Clinical Outcome of Chemoradiation Therapy in Patients with Limited-Disease Small Cell Lung Cancer with Ipsilateral Pleural Effusion

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Background: The indications for definitive thoracic radiotherapy (TRT) in limited-disease small cell lung cancer (LD-SCLC) and ipsilateral pleural effusion have not been thoroughly investigated. We retrospectively investigated the clinical outcome of LD-SCLC patients with ipsilateral pleural effusion.

Methods: The medical records of SCLC patients who received treatment at the National Cancer Center Hospital East between July 1992 and December 2006 were reviewed. Sixty-three of the 373 LD-SCLC patients (17%) had ipsilateral pleural effusion. Of these, 62 patients received chemotherapy as an initial treatment, and were included in this study. Since about 1998, definitive TRT was routinely performed if the patient's pleural effusion disappeared after induction chemotherapy. The 62 patients were divided into three subgroups: group A included patients who received chemotherapy and TRT ($n = 26$), group B included patients who did not receive TRT in spite of the disappearance of pleural effusion after first-line chemotherapy ($n = 8$), and group C included patients who did not receive TRT and whose pleural effusion persisted after first-line chemotherapy ($n = 28$).

Results: The response rate for first-line chemotherapy was 74%. Ipsilateral pleural effusion disappeared after first-line chemotherapy in 34 patients (55%). The median overall survival time was 11.8 months, and the 2 and 3-year survival rates were 21 and 10%, respectively. In groups A, B, and C, the median survival times were 19.2, 10.5, and 9.2 months, respectively, and the 2-year survival rates were 38, 25, and 7%, respectively.

Conclusion: Long-term survival was achieved by LD-SCLC patients with ipsilateral pleural effusion who successfully underwent chemoradiotherapy.

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Lung cancer is the leading cause of cancer-related deaths worldwide. In Japan, over 56,000 people died of lung cancer in 2003. Small cell lung cancer (SCLC) accounts for about 15% of all forms of lung cancer. SCLC has a more aggressive biologic behavior than non-small cell lung cancer. At the time of presentation, two-thirds of patients exhibit disseminated disease. SCLC is sensitive to chemotherapy, with a response rate of 70 to 80%. A clinical two-stage system proposed by the Veterans Administration Lung Study Group distinguishes limited-disease (LD) and extensive-disease (ED) in SCLC.[1] LD is defined as being limited to one hemithorax, including mediastinal, contralateral hilar, and ipsilateral supraclavicular lymph nodes, whereas ED represents tumor spread beyond these regions. The current standard care for LD-SCLC is a combination of chemotherapy and thoracic radiotherapy (TRT). On the other hand, ED-SCLC is treated with chemotherapy alone. The original definition of LD was a tumor volume that could be encompassed by a reasonable radiotherapy plan. According to the International Association for the Study of Lung Cancer (IASLC)'s consensus report, on the other hand, the classification of LD-SCLC includes bilateral hilar and/or supraclavicular nodal involvement and ipsilateral pleural effusion.[2] However, the indication for definitive TRT in patients with LD-SCLC and ipsilateral pleural effusion have not been thoroughly investigated. Recently, the IASLC proposed the seventh edition of the tumor, node, metastasis (TNM) classification for lung cancer. In the proposals, the presence of a pleural effusion is considered as M1 disease.[3–6]

Definitive TRT is contraindicated in lung cancer patients with malignant pleural effusion. We have sometimes treated SCLC cases in which the ipsilateral pleural effusion disappeared after induction chemotherapy. Should definitive TRT be indicated in SCLC patients if the ipsilateral pleural effusion disappears after induction chemotherapy? Since about 1998, we have routinely performed definitive TRT if the patient's pleural effusion disappeared after induction chemotherapy. In this retrospective study, we investigated the clinical course and outcome of LD-SCLC patients with ipsilateral pleural effusion and exam-

ined the overall survival in patients who received chemotherapy and TRT, comparing with that of ED-SCLC or LD-SCLC patients without ipsilateral pleural effusion. We also applied the proposed seventh edition of the TNM stage to our cohort.

PATIENTS AND METHODS

We retrospectively reviewed the medical records of lung cancer patients who received treatment at the National Cancer Center Hospital East between July 1992 and December 2006. During this period 699 patients were newly diagnosed as having SCLC. Three-hundred and seventy-three patients were diagnosed as having LD-SCLC, and 326 were diagnosed as having ED-SCLC using conventional staging procedures, including a medical history and physical examination, chest radiography, computed tomography (CT) scan of the chest, CT scan or ultrasound of the abdomen, bone scan, and CT scan or magnetic resonance imaging of the brain. In this study, LD-SCLC was defined as disease limited to one hemithorax, including mediastinal, contralateral hilar, and supraclavicular lymph nodes, ipsilateral pleural effusion, and pericardial effusion; ED-SCLC was defined as tumor spread beyond these manifestations.[2] Sixty-three of the 373 LD-SCLC patients (17, 95% confidence interval (CI): 13–21%) had ipsilateral pleural effusion. Thirty-seven SCLC patients underwent surgical resection as an initial treatment, and 13 patients received only TRT and/or best supportive care. Remaining 649 patients received chemotherapy as an initial treatment. Of these, 62 LD-SCLC patients had ipsilateral pleural effusion, and were included in this study. The patient characteristics are shown in Table 1. The breadth of the pleural effusion was measured using a CT scan of the chest (Figure 1). Cytologic examination of the pleural effusion prior to treatment was performed in 26 patients. Eleven patients had cytologically positive effusion. Ten patients also had pericardial effusion. Three patients had solid pleural tumor and pleural effusion detected on CT scan. Twenty-six patients had atelectasis. Of these, 14 patients received cytologic examination of the pleural effusion, and four patients had cytologically positive effusion.

We collected clinical data on the patients from their medical records; this data included the chemotherapy regimen that was received, the response to first-line chemotherapy, whether pleural effusion disappeared after first-line chemotherapy, and whether the patient underwent definitive TRT. The World Health Organization's response criteria were used.[7]

Overall survival was defined as the interval between the start of treatment and death or the final follow-up visit. Median overall survival was estimated using the Kaplan-Meier analysis method.[8] Survival data was compared among groups using a log-rank test. The breadth of pleural effusion was compared using the Mann-Whitney U test. All reported p values are two-sided.

RESULTS

The induction chemotherapy regimens were shown in Table 2. Most common regimen was cisplatin or carboplatin plus etoposide. In LD patients with ipsilateral pleural effusion, there were three complete responses, 43 partial responses, seven no changes, and six progressive diseases. Response was not evaluated in three patients because of early death. The response rate was 74% (95% CI: 62–84%). Ipsilateral pleural effusion disappeared after first-line chemotherapy in 34 patients (55, 95% CI: 42–68%).

TABLE 1. Patient Characteristics

	LD-SCLC without Ipsilateral Pleural Effusion	LD-SCLC with Ipsilateral Pleural Effusion	ED-SCLC
No. of patients	270	62	317
Sex			
Male	226	50	262
Female	44	12	55
Age, yr			
Median	66	67	66
Range	38–87	46–79	28–85
Performance status			
0	71	2	20
1	178	45	203
2	14	10	59
3	6	5	28
4	1	0	7
Breadth of pleural effusion on CT scan, cm			
Median		2.3	
Range		0.5–9.4	
Cytology of pleural effusion			
Positive		11	
Negative		15	
Not examined		36	

Patients who received chemotherapy as an initial treatment were included.
LD, limited-disease; SCLC, small cell lung cancer; ED, extensive-disease; CT, computed tomography.

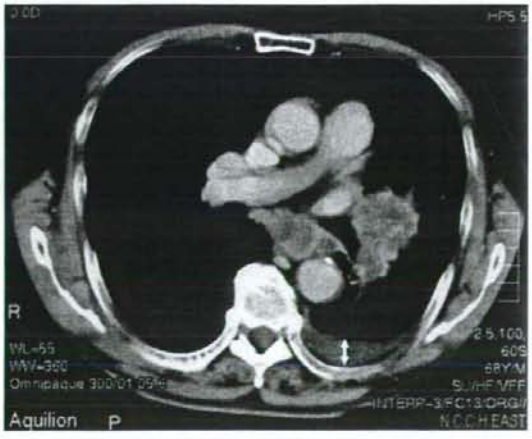

FIGURE 1. Ipsilateral pleural effusion. The arrow indicates the breadth of pleural effusion.

TABLE 2. Induction Chemotherapy Regimens and Response

	LD-SCLC without Ipsilateral Pleural Effusion	LD-SCLC with Ipsilateral Pleural Effusion	ED-SCLC
Chemotherapy regimens			
Platinum + ETP	252	54	154
Cisplatin and irinotecan containing regimens	10	2	92*
CODE	7	5	52
CAV/PE	1	1	11
Other	0	0	8
Response			
CR	64	3	28
PR	189	43	213
NC	8	7	37
PD	5	6	18
NE	4	3	21
Response rate (%) (95% CI)	94 (90–96)	74 (62–84)	76 (71–81)

*Nine patients received chemotherapy of cisplatin and topotecan.

LD, limited-disease; SCLC, small cell lung cancer; ED, extensive-disease; ETP, etoposide; CODE, weekly cisplatin, vincristine, doxorubicin, plus etoposide; CAV/PE, cyclophosphamide, doxorubicin, plus etoposide alternating with cisplatin plus etoposide; CR, complete response; PR, partial response; NC, no change; PD, progressive disease; NE, not evaluable; CI, confidence interval.

Since about 1998, definitive TRT to the primary lesion and mediastinum was routinely performed in patients whose pleural effusion disappeared after chemotherapy. We divided the 62 patients in this study into three subgroups: group A included patients who received chemotherapy and TRT (n = 26), group B included patients who did not receive TRT in spite of the disappearance of pleural effusion after first-line chemotherapy (n = 8), and group C included patients who did not receive TRT and whose pleural effusion persisted after first-line chemotherapy (n = 28).

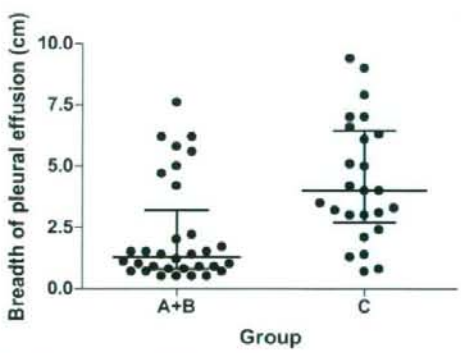

FIGURE 2. Breadth of pleural effusion in subgroup A + B, and C. Group A included patients who underwent chemotherapy and thoracic radiotherapy (TRT) (n = 26), group B included patients who did not undergo TRT in spite of the disappearance of pleural effusion after first-line chemotherapy (n = 8), and group C included patients who did not undergo TRT and whose pleural effusion persisted after first-line chemotherapy (n = 28). The line represents the median with the interquartile range.

The median (range) breadth of pleural effusion was 11.2 cm (0.5–7.6 cm) in group A, 1.8 cm (0.5–5 cm) in group B, and 4 cm (0.7–9 cm) in group C. Combining group A and B, the median breadth of pleural effusion was 1.3 cm, which was significantly lower than that of group C (p = 0.0007) (Figure 2).

In group A, all but two patients received platinum-based chemotherapy. One patient received weekly cisplatin, vincristine, doxorubicin, plus etoposide (PE) therapy, and the other patient received cyclophosphamide, doxorubicin, PE alternating with cisplatin PE therapy. Three of the 26 patients in group A underwent TRT (twice daily, 45 Gy in total) concurrently with the first course of chemotherapy. The breadths of pleural effusion in those three patients were 0.7, 0.8, and 1.0 cm. Two, seven, and one patient underwent TRT (once daily, 50 Gy in total) concurrently with the second, third, and fourth courses of chemotherapy, respectively. Thirteen patients underwent TRT (once daily, 50 Gy in total) sequentially after chemotherapy. Six patients received prophylactic cranial irradiation (PCI) of 25 Gy.

Figure 3*A* showed the survival of the all 699 SCLC patients by the proposed seventh edition of TNM stage. Figure 3*B* showed the survival of the 649 SCLC patients who received chemotherapy as an initial treatment. The survival of LD patients with ipsilateral pleural effusion was intermediate between those of LD patients without effusion and ED patients ($p < 0.0001$). The median survival time in LD patients with ipsilateral pleural effusion was 11.8 months (95% CI: 9.2–16.6), and the 1, 2, 3 and 5-year survival rates were 48, 21, 10 and 8%, respectively. Four patients have survived for over 5 years. One patient had a cytologically negative pleural effusion, and cytologic examinations were not performed for the remaining three patients. Breadth of pleural effusion of these four patients ranged from 1.0 to 1.5 cm. Two of these four patients have not shown any progression for more than 5 years. One patient who received only chemotherapy as an initial treatment developed a local recurrence 3 years after the first-line treatment. This patient received concurrent chemoradiotherapy and achieved a complete response. Unfortunately, he developed brain metastasis 9 years after the first-line chemotherapy and received whole brain radiotherapy. The other patient developed cervicular and inguinal node metastases 8 months after the initiation of first-line chemotherapy and concurrent TRT with three courses of chemotherapy. This patient received second, third, and fourth-line chemotherapy, radiotherapy to the cervicular and inguinal node metastases, and surgical resection of the recurrent inguinal node metastasis. He has not shown any signs of progression for 3 years and 3 months after the final surgical resection of the metastatic inguinal node. All three patients who had solid pleural tumor died within 31 months.

Survival analyses for the subgroups in LD patients with ipsilateral pleural effusion are shown in Figures 4, 5 and Table 3. In group A, the median survival time was 19.2 months (95% CI: 16.7–27.9) and the 1 and 2-year survival rates were 81 and

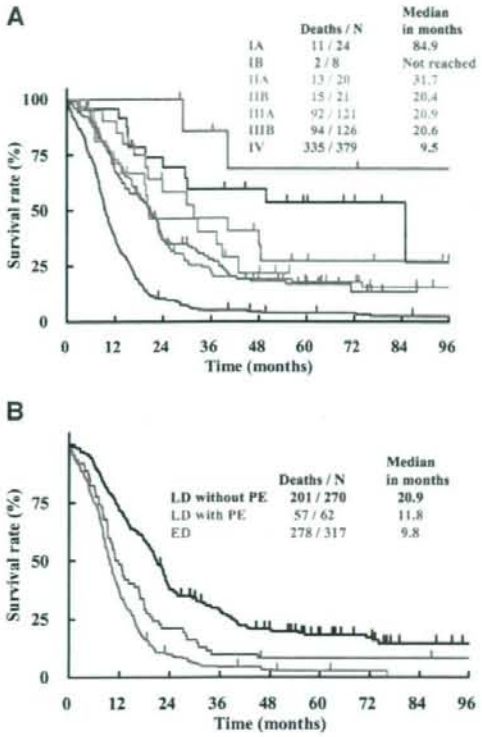

FIGURE 3. *A*, Overall survival in the all 699 patients with small cell lung cancer by the proposed seventh edition of the tumor, node, metastasis stage. *B*, Overall survival in the 649 patients who received chemotherapy as an initial treatment. LD, limited-disease; SCLC, small cell lung cancer; ED, extensive-disease.

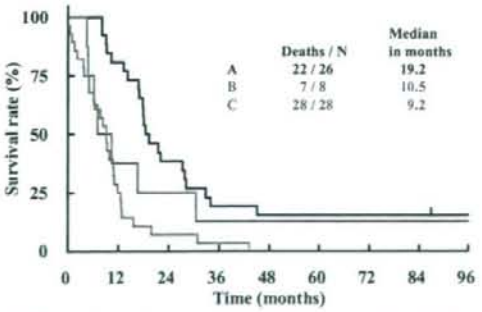

FIGURE 4. Overall survival in subgroups A, B, and C.

38%, respectively. The median survival time of patients with cytologically positive and negative pleural effusion were 9.3 months (95% CI: 3.8–14.2) and 12.7 months (95% CI: 5.1–17.9), respectively. The median survival time of those patients

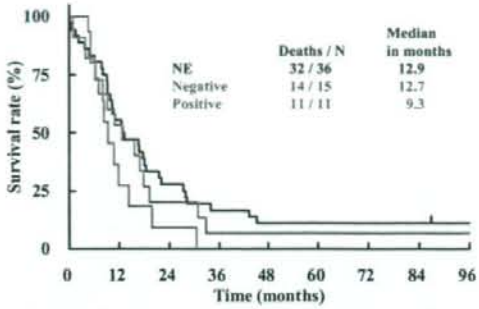

FIGURE 5. Overall survival according to the results of cytologic examination for ipsilateral pleural effusion. NE, not examined.

whose pleural effusions were not examined cytologically was 12.9 months (95% CI: 9.2–18.4). This difference was not statistically significant ($p = 0.1959$).

Disease progression was confirmed in 21 of the 26 patients in group A. The sites of first disease progression included the brain ($n = 10$), regional lymph nodes ($n = 5$), primary lesion ($n = 3$), distal lymph nodes ($n = 2$), liver ($n = 1$), adrenal gland ($n = 1$), and bone ($n = 1$). Twelve (57%) were distant, seven (33%) were local-regional, and two (10%) were both local-regional and distant. Brain metastasis was the only site of recurrence in nine patients. These nine patients had not received PCI. At the time of disease progression, ipsilateral pleural effusion recurred in 10 of the 18 patients.

DISCUSSION

LD-SCLC with ipsilateral pleural effusion accounted for 9% of all the patients with SCLC (63 of 669 patients) and 17% of all the patients with LD-SCLC (63 of 373 patients). Twenty-six (41%) of the LD-SCLC patients with ipsilateral pleural effusion received chemotherapy and definitive TRT. The median survival time of these patients was 19.2 months (95% CI: 16.7–27.9), and the 1 and 2-year survival rates were 81 and 38%, respectively. This overall survival time was comparable to that of LD patients without ipsilateral pleural effusion.

Among the LD-SCLC patients with ipsilateral pleural effusion, the median survival time was 11.8 months (95% CI: 9.2–16.6), and the 1 and 2-year survival rates were 48 and 21%, respectively. This survival was intermediate between those of LD patients without ipsilateral pleural effusion and ED patients. An analysis of 2,580 patients treated in the Southwest Oncology Group trials demonstrated that the survival of patients with LD-SCLC and ipsilateral pleural effusion was not significantly different from that of patients with ED-SCLC and a single metastatic lesion. The median survival times were 13.0 and 12.0 months ($p = 0.85$), respectively.[9] Thus, our data was compatible with that of the Southwest Oncology Group trials. Another analysis of 5,758 patients with SCLC from the IASLC database also demonstrated consistent results.[10]

According to the proposed seventh edition of the TNM classification for lung cancer, LD patients with ipsilateral

TABLE 3. Survival Data

Subgroup	No. of Patients	Median Survival Time (mo) (95%CI)	1-yr Survival Rate (%)	2-yr Survival Rate (%)	3-yr Survival Rate (%)
ED	317	9.8 (8.8–10.6)	37	10	4
LD without ipsilateral pleural effusion	270	20.9 (19.1–22.7)	72	38	29
LD with ipsilateral pleural effusion	62	11.8 (9.2–16.6)	48	21	10
Receiving TRT	26	19.2 (16.7–27.9)	81	38	19
Not receiving TRT	36	9.1 (6.0–10.8)	28	11	6
Not receiving TRT in spite of disappearance of pleural effusion	8	10.5 (4.5–30.6)	38	25	13
Not receiving TRT and persistent pleural effusion after chemotherapy	28	9.2 (5.1–10.8)	25	7	4
Cytologically positive pleural effusion	11	9.3 (3.8–14.2)	27	9	0
Cytologically negative pleural effusion	15	12.7 (5.1–17.9)	53	20	7
Without cytological examination	36	12.9 (9.2–18.4)	56	28	17

CI, confidence interval; ED, extensive-disease; SCLC, small cell lung cancer; LD, limited-disease; TRT, thoracic radiotherapy.

pleural effusion will be classified as stage IV.[3–6] However, prognosis of LD patients with ipsilateral effusion is better than that of ED patients with distant metastasis. If surgical cases such as clinical stage I cases were excluded, the simple staging system, LD or ED, seemed to be sufficient to select treatment strategy.

In our study, four LD patients with ipsilateral pleural effusion have survived for more than 5 years. Three patients received chemotherapy and TRT as an initial treatment. The remaining one patient received only chemotherapy as an initial treatment but received chemotherapy and TRT after a local recurrence. TRT probably contributed to local control and long-term survival in those LD-SCLC patients with ipsilateral pleural effusion. A previous systematic review demonstrated that an early timing of TRT contributed to a significant improvement in long-term survival, compared with a late timing.[11] In patients whose ipsilateral pleural effusion disappears after chemotherapy, definitive TRT should be considered as early as possible.

Disease progression was confirmed in 21 out 26 patients (81%) who received chemotherapy and definitive TRT. The most common site of first failure was the brain. Nine of the 10 patients had not received PCI. In these nine patients, brain metastasis was the only site of recurrence. In LD-SCLC patients with ipsilateral pleural effusion who undergo chemotherapy and definitive TRT, PCI may further improve treatment outcome.

Cytologic examinations of the pleural effusion before treatment were only performed in 26 patients (42%). These cytologic results did not significantly affect overall survival. However, all nine patients with cytologically positive pleural effusion died within 31 months. A similar observation was reported in a cohort of IASLC database.[10]

Chemotherapy regimens were heterogeneous between LD and ED patients. More patients with ED received cisplatin and irinotecan containing regimens. However, response rates were similar between LD with ipsilateral pleural effusion and ED patients (74 and 76%).

In conclusion, long-term survival was achieved by LD-SCLC patients who underwent definitive TRT after their ipsilateral pleural effusion disappeared after induction chemotherapy. A prospective randomized trial is warranted to compare chemotherapy alone with chemoradiotherapy in LD-SCLC patients with ipsilateral pleural effusion. This work was supported in part by a Grant from the Ministry of Health, Labor, and Welfare for the 3rd term Comprehensive Strategy for Cancer Control and a Grant-in-Aid for Cancer Research from the Ministry of Health, Labor, and Welfare, Japan.

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Immunohistochemical expression of BCRP and ERCC1 in biopsy specimen predicts survival in advanced non-small-cell lung cancer treated with cisplatin-based chemotherapy☆

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ABSTRACT

Purpose: The aim of this study was to determine the prognostic value of expression of ATP binding cassette (ABC) transporter proteins and DNA repair gene proteins by immunohistochemically staining tumor biopsy specimens from patients with advanced non-small-cell lung cancer (NSCLC) being treated with platinum-based chemotherapy.

Experimental design: Expression of ABC transporter proteins, including BCRP (breast cancer resistance protein) and MRP2 (multidrug resistance proteins 2), and the DNA-repair-related proteins, ERCC1 (excision repair cross-complementation group 1) and BRCA1 (breast cancer type 1 susceptibility protein) was assessed immunohistochemically in 156 tumor samples from untreated stage IV NSCLC patients. All of the patients had received platinum-based chemotherapy. Response to chemotherapy, progression-free survival (PFS), and overall survival were compared in relation to expression of each of the proteins and to clinicopathological factors.

Results: High ERCC1 expression was associated with short survival (237 days vs. 453 days, log-rank $P = 0.03$), but not with response to chemotherapy or PFS. And high BCRP expression was associated with short survival (214 days vs. 412 days, log-rank $P = 0.02$) but not with response to chemotherapy or PFS. Multivariate analysis confirmed that negativity for the expression of BCRP tends to be an independent variable related to overall survival ($P = 0.06$).

Conclusions: This study examined ERCC1 and BCRP expression in biopsy specimens as candidates for predictors of the survival of patients with advanced NSCLC treated with platinum-based chemotherapy.

1. Introduction

More than half of all patients with non-small-cell lung cancer (NSCLC) have advanced stage IIIB or IV disease when first diagnosed, and patients with advanced NSCLC are candidates for systemic chemotherapy. Despite the increasing number of active chemotherapeutic agents available, patients with advanced NSCLC still have a median survival time of only 1 year. Intrinsic or acquired tumor-mediated drug resistance is a major clinical problem that can result in lack of tumor response to chemotherapy. Clinical investigators have recognized that several genetic abnormalities underlying NSCLC contribute to the development of the chemotherapeutic patterns that influence chemotherapeutic sensitivity to certain cytotoxic drugs. If the resistance to drugs could be explained by a simple, widely applicable method, such as immunohistochemical analysis of tumor biopsy specimens, the most effective drug candidates for the treatment could be more accurately identified.

The mechanisms of chemoresistance are likely to involve multiple gene products, and understanding of the potential modes of chemotherapeutic resistance to platinum-based chemotherapy has recently been achieved through studies that have correlated cytotoxicity with DNA repair or drug efflux [1,2]. Breast cancer

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resistance protein (BCRP) and multidrug resistance protein 2 (MPR2), a member of the superfamily of ATP binding cassette (ABC) transporter proteins, are involved in membrane transport during drug metabolism, and elevated expression of BCRP and MRP2 in vitro causes resistance to anticancer drugs [3–8]. Expression of BCRP and MRP2 has been found to be characterized by a reduced intracellular drug level. Moreover, there is convincing evidence that BCRP expression in biopsy specimens from patients with advanced NSCLC predicts response to chemotherapy or outcome [9].

The cytotoxic effect of anticancer platinum drugs is principally attributable to the formation of platinum-DNA adducts. Repair of these lesions in genomic DNA is mediated by both NER and Interstrand Cross-Link Repair (ICL-R) pathways, both in whose ERCC1 is a critical element [10–15]. Further, high ERCC1 expression is associated with resistance of human cancers to platinum-containing therapy [16–20].

Mutations in Breast cancer type 1 susceptibility protein (BRCA1), another DNA repair protein, can induce resistance to cisplatin-mediated apoptosis. BRCA1 is also involved in the repair of DNA damage induced by platinum drugs [21,22].

Attempts to overcome resistance have mainly involved the use of combination therapy with different classes of drugs in this study. We focused on the two different classes of proteins involved in resistance: ABC transporter proteins and DNA damage repair proteins. We quantified expression of ABC transporter (BCRP, MRP2) proteins and DNA repair genes (ERCC1, BRCA1) proteins by immunohistochemical staining of tumor biopsy specimens collected before chemotherapy. We also evaluated the value of these proteins for predicting tumor response and survival in NSCLC patients treated with platinum-based combination therapy.

2. Materials and methods

2.1. Subjects

A total 200 of stage IV NSCLC patients received platinum-based combination chemotherapy at the National Cancer Center Hospital East between February 1996 and December 2004 because they had a PS of 0 or 1 on the Eastern Cooperative Oncology Group scale. Adequate tumor biopsy specimens collected before chemotherapy were available for 156 of these patients, and they were analyzed in this study. All tumor specimens analyzed were collected before chemotherapy. The histological classification was based on a WHO report. Clinical staging was based on an initial evaluation that consisted of a clinical assessment, chest X-ray, computed tomography of the chest and abdomen, computed tomography or magnetic resonance imaging of the brain, and bone scintigraphy. The current International Staging System was used to stage clinical disease. The clinicopathological characteristics of all of the patients are listed in Table 1. Their median age at diagnosis was 62 years (range, 39–79 years), and 44 of the 156 stage IV patients were women. All of the patients were treated with platinum-based combination chemotherapeutic regimens, which are considered standard regimens for patients with advanced NSCLC. After obtaining informed consent in accordance with institutional guidelines, all of the patients underwent tumor biopsy and chemotherapy.

2.2. Chemotherapy

All of the patients received at least 2 courses of platinum-based chemotherapy and received courses until the appearance of progressive disease. The platinum regimens were vinorelbine 25 mg/m^2 on days 1 and 8 plus cisplatin 80 mg/m^2 on day 1 of a 21-day cycle (68 patients), docetaxel 60 mg/m^2 on day 1 plus cisplatin 80 mg/m^2 on day 1 of a 21-day cycle (20 patients), irinotecan 60 mg/m^2 on days 1, 8, and 15 plus cisplatin 80 mg/m^2 on day 1 of a 28-day cycle (16 patients), gemcitabine 1000 mg/m^2 on days 1 and 8 plus cisplatin 80 mg/m^2 on day 1 of a 21-day cycle (15 patients), and paclitaxel 200 mg/m^2 administered over 3 h on day 1 plus carboplatin dosed with an area under the curve of 6 on day 1 of a 21-day cycle (28 patients). We used the standard criteria to evaluate the response to chemotherapy. Complete response was defined as the disappearance of all clinically detectable disease for at least 4 weeks. Partial response required a minimum of a 50% reduction in the sum of the products of the greatest perpendicular diameters of all measurable lesions for a minimum of 4 weeks. Progressive disease was defined as the appearance of new lesions or an increase in disease >25% measured in the same manner as for partial response. All other results were classified as "no change." The response rate was defined as the sum of the complete responses and partial responses cases expressed as a percentage of the total number of cases.

2.3. Immunohistochemistry

Immunohistochemical staining was performed on 4 μm formalin-fixed, paraffin-embedded tissue sections. The slides were deparaffinized in xylene and dehydrated in a graded ethanol series. Endogenous peroxidase was blocked with 0.3% H_2O_2 in methanol for 15 min. For antigen retrieval, the slides for BCRP (clone BXP21, dilution 1:20, Sanbio, Uden, Netherlands), MRP2 (clone M2III-6, dilution 1:20, Sanbio, Uden, Netherlands), ERCC1 (clone 8F1,dilution 1:100, Thermo Fisher, Scientific Inc., Fremont, USA), and BRCA1 (clone MS110, dilution 1:150, EMD chemicals Inc., Darmstadt, Germany) were immersed in 10 mm citric buffer solution (pH 6.0). The slides for BCRP, MRP2, and BRCA1 were heated to 95 °C by exposure to microwave irradiation for 20 min, and the slides for ERCC1 were heated to 125 °C by exposure to autoclave irradiation for 15 min. The slides were then allowed to cool for 1 h at room temperature and washed in water and PBS. Next, nonspecific binding was blocked by preincubation with 2% BSA plus 0.1% NaN_3 for 30 min. The blocking solution was drained off, and the slides were incubated overnight at 4 °C with the primary antibodies. Staining with an irrelevant mouse IgG1 or IgG2a was routinely performed as a negative control procedure. After washing three times in PBS, the slides were incubated with a labeled polymer, EnVision + Peroxidase Mouse (DAKO, Glostrup, Denmark), for 30 min. The chromogen used was 2% 3,3′-diaminobenzidine in 50 mM Tris buffer (pH 7.6) containing 0.3% hydrogen. Slides were counterstained with hematoxylin. Normal liver and lung tissue was used as a positive control. Staining with all antibodies was considered positive if >10% of the tumor cells stained, because a 10% cutoff level has been used in several studies using these antibodies. All of the slides were examined and scored independently by two observers (S,O and G,I) without any knowledge of the patient's clinical data. When their staining evaluations differed, the examiners discussed then, with or without reevaluating the slides, until agreement was reached.

2.4. Statistical analysis

The correlations between immunohistochemical expression and the clinical variables and response to chemotherapy were evaluated by the χ^2 test or Fisher's exact test, as appropriate. Overall survival was measured from the start of chemotherapy to the date of death from any cause or the date the patient was last known to be alive. Survival curves were estimated by the Kaplan–Meier method. The Cox proportional hazards model was used for multivariate

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analysis. P values < 0.05 were considered significant. Two-sided statistical tests were used in all of the analyses. Statistical analysis software (Dr SPSSII, Windows) was used to perform the analyses

3. Results

3.1. Expression of ABC transporter and DNA damage repair proteins in NSCLC

Eighty (51%) of the 156 tumors were BCRP-positive, 26 (17%) were MRP2-positive, 100 (64%) were ERCC1-positive, and 131 (84%) were BRCA1-positive. Median percentage of staining for BCRP, ERCC1, BRCA1, and MRP2 was 20%, 40%, 50%, and 10%, respectively (the range was 0–100%).

Most of the ABC-transporter-protein-positive tumors showed mixed membranous and cytoplasmic staining. An external positive control for BCRP was canalicular membrane in liver. BCRP in the apical membrane of the bronchial layer was used an internal control, and the endothelial cells of blood vessels also stained positive. An external positive control for ERCC1 was endothelial in the tonsil and an internal positive control was stroma cells. Representative immunohistochemical BCRP and ERCC1 staining is shown in Fig. 1. The relationship between expression of ABC transporter proteins and DNA damage repair proteins and the clinical variables is shown in Table 1. ERCC1 expression and BRCA1 expression were significantly greater in the patients with a smoking history (≧20 pack years) (P = 0.015). BRCA1 expression was significantly greater in the males than in the females (P = 0.027). BRCA1 expression correlated to ERCC1 expression (P = 0.003). BCRP expression correlated to ERCC1 (P = 0.012), MRP2 (P = 0.005), but not BRCA1 (P = 0.126) (data not shown).

3.2. Expression of ABC transporter and DNA damage repair proteins and clinical outcome

It was possible to assess all 156 patients for response to chemotherapy and to analyze their survival data. The relationships between clinical variables and response to chemotherapy and survival in this study are shown in Table 2. Only "smoking history" was significantly associated with both PFS (P = 0.05) and overall survival (P = 0.02). Table 3 shows the relationships between expression of ABC transporter proteins and DNA damage repair proteins and the response to chemotherapy and survival. No significant associations were found between MRP2 expression and response to chemotherapy (P = 0.63), PFS (P = 0.94), or survival (P = 0.96), and between BRCA1 expression and response to chemotherapy (P = 0.62), PFS (P = 0.67), or survival (P = 0.06). By contrast, BCRP expression was significantly associated with both PFS (P = 0.02) and survival (P = 0.02), but not with response to chemotherapy (P = 0.15). ERCC1 expression was associated with overall survival (P = 0.03) but not with response to chemotherapy (P > 0.09) or PFS (P = . 0.06).

3.3. Multivariate analysis for PFS and overall survival

Multivariate analysis was performed by using the Cox proportional hazards model to determine whether the prognostic value of BCRP or ERCC1 disappeared when other prognostic factors were considered (Tables 4 and 5). A multivariate analysis that included gender, age, smoking history, PS, histology, BCRP, and ERCC1, showed that BCRP was not a significant independent variable correlated with PFS (P = 0.13) but overall survival was marginal (P = 0.06). The BCRP-positive value for overall survival yielded a hazard ratio of 0.72, with a 95% confidence interval of 0.51–1.01. The results show that negativity for the expression of BCRP tends to be a prognostic factor in advanced NSCLC. The PFS and overall survival curves drawn by the Kaplan–Meier method are shown according to BCRP in Fig. 2. Median survival time in the BCRP-negative group was 412 days, as opposed to 214 days in the BCRP-positive group.

4. Discussion

In this study the BCRP-positive cases had a shorter overall survival time, and BCRP expression tend to be a prognostic factor overall survival in the multivariate analysis. Expression of MRP2, on the other hand, was not an independent prognostic factor, a finding that was consistent with previous studies [9,10]. MRP2 was studied within the IALT biologic program [23]. This was the largest group of NSCLC patients used for the study of MRP2 expression and the result was that MRP2 does not predict response to adjuvant cisplatin-based chemotherapy. Yoh et al. found that the expression of BCRP in stages III and IV NSCLC patients was a significant independent variable that correlated with PFS and tend to correlated

Table 1
Relationship between clinical variables and immunohistochemical expression

	n (%)	BCRP-positive patients	MRP2-positive patients	ERCC1-positive patients	BRCA1-positive patients
Total	156	80	26	100	131
Gender					
Male	112 (72)	60	22	76	99[b]
Female	44 (28)	20	4	24	32
Age					
≧70	34 (22)	22	7	25	32
<70	122 (78)	58	19	75	99
Histology					
Ad	100 (64)	50	20	61	81
Non-ad	56 (36)	30	6	39	50
PS					
0	38 (24)	15	4	23	29
1	118 (76)	65	22	77	102
Smoking history					
≧20 pack year	99 (63)	56	19	71[a]	89[c]
<20 pack year	57 (37)	24	7	29	42

[a] P = 0.015.
[b] P = 0.027.
[c] P = 0.012.

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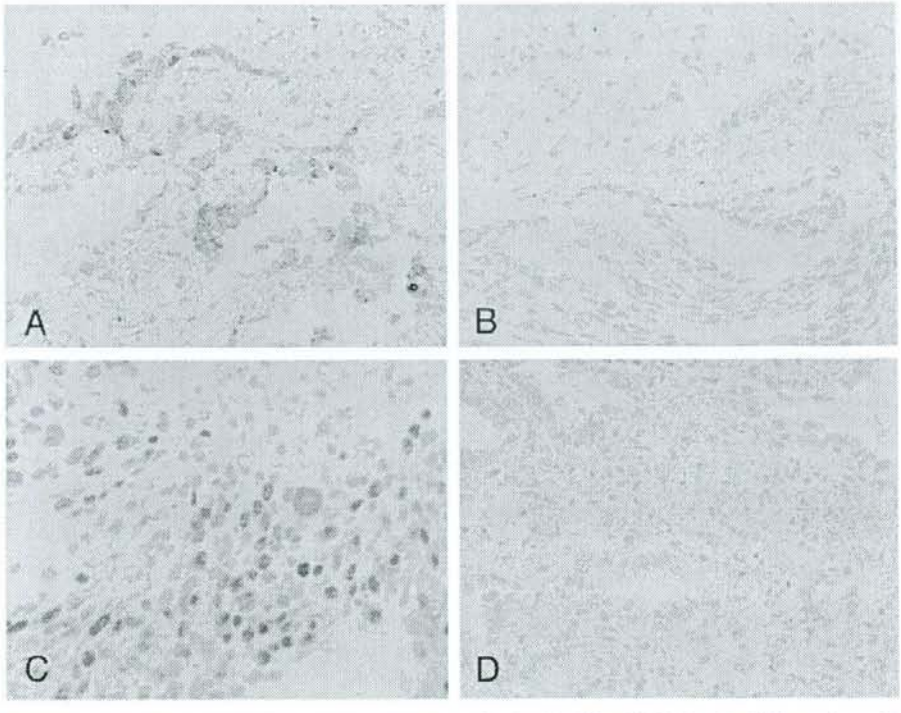

Fig. 1. Typical immunohistochemical staining patterns of NSCLC tumor biopsy specimens for BCRP (A and B) and ERCC1 (C and D). (A) Adenocarcinoma showing membrane staining for BCRP. (B) BCRP-negative adenocarcinoma. (C) Squamous cell carcinoma with positive nuclear staining for ERCC1. (D) ERCC1-negative adenocarcinoma.

with response to chemotherapy or overall survival in a multivariate analysis [9]. We think that the results of the present study reinforce the reliability of the prognostic significance of BCRP expression in stage IV NSCLC patients.

ERCC1 expression and BRCA1 expression were significantly greater in the patients with a smoking history ($P = 0.015$, $P = 0.012$ respectively). The correlation between smoking history and ERCC1, BRCA1 expression is often a lack in previous studies such as in the IALT-bio study. Fujii et al. [24] and Lee et al. [25] reported relationship between ERCC1, BRCA1 expression and smoking history, which tended to be greater in the patients with a smoking history but it was not significant statistically. Relationship between DNA repair gene protein expression and DNA damage arised from smoking could only be presumed. Interestingly, we also noticed in the present study that patients with high ERCC1 and BRCA1 double expression in tumors had shorter survival than patients who have

Table 2
Summary of relationship between clinical variables and response to chemotherapy or survival

	n	Response rate (%)	*P*	PFS (day)	*P*	MST (day)	*P*
Total	156	26		163		317	
Gender							
Male	112	24	0.32	155	0.17	307	0.23
Female	44	32		223		324	
Age							
≧70	34	18	0.27	119	0.10	261	0.14
<70	122	29		171		333	
Histology							
Ad	100	22	0.13	148	0.32	366	0.55
Non-ad	56	34		180		261	
PS							
0	38	26	>0.99	184	0.35	386	0.23
1	118	26		153		274	
Smoking history							
≧20 pack year	99	23	0.26	151	0.05	256	0.02
<20 pack year	57	32		223		426	

PFS: progression free survival, MST: median survival time.

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Table 3
Relationship between immunohistochemical expression and response to chemotherapy or survival

	n	Response rate (%)	P	PFS (day)	P	MST (day)	P
BCRP							
Positive	80	21	0.15	148	0.02	214	0.02
Negative	76	32		211		412	
MRP2							
Positive	26	31	0.63	161	0.94	344	0.96
Negative	130	25		165		304	
ERCC1							
Positive	100	26	>0.99	148	0.06	237	0.03
Negative	56	27		187		453	
BRCA1							
Positive	131	27	0.62	161	0.67	261	0.06
Negative	25	20		184		461	

PFS: progression free survival, MST: median survival time.

Table 4
Multivariate analysis for overall survival of 156 patients

Variables	Category	Risk ratio	95% CI	P
Gender	Male vs. female	1.08	0.68–1.72	0.74
Age	≧70 vs. <70	1.18	0.75–1.82	0.50
PS	0 vs. 1	1.16	0.77–1.75	0.48
Histology	Ad vs. non-ad	1.33	0.93–1.90	0.12
Smoking history	≧20 vs. <20	1.47	0.96–2.27	0.08
BCRP	(−) vs. (+)	0.72	0.51–1.01	0.06
ERCC1	(−) vs. (+)	0.75	0.52–1.07	0.12

other expression pattern for those two markers ($p = 0.0027$) (data not shown).

A relation between expression of ERCC1 mRNA and resistance to platinum-based chemotherapy has been corroborated by small, retrospective studies in patients with advanced gastric, ovarian, colorectal, and esophageal cancer and in NSCLC patients [16–20]. Simon et al. reported that patients who have undergone complete resection of NSCLC with high ERCC1 mRNA expression have a better survival than patients with low ERCC1 mRNA expression [26]. Olaussen et al. found that patients who underwent complete resection of ERCC1-negative NSCLC appeared to benefit from adjuvant cisplatin-based chemotherapy, and, showed that in the group that had not received adjuvant therapy patients with ERCC1-positive tumors had a longer overall survival than patients with ERCC1-negative tumors [27]. Our findings showed that expression of ERCC1 was significantly associated with overall survival but not with response to chemotherapy or PFS. Thus, ERCC1 expressing tumor may become a poor prognostic tumor by other factors induced by administered chemotherapy (e.g., tolerance to DNA damage).

Rosell's group in Barcelona reported overexpression of BRCA1 mRNA was strongly associated with poor survival in NSCLC patients [28]. In this study, BRCA1 protein expression was borderline significance for correlation with overall survival. The reason of this discrepantly result might be explained by the difference of study population. Rosell's group study targeted for operable early stage NCSLC. To solve this problem, further investigation was needed.

In our study, BCRP expression, alone not ERCC1 expression, was of marginal significant prognostic value for MST in the multivariate analysis. Liedert et al. reported overexpression of ABC transporter protein in a cisplatin-resistant melanoma cell line [29], and this observation was accompanied by reduced formation of platinum-DNA adducts measured by an immunocytologic assay. The overexpression of ABC transporter may regulate the formation of platinum-DNA adducts in tumor cells, and BCRP may act upstream during the chemoresistance process. This mechanism may have been responsible for BCRP expression alone, not ERCC1 expression, being of marginal significant prognostic value for MST in the multivariate analysis in this study.

It is noteworthy that there was no significant association between any of the markers and response to chemotherapy. One explanation might be that response rate by itself did not correlate to survival in our study. Another consideration to answer to the discrepancy between response, PFS and overall survival, is that ERCC1 expressing tumor may become a poor prognostic tumor due to other factors than those induced by chemotherapy. Illustration of this notion came from a recent study by Hadnagy et al. [30] who recently reported a relationship between BCRP expression and cancer stem cells that seemed to play a pivotal role in tumor progression. BCRP expression by itself may be poor prognostic factor regardless of chemotherapy. A widely used flow cytometry assay for identifying cancer stem cells defines a "side-population" (SP) of cells that display Hoechst 33342 [31]. Cancer stem cells can be puri-

Table 5
Multivariate analysis for progression free survival of 156 patients

Variables	Category	Risk ratio	95% CI	P
Gender	Male vs. Female	0.90	0.56–1.49	0.70
Age	≧70 vs. < 70	1.24	0.78–1.98	0.37
PS	0 vs. 1	1.10	0.70–1.74	0.68
Histology	Ad vs. Non-ad	1.39	0.93–2.07	0.11
Smoking history	≧20 vs. <20	1.41	0.91–2.21	0.12
BCRP	(−) vs.(+)	0.72	0.48–1.10	0.13
ERCC1	(−) vs.(+)	0.82	0.54–1.26	0.37

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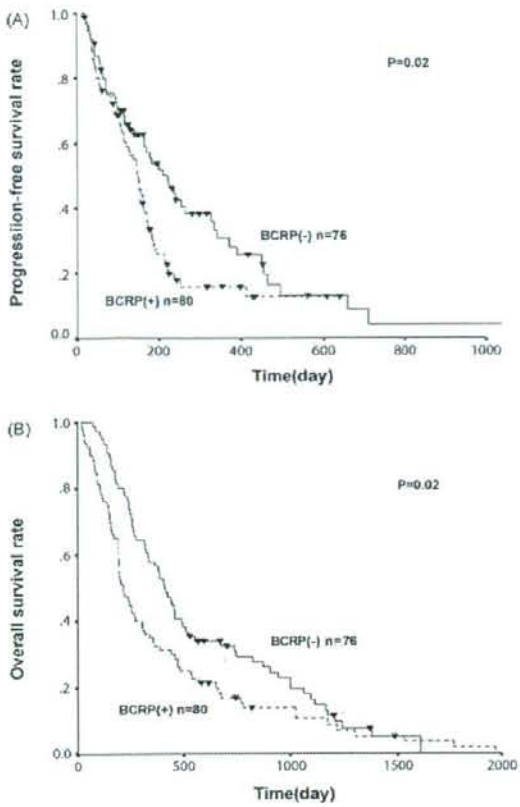

Fig. 2. (A) Progression-free survival curve of 156 patients with advanced non-small-cell lung cancer, according to BCRP expression. The median progression-free survival period of the BCRP-negative patients and BCRP-positive patients was 211 and 148 days, respectively. (B) Overall survival curves of 156 patients with advanced non-small-cell lung cancer, according to BCRP expression. Patients with BCRP-negative tumors survived longer than those with BCRP-positive tumors, and the difference was statistically significant ($P = 0.02$).

fied based on the efflux of dyes and Hoechst 33342 [32,33]. Zhou et al. reported that BCRP mRNA is expressed in a wide variety of stem cells and is a molecular determinant of the SP cells, and moreover, dyes and Hoechst 33342 efflux activity was provided by BCRP expression in mice [34]. Haraguchi et al. [35] reported significantly increased BCRP expression in SP cells in human gastrointestinal system cancer cell lines and that SP cells exhibited greater resistance to chemotherapy. The self-renewal and chemoresistance capacities of these cancer SP cells may also play important roles in maintaining cancer foci to proliferate after chemotherapy and radiotherapy. Investigating whether BCRP-positive cells have the characteristics, as cancer stem cells in NSCLC will be a future task.

We speculate, that patients with tumors that are positive for BCRP expression show drug resistance to platinum-based chemotherapy. We suggest that BCRP serve as molecular target for reducing drug resistance. Kuppens et al. reported a phase I study of Elacridar (GF120918) [36,37]. Minderman et al. [38] reported Bricodar (VX710) increases drug retention and enhances chemosensitivity in resistant cells expressing BCRP. Another approach to solving platinum-based chemotherapeutic resistance, non-platinum chemotherapy will be alternative regimen for the subgroup which over express BCRP protein.

Conflict of interest

The authors certify that there are no potential conflicts of interest.

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Marital status and non-small cell lung cancer survival: the Lung Cancer Database Project in Japan

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Abstract

***Objective*: Previous studies have suggested that marital status is associated with survival from lung cancer; however, its association is not conclusive. The association between marital status and survival in Japanese patients with non-small cell lung cancer (NSCLC) was prospectively investigated.**

***Methods*: Between July 1999 and July 2004, a total of 1230 NSCLC patients were enrolled. The baseline survey consisted of the collection of clinical information and various demographic data, including marital status. A Cox regression model was used to estimate the hazards ratio (HR) of all-cause mortality adjustments for age, BMI, education level, performance status, histology type, clinical stage, smoking status, choice of definitive treatment, and depression.**

***Results*: The multivariable adjusted HR of male widowed patients versus male married patients was 1.7 (95% confidence interval = 1.2–2.5, $p = 0.005$). However, no significant increased risk of death in female widowed patients compared with female married patients was observed (HR = 0.7, 95% confidence interval = 0.5–1.1, $p = 0.15$). With regard to separated/divorced and single patients no significant increased risk of death in male and/or female compared with married patients was observed.**

***Conclusions*: The present data suggest that male widowed patients with NSCLC have a higher mortality rate than male married patients with NSCLC, after controlling for various factors.**

Keywords: marital status; non-small cell lung cancer; prospective study; survival

Introduction

Lung cancer is among the most common forms of cancer and is the most common cause of cancer-related death in the world [1,2]. Many studies have suggested that marital status is associated with survival from lung cancer; however, its association is not conclusive. Having a spouse die can significantly increase a person's risk of death; this 'widow/widower effect' is especially pronounced in men [3–6]. Therefore, the association between marital status and lung cancer survival should be clarified according to sex and subdivided marital status, such as married, widowed, separated/divorced, or single. However, only two studies have examined the association between marital status and lung cancer survival according to sex and subdivided marital status [7,8]. One study suggested that separated/divorced, single, and widowed patients had a higher risk of death compared with married patients, for both sexes [7]. The other one found no association between marital status and survival among divorced and widowed patients [8]. However, these studies were limited by small sample sizes [8] and a lack of differentiation between small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [7,8].

Possible associations between marital status and survival from lung cancer may be mediated by several factors. An unmarried status has been associated with an increased frequency of unhealthy life-style behaviors (especially with regard to smoking habits), maladjustment to the cancer diagnosis (especially among subjects who continue smoking even after they have been diagnosed as having cancer), psychological reactions (especially depression), delays in seeking treatment (more

advanced stages at the time of cancer diagnosis), and a lower likelihood of receiving definitive treatment [9–18]. However, previous studies did not consider these variables and did not clarify the effects of each factor on the associations between marital status and sex-specific survival from NSCLC.

In this prospective study, we investigated the influence of marital status on survival in patients with NSCLC in Japan. We were able to evaluate survival according to each sex and marital status in view of potential confounding factors and to clarify the effects of each modifying factor, such as smoking habits, psychological reactions, delays in seeking treatment, and likelihood of receiving definitive treatment, on the associations between marital status and survival. If several intermediate factors are provided, the physician could suggest possible means of improving the prognosis to their patients.

Methods

Participants

The design of this study, which was included as part of The Lung Cancer Database Project in Japan, has been reported in detail elsewhere [19]. Briefly, consecutive newly diagnosed lung cancer patients were invited to participate in the study, which was conducted at the Thoracic Oncology Division, National Cancer Center Hospital East, Kashiwa, Japan. Patients were included in the database study if they met all of the following criteria: informed of their lung cancer diagnosis; newly diagnosed patients with primary lung cancer; physically capable of completing the questionnaires; absence of cognitive impairment, such as dementia and delirium; ability to provide written consent; and no problems regarding the patients' participation in this project, as judged by their physicians.

In total, the project was explained to 2506 patients, and 2036 (81.3%) patients with newly diagnosed, untreated primary lung cancer were admitted during the project enrolment period. A total of 470 cases were ineligible for the following reasons: could not be contacted (49 cases), lung cancer diagnosis not confirmed at the time of admission (175 cases), non-lung cancer (120 cases), poor physical state (77 cases), refusal to participate in the project (43 cases), treated for lung cancer at another hospital (5 cases), and not yet informed of the diagnosis (1 case). For 40 of the 2036 patients, written informed consent could not be confirmed, and one patient withdrew consent during the follow-up period. Finally, the analyzed cohort consisted of 1995 patients.

As a result, the analytic cohort consisted of 1995 patients who were enrolled in the study between July 1999 and July 2004. The study protocol was approved by the institutional review board of the National Cancer Center, Japan. Each patient was fully informed of the purpose of the study before obtaining written consent and prior to participation in the study.

Exposure data

The patients completed the questionnaires during the waiting period prior to admission, and the questionnaires were collected after the patients were admitted. Questionnaires on demographic data and health habits (excluding the questionnaires on psychological factors) were distributed to all patients who had been registered by July 2004. Questionnaires on psychological factors were distributed only to patients who had registered by July 2003.

Demographic factors (age at cancer diagnosis, sex, education level, marital status, body mass index [BMI], smoking status, past history of cancer) and medical information (histology, clinical stage, PS, and first treatment) were obtained from the self-administered questionnaires and the patients' medical charts. PS was assessed by each attending physician using the Eastern Cooperative Oncology Group criteria [20].

To examine patient characteristics associated with variations in best-treatment practices, we defined, *a priori*, the minimally recommended initial therapies for each cancer stage at the time of diagnosis. As a practical matter, therapy for lung cancer is mainly decided, which take into account not only clinical stage but also age, comorbid illness, organopathy, and physical status. For the purposes of this analysis, the determination of the recommended therapies was based on pertinent information from medical literature published before 2004, including both randomized trials and meta-analyses of randomized trials, as well as the definitions of accepted therapy reflected in the Japan Lung Cancer Society clinical practice guidelines for the treatment of lung cancer, published in 2005 [21]. For tumor-node metastasis system stages I, II, and IIIA N0-1 surgical resection was considered the recommended initial therapy. For stage IIIA N2 patients, combination chemoradiotherapy was defined as the recommended therapy. For stage IIIB patients, combination chemoradiotherapy or chemotherapy alone was defined as the recommended therapy. For patients with stage IV disease, chemotherapy alone was considered the recommended therapy.

Depression symptoms were evaluated using the depression subscale of the Hospital Anxiety and Depression Scale (HADS) [22]. The HADS has

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been used as a reliable and valid method of screening for depression in patients with cancer. Each item is rated on a scale of 0–3, with higher scores denoting a greater mood disturbance. The reliability and validity of the Japanese version of this questionnaire has been established in Japanese cancer patients [23]. The present study used a cutoff point of four out of five [23].

Follow-up

In order to follow up the subjects for vital status, confirmation was made by medical records, normal postal mail, and municipality registration data. The survival of subjects was followed from July 1999 to December 2004. The psychological questionnaire was only distributed to the patients who had registered by July 2003. In this study, we analyzed the subject who answered psychological questionnaire. Out of the remaining 1995 patients, 414 patients were excluded from the analysis because of lack of psychological questionnaires. A total of 351 cases were excluded from the analysis for the following reasons: double cancer (188 cases) or SCLC (163 cases). Finally, 1230 patients were included in the subsequent analyses.

The person-months of follow-up were counted for each subject from the date of enrollment in the study until death or the end of the study period (December 2004), whichever occurred first, and a total of 31 508 person-months (median, 24 months; range, 0–67 months) were accrued. During the follow-up period, 716 deaths from all causes were identified.

Statistical analysis

All statistical analyses were performed according to sex. Standard descriptive statistics were used to characterize the marital status. Thus, marital status was categorized into married, widowed, separated/divorced, and single. Intergroup comparisons of categorical and continuous variables were performed using chi-square tests and one-way analyses of variance, respectively. Hazard ratios (HRs) were computed as the number of deaths from all causes among the subjects in each marital status category versus the number of deaths from all causes among the respective reference category (married patients). A Cox proportional-hazards regression analysis was conducted to adjust for age at the time of cancer diagnosis, BMI in kg/m^2 (<18.5, >18.5, or unknown), education level (college/university or higher, or not), PS (0, 1, or 2–4) histological type (adenocarcinoma, squamous carcinoma, large, or other), smoking status (never-smoker, ex-smoker, or current smoker), clinical stage (IA–IIB, IIIA–IIIB, or IV), HADS depression score (<5, $\geqslant 5$, or unknown), and choice of cancer treatment (definitive treatment or non-definitive treatment) using the SAS PHREG procedure included in the SAS version 8.2 statistical software package (Cary, NC, USA). The assumption of proportional hazards was verified graphically. In all the statistical evaluations, p-values of less than or equal to 0.05 were considered to denote a significant difference. All p-values were two-tailed.

In secondary analyses, we also conducted stratified analyses to examine factors that markedly modified the associations between marital status and survival, such as smoking status, clinical stage, HADS-depression, or definitive treatment.

Results

The mean age of the subjects was 63.9 years, and the percentage of men was 70%. The proportions of married, widowed, separated/divorced, and single patients were 84, 9, 4, and 3%, respectively. The mean age differed significantly according to marital status for both male and female patients (Table 1). Moreover, the smoking status also differed significantly according to marital status for both male and female patients. In women, BMI, histology, and definitive treatment differed significantly according to marital status. No significant associations between marital status and any other variables were seen.

According to the univariate Cox proportional-hazards regression analyses, six demographic or clinical variables were significantly associated with increased HRs of lung cancer survival for male and female subjects versus their respective reference categories: BMI (<18.5), smoking status (ex-smoker and current smoker), clinical stage (IIIA–IIIB or IV), PS (1 or 2–4), histology type (squamous cell carcinoma or large cell carcinoma), definitive treatment (non-definitive), and HADS depression score ($\geqslant 5$) (Table 2).

Table 3 shows the HRs for lung cancer survival according to marital status. A univariate Cox proportional-hazards regression analysis showed no significant association between survival and marital status for male and female subjects (Table 3). These findings remained basically unchanged even after multivariate adjustments for age, BMI, education level, PS, histology type, clinical stage, smoking status, choice of definitive treatment, and HADS depression score. For male patients, however, a multivariate Cox proportional-hazards regression analysis showed a significant association between survival and marital status. The multivariable adjusted HRs of widowed, separated/divorced, and single patients versus married patients were 1.7 (95% confidence interval (CI), 1.2–2.5; $p = 0.005$), 1.1 (0.7–1.7; $p = 0.72$), and 0.9 (0.5–1.5; $p = 0.61$), respectively.

Table 1. Demographic, medical, and psychological characteristics in NSCLC patients to marital status

	Male				Female			
	Marital status				Marital status			
	Married	Widowed	Separate/ divorced	Single	Married	Widowed	Separate/ divorced	Single
No. of subjects	774	41	26	24	262	72	19	12
Demographic characteristics								
Mean age in years (SD)	64.3 (8.9)	70.5 (8.3)	62.7 (8.4)	50.0 (10.1)	61.9 (9.3)	69.6 (8.2)	59.6 (8.1)	59.7 (14.6)
Body mass index (kg/m^2) (%)								
<18.5	11	10	12	8	8	3	26	25
$\geqslant 18.5$	88	90	85	92	91	96	74	75
Unknown	1	0	4	0	1	1	0	0
Duration of education (%)								
>15 yr	23	20	27	29	6	4	0	17
$\leqslant 15$ yr	77	78	73	71	94	96	100	83
Unknown	1	2	0	0	0	0	0	0
Smoking status (%)								
Never-smoker	4	0	0	25	76	71	42	58
Ex-smoker	33	44	23	4	7	11	16	8
Current smoker	62	56	77	71	17	18	42	33
Medical characteristics								
Clinical stage[a] (%)								
IA–IIB	44	44	38	25	57	71	53	50
IIIA–IIIB	29	39	42	29	18	10	26	8
IV	27	17	19	46	25	19	21	42
Performance status[b] (%)								
0	39	39	27	21	56	63	47	50
1	55	59	65	79	39	36	42	42
2–4	6	2	8	0	5	1	11	8
Histology type (%)								
Adenocarcinoma	57	49	62	67	86	88	68	75
Squamous cell carcinoma	28	44	35	25	8	6	26	17
Large cell carcinoma	12	7	0	8	6	7	5	0
Other	3	0	4	0	1	0	0	4
Definitive treatment (%)								
Definite	85	85	73	83	91	94	74	83
Non-definitive	15	15	27	17	9	6	26	17
Psychological characteristics								
HADS depression (%)								
<5	42	37	23	54	43	44	47	50
$\geqslant 5$	53	51	69	38	54	51	47	42
Unknown	5	12	8	8	3	4	5	8

[a] Defined by TNM classification: International Union Against Cancer.
[b] Defined by Eastern Cooperative Oncology Group (ECOG).

For female patients, however, a multivariate Cox proportional-hazards regression analysis showed no significant association between survival and marital status. The multivariable HRs of widowed, separated/divorced, and single patients versus married patients were 0.7 (0.5–1.1; $p = 0.15$), 0.5 (0.3–1.1; $p = 0.10$), and 1.2 (0.5–2.7; $p = 0.71$), respectively.

In addition, we conducted an effect modification analysis to assess the effects of clinical stage, smoking status, choice of definitive treatment, and HADS depression score on the relationship between marital status and survival in male widowed patients. All of these factors had no significant effect on the association between male widowed patients and survival ($p > 0.05$ for all variables).

No survival differences were seen between married and unmarried (including widowed, separated/divorced, and single) patients. The multivariable adjusted HR of unmarried patients versus married patients was 1.0 (0.8–1.2; $p = 0.91$).

Discussion

In this prospective study conducted in Japan, a significant association was found between marital status and survival in male patients with NSCLC. Male widowed patients had a higher mortality risk

Table 2. Results of univariate analysis for survival from lung cancer

	Male				Female			
	No. of subjects	Person-months median (range)	Cases	Univariate HR (95% CI)	No. of subjects	Person-months median (range)	Cases	Univariate HR (95% CI)
No. of subjects	865	21.6 (0.6–66.3)	548		365	26.8 (0.5–66.7)	168	
Demographic characteristics								
Age								
<49	58	20.5 (0.9–64.4)	38	1.0 (referent)	30	25.7 (1.8–56.7)	16	1.0 (referent)
50–59	193	21.8 (1.7–65.7)	125	1.0 (0.7–1.5)	95	28.1 (2.9–66.1)	42	0.7 (0.4–1.3)
60–69	350	20.7 (0.8–65.9)	221	1.0 (0.7–1.5)	136	28.2 (1.9–66.7)	63	0.8 (0.4–1.3)
70<	264	22.1 (0.6–66.3)	164	0.9 (0.7–1.4)	104	26.7 (0.5–63.7)	47	0.8 (0.4–1.4)
Body mass index (kg/m^2)								
⩾18.5	765	22.1 (0.6–66.3)	469	1.0 (referent)	330	27.3 (0.5–66.7)	146	1.0 (referent)
<18.5	93	14.4 (0.8–66.3)	73	1.6 (1.2–2.0)	31	18.6 (3.7–62.8)	20	1.9 (1.2–3.0)
Unknown	7	15.7 (5.6–58.6)	6	1.6 (0.7–3.6)	4	25.7 (11.7–30.7)	2	1.4 (0.3–5.5)
Duration of education								
>15 yr	197	20.3 (0.9–65.8)	122	1.0 (referent)	22	29.3 (3.4–45.2)	6	1.0 (referent)
⩽15 yr	661	21.9 (0.8–66.3)	423	0.9 (0.8–1.2)	343	26.7 (0.5–66.7)	162	1.8 (0.8–4.0)
Unknown	7	29.7 (0.6–63.9)	3	0.7 (0.2–2.3)	0	—	0	—
Smoking status								
Never-smoker	39	28.4 (2.4–63.9)	18	1.0 (referent)	265	28.3 (0.9–66.7)	110	1.0 (referent)
Ex-smoker	283	21.3 (0.8–66.3)	179	1.6 (0.9–2.6)	34	26.7 (0.5–54.8)	15	1.1 (0.7–1.9)
Current smoker	543	20.3 (0.6–66.3)	351	1.7 (1.1–2.8)	66	22.1 (1.8–63.6)	43	2.0 (1.4–2.9)
Medical characteristics								
Clinical stage[a]								
IA, IB, IIA, IIB	371	34.2 (3.1–66.3)	121	1.0 (referent)	216	35.2 (0.9–66.7)	45	1.0 (referent)
IIIA, IIIB	259	16.1 (0.6–65.9)	201	4.1 (3.3–5.2)	61	23.1 (3.2–65.6)	46	6.1 (4.0–9.3)
IV	235	8.0 (0.8–63.8)	226	9.8 (7.8–12.3)	88	11.3 (0.5–62.1)	77	12.0 (8.2–17.7)
Performance status[b] (%)								
0	336	29.7 (3.1–66.3)	140	1.0 (referent)	207	33.9 (0.9–66.7)	51	1.0 (referent)
1	482	15.6 (0.8–66.3)	363	2.8 (2.3–3.4)	141	21.5 (2.4–66.1)	100	4.2 (2.9–5.9)
2–4	47	4.1 (0.6–25.2)	45	12.7 (8.9–17.9)	17	5.7 (0.5–23.2)	17	28.9 (16.1–52.0)
Histology type								
Adenocarcinoma	490	23.0 (0.6–66.3)	306	1.0 (referent)	309	27.9 (0.5–66.7)	130	1.0 (referent)
Squamous cell carcinoma	252	20.7 (0.9–66.3)	157	0.9 (0.8–1.2)	32	24.9 (3.4–60.8)	23	2.2 (1.4–3.4)
Large cell carcinoma	99	14.6 (1.4–65.8)	72	1.4 (1.0–1.8)	21	22.5 (2.9–61.9)	14	1.8 (1.0–3.1)
Other	24	29.0 (2.8–65.6)	13	0.8 (0.4–1.3)	3	29.7 (22.9–57.6)	1	0.7 (0.1–4.8)
Definitive treatment								
Definitive	733	23.0 (0.8–66.3)	445	1.0 (referent)	331	27.9 (0.9–66.7)	140	1.0 (referent)
Non-definitive	132	10.1 (0.6–65.9)	103	1.9 (1.5–2.3)	34	12.9 (0.5–56.7)	28	3.2 (2.1–4.8)
Psychological characteristics								
HADS depression								
<5	452	23.5 (0.9–66.3)	265	1.0 (referent)	189	28.7 (0.9–66.7)	71	1.0 (referent)
⩾5	364	16.7 (0.8–65.9)	251	1.3 (1.1–1.6)	163	25.1 (0.5–66.1)	89	1.7 (1.2–2.4)
Unknown	49	24.4 (0.6–60.6)	32	1.1 (0.8–1.6)	13	38.6 (4.9–60.9)	8	1.6 (0.8–3.4)

[a] Defined by TNM classification: International Union Against Cancer.
[b] Defined by Eastern Cooperative Oncology Group (ECOG)

than male married patients. Our study had some methodological advantages over previous studies in that we were able to take into account differences in sex and marital status as well as potential modifying factors, such as smoking status, psychological variables, choice of definitive treatment, and disease stage at the time of diagnosis. The present study indicates that these potential modifying factors did not participate in association between marital status and survival in male patients with NSCLC. Further examinations are needed to clarify the details of this association.

Of the three studies that examined the association between marital status and lung cancer survival according to sex and subdivided marital status [7,8]. Kravdal [7] followed up SCLC and NSCLC patients (number of patients were not specified) and documented 15 882 deaths in males and 3944 deaths in females. Single female patients had a higher risk of death than married patients. Lastly, Kvikstad *et al.* [8] followed up 333 female married, divorced, and widowed cases of SCLC and NSCLC for 6 years, revealing 268 deaths. No significant associations were found between marital

Table 3. Hazard ratios (HR) of cancer survival according to the marital status

	Male				Female				Total			
	Married	Widowed	Separate/ divorced	Single	Married	Widowed	Separate/ divorced	Single	Married	Widowed	Separate/ divorced	Single
No. of subjects	774	41	26	24	262	72	19	12	1036	113	45	36
Person-months of follow-up	21.8 (0.6–66.3)	17.0 (2.5–57.9)	23.7 (0.9–65.9)	19.1 (3.0–65.1)	26.5 (0.5–66.7)	30.5 (4.4–63.6)	28.1 (5.9–62.2)	23.6 (5.2–50.2)	23.6 (0.5–66.7)	25.9 (2.5–63.6)	27.2 (0.9–65.9)	21.0 (3.0–65.1)
No. of death from all causes	481	31	20	16	121	31	9	7	602	62	29	23
Unadjusted HR	1.0 (referent)	1.4 (0.9–1.9)	1.3 (0.8–2.0)	1.1 (0.7–1.9)	1.0 (referent)	0.9 (0.6–1.3)	0.9 (0.5–1.8)	1.8 (0.9–3.4)	1.0 (referent)	0.9 (0.7–1.1)	1.1 (0.7–1.5)	1.2 (0.8–1.8)
p-Value		0.08	0.26	0.62		0.45	0.84	0.23		0.32	0.77	0.39
Multivariable adjusted HR1	1.0 (referent)	1.4 (0.9–2.1)	1.1 (0.7–1.7)	1.1 (0.7–1.9)	1.0 (referent)	0.8 (0.5–1.3)	0.7 (0.3–1.4)	1.8 (0.8–3.9)	1.0 (referent)	0.9 (0.7–1.3)	0.9 (0.6–1.3)	1.2 (0.8–1.8)
p-Value		0.06	0.81	0.69		0.43	0.27	0.17		0.81	0.57	0.52
Multivariable adjusted HR2	1.0 (referent)	1.7 (1.2–2.5)	1.1 (0.7–1.7)	0.9 (0.5–1.5)	1.0 (referent)	0.7 (0.5–1.1)	0.5 (0.3–1.1)	1.2 (0.5–2.7)	1.0 (referent)	1.1 (0.9–1.5)	0.9 (0.6–1.3)	0.9 (0.6–1.4)
p-Value		0.005	0.72	0.61		0.15	0.10	0.71		0.41	0.42	0.65

HR1: age, BMI, education, PS, and histology type adjusted.
HR2: age, BMI, education, PS, histology type, smoke stage, definitive treatment, and HADS-depression adjusted.

status and survival among female divorced and widowed patients. The present study showed no significant association between marital status and survival when male and female patients were examined as a single group. On the other hand, when the subjects were divided into male and female patients, only the male widowed patients had a higher mortality risk than the male married patients. Having a spouse die significantly increases a person's risk of death in the general population, and this 'widow/widower effect' is especially pronounced in men [3–6]. In the present study, the findings for male patients with NSCLC are consistent with these previous results.

Possible associations between marital status and survival may be mediated by several factors. An unmarried status has been associated with an increased frequency of smoking, depression, advanced disease stage at the time of diagnosis, and a lower likelihood of receiving definitive treatment [9–13,15–18]. Previous studies did not consider possible modifying factors' effects to examine differences in sex and marital status [7,8]. Therefore, it is not clarified why single, separate/divorced, and widowed patients have a higher mortality compared with married patients. This is the first study to examine differences in sex and subdivided marital status as well as the effects of potential modifying factors, such as smoking status, psychological variables, choice of definitive treatment, and disease stage at the time of diagnosis, on the association between marital status and survival from NSCLC. In the present study, smoking status, disease stage at the time of diagnosis, choice of definitive treatment, and the HAD depression score did not have a significant modifying effect on the relationship between male widowed patients and survival. Thus, smoking status, disease stage at the time of diagnosis, choice of definitive treatment, and the HAD depression score might not have a major impact on the association between marital status and survival. However, an unmarried status has been associated with an increased chance of the patient continuing to smoke even after a diagnosis of cancer has been made [12]. The continuation of smoking even after a diagnosis of cancer has been made is known to be significantly associated with survival [12,14]. In this study, we could not evaluate this association because information on smoking continuation after cancer diagnosis was not available.

Our study had several limitations. First, the study was performed at a single National Cancer Center. Whether our results can be generalized to reflect other institutions remains unclear. Thus, further studies performed at multiple institutions are necessary to clarify the prognostic effects of marital status on the survival of lung cancer patients. Second, in this study the subjects were only NSCLC patients. Histological classification of

the lung cancers in our database at the National Cancer Center Hospital East (NCCHE), Japan, revealed that small cell carcinomas were much less common (11%) than NSCLC (89%); other reports have suggested that these cancers account for nearly 80 and 20% of all lung cancers, respectively [24]. Moreover, NSCLC and SCLC differ in terms of their prognosis as well as the therapeutic strategies employed [25]. Therefore, we clarified the association between marital status and survival using a homogeneous group, focusing only on NSCLC patients. Third, data on unhealthy lifestyle behaviors after a cancer diagnosis had been made were unavailable. An unmarried status has been associated with an increased frequency of maladjustment to the cancer diagnosis (especially among subjects who continue to smoke even after they have been diagnosed as having cancer) [12]. There is some possibility that the association between marital status and survival may be mediated by this factor. If data on unhealthy lifestyle behaviors after cancer diagnosis were made available, then the mechanism responsible for the association between marital status and survival could be clarified, and the physician could suggest possible means to improve the prognosis to their cancer patients.

In conclusion, our data indicated that marital status might influence survival among male widowed NSCLC patients in Japan. The present results indicate that potential modifying factors, such as smoking status, disease stage at the time of diagnosis, choice of definitive treatment, and the HAD depression score, did not participate in association between marital status and survival in male patients with NSCLC. Further research on marital status and survival in male patients with NSCLC within the potential modifying factors such as continued smoking and including a large population is needed to clarify the details of this association.

Conflict of interest

None of the authors have any conflict of interest with any aspect of submitting this article for publication.

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