variables were compared using the χ^2 test, and continuous variables were compared using the nonparametric Wilcoxon test or the parametric t test. Logistic regression analyses were performed to identify independent risk factors. A value of $p < 0.050$ was considered statistically significant.

Results

Patients

A total of 222 patients without a preoperative diagnosis of PVT underwent hepatectomy between January 2009 and June 2012. Six of the patients were excluded because they underwent simultaneous splenectomy, and eight were excluded because they did not undergo contrast-enhanced CT on POD 7. The remaining 208 patients were enrolled in this study.

The patients included 153 men and 55 women, with a mean age of 66.7 ± 0.8 years. The indications for hepatectomy were primary liver tumor in 160 patients and metastatic liver tumor in 48 patients. The operative procedure was trisectionectomy in 2 patients (1.0 %), bisectionectomy in 38 patients (18.3 %), monosectionectomy in 35 patients (16.8 %), subsectionectomy in 30 patients (14.4 %), and partial hepatectomy in 103 patients (49.5 %). A total of 43 patients (20.7 %) underwent laparoscopic hepatectomy.

PVT after hepatectomy

Postoperative PVT occurred in 19 patients (9.1 %) who had undergone hepatectomy, including MPV thrombosis in 7 patients and PPV thrombosis in 12 patients (Fig. 1). In patients with MPV thrombosis, thrombus was limited to the MPV in five cases and extended from the MPV to the superior mesenteric vein in two cases. In patients with PPV thrombosis, thrombus was in the umbilical portion of the portal vein in four cases and in the portal vein stump in eight cases. Details of the 19 patients with PVT are shown in Table 1.

Comparison of patients with and without MPV thrombosis

Univariate analyses showed that patients with MPV thrombosis ($n = 7$) had a significantly higher proportion of right hepatectomy (85.7 vs. 6.9 %; $p < 0.001$), larger resection volume (665 ± 138 vs. 243 ± 27 g; $p = 0.003$), and longer operation time (385 ± 37 vs. 335 ± 9 min; $p = 0.021$) than patients without PVT ($n = 189$; Table 2). There were no significant differences in preoperative clinical characteristics between patients with MPV thrombosis and patients without PVT. Multivariate analysis identified right hepatectomy as a significant independent risk factor for MPV thrombosis [odds ratio (OR) 108.886 (95 % confidence interval 10.54–2,906.57); $p < 0.001$]. On the other hand, resection volume [OR 1.001 (0.999–1.004); $p = 0.413$] and operation time [OR 1.001 (0.993–1.005); $p = 0.728$] were not significantly associated.

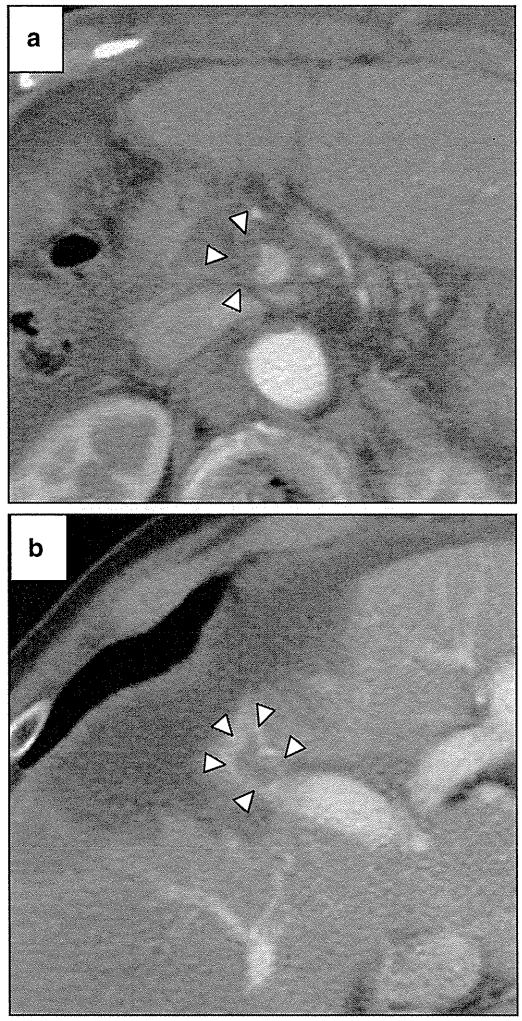

Fig. 1 Postoperative portal vein thrombosis (PVT) after hepatectomy was classified as main portal vein (MPV) thrombosis when there was thrombus in the MPV and superior mesenteric vein (**a**). It was classified as peripheral portal vein (PPV) thrombosis when there was thrombus in the portal vein stump or branches of the portal vein (**b**). *Arrowheads* indicate the PVT

Comparison of patients with and without PPV thrombosis

Univariate analyses showed that patients with PPV thrombosis ($n = 12$) had a significantly longer duration of the Pringle maneuver than patients without PVT (76 ± 11 vs. 43 ± 3 min; $p = 0.002$; Table 2).

Regeneration

To evaluate the impact of PVT on early clinical outcomes, laboratory data and liver regeneration rate on POD 7 were investigated in patients who underwent right hepatectomy ($n = 19$). There were no significant differences in preoperative clinical characteristics, including the estimated tumor volume and resection with or without the middle hepatic vein, between patients with PVT ($n = 6$) and without PVT ($n = 13$). Interestingly, patients with PVT had a significantly lower rate of liver regeneration than patients without PVT (46.9 ± 3.4 vs. 56.4 ± 2.4 %;

Table 1 Characteristics of patients with PVT

Cases	Age (years)	Sex	Extent of resection	PVT location	Anticoagulation	Outcome
1	63	Male	Right lobe	MPV + SMV	Yes	Resolved
2	77	Male	Left lateral section + S8	MPV + SMV	Yes	Resolved
3	70	Male	Right lobe	MPV	Yes	Resolved
4	71	Male	Right lobe	MPV	No	Resolved
5	37	Male	Right lobe	MPV	No	Resolved
6	77	Female	Right lobe	MPV	No	Resolved
7	75	Male	Right lobe	MPV	No	Resolved
8	73	Male	Anterior section	PPV, UP	Yes	Resolved
9	65	Male	S4	PPV, UP	Yes	Resolved
10	76	Female	Partial S3/4	PPV, UP	Yes	Resolved
11	65	Female	S4	PPV, UP	Yes	Resolved
12	40	Male	Posterior section	PPV, Stump-Rt	Yes	Resolved
13	76	Male	S8	PPV, Stump-Rt	Yes	Resolved
14	64	Male	S8	PPV, Stump	No	Resolved
15	81	Male	Left lateral section	PPV, Stump	No	Resolved
16	77	Male	Left lobe + partial S5	PPV, Stump	No	Stable
17	71	Male	Left lateral section	PPV, Stump	No	Stable
18	65	Male	S8	PPV, Stump	No	Stable
19	64	Female	Partial S2 + S7	PPV, Stump	No	Stable

MPV main portal vein, *PPV* peripheral portal vein, *PVT* portal vein thrombosis, *Rt* right branch of the portal vein, *S* segment, *Stump* stump of the portal vein, *SMV* superior mesenteric vein, *UP* umbilical portion of the portal vein

Table 2 Univariate analysis of relations between clinical factors and PVT

Factors	Without PVT ($n = 189$)	MPV thrombosis ($n = 7$)	PPV thrombosis ($n = 12$)	p^a	p^b
Age (years)	66.6 ± 0.8	67.1 ± 4.3	68.1 ± 3.3	0.895	0.652
Male sex	138 (73.0 %)	6 (85.7 %)	9 (75.0 %)	0.427	0.880
Primary tumor, yes	144 (76.2 %)	6 (85.7 %)	10 (83.3 %)	0.538	0.557
Albumin (g/dl)	4.0 ± 0	4.1 ± 0.2	3.9 ± 0.1	0.544	0.640
AST (IU/l)	39 ± 2	48 ± 10	38 ± 7	0.388	0.861
ALT (IU/l)	37 ± 2	39 ± 11	37 ± 9	0.824	0.997
Total bilirubin (mg/dl)	0.8 ± 0	0.7 ± 0.2	0.8 ± 0.2	0.677	0.990
Platelet count ($\times 10^4/\mu l$)	17.4 ± 0.7	18.7 ± 3.9	17.4 ± 2.9	0.742	0.999
PT-INR	1.05 ± 0.01	1.07 ± 0.03	1.02 ± 0.03	0.541	0.370
$ICGR_{15}$ (%)	13.2 ± 0.6	9.1 ± 2.8	16.3 ± 2.2	0.159	0.176
Liver cirrhosis, yes	35 (18.5 %)	0	3 (25.0 %)	0.094	0.591
Rt. hepatectomy, yes	13 (6.9 %)	6 (85.7 %)	0	**<0.0001**	0.181
Resection volume (g)	243 ± 27	665 ± 138	232 ± 103	**0.003**	0.916
Operation time (min)	335 ± 9	451 ± 49	385 ± 37	**0.021**	0.194
Blood loss (g)	541 ± 42	562 ± 226	406 ± 170	0.927	0.442
Duration of Pringle maneuver (min)	43 ± 3	66 ± 14	76 ± 11	0.078	**0.002**
Number of intraoperative Pringle maneuvers	133 (70.4 %)	6 (85.7 %)	10 (83.3 %)	0.348	0.312

Boldface numbers indicate significance in Tables 2 and 3

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *$ICGR_{15}$* indocyanine green retention rate at 15 min, *PT-INR* prothrombin time-international normalized ratio, *Rt.* right

[a] Comparisons between patients with MPV thrombosis and without PVT

[b] Comparison between patients with PPV thrombosis and without PVT

Table 3 Impact of PVT on recovery of liver function and early liver regeneration after right hepatectomy

Factors	Without PVT ($n = 13$)	With PVT ($n = 6$)	p
Liver regeneration (%)	56.4 ± 2.4	46.9 ± 3.4	**0.040**
Albumin (g/dl)	3.4 ± 0.1	2.9 ± 0.1	**0.019**
Total bilirubin (mg/dl)	1.2 ± 0.2	1.8 ± 0.2	**0.034**
AST (IU/l)	42 ± 6	36 ± 6	0.461
ALT (IU/l)	109 ± 20	89 ± 29	0.581
PT-INR	1.13 ± 0.03	1.36 ± 0.05	**0.002**
PHLF, yes	2 (15.4 %)	5 (83.3 %)	**0.004**
PHLF grades (A/B/C)	2/0/0	1/4/0	**0.033**

PHLF posthepatectomy liver failure

$p = 0.040$). Laboratory data on POD 7 also indicated delayed liver regeneration in patients with PVT compared with patients without PVT (Table 3). Patients with PVT had a significantly lower serum albumin level (2.9 ± 0.1 vs. 3.4 ± 0.1 g/dl; $p = 0.019$), higher serum total bilirubin level (1.8 ± 0.2 vs. 1.2 ± 0.2 mg/dl; $p = 0.034$), and higher PT-INR (1.36 ± 0.05 vs. 1.13 ± 0.03; $p = 0.002$) than patients without PVT. There were no significant differences between patients with and without PVT regarding the aspartate aminotransferase level (36 ± 6 vs. 42 ± 6 IU/l; $p = 0.461$) or alanine aminotransferase level (89 ± 29 vs. 109 ± 20 IU/l; $p = 0.581$). Posthepatectomy liver failure, PHLF [7] occurred significantly more frequently in patients with PVT than without PVT (83.3 vs. 15.4 %; $p = 0.004$). Among patients with PVT, four had grade B PHLF and one had grade A PHLF. Among the patients without PVT, two had grade A PHLF ($p = 0.033$). There were no postoperative deaths of patients with or without PVT.

Clinical course of PVT

Nine patients received anticoagulation therapy for PVT (Table 1). These patients had a mean follow-up of 4.6 ± 1.9 months, and the PVT resolved in all patients after a mean treatment period of 1.6 ± 0.5 months. Interestingly, the PVT also resolved after a mean period of 3.0 ± 0.6 months in the six patients who did not receive anticoagulation therapy. There were no cases of PVT progression.

Discussion

Although PVT is widely recognized as a common complication of liver cirrhosis, it is unclear whether postoperative PVT is a complication of hepatectomy, and the incidence of PVT after hepatectomy is unknown. In the current study, the rate of postoperative PVT occurring after hepatectomy was 9.1 %. Previous studies have reported a postoperative pneumonia rate of 13 % (17/555) [9] and a venous thromboembolism rate of 2.9 % (167/5,706) [11] after hepatectomy. Compared with other posthepatectomy complications it is clear that postoperative PVT after hepatectomy is not rare.

For diagnosis of PVT, abdominal CT is preferable to color Doppler ultrasonography because of its high sensitivity (90 %) and specificity (99 %) [6, 16]. Although the point at which the postoperative PVT starts to develop is not known, the results of the current study showed that it is reasonable to screen patients on POD 7 because those with PVT did not have symptoms indicating mesenteric ischemia (e.g., acute or colicky abdominal pain or bloody stools [17]) at that time. Contrast-enhanced CT on POD 7 is therefore recommended for screening patients for PVT.

The etiology of PVT can be categorized based on Virchow's triad of venous stasis, the hypercoagulable state, and endothelial injury. These three factors may be interdependent and often coexist [6, 18]. In the current study, PPV was detected in the portal vein stump in 8 of 12 patients, suggesting that venous stasis and endothelial injury at the stump induced PPV thrombosis. The Pringle maneuver can result in portal vein endothelial injury and stasis, and the duration of the Pringle maneuver was a significant risk factor for PPV thrombosis. It is also hypothesized that blood clots may be formed during clamping of the hepatoduodenal ligament, which embolize to the stumps of PPVs to form PPV thrombosis. Patients who underwent right hepatectomy tended to have a larger resection volume, smaller remnant liver volume, and more frequent Pringle maneuver than patients who underwent other hepatectomy procedures. Recently, a correlation was reported between small remnant liver volume and an increased von Willebrand factor/disintegrin ratio and metalloproteinase with thrombospondin type 1 motif (ADAMTS13), which may induce thrombogenesis [18]. Patients who undergo right hepatectomy therefore have increased risks of thrombogenesis, portal venous stasis, and endothelial injury. Also, right hepatectomy may be an independent risk factor for MPV thrombosis.

Among patients who underwent right hepatectomy, the liver regeneration rate was 46.9 % in patients with PVT. In contrast, the liver regeneration rate was 56.4 % in patients without PVT, which is consistent with previously reported rates [19, 20]. As many studies have indicated the importance of portal venous flow for liver regeneration [21–23], it is possible that reduced portal venous flow due to PVT results in delayed liver regeneration. Smaller liver volume also results in decreased portal venous flow and increased

intrahepatic vascular resistance [24], which may result in progression of the PVT and deterioration of liver function. In the current study, 83 % (5/6) of patients with PVT who underwent right hepatectomy had PHLF according to the consensus definition of the International Study Group of Liver Surgery [7].

Luca et al. [25] studied the natural course of PVTs in patients who had cirrhosis but no malignancy and who did not receive anticoagulation therapy. They reported that partial PVT worsened in 48 % of patients (20/42), improved in 45 % of patients (19/42), and was stable in 7 % of patients (3/42). Spontaneous resolution of PVT was thought to be due to thrombus shrinkage rather than lysis because of changes in vessel size. In the current study, PVT resolved spontaneously in the six patients (60 %) who did not receive anticoagulation therapy, which is consistent with the previous literature [25, 26]. In all patients who received anticoagulation therapy, the PVT resolved during follow-up. However, a recent prospective study reported that recanalization of the portal vein occurred in only 38 % of patients with symptomatic PVT who received anticoagulation therapy [27]. Portal vein occlusion in patients with PVT with extension into the superior mesenteric vein may result in mesenteric ischemia, sepsis, and death [6]. Turnes et al. [28] reported that among patients with acute PVT who had cirrhosis but no malignancy those who received early anticoagulation therapy had a higher frequency of recanalization than those who did not receive early anticoagulation therapy. As many reports have recommended immediate initiation of anticoagulation therapy after a definitive diagnosis of PVT [6, 28–30], the high rate of recanalization in this study may be a result of the early initiation of anticoagulation therapy in patients with PVT.

The main limitation of this study is its retrospective nature, which limited the data available for analysis. Portal hemodynamics may have had an impact on PVT, and it was unclear how the local hemodynamics affected changes in PVT. This study also included a relatively small number of inhomogeneous cases. Although various surgical procedures were included in this study, the analyses did not account for potential differences in background characteristics among patients who underwent different procedures. Further analysis of a larger number of patients from multiple centers, and of differences between procedures, is necessary to confirm these findings.

Conclusions

The findings of this study suggest that most cases of PVT can be stabilized or improved, with recovery of liver function. However, the small number of patients with severe PVT indicates that these patients should be carefully observed because of the possibility of worsening liver function.

Conflict of interest None.

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Carboplatin plus Either Docetaxel or Paclitaxel for Japanese Patients with Advanced Non-small Cell Lung Cancer

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Abstract. *Aim: Assessment of the efficacy of docetaxel plus carboplatin vs. paclitaxel plus carboplatin in Japanese patients with advanced non-small cell lung cancer (NSCLC). Patients and Methods: Chemotherapy-naïve patients were randomly assigned at a ratio of 2 to 1 to receive six cycles of either docetaxel (60 mg/m^2) plus carboplatin [area under the curve (AUC)=6 mg/ml min] or paclitaxel (200 mg/m^2) plus carboplatin (same dose), on day 1 every 21 days. The primary end-point was progression-free survival (PFS). Results: A total of 90 patients were enrolled. Overall response rate, median PFS and median survival time in the docetaxel-plus-carboplatin group and the paclitaxel-plus-carboplatin group were 23% vs. 33%, 4.8 months vs. 5.1 months, and 17.6 months vs. 15.6 months, respectively. The docetaxel-plus-carboplatin group had a higher incidence of grade 3 or 4 neutropenia (88% vs. 60%). Conclusion: Both regimens were similarly effective in Japanese patients with advanced NSCLC.*

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Key Words: Non-small cell lung cancer, chemotherapy, randomized phase 2, ethnicity, docetaxel, paclitaxel carboplatin.

Lung cancer is one of the most common malignancies and is the leading cause of cancer-related death worldwide (1). Non-small cell lung cancer (NSCLC) accounts for 85% of all cases of lung cancer. Platinum-based chemotherapy has been considered a standard treatment for advanced NSCLC. In addition, molecular-targeted therapy, including vascular endothelial growth factor (VEGF) inhibitors such as bevacizumab, epidermal growth factor receptor (EGFR) inhibitors such as gefitinib or erlotinib, and anaplastic lymphoma kinase (ALK) inhibitors, has recently become a treatment option for specific subsets of patients, especially those with non-squamous cell lung cancer (2-5). These molecular targeted therapies have led to a paradigm shift of

treatment. Unfortunately, all patients with *EGFR*-mutant or ALK-positive lung cancer who receive EGFR or ALK inhibitors eventually experience disease relapse and require chemotherapy at some point during the course of treatment (4). Chemotherapy thus continues to play an important role in the management of NSCLC.

Docetaxel has been demonstrated to be effective against previously-untreated advanced NSCLC. Results of a large phase III trial found that docetaxel plus cisplatin was significantly superior to vindesine plus cisplatin in terms of overall response rate and overall survival (6). Carboplatin has shown broad equivalence to cisplatin in combination with chemotherapy for advanced NSCLC. To our knowledge, however, no clinical trial has directly compared docetaxel + carboplatin (DCarb) with paclitaxel plus carboplatin (PCarb) in patients with advanced NSCLC.

Fossella *et al.* reported a phase III study comparing docetaxel plus a platinum agent with vinorelbine plus cisplatin, performed by the TAX 326 Study Group (7). Docetaxel with cisplatin led to a better overall response and higher survival rate than docetaxel plus carboplatin, with a median survival time (MST) of 11.3 months, as compared with 9.4 months, respectively. However, that study was not designed to directly compare docetaxel plus cisplatin with docetaxel plus carboplatin. The therapeutic value of docetaxel with carboplatin as a front-line regimen for advanced NSCLC, thus remains unclear.

Millward *et al.* conducted a phase II study of docetaxel plus carboplatin in white and Asian patients with advanced NSCLC (8). The MST was 12.9 months, and multivariate analysis showed that ethnicity was a significant independent predictor of response and survival. Two clinical trials have evaluated docetaxel with carboplatin in Japanese patients with advanced NSCLC (9, 10). These trials reported a good MST of 12 months and 12.9 months, respectively. However, randomized phase II studies comparing docetaxel plus carboplatin with a standard regimen have yet to be performed on Asian patients with NSCLC. We therefore designed a randomized phase II study to compare the newer combination of DCarb with PCarb as standard treatment in patients with advanced NSCLC.

Patients and Methods

All patients enrolled in this study had cytologically- or histologically-confirmed diagnoses of NSCLC (adenocarcinoma, squamous cell carcinoma, large cell carcinoma, or NSCLC not otherwise specified) with advanced stage IIIB or stage IV disease or relapse after surgical resection of NSCLC (regarded as stage IV). Other eligibility criteria were as follows: chemotherapy-naïve status; an Eastern Cooperative Oncology Group performance status (PS) of 0 or 1; a neutrophil count of at least 2.0×10^9 cells/l; a platelet count higher than 100.0×10^9 cells/l; a hemoglobin concentration of at least 90 g/l; serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) concentrations of less than two-times the upper limit of normal (ULN); serum total bilirubin and creatinine concentrations of less than the ULN; a creatinine clearance of 50 ml/min or higher (as calculated by the Cockcroft-Gault equation) (11); and an alveolar partial pressure of oxygen (PaO_2) of 70 Torr or higher or an oxygen saturation on pulse oximetry (SpO_2) of 94% or higher (while breathing room air). Patients were excluded if they had any of the following conditions: severe infection, pregnancy or breastfeeding; a previous malignancy within the previous five years (except for patients with cured carcinoma *in situ*); another active cancer; an allergy to polysorbate 80 or polyoxyethylene castor oil; evidence of interstitial lung disease on a plain chest x-ray film; uncontrolled co-morbidities such as malignant hypertension, congestive heart failure, myocardial infarction within the previous six months, arrhythmia requiring treatment, bleeding tendency, or diabetes mellitus; pleural or pericardial effusion requiring drainage; symptomatic brain metastasis; or peripheral neuropathy of more than grade 1.

All patients provided written informed consent. The study protocol was approved by the Institutional Review Boards of all participating institutions and by the Japan Multinational Trial Organization (JMTO) ethical committee. This study was conducted in accordance with the Declaration of Helsinki and was registered with UMIN 000001225 on June 30, 2008.

Study design and treatment. This was a randomized, phase II, open-label study. The primary end-point was the determination of progression-free survival (PFS). The secondary end-points were tumor response, survival (1-year survival rate, overall survival), and toxic effects. Patients were randomly assigned at a ratio of 2 to 1 to receive either DCarbo or PCarbo. Central randomization to each arm was performed with the use of Pocock and Simon's method (12). Stratification factors were PS (0 or 1), more than 5% weight loss within the previous six months (yes or no), and serum lactic dehydrogenase (LDH) concentration (abnormally high or not).

Patients in the DCarbo group received intravenous docetaxel (60 mg/m^2) over the course of 60 to 90 min and carboplatin [area under the curve (AUC) 6 mg/ml min] over the course of three hours on day 1 every 21 days for six cycles. Pre-medication, such as anti-emetic agents or corticosteroids, was given as required. In the PCarbo group, patients received intravenous paclitaxel (200 mg/m^2) and carboplatin (AUC 6 mg/ml min, same as in the DCarbo group) on day 1 every 21 days for six cycles. Creatinine clearance was calculated using the Cockcroft-Gault equation. The serum creatinine level (mg/dl) used in this equation was modified by adding 0.2 mg/dl, because an enzyme assay is used in Japan, whereas Jaffe's non-enzyme assay was used to develop this equation. Patients in the PCarbo group were given pre-medication with dexamethasone, diphenhydramine, and ranitidine or cimetidine. The use of additional antiemetics was left at the physician's discretion. Use of granulocyte-colony stimulating factor (G-CSF) was permitted any time during the study (except for prophylactic use) in both groups. In the absence of progressive disease or intolerable toxicity, patients in both groups received six cycles of chemotherapy.

Treatment could be delayed for up to 14 days if the neutrophil count was less than 1.5×10^9 cells/l and the platelet count was less than 75×10^9 cells/l on day 1 of each course. In the event of prolonged or complicated grade 4 neutropenia or thrombocytopenia, the dose of docetaxel was reduced by 10 mg/m^2, that of paclitaxel by 25 mg/m^2, or that of carboplatin by AUC 1 mg/ml min for the subsequent cycle of chemotherapy. Dose reduction was allowed

twice. Treatment could be delayed for up to 14 days if AST or ALT (or both) was more than 2.5-times higher than the ULN, the serum creatinine concentration was more than 1.5-times higher than the institutional ULN, or nonhematological toxicity of grade 2 or higher developed (except for nausea, vomiting, fatigue, loss of appetite, mild electrolyte abnormalities, and alopecia) developed.

Patients were assessed every two cycles, and the objective response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0 (13). The best response in individual patients was derived from investigator-reported data. Objective response rates were confirmed by at least one sequential tumor assessment. Toxic effects were graded in accordance with the National Cancer Institute Common Toxicity Criteria, version 2.0 (14). The numbers and frequencies of each adverse event were respectively summarized for any grade and for grade 3 or higher in each treatment group. The MST with 95% confidence intervals (CI) and the probability of 1-year survival with 95% CI were calculated by the Kaplan-Meier method for each group.

Statistical plan and analysis. The primary end-point was PFS. The main objective of the study was to estimate the PFS rate at six months in the DCarbo group. The median PFS in the DCarbo group was predicted to be about 150 days on the basis of the results of previous studies. The PFS rate at six months was thus assumed to be 45%. Given that the range of the 90% CI at six months is 0.1 or less, we estimated that at least 60 patients would be required in the DCarbo group. Because patients were randomly assigned to either the DCarbo group or PCarbo group at a ratio of 2:1, the target number of patients in the latter group (calibration group) was 30. Hazard ratios (HR) and 95% CIs were calculated with a Cox proportional-hazards model.

Results

Patients' characteristics. A total of 90 patients were enrolled between June 2007 and September 2008 at 15 institutions in Japan. All patients were eligible for analysis. Sixty patients were assigned to the DCarbo group and 30 were assigned to the PCarbo group (Figure 1). The patients' characteristics for both groups were shown in Table I. The baseline characteristics of patients in the DCarbo group were similar to those in the PCarbo group.

Tumor response and survival. The total number of administered cycles of chemotherapy was 230 in the DCarbo group and 139 in the PCarbo group. The median follow-up time was 15.8 months.

Sixty patients began chemotherapy in the DCarbo group, and 19 completed six cycles according to protocol. The mean number of administered cycles of chemotherapy was 4.0 (range, 1 to 6). Dose modification was carried out once in 17 patients (28%) and more than once in 23 patients (38%). Treatment was delayed in 11 patients (18%). The reasons for treatment discontinuation before the completion of six cycles of DCarbo were disease progression (n=18), dose modification necessitated by adverse events more than twice (n=12), and withdrawal of treatment by the patient (n=6) or investigator (n=5). In the PCarbo group, 30 patients began chemotherapy, and 14 completed six cycles. The mean number of administered cycles was 4.6 (range, 1 to 6). Dose modification was carried out once in seven patients (23%) and more than once in seven patients (23%). Treatment was delayed in 10 patients (33%). The reasons for discontinuation of PCarbo before the completion of six cycles were disease progression (n=6), withdrawal of treatment by the patient (n=5), dose modification necessitated by adverse events more than twice (n=4), and withdrawal of treatment by the investigator (n=1).

The overall response rate (based on the best confirmed response during study treatment) was 23% [14 out of 60 patients with partial response (PR); 95% CI=13%-36%] in the DCarbo group and 33% (10 out of 30 patients with PR; 95% CI=17%-53%) in the PCarbo group (Table II). No patient had a complete response. Stable disease was obtained in 31 patients (52%; 95% CI=38%-65%) in the DCarbo group and 15 patients (50%; 95% CI=31%-69%) in the PCarbo group. The Median PFS was 4.8 months (95% CI=3.9-7.2 months) in the DCarbo group and 5.1 months (95% CI=4.4-6.4 months) in the PCarbo group. The PFS rate at six months was 42% (90% CI=31%-52%) in the DCarbo group and 40% (90% CI=25%-54%) in the PCarbo group (Figure 2). The hazard ratio of DCarbo referenced to PCarbo was 0.86 (95% CI=0.55-1.36). The MST was 17.6 months (95% CI=10.2-22.9 months) in the DCarbo group and 15.6 months (95% CI=9.3-20.8 monts) in the PCarbo group (Figure 3). The 1-year survival rate was 60% in both groups (90% CI=49%-70% in the DCarbo group and 44%-73% in the PCarbo group). The hazard ratio of DCarbo compared to PCarbo was 0.77 (95%CI=0.47-1.26).

Toxicity. All patients were assessable for toxicity (Table III). Patients in the DCarbo group had a higher incidence of grade 3 or 4 neutropenia than those in the PCarbo group (88% *vs*. 60%, 95% CI=77%-95% *vs*. 41%-77%). The PCarbo group had a higher incidence of grade 2 or more sensory neuropathy (37% *vs*. 3%, 95% CI=20%-56% *vs*. 0%-12%), myalgia (13% *vs*. 0%, 95% CI=4%-31% *vs*. 0%-6%), and arthralgia (20% *vs*. 2%, 95% CI=8%-39% *vs*. 0%-9%) than the DCarbo group. There were no major differences between the two groups regarding any other toxic effects (Table III).

One treatment-related death was reported in the DCarbo group. Acute respiratory distress syndrome (ARDS) developed in a 76-year-old woman two months after the end of the fifth, final cycle of treatment. Five days after the onset of respiratory failure, the patient had an acute myocardial infarction and died two days later. The patient's attending physician judged that the relation to treatment was "not definite." An independent data monitoring committee judged

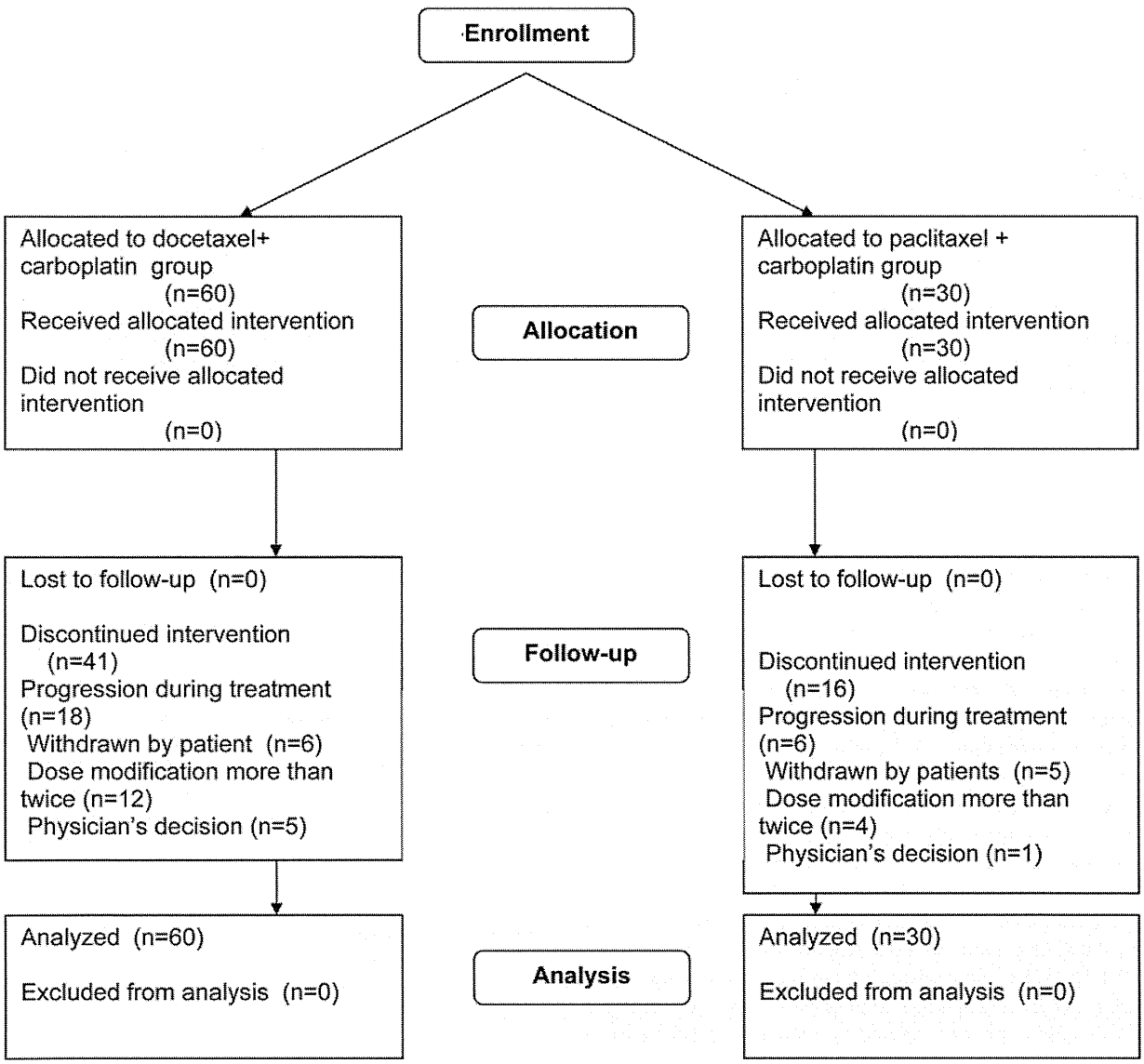

Figure 1. *Study design and patient flow. n: Number of patients.*

that the relation of death to the study treatment was not definite, but possible.

Discussion

This randomized phase II trial comparing DCarbo with PCarbo is the first of this kind to be performed in Asia. Our results suggest that both regimens are similar in terms of PFS and overall survival. The PFS of 4.8 (95% CI=3.9-7.2) months and MST of 17.6 (95% CI=10.2-22.9) months in the DCarbo group were favorable.

Asian ethnicity may contribute to some degree to better results in patients who receive DCarbo, as reported by Millward *et al.* (8). Three large phase III trials performed on Japanese patients with advanced NSCLC have included paclitaxel + carboplatin as one treatment arm (15-17). In these studies, the number of patients who received PCarbo was 281 (Okamoto *et al.*) (15), 197 (JMTO LC 00-03 study) (16), and 145 (Four-Arm Cooperative Study) (17), respectively. The dose of carboplatin was AUC 6 mg/ml min, with paclitaxel given at a dose of 200 mg/m^2 in two studies (15, 17) and 225 mg/m^2 in the other (16). The median PFS

Table I. *Patients' characteristics.*

	Docetaxel + carboplatin c (%) (n=60)	Paclitaxel + arboplatin (%) (n=30)
Age (median) (years)	67.5	65.5
Male/female	43/13 (78/22)	22/8 (73/27)
Body weight loss>5% Yes /no	11/49 (18/82)	5/25 (17/83)
Performance status 0/1	19/41 (32/68)	7/23 (23/77)
Histology Sq/Ad/La/Other	13/36/2/9 (22/60/3/15)	10/17/0/3 (33/57/0/10)
Stage IIIB/IV	24/36 (40/60)	10/20 (33/67)
Naïve/relapsed	53/7 (88/12)	26/4 (87/13)
LDH Normal/abnormally high	44/16 (73/27)	21/9 (70/30)
Prior radiotherapy	3 (5)	3 (10)

Sq: Squamous cell carcinoma, Ad: adenocarcinoma, La: large cell carcinoma, LDH: lactate dehydrogenase.

Table II. *Overall response and survival data.*

Regimen	Docetaxel + carboplatin	Paclitaxel + carboplatin
Number of patients	60	30
Response rate (95%CI)	23% (13-36%)	33% (17-53%)
Median PFS (95% CI), months	4.8 (3.9-7.2)	5.1 (4.4-6.4)
PFS rate (90% CI)*	42% (31-52)	40% (25-54)
HR (95% CI)	0.86 (0.55-1.36)	Referent
Median OS (95% CI), months	17.6 (10.2-23.0)	15.5 (9.4-20.8)
HR (95% CI)	0.77 (0.47-1.26)	Referent
1-Year survival rate (90% CI)	60% (49-70)	60% (44-73)

MST: Median survival time, CI: confidence interval, HR: hazard ratio, PFS: progression-free survival , OS: overall survival. *At six months.

Table III. *Toxicities experienced during study period.*

Toxicity	Docetaxel+ carboplatin % (95% CI) N=60	Paclitaxel+ carboplatin % (95% CI) N=30
Grade 3 or more Neutropenia	88 (77-95)	60 (41-77)
Grade 3 or more Anemia (hemoglobin)	12 (5-23)	7 (1-22)
Grade 3 or more Thrombocytopenia	0	3 (0-17)
Grade 3 or more Frbrile neutropenia	17 (8-29)	13 (4-31)
Grade 2 or more Nausea	28 (18-41)	17 (6-35)
Grade 2 or more Vomiting	12 (5-23)	10 (2-27)
Grade 2 or more Sensory neuropathy	3 (0-12)	37 (20-56)
Grade 2 or more Myalgia	0	13 (4-31)
Grade 2 or more Arthralgia	2 (0-9)	20 (8-39)
Possible TRD (ARDS)	1	0

CI: Confidence interval, TRD: treatment-related death, ARDS: acute respiratory distress syndrome.

or time to progression was 4.8, 5.8, and 4.5 months, and the MST was 13.3, 14.1, and 12.3 months, respectively. These results are similar to those of the present trial, obtaining a PFS of 5.1 months and an MST of 15.6 months, and suggest that Japanese patients have a good response to taxane-based chemotherapy. C1236T polymorphism in the ATP-binding cassette sub-family B member-1 (*ABCB1*) gene is significantly related to docetaxel clearance (18). Gandara *et al*. reported ethnic differences in the metabolism of taxanes between American and Japanese patients with lung cancer in a common-arm analysis of PCarbo, performed jointly in the United States and Japan (19).

Differences in the allelic distribution of genes involved in paclitaxel disposition or DNA repair [cytochrome *P450* 3A4 (CYP3A4)*1B and excision repair cross-complementation group 2 (*ERCC2*) K751Q] were observed between Japanese and American patients. Resulting metabolic differences in taxane metabolism may consequently contribute to better outcomes in Asian patients with lung cancer who receive taxanes.

In our study the dose of docetaxel was 60 mg/m^2 and that of carboplatin was AUC 6 mg/ml min. This dose of docetaxel is generally used in Japan to treat NSCLC. When combined with cisplatin, the dose of docetaxel used in Japan may be slightly lower the one that used in other countries (6). However, the results of Japanese studies in terms of PFS or overall survival are not inferior to those of studies performed in other countries, where docetaxel is usually given at a dose of 75 mg/m^2 (7). On the other hand, most Japanese studies have used cisplatin at a dose of 80 mg/m^2, which is slightly higher than that used in other countries (75 mg/m^2). The modest differences in the doses of chemotherapeutic agents may not have had a major influence on PFS or overall survival. Brunetto *et al*. reported that the dose intensity of platinum-doublet regimens including cisplatin or carboplatin with either vinorelbine or gemcitabine did not have an impact on survival or time-to-progression in patients with NSCLC (20).

A phase III study comparing DCarbo with PCarbo as first-line chemotherapy was performed in 1,077 patients with ovarian cancer (21). Docetaxel (75 mg/m^2) or paclitaxel (175 mg/m^2) with carboplatin to (AUC 5 mg/ml min) was administered every three weeks for six cycles.

The study also concluded that DCarbo is similar to PCarbo in terms of PFS and response, but recommended that longer follow-up is required before making a definitive statement on survival. DCarbo was considered an alternative first-line regimen for chemotherapy in patients with ovarian cancer. As for toxicity, DCarbo was associated with

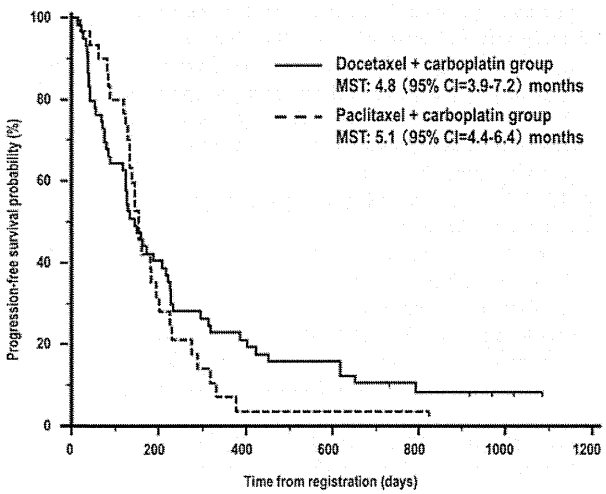

Figure 2. *Progression-free survival. MST: Median survival time, CI: confidence interval.*

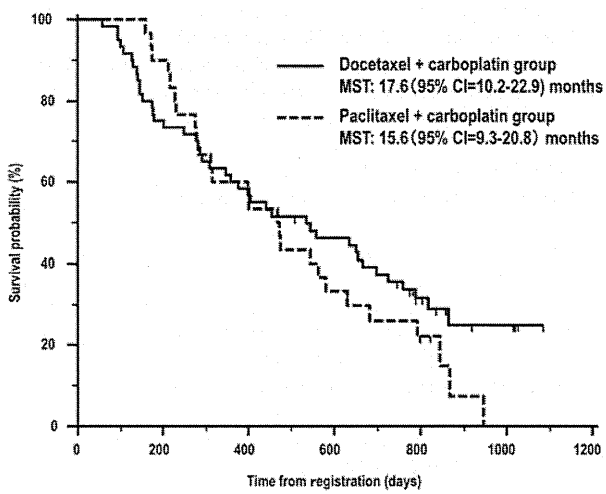

Figure 3. *Overall survival. MST: Median survival time, CI: confidence interval.*

substantially less overall and grade 2 or more neurotoxicity than PCarbo. On the other hand, DCarbo led to a higher incidence of grade 3 or 4 neutropenia than did PCarbo. Similar trends were noted in our study: DCarbo had a lower incidence of grade 2 or more sensory neuropathy (3% *vs.* 37%), but a higher incidence of grade 3 or more neutropenia (87% *vs.* 60%) as compared with PCarbo. Although myelosuppression was also frequently associated with DCarbo in our study, this adverse effect was not dose-limiting.

Recently, the survival of patients with NSCLC has improved, in part because of improved treatments or perhaps because of selection bias. The longer the survival, the more problematic is chronic toxicity such as neurotoxicity. Such toxicity negatively affects the quality of life of patients with NSCLC. This is especially true for those tested with PCarbo regimens (22). Even if the dose of paclitaxel is reduced from 225 mg/m^2 to 200 mg/m^2, the problem of neurotoxicity persists. DCarbo would, thus, be the preferred regimen to avoid severe neurotoxicity.

The treatment-related death in the DCarbo group in our study was reviewed by a safety committee. ARDS occurred as late as two months after the end of the patient's fifth, final cycle of treatment. The relation of death to chemotherapy with DCarbo was considered not definite, but possible.

Our study had several important limitations. We studied only Japanese patients, and it remains unclear whether our results can be extrapolated to other ethnic groups. Our study group comprised of patients with all histological types of NSCLC, and information on mutations in the *EGFR* gene was not obtained. In addition, the doses of docetaxel and carboplatin differed from those used in Western studies of patients with NSCLC.

Conclusion

Docetaxel plus carboplatin is considered an alternative first-line chemotherapeutic regimen for patients with newly-diagnosed advanced NSCLC, at least in Asia. In the future, this regimen might be combined with other treatments, such as molecular targeted therapy.

Conflicts of Interest

None.

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ORIGINAL ARTICLE

Evaluating the 21-gene assay Recurrence Score® as a predictor of clinical response to 24 weeks of neoadjuvant exemestane in estrogen receptor-positive breast cancer

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Abstract
Background The aim of this study was to investigate the association between the results of the Recurrence Score (RS) assay and the clinical response to neoadjuvant endocrine therapy in postmenopausal women with breast cancer.
Methods Core biopsy samples at baseline and post-treatment surgical samples were obtained from 80 and 77 of 116 patients, respectively, enrolled in the multicenter prospective study of neoadjuvant exemestane therapy (JFMC34-0601). The 21-gene assay was performed after appropriate manual microdissection. The estrogen receptor (ER), progesterone receptor, HER2 and Ki-67 were assayed by immunohistochemistry at a central laboratory. Clinical response was assessed based on the RECIST (Response Evaluation Criteria In Solid Tumors) guideline.
Results Sixty-four core biopsy samples and 52 resection samples met the RS quality requirements. The clinical response rate in those patients with a low RS result (low RS group; 19/32, 59.4 %) was significantly higher than that in those patients with a high RS result (high RS group; 3/15, 20.0 %) ($P = 0.015$) and similar to that in patients with an intermediate RS result (intermediate RS group; 10/17, 58.8 %). The rates of breast-conserving surgery (BCS) were 90.6 % (29/32) in the low RS group, 76.5 % (13/17) in the intermediate RS group and 46.7 % (7/15) in the high RS group. The odds ratio for BCS adjusted for continuous

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baseline Ki-67 was 0.114 [95 % confidence interval (CI) 0.014–0.721; $P = 0.028$] between the high and low RS groups. RS values in pre-treatment samples were highly correlated with those in post-treatment samples (Spearman correlation coefficient 0.745, 95 % CI 0.592–0.846).

Conclusion Our results demonstrate the predictive value of the RS for clinical response to neoadjuvant exemestane therapy in postmenopausal women with ER-positive breast cancer.

Keywords Recurrence Score · Neoadjuvant endocrine therapy · Ki-67 · Clinical response · Breast-conserving surgery rate

Introduction

There are several potential advantages to neoadjuvant therapy of breast cancer in terms of improving outcomes in women with operable and inoperable early-stage disease [1, 2]. Both neoadjuvant chemotherapy and endocrine therapy have been shown to enable less extensive resection and improve rates of breast-conserving surgery (BCS) [3–6]. The ACOSOG Z1031 trial, which compared three aromatase inhibitors (AIs) in neoadjuvant settings, showed that 51 % (81/159) of the patients who were designated candidates for mastectomy experienced downstaging to BCS [7]. Neoadjuvant endocrine therapy is now an acceptable option for postmenopausal patients with endocrine-responsive disease [8].

Despite the use of standard biomarkers, the considerable heterogeneity of response to therapy still represents a challenge to clinicians in terms of choosing the most suitable neoadjuvant therapy. As such, tools to improve the identification of those patients who will respond to therapy would represent a major clinical advance. Although the Ki-67 labeling index (LI) shows some consistency in predicting response to chemotherapy, its ability to predict response to neoadjuvant endocrine therapy is controversial [9, 10].

We previously reported results from a neoadjuvant exemestane study in postmenopausal women [11]. In that study, the target response rate was 51 % (59/116), and 40 (77 %) of 59 patients who would have required mastectomy were converted to BCS. Neither baseline Ki-67 LI nor changes in Ki-67 LI were associated with clinical response in the study.

The Onco*type* DX® assay (Genomic Health, Redwood City, CA) has been shown to be able assess recurrence risk in women with hormone receptor-positive (HR+), lymph node-negative or -positive, early stage breast cancer who are treated with adjuvant endocrine therapy [12–15]. It has also been shown to predict the likelihood of benefit from adjuvant chemotherapy [12, 16]. Accordingly, the assay is included in clinical guidelines for use in patients with HR+ lymph node-negative disease; however, its applicability to HR+ postmenopausal women with lymph node positive disease is considered controversial, pending results of the RxPONDER trial [8, 17–19]. Additionally, studies in the neoadjuvant setting have shown that the test can be used to predict the response to chemotherapy [20, 21]. More recently, a study suggested that the Recurrence Score (RS) value may predict responses to neoadjuvant endocrine therapy with either tamoxifen or anastrozole [22]. The Onco*type* DX assay may improve the clinician's ability to discriminate between clinically similar tumors based on the tumor's underlying biology. Consequently, the aim of this study was to investigate the clinical usefulness of the RS assay results in the prediction of response to neoadjuvant endocrine therapy.

Methods

Study design

This was a prospectively designed study using archived tumor tissues from the previously conducted JFMC34-0601 study. The primary objective was to assess the association between the results of the RS assay at baseline and clinical response, by comparing the response rates between patients with a low RS result (<18; low RS group) and those with a high RS result (≥31; high RS group). Secondary objectives included assessment of the associations of continuous baseline RS, quantitative estrogen receptor (ER) by reverse transcriptase (RT)-PCR and Ki-67 with clinical response and with BCS, as well as associations of changes from baseline to post-treatment values of these markers with clinical response. The study protocol was approved by the Ethics Committee of each participating institution. Informed consent was obtained from all patients. The study was performed in accordance with the Helsinki Declaration.

Patient cohort and tumor samples

Eligibility criteria for the parent JFMC34-0601 study included age 55–75 years, ER+ and stage II or IIIa invasive breast cancer (T2-3, N0-2, M0). Patients were confirmed positive for ER or progesterone receptor (PgR) by immunohistochemistry (≥10 % nuclear staining). The study treatment was 25 mg/day exemestane for 16 weeks, with a possible 8-week extension based on the assessment of clinical response. Patients with progressive disease (PD) were withdrawn from the study. At week 24, patients underwent surgery, except those with PD, who had the option of selecting another treatment approach.

Clinical outcomes measures

Clinical response was assessed by comparing the longest diameter of the target lesions with the baseline measurement, based on the Response Evaluation Criteria in Solid Tumors (RECIST) guideline version 1.0, by caliper measurement of palpable lesions and ultrasound as previously described [11]. Briefly, complete response (CR) was defined by the disappearance of all target lesions; partial response (PR) by at least a 30 % decrease in the sum of diameters of the target lesions; PD by at least a 20 % increase in the sum of diameters of the target lesions; stable disease (SD) by neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.

Biomarker assessments

The Onco*type* DX® 21-gene assay was performed on core biopsy and resection samples by Genomic Health [14].

Immunohistochemistry assays of Ki-67, ER and PgR were performed at one central location and the results assessed by three independent pathologists as described previously [11]. In brief, immunohistochemistry staining was performed using a Histofine kit (Nichirei, Tokyo, Japan). Ki-67 was stained using the following antibody dilution: 1:100 (Dako, Glostrup, Denmark), and the Ki-67 LI was obtained by counting 500–1,000 tumor cells at the sites of hot spots. Ki-67 groups were defined post hoc as <10, 10–30 and >30 %, respectively. ER and PgR immunoreactivity were scored according to Allred's procedure.

Expression of HER2 was determined by the HercepTest (Dako, Glostrup, Denmark). Positive HER2 status was defined as either 3+ or 2+ with confirmed c-erbB2 gene amplification by the fluorescence in situ hybridization (FISH) test.

Statistical analyses

Analyses of baseline markers included all patients with an evaluable RT-PCR result from core biopsies. Analyses of changes from baseline to post-treatment markers included the subset of patients with results from both core biopsies and surgical resections. Changes in continuous markers were defined as "post-treatment value–pre-treatment value". In the primary analysis, the rates of clinical response were compared between the high and low baseline RS groups using Fisher's exact test. Logistic regression models were fit to both clinical response and surgery type. Odds ratio (OR) estimates are presented with Wald p values and 95 % confidence intervals (CIs). All P values are two-sided. In exploratory analyses, the Spearman rank correlation coefficient (and associated 95 % CI) was calculated for the baseline continuous RS and either the post-treatment RS or baseline continuous Ki-67 as determined by immunohistochemistry. A paired t test was applied to compare the baseline and post-treatment RS values. A two-sample t test was used to compare the percentage reduction in tumor size between the high and low RS groups. Fisher's exact test was used to compare the conversion rate from mastectomy to BCS among risk groups.

Results

A total of 116 patients were enrolled in JFMC34-0601 between March 2006 and December 2007, of whom 102 completed 24 weeks of neoadjuvant exemestane treatment [11]. Core biopsy and resection samples were obtained for 80 (69 %) and 77 (66 %) patients, respectively. Of the 157 samples sent for Onco*type* DX testing, two were deemed ineligible based on the blinded Genomic Health pathology review, insufficient RNA (<375 ng) was extracted from 18 samples (15 core biopsy and 3 resection samples), and standard quality metrics were not met for eight samples (all resections). This left 64 core biopsy samples, of which 52 had matching resection samples with evaluable RT-PCR results.

Baseline characteristics and clinical outcomes for the 64 patients are shown in Table 1. Forty-nine (76.6 %) patients had BCS, and 32 patients (50 %) had been candidates for BCS before the treatment. Four patients refused surgery after exemestane therapy and are treated as not BCS patients.

In the primary analysis, the clinical response rate in the low RS group (19/32, 59.4 %) was significantly higher than that in the high RS group (3/15, 20.0 %) ($P = 0.015$) (Table 2). The clinical response rate in the intermediate risk group (10/17, 58.8 %) was similar to that in the low risk group. Logistic regression revealed that the OR for clinical response between the intermediate and low RS groups was 0.977 (95 % CI 0.296–3.233, $P = 0.970$) and that the OR between the high and low RS groups was 0.171 (95 % CI 0.040–0.728, $P = 0.017$). In an exploratory analysis, the percentage reduction in tumor size determined by ultrasound was compared between the low and high RS groups. Patients in the low RS group showed an average reduction in tumor size of 31.8 % while those in the high RS group showed an average reduction of 12.5 %; this difference was significant between the groups ($P = 0.045$). The average reduction (27.6 %) in patients in the intermediate risk group was similar to that in the low risk group.

When treated as a continuous variable, the baseline RS Score was significantly associated with clinical response in a logistic regression analysis ($P = 0.042$; Table 3). There

Table 1 Baseline patient characteristics and clinical outcomes ($n = 64$)

Feature	*n* (%)
Age (years)	
55–64	34 (53.1)
65–74	25 (39.1)
75–77	5 (7.8)
Tumor stage at baseline	
T2	62 (96.9)
T3	2 (3.1)
Stage	
IIA	47 (73.4)
IIB	15 (23.4)
IIIA	2 (3.1)
ER by IHC (Allred score)	
4	1 (1.6)
5	3 (4.7)
6	5 (7.8)
7	14 (21.9)
8	41 (64.1)
ER status by RT-PCR	
ER− ($\leq 6.5 C_T$)	1 (1.5)
ER+ ($>6.5 C_T$)	63 (98.4)
PgR by IHC (Allred score)	
0	4 (6.25)
4	7 (10.94)
5	4 (6.25)
6	8 (12.5)
8	12 (18.75)
NE	10 (15.63)
PgR status by RT-PCR	
PgR− (≤ 5.5 C_T)	14 (21.9)
PgR+ (>5.5 C_T)	50 (78.1)
HER2 by IHC/FISH	
Negative	50 (78.1)
Positive	2 (3.1)
Unknown	12 (18.8)
RS risk group	
Low (<18)	32 (50.0)
Intermediate (18–30)	17 (26.6)
High (≥31)	15 (23.4)
Ki-67 by IHC (%)	
<10	28 (43.8)
10–30	23 (35.9)
>30	13 (20.3)
Clinical response	
Complete response (CR)	0
Partial response (PR)	32 (50.0)
Stable disease (SD)	24 (37.5)
Progressive disease (PD)	5 (7.8)
NE	3 (4.7)
Surgery type	
Breast-conserving	49 (76.6)
Mastectomy	11 (17.2)
No surgery	4 (6.3)

ER estrogen receptor, *IHC* immunohistochemistry, *RT* reverse transcriptase, *PgR* progesterone receptor, *NE* not evaluable, *FISH* fluorescence in situ hybridization, C_T cycling threshold score, *RS* recurrence Score

was a trend between continuous baseline ER as determined by RT-PCR and clinical response ($P = 0.076$). Continuous baseline Ki-67 by IHC was not associated with clinical response ($P = 0.273$).

The associations between changes from baseline to post-treatment values of continuous markers and clinical response were examined in logistic regression analyses. Changes in the RS, ER as determined by RT-PCR, and Ki-67 as determined by IHC were not associated with clinical response ($P = 0.240$, 0.343 and 0.629, respectively).

Analysis of the RS categories and BCS is shown in Table 2. The OR for BCS between the intermediate and low RS groups was 0.336 (95 % CI 0.066–1.722, $P = 0.19$) and that between the high and low RS groups was 0.091 (95 % CI 0.019–0.432, $P = 0.003$). The logistic regression analyses of continuous baseline RS, ER by RT-PCR and Ki-67 by IHC with BCS are shown in Table 3. The continuous baseline RS was significantly associated with BCS in both the unadjusted ($p = 0.001$) and covariate-adjusted (for tumor size and PgR) ($P = 0.004$) analyses. The continuous baseline ER by RT-PCR was also significantly associated with BCS in both the unadjusted ($P = 0.001$) and covariate-adjusted ($P = 0.023$) analyses. Continuous baseline Ki-67 by IHC was significantly associated with BCS in the unadjusted analysis ($P = 0.024$) but lost its significance when adjusted for tumor size and PgR ($P = 0.060$). When both the continuous RS values and continuous Ki-67 were included in the logistic regression model for BCS, the RS retained its statistical significance ($P = 0.012$) whereas Ki-67 did not ($P = 0.868$). The conversion rate from mastectomy planned at baseline to BCS performed after the treatment was 88 % (15/17) in the low RS group, 70 % (7/10) in the intermediate RS group and 20 % (1/5) in the high RS group. The rate was significantly different among groups ($P = 0.010$).

The associations between RS and Ki-67, and their respective and joint associations with BCS were examined in exploratory analyses. Figure 1a shows a scatterplot of baseline Ki-67 as determined by IHC versus the baseline RS results. The Spearman correlation coefficient was 0.672 (95 % CI 0.506–0.785). All patients with PD had a high RS

Table 2 Clinical response and breast-conserving surgery according to categorical baseline Recurrence Score

RS risk group	Clinical response		
	Proportion (response rate)[a] (%)	Odds ratio (95 % CI)	*P* value
Low (RS <18)	19/32 (59.4)	1	n/a
Intermediate (RS 18–30)	10/17 (58.8)	0.977 (0.296, 3.233)	0.970
High (RS ≥31)	3/15 (20.0)	0.171 (0.040, 0.728)	0.017
RS risk group	Breast-conserving surgery		
	Proportion (BCS rate) (%)	Odds ratio (95 % CI)	*P* value
Low (RS <18)	29/32 (90.6)	1	n/a
Intermediate (RS 18–30)	13/17 (76.5)	0.336 (0.066, 1.722)	0.19
High (RS ≥31)	7/15 (46.7)	0.091 (0.019, 0.432)	0.003

Data are presented as the number of patients with the percentage in parenthesis

CI confidence interval, *BCS* breast-conserving surgery, *n/a* not available

[a] Primary analysis: $P = 0.015$ by Fisher's exact test for comparison of clinical response rates between the low and high RS groups

Table 3 Continuous baseline Recurrence Score and estrogen receptor by reverse transcriptase-PCR and Ki-67 by immunohistochemistry and clinical response and breast-conserving surgery

Endpoint/analysis	Continuous marker					
	RS (50 units)		ER by RT-PCR (log2 increase)		Ki-67 by IHC (%)	
	Odds ratio (95 % CI)	*P* value	Odds ratio (95 % CI)	*P* value	Odds ratio (95 % CI)	*P* value
Clinical response/unadjusted	0.205 (0.044, 0.946)	0.042	1.436 (0.963, 2.141)	0.076	0.981 (0.948, 1.015)	0.273
BCS/unadjusted	0.055 (0.009, 0.323)	0.001	1.786 (1.150, 2.774)	0.001	0.957 (0.921, 0.994)	0.024
BCS/covariate-adjusted[a]	0.016 (<0.001, 0.259)	0.004	1.881 (1.090, 3.245)	0.023	0.953 (0.907, 1.002)	0.060

RT reverse transcriptase

[a] Adjusted for tumor size and PgR Allred score, which were significantly associated with BCS in the univariable analyses

(range 32–73) while three of five PD patients had an intermediate Ki-67 LI (Fig. 1a).

No statistically significant difference was observed between baseline and post-treatment RS values ($P = 0.484$). A scatterplot is shown in Fig. 1b. The Spearman correlation analysis showed a high correlation (correlation coefficient 0.745, 95 % CI 0.592–0.846).

Discussion

In this study, we demonstrated the predictive value of the RS results for response to neoadjuvant endocrine therapy. Among our patient cohort, those with low scores showed a better response to neoadjuvant endocrine therapy than those with high scores. Since patients with high RS results have been shown to benefit from chemotherapy, the 21-gene assay may provide additional information that could facilitate the selection of neoadjuvant treatment with endocrine therapy for cancer patients with a low RS and chemotherapy for those with a high RS.

ER Allred scores have been reported to correlate with response rates to neoadjuvant letrozole or tamoxifen. The P024 trial of neoadjuvant letrozole or tamoxifen showed that tumors with low ER Allred scores still responded to letrozole [23]. Conversely, some tumors with higher ER levels did not respond to endocrine therapy [23, 24]. Gene expression-based profiles categorize HR+, HER2− breast cancers into two subtypes: luminal-A and -B [25]. However, the classification, which is based on PAM50, has been reported not to relate to clinical response or the likelihood of BCS after neoadjuvant AI treatment [7].

In our study, the RS was the only predictive factor for clinical responses to neoadjuvant endocrine therapy and the most potent predictive factor for BCS in the covariate-adjusted analysis. These results are consistent with those from other studies which suggest that a low RS can predict benefit from endocrine therapy [22, 24]. The study by Kim et al. [24] compared the outcomes of the tamoxifen and

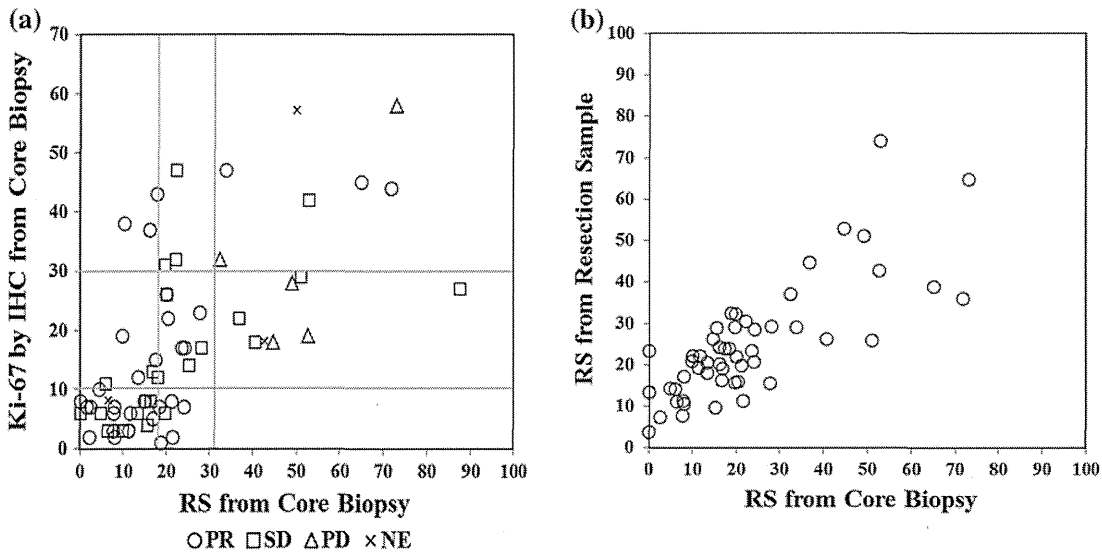

Fig. 1 **a** Scatterplot of the baseline Recurrence score (*RS*) and baseline Ki-67, with the Spearman correlation coefficient. The Spearman correlation coefficient between the baseline RS and baseline Ki-67 was 0.672 [95 % confidence interval (CI) 0.506–0.785]. None of five patients with tumor progression was in the low or intermediate RS groups. **b** Scatterplot of the baseline RS and post-treatment RS, with the Spearman correlation coefficient. The baseline RS was highly correlated with RS in the post-treatment samples (Spearman correlation coefficient 0.745, 95 % CI 0.592–0.846). *PR* Partial response, *SD* stable disease, *PD* progressive disease, *NE* Not evaluable

placebo arms of the NSABP B14 trial and demonstrated that higher levels of quantitative ER expression, as determined by RT-PCR, correlated with a greater benefit from adjuvant tamoxifen, as measured by distant recurrence.

Our results indicate that the values of the RS before and after endocrine therapy were highly correlated. Since a number of studies have suggested that post-treatment biomarkers such as Ki-67 LI and ER have better prognostic values than pre-treatment biomarkers, post-treatment biomarkers are receiving increasing interest in clinical trials as a tool for patient stratification [26–28]. Dowsett et al. [26] reported the results of an unplanned, exploratory investigation of the relationship between post-treatment Ki-67 (2 weeks) and recurrence-free survival (RFS) using archived tumors from the IMPACT study. Their results indicate that post-treatment Ki-67, larger baseline tumor size and post-treatment ER level are significantly correlated with DFS. Ellis et al. [27] analyzed the ability of post-treatment Ki-67 and other factors (tumor size, grade, nodal status, and post-treatment ER expression) to predict RFS and breast cancer-specific survival using archived tumors from the P024 study. Another interesting study (ACOSOG Z1031, Cohort B) has been conducted to determine whether patients with a high Ki-67 value after 2 weeks of neoadjuvant AI treatment show a higher than expected pathogenic CR rate to neoadjuvant chemotherapy than would be typically observed for those patients with unselected ER-rich tumors. The results will tell us whether an assessment of Ki-67 2 weeks after neoadjuvant AI treatment will be useful for the identification of a chemotherapy-sensitive subgroup of ER+ tumors. However, even if this is the case, intervention of a 2-week AI treatment and re-biopsy are necessary. Although further investigations are needed, the comparative stability of the RS would improve the overall decision-making process regarding the complete treatment before the initiation of treatment.

The main limitation of this was its small sample size. The availability of tumor samples from the parent study was limited and recovery of mRNA was not uniformly adequate. Further investigation in larger prospective studies would better define candidates for neoadjuvant endocrine therapy. Another limitation was the absence of any assessment of lymph node response. Although nodal response is clinically relevant, one of the major purposes of neoadjuvant endocrine therapy is improvement in surgical outcome. That said, however, the clinical response at the primary site and the BCS rate are also of clinical importance for the assessment of the effect of neoadjuvant endocrine therapy.

In conclusion, this study showed that RS results have predictive value for the clinical response to neoadjuvant exemestane therapy. The 21-gene assay would appear to be a promising tool for providing useful information to guide the clinician in choosing neoadjuvant treatment for systemic therapy, with neoadjuvant endocrine treatment for patients with low RS disease and neoadjuvant chemotherapy treatment for patients with high RS disease.

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Conflict of interest The authors declare that they have no conflict of interest.

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Original article

Sarcopenia as a predictor of prognosis in patients following hepatectomy for hepatocellular carcinoma

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Background: Sarcopenia was identified recently as a poor prognostic factor in patients with cancer. The present study investigated the effect of sarcopenia on short- and long-term outcomes following partial hepatectomy for hepatocellular carcinoma (HCC), and aimed to identify prognostic factors.

Methods: Data were collected retrospectively for all consecutive patients who underwent hepatectomy for HCC with curative intent between January 2004 and December 2009. Patients were assigned to one of two groups according to the presence or absence of sarcopenia, assessed by computed tomographic measurement of muscle mass at the level of the third lumbar vertebra. Clinicopathological, surgical outcome and long-term survival data were analysed.

Results: Sarcopenia was present in 75 (40·3 per cent) of 186 patients, and was significantly correlated with female sex, lower body mass index and liver dysfunction, as indicated by abnormal serum albumin levels and indocyanine green retention test at 15 min values. In patients with, and without sarcopenia, the 5-year overall survival rate was 71 and 83·7 per cent respectively, and the 5-year recurrence-free survival rate was 13 and 33·2 per cent respectively. Multivariable analysis revealed that reduced skeletal muscle mass was predictive of an unfavourable prognosis.

Conclusion: Sarcopenia was predictive of worse overall survival even when adjusted for other known predictors in patients with HCC after partial hepatectomy.

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world[1,2]. As a consequence of advances in the diagnosis and management of HCC, major improvements in overall and disease-free survival rates for HCC after partial hepatectomy have been achieved. However, even when curative resection is performed, a considerable number of patients develop intrahepatic or extrahepatic recurrence[3,4]. The prognostic assessment of patients with HCC after hepatic resection and recurrence is an important clinical issue in this population[5–7]. Both tumour- and host-related factors are related to clinical outcome, and general condition and liver function are important in this context. Unfortunately, it is difficult to evaluate the general condition of patients excluding liver function before hepatectomy. Conventional methods, such as the Child–Pugh classification, have been used initially to determine the severity of cirrhosis and to select patients who might tolerate hepatic resection. However, these methods do not reflect the patient's general condition. The American Society of Anesthesiologists (ASA) grade was reported to predict the prognosis of HCC after hepatectomy[8], but this classification is not always objective.

Recently, loss of skeletal muscle mass, termed sarcopenia, was identified as a poor prognostic factor for patients with pancreatic cancer, colorectal liver metastases, melanoma, liver cirrhosis and liver transplantation[9–14]. Sarcopenia is a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength, with a risk of adverse outcomes such as physical disability, poor quality of life and death[15,16]. To date, there have been no reports on the relationship between sarcopenia and the prognosis of patients with HCC following hepatic resection.