 95 % CI 1.53–13.14, $P = 0.005$ for cN2–3 vs. cN0), and young age (HR 2.40, 95 % CI 1.12–4.94, $P = 0.026$ for ≤40 vs. >40) were negative predictors for DFS. Strict pCR (ypT0 + ypN0) was an independent predictor for DFS in

On behalf of the JBCRG-C03 Collaborative Group.

**Electronic supplementary material** The online version of this article (doi:10.1007/s10549-014-2907-9) contains supplementary material, which is available to authorized users.

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both the ER/PgR-negative and -positive datasets (HR 2.66, 95 % CI 1.31–5.97, $P = 0.006$ and HR 3.86, 95 % CI 1.13–24.21, $P = 0.029$, respectively). These results may help assure a more accurate prognosis and personalized treatment for HER2-positive breast cancer patients.

**Keywords** Breast cancer · HER2 · Neoadjuvant chemotherapy · Pathologic complete response · Prognostic factors · Trastuzumab

## Introduction

Amplification or overexpression of human epidermal growth factor receptor-2 (HER2) is associated with a high risk of breast cancer recurrence and metastasis [1]. Adjuvant use of cytotoxic chemotherapy and trastuzumab, a recombinant humanized monoclonal antibody that targets HER2, improves the overall survival (OS) and disease-free survival (DFS) of patients with HER2-positive primary breast cancer [2, 3].

Neoadjuvant chemotherapy (NAC) reduces tumor size, which improves the rate of breast-conserving surgery, and provides information about chemosensitivity that helps with the design of postoperative therapy. Several meta-analyses have revealed that patients with a pathologic complete response (pCR) after NAC had higher survival rates than those without pCR, indicating that pCR represents a surrogate prognostic indicator [4–6].

Adding trastuzumab to NAC doubles the rate of pCR in patients with HER2-positive primary breast cancer [7–9]. The NOAH trial showed better 3-year event-free survival for chemotherapy plus trastuzumab versus chemotherapy alone [8]. In the TECHNO trial, patients with pCR after NAC plus trastuzumab showed better 3-year DFS than patients without pCR [10]; however, predictors for pCR and survival after treatment are unknown.

This multicenter retrospective study investigated the survival after NAC with trastuzumab among patients with HER2-positive primary breast cancer in efforts to identify predictive factors.

## Patients and methods

### Patients

In this multicenter retrospective cohort study, the inclusion criteria were female sex, histologically confirmed HER2-positive invasive breast cancer diagnosed between 2001 and 2010, no distant metastasis, age 20–70 years, and received NAC containing trastuzumab. Eligible patients were identified from the institutional databases. Data were managed by the data center of the Japan Breast Cancer Research Group (JBCRG).

The study protocol was approved by the Institutional Review Board at Kyoto University Hospital and participating institutions. All patient data were anonymized and

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allocated numbers according to Japanese ethics guidelines for epidemiologic research.

### Pathological assessment

Pathology specialists at each institution performed the pathological investigation. HER2-positive status was defined as 3+ overexpression by immunohistochemical testing or HER2 amplification by fluorescent in situ hybridization (HER2/CEP17 ratio $\geq$2.0). At each institution, surgical specimens obtained following NAC were serially sectioned, stained with hematoxylin and eosin (H&E), and diagnosed by experienced pathologists. pCR was defined as the absence of residual invasive cancer cells in the breast and axillary lymph nodes (ypT0/is + ypN0). Strict pCR (spCR), another pCR definition, was defined as no invasive and non-invasive residuals in the breast and axillary nodes (ypT0 + ypN0).

### Statistical analysis

All survival outcomes were measured from the date of starting NAC to the date of first event. The primary survival outcome was DFS defined as time to occurrence of recurrence, secondary malignancy (including contralateral breast cancer, hematological malignancy, and sarcoma), or death as a result of any cause. Secondary survival outcomes were OS defined as time to death as a result of any cause, distant recurrence-free survival (DRFS) defined as time to any recurrence except for ipsilateral breast or regional lymph node, and death as a result of any cause.

The Kaplan–Meier method was used to estimate survival outcomes. $\chi^2$ tests for categorical data and log-rank tests for time-to-event endpoints provided two-sided $P$ values, and $P$ values $< 0.05$ were considered statistically significant. Cox proportional hazards regression analysis was used to estimate hazard ratios (HRs) and 95 % confidence intervals (CIs). Logistic regression was used to estimate odds ratios (ORs) and 95 % CIs. Covariates used in the multivariate model were age, body mass index, clinical tumor stage, clinical nodal stage, estrogen receptor (ER)/progesterone receptor (PgR) status, histological/nuclear grade, pCR/spCR, surgery type, radiation therapy, adjuvant hormonal therapy, adjuvant chemotherapy, and adjuvant trastuzumab. Menopausal status was not included in the model because of collinearity with age. Patients with missing data were excluded from the multivariate analysis (e.g., patients whose adequate pathologic responses were not confirmed due to insufficient local therapy or lack of information regarding local therapy type). All statistical analyses were performed using JMP® (ver. 10.0.2, SAS Institute Inc. Cary, NC, USA). All analyses were supervised by a statistician (SM).

## Results

### Patient characteristics

Data of 829 patients from 38 institutions in Japan were collected. Among them, 53 did not meet the inclusion criteria and were excluded, leaving a total of 776 patients for analysis (whole dataset). HER2-positive tumors could be subdivided into ER/PgR positive and negative, and we therefore divided the patients into an ER/PgR-positive dataset ($N = 334$) and ER/PgR-negative dataset ($N = 439$) and also performed the analyses for each dataset (Fig. 1).

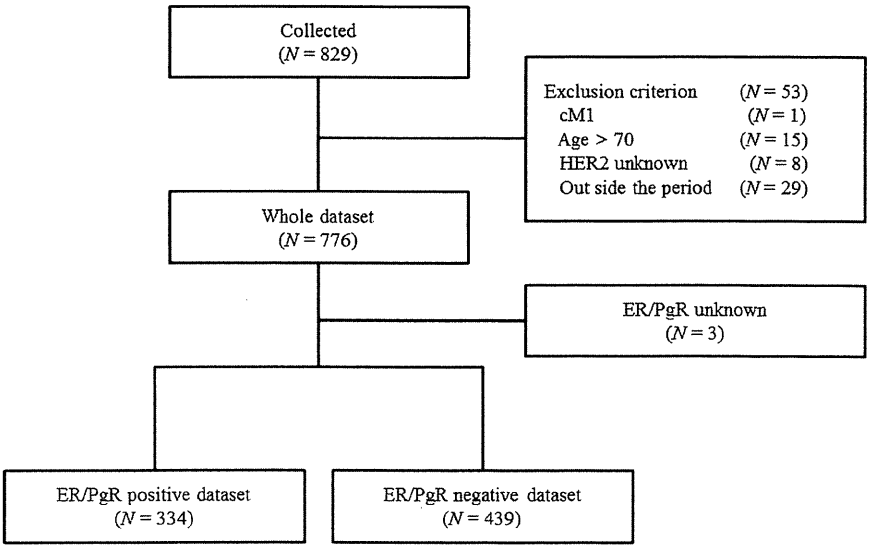


**Fig. 1** Flowchart of data collection and analysis

**Table 1** Patient, disease, and treatment characteristics

| Factors | *n* | (%) |
|---|---|---|
| All cases | 776 | (100) |
| Age | | |
| Median (min–max) | 53 | (25–70) |
| BMI | | |
| Median (min–max) | 22.0 | (15.0–47.3) |
| Unknown | 2 | (0.3) |
| Menopausal status | | |
| Pre-menopausal | 335 | (43.2) |
| Post-menopausal | 422 | (54.4) |
| Unknown | 19 | (2.4) |
| Clinical tumor size | | |
| T1b | 9 | (1.2) |
| T1c | 77 | (9.9) |
| T2 | 476 | (61.3) |
| T3 | 122 | (15.7) |
| T4 | 91 | (11.7) |
| Unknown | 1 | (0.1) |
| Clinical nodal status | | |
| N0 | 252 | (32.5) |
| N1 | 366 | (47.2) |
| N2 | 103 | (13.3) |
| N3 | 54 | (7) |
| Unknown | 1 | (0.1) |
| ER/PgR status | | |
| Positive | 334 | (43) |
| Negative | 439 | (56.6) |
| Unknown | 3 | (0.4) |
| Histological/nuclear grade | | |
| 1 | 107 | (13.8) |
| 2 | 184 | (23.7) |
| 3 | 350 | (45.1) |
| Unknown | 135 | (17.4) |
| NAC regimen | | |
| Anthracycline and taxane | 676 | (87.1) |
| Taxane only | 78 | (10.1) |
| Anthracycline only | 7 | (0.9) |
| Others | 1 | (0.1) |
| Unknown | 14 | (1.8) |
| Local therapy | | |
| Mastectomy + XRT | 96 | (12.4) |
| Mastectomy alone | 181 | (23.3) |
| BCS + XRT | 449 | (57.9) |
| BCS alone | 44 | (5.7) |
| Needle biopsy + XRT | 1 | (0.1) |
| Needle biopsy alone | 1 | (0.1) |
| Unknown | 4 | (0.5) |
| pCR (ypT0/is + ypN0) | | |
| Yes | 399 | (51.4) |
| No | 365 | (47) |
| Unknown | 12 | (1.5) |
| spCR (ypT0 + ypN0) | | |
| Yes | 240 | (30.9) |
| No | 525 | (67.7) |
| Unknown | 11 | (1.4) |
| Adjuvant hormonal therapy | | |
| Yes | 281 | (36.2) |
| No | 440 | (56.7) |
| Unknown | 55 | (7.1) |
| Adjuvant trastuzumab therapy | | |
| Yes | 697 | (89.8) |
| No | 65 | (8.4) |
| Unknown | 14 | (1.8) |
| Adjuvant chemotherapy | | |
| Yes | 45 | (5.8) |
| No | 720 | (92.8) |
| Unknown | 11 | (1.4) |

*BMI* body mass index, *ER/PgR* estrogen receptor/progesterone receptor, *NAC* neoadjuvant chemotherapy, *XRT* radiation therapy, *BCS* breast-conserving surgery, *pCR* pathologic complete response

Baseline characteristics and treatment of the whole dataset are summarized in Table 1. Median age was 53 (range 25–70) years. Most patients had tumor stage T2 (61 %) and were clinically node positive (67 %). ER and PgR were negative in 57 % of the patients. Most patients received anthracycline- and taxane-containing chemotherapy (87 %), and trastuzumab was administered concurrently with taxane (80 %). Breast-conserving surgery was performed in 64 % of the patients, most of whom (91 %) received radiation therapy. Radiation therapy was performed in 35 % of the patients who received mastectomy. Adjuvant hormonal therapy was performed in 86 % of the ER/PgR-positive patients. Most patients received adjuvant trastuzumab (90 %).

## Clinical outcomes

The median follow-up period was 42 (interquartile range 30–58) months. For the whole dataset, the 3-year DFS rate was 87 % (95 % CI 85–90) (Fig. 2a). 3-year OS and DRFS were 97 % (95 % CI 96–98) and 91 % (95 % CI 89–93), respectively. pCR was achieved in 399 (51 %) patients and spCR in 240 (31 %) patients.

The 3-year DFS rate was almost the same among patients in the ER/PgR-positive and -negative datasets (87 vs. 88 %, $P = 0.888$) (Fig. 2B). The pCR and spCR rates were higher in the ER/PgR-negative patients than in the ER/PgR-positive

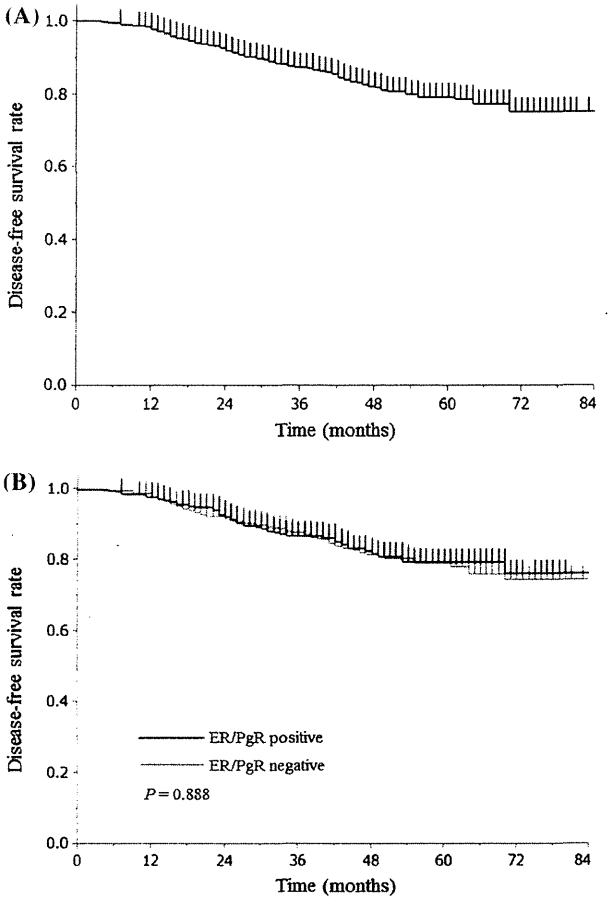


**Fig. 2** DFS curves of the **a** whole dataset and **b** ER/PgR-positive and -negative datasets

patients (64 vs. 36 % for pCR, $P < 0.001$; 38 vs. 23 % for spCR, $P < 0.001$, respectively).

### Prognostic factors for survival outcomes

The results of Cox proportional hazard regression performed to evaluate the prognostic effect of baseline characteristics and pathologic tumor response to NAC with trastuzumab are shown in Table 2. In the whole dataset, independent predictors for poorer DFS were advanced clinical nodal stage (adjusted HR 2.63, 95 % CI 1.36–5.21, $P = 0.004$ for cN2–3 vs. cN0; adjusted HR 1.64, 95 % CI 0.91–3.09, $P = 0.100$ for cN1 vs. cN0), histological/nuclear grade 3 (adjusted HR 1.81, 95 % CI 1.15–2.91, $P = 0.011$), and failure to achieve pCR (adjusted HR 1.98, 95 % CI 1.22–3.24, $P = 0.005$). Neither age nor ER/PgR status was an independent predictor for DFS. Multivariate analysis including spCR yielded the same results. The DFS rate was higher among patients with pCR than those without pCR (93 vs. 82 %, $P < 0.001$) (Fig. 3a). Patients who achieved spCR had a higher DFS rate than those who did not (96 vs. 84 %, $P < 0.001$) (Fig. 3b).

In the ER/PgR-positive dataset, independent predictors for poorer DFS were advanced clinical nodal stage, histological/nuclear grade 3, young age (≤40), and not achieving spCR. pCR was not an independent predictor for DFS on multivariate analysis (Table 2; Fig. 3c, d). For the ER/PgR-negative dataset, clinical tumor stage and both pCR and spCR were independent predictors for DFS (Table 2; Fig. 3e, f).

Predictors for other survival outcomes are listed in Supplementary Table S1. Predictors for OS were clinical nodal stage, histological/nuclear grade, and spCR, but pCR was not an independent predictor. Predictors for DRFS were clinical nodal stage, histological/nuclear grade, young age, pCR, and spCR.

### Predictive factors for pCR

The association of baseline characteristics with pCR/spCR following NAC plus trastuzumab was evaluated by multivariate logistic regression (Table 3). In the whole dataset, independent predictors for pCR were negative ER/PgR status (adjusted OR 3.42, 95 % CI 2.42–4.86, $P < 0.001$) and clinical tumor stage T1–2 compared with T3–4 (adjusted OR 1.88, 95 % CI 1.27–2.79, $P = 0.002$). Histological/nuclear grade 3 showed a statistically marginal association with pCR (adjusted OR 1.39, 95 % CI 0.99–1.95, $P = 0.060$). The same factors were selected as independent predictors in the multivariate model for spCR.

In the ER/PgR-positive dataset, clinical tumor stage was a predictor for pCR and spCR. In the ER/PgR-negative dataset, clinical tumor stage was an independent predictor for both pCR and spCR. Histological/nuclear grade was marginally predictive of pCR and spCR.

## Discussion

In this analysis, we assessed survival after NAC plus trastuzumab among patients with HER2-positive breast cancer. Although clinical nodal status, histological/nuclear grade, and pCR/spCR were independent predictors for DFS, the prognostic impact differed depending on ER/PgR status. pCR was a predictor for DFS particularly in patients with ER/PgR-negative tumor, and spCR—a stricter definition of pCR—was an independent prognostic factor regardless of ER/PgR status.

Our data included more patients with clinical tumor stage T2 or higher (89 %) and clinically node positive (67 %). In this population, a 3-year DFS rate of 87 % was relatively good; however, a considerable number of patients experienced disease relapse during the follow-up period. Risk factors associated with disease relapse need to

**Table 2** Adjusted hazard ratios of factors predicting DFS

| Factor | pCR (ypT0/is + ypN0) | | | spCR (ypT0 + ypN0) | | |
|---|---|---|---|---|---|---|
| | HR | 95 % CI | *P* value | HR | 95 % CI | *P* value |
| Whole dataset | | | | | | |
| Age | | | | | | |
| ≤40 vs. >40 | 1.67 | (0.95–2.81) | 0.074 | 1.63 | (0.93–2.75) | 0.088 |
| BMI | | | | | | |
| 25≤ vs. <22 | 1.31 | (0.74–2.24) | 0.351 | 1.31 | (0.74–2.24) | 0.348 |
| 22≤ , <25 vs. <22 | 0.96 | (0.56–1.61) | 0.891 | 1.00 | (0.58–1.67) | 0.993 |
| Clinical tumor size | | | | | | |
| T3–4 vs. T1–2 | 1.53 | (0.93–2.49) | 0.093 | 1.42 | (0.87–2.32) | 0.160 |
| Clinical nodal status | | | | | | |
| N2–3 vs. N0 | 2.63 | (1.36–5.21) | 0.004 | 2.58 | (1.34–5.12) | 0.004 |
| N1 vs. N0 | 1.64 | (0.91–3.09) | 0.100 | 1.73 | (0.96–3.26) | 0.070 |
| ER/PgR | | | | | | |
| Negative vs. positive | 0.97 | (0.47–2.08) | 0.933 | 0.93 | (0.46–1.96) | 0.842 |
| Histological/Nuclear grade | | | | | | |
| 3 vs. 1&2 | 1.81 | (1.15–2.91) | 0.011 | 1.77 | (1.12–2.84) | 0.014 |
| pCR/spCR | | | | | | |
| Non-pCR vs. pCR | 1.98 | (1.22–3.24) | 0.005 | 2.90 | (1.57–5.90) | <0.001 |
| ER/PgR-positive dataset | | | | | | |
| Age | | | | | | |
| ≤40 vs. >40 | 2.40 | (1.12–4.94) | 0.026 | 2.33 | (1.08–4.80) | 0.031 |
| BMI | | | | | | |
| 25≤ vs. <22 | 1.49 | (0.63–3.38) | 0.354 | 1.54 | (0.66–3.45) | 0.313 |
| 22≤, <25 vs. <22 | 0.69 | (0.25–1.67) | 0.419 | 0.69 | (0.25–1.68) | 0.433 |
| Clinical tumor size | | | | | | |
| T3–4 vs. T1–2 | 0.83 | (0.35–1.88) | 0.653 | 0.69 | (0.28–1.62) | 0.399 |
| Clinical nodal status | | | | | | |
| N2–3 vs. N0 | 4.26 | (1.53–13.14) | 0.005 | 4.54 | (1.62–14.13) | 0.004 |
| N1 vs. N0 | 2.55 | (0.99–7.43) | 0.053 | 2.83 | (1.08–8.39) | 0.034 |
| Histological/Nuclear grade | | | | | | |
| 3 vs. 1&2 | 3.09 | (1.48–6.62) | 0.003 | 3.14 | (1.49–6.85) | 0.003 |
| pCR/spCR | | | | | | |
| Non-pCR vs. pCR | 1.20 | (0.57–2.69) | 0.634 | 3.86 | (1.13–24.21) | 0.029 |
| ER/PgR-negative dataset | | | | | | |
| Age | | | | | | |
| ≤40 vs. >40 | 0.95 | (0.35–2.18) | 0.913 | 1.01 | (0.38–2.28) | 0.979 |
| BMI | | | | | | |
| 25≤ vs. <22 | 0.94 | (0.39–2.05) | 0.886 | 0.97 | (0.40–2.11) | 0.942 |
| 22≤, <25 vs. <22 | 1.10 | (0.56–2.08) | 0.774 | 1.10 | (0.56–2.08) | 0.779 |
| Clinical tumor size | | | | | | |
| T3–4 vs. T1–2 | 2.20 | (1.16–4.20) | 0.017 | 2.11 | (1.11–4.04) | 0.024 |
| Clinical nodal status | | | | | | |
| N2–3 vs. N0 | 2.04 | (0.85–5.07) | 0.112 | 1.73 | (0.73–4.27) | 0.217 |
| N1 vs. N0 | 1.49 | (0.70–3.38) | 0.306 | 1.39 | (0.66–3.13) | 0.398 |
| Histological/Nuclear grade | | | | | | |
| 3 vs. 1&2 | 1.33 | (0.74–2.48) | 0.354 | 1.29 | (0.72–2.41) | 0.393 |
| pCR/spCR | | | | | | |
| Non-pCR vs. pCR | 2.63 | (1.43–4.90) | 0.002 | 2.66 | (1.31–5.97) | 0.006 |

*BMI* body mass index, *ER/PgR* estrogen receptor/progesterone receptor, *pCR* pathologic complete response, *spCR* strict pathologic complete response, *HR* hazard ratio

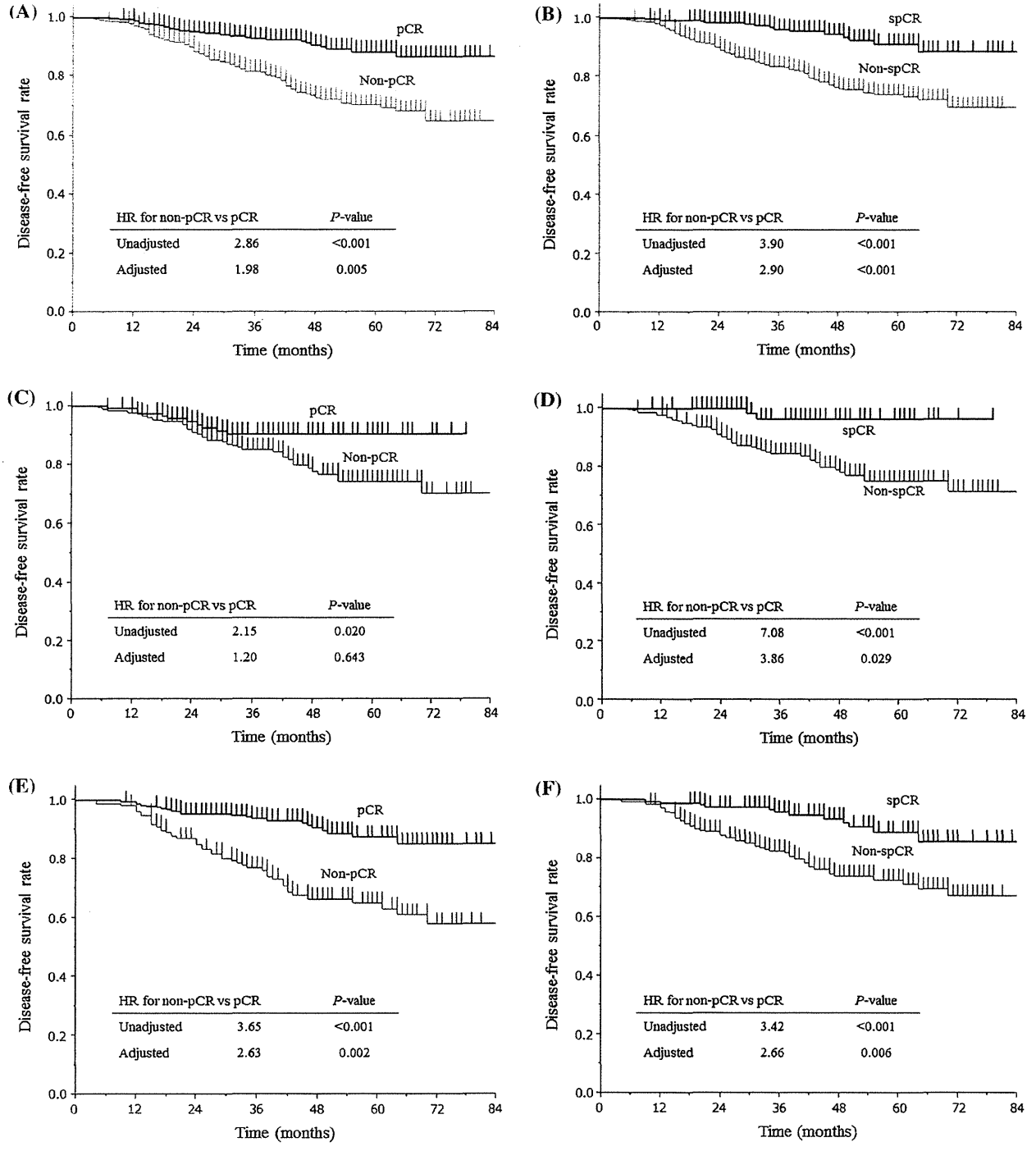


**Fig. 3** DFS curves of patients with pCR (ypT0/is + ypN0) versus non-pCR in the **a** whole dataset, **c** ER/PgR-positive dataset, and **e** ER/PgR-negative dataset. DFS curves of patients with spCR (ypT0 + ypN0) versus non-spCR in the **b** whole dataset, **d** ER/PgR-positive dataset, and **f** ER/PgR-negative dataset

be clarified to conduct a clinical trial aimed at improving these patients' prognosis.

In two phase-III trials in which patients with HER2-positive disease were randomly allocated to NAC with trastuzumab or NAC only, the addition of trastuzumab to NAC resulted in a higher pCR rate and improved DFS [8, 11]. The pCR rate in our study (51 %) is comparable to those reported in previous trials of NAC with trastuzumab