on days 1 and 2 and peaked on day 2 in both the SI and the RI groups. However, on day 3, IL-17 was only detected in the RI group ($P<0.01$) (Fig. 3a). KC levels were significantly higher in the RI group than in the SI group on day 1 ($P<0.05$) (Fig. 3b). TNF-α levels were significantly higher in the RI group than in the SI group on days 0.5 and 1 ($P<0.01$) (Fig. 3c). IL-6 levels were significantly higher in the RI group than in the SI group on days 1 ($P<0.05$) and 2 ($P<0.01$) (Fig. 3d). The IFN-γ levels were low in both groups, and no difference was observed between the SI and RI groups (Fig. 3e). IL-4 levels were significantly higher in the RI group than in the SI group on day 0.5 ($P<0.05$) (Fig. 3f). IL-12 was not detected in either group on any day (data not shown). In preliminary experiments, these cytokines were not detected in the BALF collected on day 7.

Immunohistochemical Analysis of IL-23

Immunohistochemical analysis of lung tissue collected on day 1 indicated the focal distribution of IL-23-positive cells in the alveolar lumen in both groups (SI, Fig. 4a; RI, Fig. 4b). These IL-23-positive cells were morphologically identified as macrophages. The percentage of these cells among the total cell counts tended to be higher in the RI group (16.0±12.5 %) than in the SI group (5.9± 10.3 %).

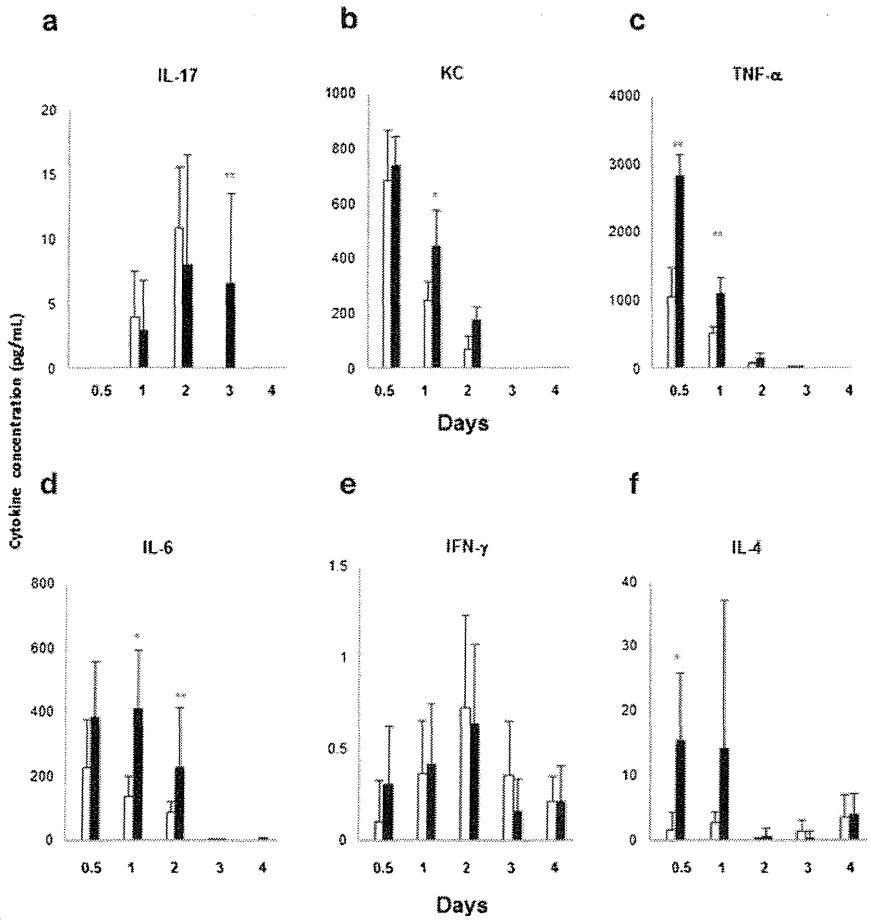

Fig. 3. Cytokine levels in the BALF of the mice. Cytokines in the BALF collected from SI (*open bars*) and RI (*solid bars*) mice on the indicated days following the final Mp extract inoculation were analyzed using a multiplex bead array. The concentrations of IL-17 (**a**), KC (**b**), TNF-α (**c**), IL-6 (**d**), IFN-γ (**e**), and IL-4 (**f**) are shown ($n=5$ per each point). Significant differences between SI and RI groups at specific times are indicated by asterisks: $^{*}P<0.05$, $^{**}P<0.01$. Data are expressed as means ± standard deviation.

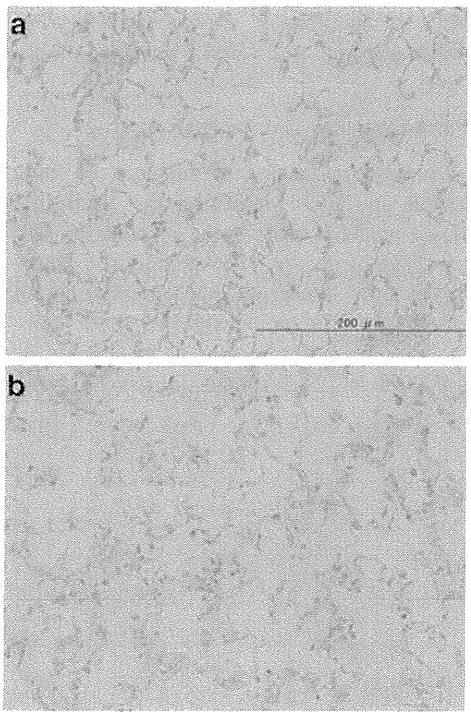

Fig. 4. Immunohistochemical analysis of IL-23 expression. Typical sections of the lungs for IL-23 expression are shown from **a** mice that were *SI* (n=3) or **b** *RI* (n=3) with Mp extract.

Analysis of the Cells in BALF

We assayed the effect of single or repeated inoculation of Mp extract into mice on the cell types present in BALF. Total cell counts were significantly higher in the RI group than in the SI group on days 2 (P <0.05), 3 (P<0.01), and 4 (P<0.01) (Fig. 5a). The number of each cell type in BALF is shown in Fig. 5b–d, and the percentages of each cell type comprising the total cell counts are shown in Fig. 5e. Neutrophil counts peaked on day 2 in both groups but were significantly higher in the RI group than in the SI group on days 2 (P <0.05), 3 (P<0.01), and 4 (P<0.01) (Fig. 5b). Macrophage counts were significantly higher in the RI group than in the SI group on days 0.5 and 1 (P<0.05) (Fig. 5c), and lymphocyte counts were significantly higher in the RI group than in the SI group on days 4 and 7 (P<0.05) (Fig. 5d). Neutrophils were the predominant cell type in both the SI and RI groups until day 3 (Fig. 5e).

DISCUSSION

This study established a novel mouse model of Mp pneumonia without the use of alum-adjuvant treatment. We analyzed this mouse model, as well as primary cultured cells prepared from mouse lungs that were stimulated with Mp extract, in order to address our hypothesis that some components of the Mp extract induce IL-17, which is associated with excessive inflammatory cell reactions in Mp pneumonia. We used Mp extract instead of live Mp in order to avoid the infectious aspect of Mp pneumonia.

We confirmed that levels of IL-23 and IL-17 were elevated at 72 h after treatment of mouse lung-derived primary cell cultures with Mp extract (Fig. 2b, c). This is the first report to demonstrate that levels of IL-23 in the media of cultured cells increased in response to the Mp extract (50 μg/mL), followed by an increase in IL-17 levels (Fig. 2a). Although we did not define which kind of cells of lung-derived primary cells produced IL-17, several reports [19–21] have demonstrated that lymphocytes and epithelial cells are the possible sources of IL-17. Previous reports indicated that some components of Mp induced IL-8 production in A549 and BEAS-2B cells [22, 23], which are derived from human alveolar or bronchial epithelial cells. Mp extract also induced IL-6 and TNF-α in RAW 264.7 cells that were derived from murine macrophages [13].

We also demonstrated that the levels of IL-17, KC, TNF-α, IL-6, and IL-4 were higher in the BALF from the RI group of mice than that from the SI group of mice (Fig. 3). IL-17 appears to be mainly generated by Th17 cells, which differentiate from naïve T cells in response to IL-6 and are maintained by IL-23 [19, 20]. Our results suggested that Mp extract might increase the levels of IL-23-expressing cells in the lungs as assessed by immunohistochemistry (IHC) and IL-6 production in BALF from RI mice, and both cytokines led to a Th17-skewed condition. KC stimulation potently induces neutrophil recruitment to the lungs. IL-17 was reported to increase the release of KC [24], and a combination of IL-17 and TNF-α appeared to synergistically enhance KC production [25]. In the present study, the KC levels in BALF were higher in the RI mice than in the SI mice. IL-17 and TNF-α levels were also higher in the RI mice. These data appear to be consistent with a scenario in which Mp increases IL-17, together with KC and TNF-α

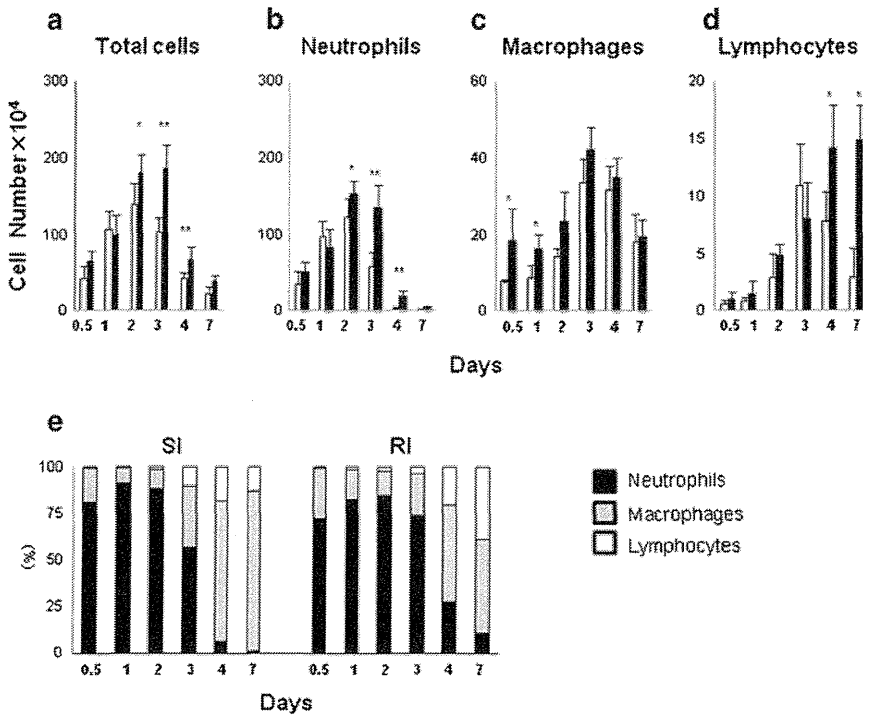

Fig. 5. Cell populations in the BALF of mice. Cell populations in the BALF of SI (*open bars*) and RI groups (*solid bars*) on days 0.5, 1, 2, 3, 4, and 7 after the final Mp extract inoculation are shown. The *vertical axis* represents average cell counts (n=5 each on days 0.5, 1, 2, 3, and 4; n=6 on day 7). Total cell counts (**a**), neutrophil counts (**b**), macrophage counts (**c**), and lymphocyte counts (**d**) are shown. Percentages of neutrophil (*black column*), macrophage (*gray column*), and lymphocyte (*white column*) are shown (**e**). Significant differences are indicated by asterisks: $*P<0.05$, $**P<0.01$. Data are expressed as means ± standard deviation.

and results in the accumulation of neutrophils in the lungs.

The total cell count in the BALF collected from RI mice was significantly higher than that in SI mice (Fig. 5a). In particular, the number of macrophages was higher in the RI mice than in the SI mice on day 0.5 and day 1 (Fig. 5c). This increase in macrophage number was followed by an increase in the number of neutrophils. The number of neutrophils was higher in the RI mice than in the SI mice at the later stages (days 2, 3, and 4) following tracheal inoculation (Fig. 5b). A previous report showed that repeated inoculation of live Mp induced neutrophilic inflammation in BALF similar to that observed in our experiment [26]. In that report, the accumulation of neutrophils in the BALF of mice repeatedly inoculated with live Mp persisted for a longer time when compared with that in mice inoculated with a single live Mp. Thus, repeated live Mp inoculation induced persistent neutrophilic inflammation similar to that observed in the RI mice in the present study.

Neutrophilic accumulation in BALF was observed in humans suffering from Mp pneumonia [9] and in mice inoculated with either live Mp [15] or with Mp extract. Few studies have assessed IL-17 levels in human BALF, although IL-17 levels in the serum of patients suffering from Mp pneumonia were higher than those suffering from streptococcal pneumonia [16]. The mechanisms of how Mp extract induced excessive infiltration of neutrophils in the lung remain not elucidated. In the mice inoculated with the Mp extract in the present study, elevation of IL-17 and associated neutrophil accumulation was observed. Previous studies of mice inoculated with live Mp reported that Mp induced an increase in the concentration of IL-17, KC, TNF-α, IL-6, IFN-γ, and IL-12 in BALF [15, 26–29]. Our current findings are generally consistent with the inflammatory reactions found in humans and mice with different experimental designs and thus suggest that lung inflam-

mation in the RI group, similar to the lung inflammation in human Mp pneumonia [5], seemed exacerbated by repeated Mp stimulation. Some of these reports [28, 29] have also suggested that IL-12 (and the IFN-γ production that it stimulated) plays a role in the increase in neutrophilic alveolar infiltration in mice infected with live Mp, whereas we did not observe an elevation in IL-12 levels.

This study had several limitations. Firstly, it was based on the evaluation of sequential changes of inflammatory mediators and cells. It should be borne in mind that very small concentrations of pro-inflammatory cytokines and mediators may produce biologic actions in a variety of different cell types. Thus, although our result failed to detect IL-12 in BALF perhaps due to dilution effect, IL-12 may still play a role in pro-inflammatory reactions induced by Mp extract.

Secondly, the initial aim of the investigation was to elucidate the noninfectious effect of modulating neutrophilic reactions by Mp extract, in association with IL-17. Thus, we focused on the levels of IL-17 and its related mediators on days 0.5, 1, 2, and 3, which is early in the time frame at which neutrophils were recruited to the lungs of RI mice. Although we did not define which cells of lung-derived primary cells produced IL-17, several reports have demonstrated that lymphocytes and epithelial cells are the possible sources of IL-17 [19–21]. Our result also demonstrated that lymphocytes were recruited to the lung on day 7, which could be linked to the modulatory effects on immune and inflammatory reactions induced by Mp extract. It was possible that increased lymphocytes in BALF after inoculation of Mp extract were Th17 cells, but we did not analyze subpopulations of BALF lymphocytes. Further analysis of the role of IL-17 may help elucidate the role of lymphocytes in this disease.

In summary, we have established a novel mouse model of Mp pneumonia by repeated inoculations of Mp extract without the use of alum-adjuvant treatment. Our current data further indicated that repeated inoculation of the Mp extract (RI group mice) results in increased levels of inflammatory cytokines including IL-17, associated with exacerbated neutrophilic lung inflammation. Although our study did not address the mechanism by which the IL-17 was activated by the Mp extract, or which component of the Mp extract exacerbates the lung lesions, the inflammatory reactions observed upon repeated inoculation of the Mp extract might help in understanding some of the important phenomena in the cellular and molecular pathogenesis of Mp pneumonia in humans.

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Original article

Perioperative factors affecting the occurrence of acute complex regional pain syndrome following limb bone fracture surgery: data from the Japanese Diagnosis Procedure Combination database

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Abstract

Objective. Complex regional pain syndrome (CRPS) describes a broad spectrum of symptoms that predominantly localize to the extremities. Although limb fracture is one of the most frequently reported triggering events, few large-scale studies have shown the occurrence of and factors associated with CRPS following limb fracture. This study aimed to show the occurrence and identify of those factors.

Methods. Using the Japanese Diagnosis Procedure Combination database, we identified 39 patients diagnosed with CRPS immediately after open reduction and internal fixation (ORIF) for limb fracture from a cohort of 185 378 inpatients treated with ORIF between 1 July and 31 December of each year between 2007 and 2010. Patient and clinical characteristics such as age, gender, fracture site, duration of anaesthesia and use of regional anaesthesia were investigated by logistic regression analyses to examine associations between these factors and the in-hospital occurrence of CRPS after ORIF.

Results. The occurrence of CRPS was relatively high in fractures of the distal forearm, but low in fractures of the lower limb and in patients with multiple fractures. Generally females are considered to be at high risk of CRPS; however, we found a comparable number of male and female patients suffering from CRPS after ORIF for limb fracture. In terms of perioperative factors, a longer duration of anaesthesia, but not regional anaesthesia, was significantly associated with a higher incidence of CRPS.

Conclusion. Although a limited number of CRPS patients were analysed in this study, reduced operative time might help to prevent the development of acute CRPS following limb fracture.

Key words: complex regional pain syndrome, bone fracture, open reduction and internal fixation, factors, regional anaesthesia.

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Introduction

Complex regional pain syndrome (CRPS) is a poorly understood syndrome that describes a broad spectrum of sensory, motor and autonomic-like features that predominantly manifest in the extremities. CRPS is usually encountered in patients recovering from limb trauma and they complain of intense pain that is disproportionate to the inciting event. Furthermore, the activities of daily living are significantly impaired in many CRPS patients because of this intense perceived pain [1]. While the underlying pathophysiological mechanisms of CRPS remain controversial, the autonomic-like, trophic and

motor disturbances are key features of CRPS, with the exception of spontaneous pain and allodynia. To elucidate the mechanisms of CRPS onset, some investigations have categorized CRPS into several subgroups based on the key features or somatosensory abnormalities [2, 3]. Since CRPS patients frequently present with different features (e.g. redness or pallor of the skin) and/or somatosensory abnormalities (e.g. hyperalgesia or hypoaesthesia) at various intervals, CRPS is often categorized on the basis of disease duration and the presence of certain features related to these intervals [4, 5]. Given this close temporal clustering of CRPS symptoms and signs, it may be assumed that focusing on a particular time frame for patients with CRPS provides a relatively homogeneous CRPS subgroup for investigations as to its aetiology.

Since the clinical features of CRPS are so diverse, another approach would be to analyse patient demographic data, particularly in terms of a potential triggering event. Limb fracture is one of the most frequently reported triggering events [6]. In attempting to gather a homogeneous CRPS study population, Schurmann *et al.* [7] recruited patients with a limb fracture and demonstrated that the sympathetic nervous system in the affected limb could possibly predict the development of CRPS. Another investigation that included patients with a fracture reported that no psychological factors could predict the development of CRPS, indicating that anxiety and depression are not predisposing factors for CRPS [8]. Furthermore, a prospective evaluation of CRPS patients after fracture revealed that specific bone fractures (i.e. dislocation and intra-articular fracture) are associated with an elevated incidence of CRPS [9]. Therefore, focusing on the triggering events for CRPS, in addition to the time frame, may help to disentangle the underlying mechanisms of its aetiology. However, the precedent study included a mixed-fracture patient cohort, with varied disease duration (within 1 year) and varied treatments for the fracture. As a result, the study identified a diverse array of features for CRPS; for instance, the prevalence of CRPS at 3 months after fracture increased significantly from that at the time of plaster removal (~6 weeks after the fracture), but decreased again by the 1-year mark [9]. Therefore a focus on the triggering events for CRPS proved inadequate as an independent method to elucidate the mechanisms of CRPS onset.

In the present study we sought to find a close-to-homogeneous population and thus sampled data from CRPS patients with at least one common aetiology (i.e. limb bone fracture), one common treatment [i.e. open reduction and internal fixation (ORIF)] and one common phase of disease (i.e. the acute phase of CRPS immediately after bone fracture). We used a nationwide, inpatient database to collect the data because the incidence of CRPS is very low: 5.5 and 26.2 cases per 100 000 person-years in the USA [10] and the Netherlands [6], respectively. Our objectives were to specify the demographic and medical factors that most likely constitute a risk of developing CRPS and to search for potential interventions to reduce the occurrence of CRPS in these limb fracture patients.

Materials and methods

DPC database

We used the nationwide inpatient database, the Japanese Diagnosis Procedure Combination (DPC) database, to collect the data. The details of the DPC inpatient database are described elsewhere [11, 12]. Briefly, the DPC is a Japanese case-mix classification system linked with a lump-sum payment system. All 82 academic hospitals are obliged to adopt the DPC system, while community hospitals can voluntarily adopt it. A survey of DPC hospitals is conducted between July 1 and December 31 each year by the DPC Research Group, funded by the Ministry of Health, Labour, and Welfare, Japan. The survey includes anonymous data of 3.19 million discharged cases from 952 acute care hospitals in 2010, representing ~45% of all admissions to acute care hospitals in Japan. The database includes the following data: the unique identifier of each hospital; patients' age and sex; main diagnoses, co-morbidities at admission and complications after admission that are coded by the International Classification of Diseases 10th Revision (ICD-10) codes; procedures coded by Japanese original codes; duration of anaesthesia (min); length of stay (days) and in-hospital mortality. Co-morbidities present at admission are clearly differentiated from complications after admission. Attending physicians are obliged to record the diagnoses for each patient at discharge with reference to medical charts to optimize the accuracy of the recorded diagnoses. Data compliance is mandatory to obtain reimbursement of medical fees.

The DPC database is a secondary database of administrative claims data. All the data were de-identified in each hospital and the anonymized data were sent to the study group. Informed consent to each patient was therefore waved because of the anonymous nature of the data. Study approval was obtained from the Institutional Review Board at the University of Tokyo.

Data extraction

We identified the records of all patients in the DPC database who underwent surgical repair (i.e. ORIF) for fracture of upper and lower limbs during 2007–10, including fracture of the shoulder and upper arm (ICD-10 code, S42), fracture of the forearm (S52), fracture at the wrist and hand level (S62), fracture of the femur (S72), fracture of the lower leg (S82), fracture of the foot (S92) and fractures involving multiple regions of the upper and lower limbs (T02.2–T02.6). For each patient we extracted the following data: sex, age, main diagnosis, type of surgery, duration of anaesthesia (ranging from the time of induction of anaesthesia to awakening in recovery), type of anaesthesia, length of stay and discharge status.

Fracture sites were categorized into the following three groups: upper limbs (ICD-10 codes, S42, S52 and S62), lower limbs (S72 and S82 and S92) and multiple regions (T02.2–T02.6). The duration of anaesthesia was categorized into three subgroups: $\leqslant$119 min, 120–179 min or $\geqslant$180 min. Regional anaesthesia included spinal

TABLE 1 Patient characteristics

	All	Upper limb	Lower limb	Both	*P*-value
Total, *n* (%)	185 378 (100.0)	49 650 (100.0)	133 030 (100.0)	2698 (100.0)	—
Age, *n* (%), years					
⩽59	45 444 (24.5)	24 645 (49.6)	19 758 (14.9)	1041 (38.6)	<0.001
60–79	59 846 (32.3)	17 869 (36.0)	40 961 (30.8)	1016 (37.7)	—
⩾80	80 088 (43.2)	7136 (14.4)	72 311 (54.4)	641 (23.8)	—
Sex, *n* (%)					
Male	63 898 (34.5)	24 283 (48.9)	38 376 (28.8)	1239 (45.9)	<0.001
Female	121 480 (65.5)	25 367 (51.1)	94 654 (71.2)	1459 (54.1)	—
Duration of anaesthesia, *n* (%)					
⩽119 min	101 220 (54.6)	23 806 (48.0)	76 769 (57.7)	645 (23.9)	<0.001
120–179 min	53 413 (28.8)	15 958 (32.1)	36 869 (27.7)	586 (21.7)	—
⩾180 min	30 745 (16.6)	9886 (19.9)	19 392 (14.6)	1467 (54.4)	—
Regional anaesthesia	83 945 (45.9)	7861 (16.4)	75 218 (56.9)	866 (32.4)	—
Length of stay, median (IQR), days	26 (14–44)	8 (4–18)	31 (21–50)	36 (21–60)	<0.001
In-hospital death, *n* (%)	2364 (1.3)	120 (0.2)	2215 (1.7)	29 (1.1)	—

IQR: interquartile range.

anaesthesia, epidural anaesthesia and peripheral nerve blocks.

We identified patients who were postoperatively diagnosed with CRPS during hospitalization with an ICD-10 code-based searching algorithm, including the ICD-10 code M89.0 (algodystrophy, shoulder–hand syndrome, Sudeck's atrophy or reflex sympathetic dystrophy) and G56.4 (causalgia). Japanese physicians have quite strictly diagnosed CRPS since 2005 on the basis of the original Japanese criteria [i.e. (i) continuing pain disproportionate to any inciting event and (ii) the presence of at least two symptoms and at least two signs of sensory, sudomotor, oedema, motor and trophic abnormalities] [13]; this criteria follows that set by Bruehl *et al.* [14] and was first published in 2005. Among these patients, a diagnosis of a peripheral nerve injury was further identified by the ICD-10 code that indicates a specified peripheral nerve injury (G56.1, G56.2, G56.3, G57.0, G57.2, G57.3, G57.4, G57.6, S44$, S54$, S64$, S74$, S84$, and S94$). The ICD-10 code includes symptom diagnosis, which sometimes indicates persistent pain itself but does not always indicate the presence of nerve injury [e.g. other nerve root and plexus disorders (G54.8)]. Therefore we gathered data on patients reliably coded with M89.0 and G56.4 to minimize the risk of including non-CRPS patients in a CRPS research sample.

Statistical analyses

We performed univariate comparisons of proportions using the chi-square test. Univariate and multivariate logistic regression analyses were performed to examine the relationships of each factor with the occurrence of postoperative CRPS. Differences were considered to be statistically significant at $P < 0.01$. All analyses were performed using SPSS software, version 19 (IBM SPSS, Armonk, NY, USA).

Results

Of the 11.6 million inpatients included in the DPC database during 2007–10, we identified 185 378 eligible patients. The background characteristics of the patients are shown in Table 1. The mean age (S.D.) of the patients was 68.6 years (23.2). Females suffered from limb fractures more frequently than males. The average duration of anaesthesia was 137 min (S.D. 116). Overall, 45.9% of patients received regional anaesthesia for ORIF. The number of lower limb fractures ($n = 133\,030$) was larger than that of upper limb fractures ($n = 49\,650$) and combined upper and lower limb fractures ($n = 2698$). The median postoperative length of stay was 8 days [interquartile range (IQR) 4–18] and 31 days (IQR 21–50) following upper and lower limb fracture surgery, respectively. Among these, 39 patients matched the ICD-10 code criteria of CRPS and 4 patients were diagnosed with a co-morbid specified nerve injury in addition to CRPS.

Table 2 shows the relationships between various factors and the occurrence of postoperative CRPS. Patients with upper limb fractures had a significantly higher rate of developing CRPS than patients with lower limb fractures (0.058% *vs* 0.006%, $P < 0.001$). Age and sex were not significantly related to the occurrence of CRPS. A longer duration of anaesthesia was significantly associated with more frequent postoperative CRPS. Regional anaesthesia was also significantly related to the development of CRPS.

The results of the logistic regression analyses are shown in Table 3. In the multivariate model, patients with fractures of the forearm, wrist and hand tended to show a relatively higher rate of CRPS [odds ratio (OR) 2.81, $P = 0.012$] as compared with those patients with fractures of the shoulder or upper arm. In contrast, patients with femoral fractures showed a significantly lower rate of CRPS (OR 0.05, $P < 0.001$). Patients ages

TABLE 2 The occurrence of postoperative CRPS

		n	CRPS	(%)	*P*-value
Total		185 378	39	(0.021)	—
Fracture site	Upper limb	49 650	29	(0.058)	<0.001
	Fracture of shoulder and upper arm (S42)	23 971	9	(0.038)	—
	Fracture of forearm (S52)	20 329	17	(0.084)	—
	Fracture of lower end of radius (S525)	*12 485*	*10*	*(0.080)*	—
	Fracture of lower end of both radius and ulna (S526)	*2058*	*4*	*(0.194)*	—
	Fracture of shafts of both radius and ulna (S524)	*1497*	*3*	*(0.200)*	—
	Fracture of forearm, others	*4289*	*0*	*(0)*	—
	Fracture at wrist and hand level (S62)	5350	3	(0.056)	—
	Lower limb	133 030	8	(0.006)	—
	Fracture of femur (S72)	106 880	2	(0.002)	—
	Fracture of lower leg (S82)	21 801	5	(0.023)	—
	Fracture of foot (S92)	4349	1	(0.023)	—
	Multiple regions of upper and lower limbs (T02.2–T02.6)	2698	2	(0.074)	—
Age, years	⩽9	3398	0	(0)	0.140
	10–19	9365	0	(0)	—
	20–29	6451	4	(0.062)	—
	30–39	6841	2	(0.029)	—
	40–49	7553	3	(0.040)	—
	50–59	11 836	2	(0.017)	—
	60–69	21 800	10	(0.046)	—
	70–79	38 046	11	(0.029)	—
	80–89	55 347	6	(0.011)	—
	90–99	23 856	1	(0.004)	—
	⩾100	885	0	(0)	—
Sex	Male	63 898	14	(0.022)	0.851
	Female	121 480	25	(0.021)	—
Duration of anaesthesia, min	⩽119	101 220	8	(0.008)	<0.001
	120–179	53 413	14	(0.026)	—
	⩾180	30 745	17	(0.055)	—
Nerve block	No	98 984	30	(0.030)	0.004
	Yes	83 945	9	(0.011)	—

Values in brackets following the fracture descriptions are International Classification of Diseases 10th Revision codes (ICD-10 codes). CRPS: complex regional pain syndrome.

60–79 years showed a higher rate of CRPS, but this result was not significant (OR 2.15, $P=0.062$). The rates of CRPS were not significantly different between males and females. The group with a long duration of anaesthesia (⩾180 min) showed a higher rate of CRPS as compared with the group with a short duration of anaesthesia (⩽119 min) (OR 5.73, $P<0.001$); however, the use of regional anaesthesia did not significantly contribute to a decrease in the rate of CRPS.

Discussion

In the present study we found that the demographic data of Japanese CRPS patients were different from those of American and Dutch CRPS patients [6, 10]. While the age distribution of our CRPS patients showed one peak around 60 years of age, which is almost compatible with the American and Dutch CRPS populations, a second peak was observed at ~20 years of age, which is quite disparate. We found that patients with upper limb fractures developed CRPS more frequently than those with lower limb fractures. Some previous reports support this observation; e.g. several studies report that Colles' fracture of the radius has a much higher association with CRPS than other fracture sites [6, 10, 15]. However, the precedent prospective study showed a higher incidence of CRPS in patients with lower limb fracture than those with upper limb fracture [9]. A common characteristic among these previous studies and our present study is that fractures to the distal end of either the upper or lower limbs are at a higher risk of co-morbid CRPS.

It has been generally considered that CRPS occurs more frequently in females than in males [6, 10], but our study showed no significant difference between males and females in the rate of CRPS following limb fracture. Because middle-aged and elderly female patients usually also suffer from osteoporosis, the absolute number of patients with bone fracture is much larger for females than for males. As such, this high incidence of female CRPS patients in older cohorts of previous epidemiological studies would be more noticeable [1, 16]. Furthermore, large populations of female CRPS patients might have other

TABLE 3 Logistic regression analyses for the occurrence of postoperative CRPS

	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Fracture site						
Shoulder or upper arm (S42)	Reference			Reference		
Forearm, wrist or hand (S52 or S56)	2.08	0.94, 4.56	0.069	2.81	1.25, 6.30	0.012
Femur (S72)	0.05	0.01, 0.23	<0.001	0.05	0.01, 0.28	<0.001
Lower leg or foot (S82 or S92)	0.61	0.22, 1.72	0.350	0.66	0.21, 2.05	0.469
Multiple regions (T02.2–T02.6)	1.98	0.43, 9.15	0.384	1.40	0.30, 6.66	0.671
Age, years						
⩽59	Reference			Reference		
60–79	1.45	0.70, 3.01	0.318	2.15	0.96, 4.79	0.062
⩾80	0.36	0.14, 0.93	0.035	1.75	0.61, 5.04	0.300
Sex						
Male	Reference			Reference		
Female	0.94	0.49 ,1.81	0.851	1.21	0.58, 2.52	0.613
Duration of anaesthesia, min						
⩽119	Reference			Reference		
120–179	3.32	1.39, 7.91	0.007	3.15	1.24, 7.97	0.016
⩾180	7.00	3.02, 16.22	<0.001	5.73	2.31, 14.24	<0.001
Regional anaesthesia						
No	Reference			Reference		
Yes	0.35	0.17, 0.75	0.006	1.11	0.46, 2.68	0.817

Values in brackets following the fracture descriptions are International Classification of Diseases 10th Revision codes (ICD-10 codes). CRPS: complex regional pain syndrome.

causative aetiologies aside from bone fracture as compared with male patients. Our present findings show that females with bone fractures do not appear to have a higher risk of developing CRPS as compared with their male counterparts.

The estimated overall incidence of CRPS was 0.026% and 0.0055% per year in the Dutch and American general populations, respectively [6, 10]. Although bone fracture is one of the major causes of CRPS and patients with bone fracture are generally at increased risk for the development of CRPS, the rate of CRPS in patients with ORIF for limb fracture in our study was 0.021%. This is lower than the estimated 22% following the International Association for the Study of Pain (IASP) criteria [17] and ~5% following the criteria of Bruehl *et al.* [14] for the only other study to prospectively evaluate the development of CRPS in ~600 patients with limb bone fracture within 6 weeks after injury [9]. The IASP criteria include a combination of (i) the presence of an initiating noxious event or a cause of immobilization; (ii) continuous pain, allodynia or hyperalgesia with which pain is disproportionate to any inciting event; (iii) evidence at some time of oedema, changes in skin blood flow or abnormal sudomotor activity in the region of the pain and (iv) the absence of a condition that would otherwise account for the degree of pain and dysfunction [17]. Comparatively the criteria by Bruehl *et al.* [14] include (i) continuing pain disproportionate to any inciting event and (ii) the presence of at least one symptom and two signs each of a sensory, vasomotor, sudomotor/oedema and motor/trophic nature. The IASP criteria are highly sensitive but with a low specificity, whereas the criteria by Bruehl *et al.* have a moderate-to-high sensitivity as well as a relatively high specificity. The fact that Japanese physicians have quite strictly diagnosed CRPS since 2005 on the basis of the original Japanese criteria [13] might contribute to the different prevalence rates between our study and the earlier prospective study [9]. In addition, a genetic contribution to pain perception in CRPS patients has been reported [18], and the genetic differences between Japanese and Dutch patients might contribute to the differences in the incidence of CRPS between these two investigations.

We consider that such distinct characteristics of our patients are possibly attributable to our efforts to gather a close-to-homogeneous population and thus recruiting only patients who were diagnosed with CRPS during hospitalization immediately after ORIF for limb bone fracture. Early in the postoperative period, physicians are generally reluctant to assign a diagnosis of CRPS. This may explain why the prevalence of the diagnosis of CRPS increased between 6 weeks to 3 months in the precedent prospective study [9]. We sampled data from CRPS patients within the very acute phase of the postoperative period. Such a limited observation period might result in a possible underestimation of the true prevalence of CRPS. Because of the difference in health care systems between Japan and Western countries, our length of hospital stay was generally longer than those in other reports [19].

There will be a certain number of patients with false-negative diagnoses of CRPS. Approaching this topic

from a different angle, in many patients, complaints begin and continue after the inciting event. However, it is clinically uncommon for patient complaints to begin during the period of time elapsed from the inciting event [16]. In addition, there was close temporal clustering of CRPS in ours and other cohorts [4, 5]. Thus it is unlikely that our data would include a considerable number of the false-negative cases. We should consider the likelihood of false-positive cases in the present study. Since the DPC system did not include any records of signs and symptoms of CRPS, we cannot confirm the diagnostic validity of our study retrospectively. However, to enhance specificity in the diagnosis of CRPS, we sampled patients who were reliably coded with CRPS (ICD-10 codes M89.0 and G56.4) and did not include those who were coded with a symptomatic diagnosis of neural disorders. This was because CRPS should be diagnosed in the absence of an alternative condition that would otherwise account for the pain. In addition to these considerations, and because of the reluctance of physicians to assign a diagnosis of CRPS early in the postoperative period, we assume that our data describe a well-selected, albeit small, cohort with few false-positive cases.

To cluster a close-to-homogeneous CRPS subgroup, we gathered only patients who were diagnosed with CRPS during hospitalization immediately after ORIF for limb fracture. Our approach may thus permit better identification of risk factors for the development of CRPS after ORIF for limb fracture early during the postoperative period with distinct characteristics. It should be noted therefore that the results cannot be generalized across all CRPS patients, but is applicable to those patients within a specific CRPS subgroup.

The underlying pathophysiological mechanisms of CRPS remain controversial. One possible explanation is that CRPS stems from neuropathic pain. Limb fractures, including dislocations, can induce peripheral nerve injuries. We found 4 of 39 patients diagnosed as a specified nerve injury; this figure is much larger than the reported 1–2% incidence of nerve injuries induced by limb trauma [20]. In the majority of reported cases, a diagnosis of peripheral nerve injury was made within 4 days of admission [21]. Because our observation period was sufficient, with a median length of stay of at least 8 days, our data would have been satisfactory to detect most of the peripheral nerve injuries occurring subsequent to fracture. Here we found that a longer duration of anaesthesia was associated with a higher incidence of CRPS. This longer requirement for anaesthesia might suggest the occurrence of more severe fractures in our patients and thereby a higher rate of nerve injury. Supporting evidence for this consideration comes from the fact that more severe traumas, such as crushing injuries, can lead to a higher rate of nerve injury [20]. Indeed, in the present study, patients with multiple fractures to the upper and lower limbs were plausibly considered to be the result of high-energy trauma. However, these multiple-fracture patients showed a relatively low association with CRPS. In contrast, we found that patients with fractures to the distal end of either the upper or lower limbs were at a higher risk of co-morbid CRPS. The frequency of nerve injuries to the humerus or femur in response to fracture is reported to be similar to that of the ulna or tibia [20]. These results argue against the hypothesis that more severe traumatic injury is followed by an increased prevalence of nerve injury and suggest that neuropathic pain alone cannot explain the underlying causes of CRPS.

This study aimed to search for any interventions that could contribute to a reduction in the development of CRPS patients in our subgroup of patients. We focused on the relationship between regional anaesthesia and the occurrence of CRPS because regional anaesthesia combined with general anaesthesia may be better than general anaesthesia alone for treating bone fracture patients with ORIF by blocking afferent nociceptive signals from the wound and the bone fracture into the central nervous system and helping to prevent the development of CRPS that can persist long after healing [22]. However, we did not find this to be the case. Instead, in perioperative factors we found that a longer duration of anaesthesia was associated with a higher occurrence of CRPS. In Japan, tourniquet inflation is used to decrease blood loss and improve the operative field visually during standard ORIF for distal end fractures of the upper and lower limbs. We can assume that the longer duration of anaesthesia implies not only a longer operation time, but also a longer inflation period using the tourniquet. Nerve compression and ischaemia by tourniquet inflation induces an increase in spontaneous activity and expansion of the receptive fields of the spinal nociceptive neurons, especially those with receptive fields located proximal to the tourniquet. This results in widely expanded areas of hyperalgesia and allodynia, such as CRPS, in the exposed limb [23]. If we consider that regional anaesthesia could prevent CRPS, one possible underlying cause of the pathological pain associated with CRPS is the central sensitization of the spinal nociceptive neurons induced by continuous nociceptive inputs from wound, bone fracture and ischaemic tissue and neuropathic inputs from nerve compression by tourniquet inflation. However, this mechanism is unlikely, given the present result that regional anaesthesia showed no relationship to the prevalence of CRPS. We propose the ischaemia–reperfusion injury theory as an alternative explanation for the relationship between a higher rate of CRPS and the longer duration of anaesthesia. Reperfusion subsequent to prolonged occlusion of the blood flow to one limb results in hyperalgesia and allodynia, and moreover the exposed limb exhibits an initial phase of CRPS features, such as hyperaemia and oedema [24]. A large body of clinical evidence suggests that, in at least a subset of CRPS patients, the fundamental cause of abnormal pain sensations and CRPS-based symptomatology is ischaemia–reperfusion injury followed by sustained inflammation due to microvascular pathology in deep tissues [25]. Our proposal is supported by the present results that, although there was a longer duration of anaesthesia with ORIF procedures for both upper and lower limb fractures

than with a single ORIF procedure for either an upper or lower limb fracture, multiple fractures showed a relatively low association with CRPS. Therefore a longer inflation period with a tourniquet on an exposed limb may be the cause of CRPS.

Several limitations in this study should be acknowledged. First, the recorded diagnoses in the administrative claims database are generally less well validated than those in planned prospective surveys. Second, because the database does not include information on the patients' signs and symptoms or laboratory data, underreporting or biased reporting (withholding sensitive cases) may have potentially led to over- and underestimation of the true occurrence rate of CRPS. Third, although the database represents 45% of acute care in patients in Japan, community hospitals voluntarily participated in the DPC system and patients were not randomly sampled. Fourth, our database lacks the actual duration of the tourniquet time during ORIF. We speculate that ischaemia–reperfusion injury may be a critical event for the initiation of CRPS in a specific subset of patients with limb fracture treated with ORIF. Prospective studies are required to confirm this hypothesis to develop treatment and preventative strategies for CRPS. Our approach of sampling CRPS patients with specified disease duration and aetiology may help to disentangle the underlying pathophysiological mechanisms of our specific subgroups of CRPS patients in a stepwise manner.

Rheumatology key messages

- Forearm fracture is associated with a higher risk of complex regional pain syndrome (CRPS).
- The risk of developing CRPS is equal in male and female limb fracture patients.

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OPEN

Effectiveness of Gefitinib against Non–Small-Cell Lung Cancer with the Uncommon EGFR Mutations G719X and L861Q

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Introduction: In non–small-cell lung cancer, an exon 19 deletion and an L858R point mutation in the epidermal growth factor receptor (EGFR) are predictors of a response to EGFR-tyrosine kinase inhibitors. However, it is uncertain whether other uncommon EGFR mutations are associated with sensitivity to EGFR-tyrosine kinase inhibitors.

Methods: A post-hoc analysis to assess prognostic factors was performed with the use of patients with EGFR mutations (exon 19 deletion, L858R, G719X, and L861Q) who were treated with gefitinib in the NEJ002 study, which compared gefitinib with carboplatin-paclitaxel as the first-line therapy.

Results: In the NEJ002 study, 225 patients with EGFR mutations received gefitinib at any treatment line. The Cox proportional hazards model indicated that performance status, response to chemotherapy, response to gefitinib, and mutation types were significant prognostic factors. Overall survival (OS) was significantly shorter among patients with uncommon EGFR mutations (G719X or L861Q) compared with OS of those with common EGFR mutations (12 versus 28.4 months; $p = 0.002$). In the gefitinib group (n = 114), patients with uncommon EGFR mutations had a significantly shorter OS (11.9 versus 29.3 months; $p < 0.001$). By contrast, OS was similar between patients with uncommon mutations and those with common mutations in the carboplatin-paclitaxel group (n = 111; 22.8 versus 28 months; $p = 0.358$).

Conclusions: The post-hoc analyses clearly demonstrated shorter survival for gefitinib-treated patients with uncommon EGFR mutations compared with the survival of those with common mutations and suggest that the first-line chemotherapy may be relatively effective for non–small-cell lung cancer with uncommon EGFR mutations.

Key Words: Gefitinib, G719X, L861Q, NEJ002, Uncommon epidermal growth factor receptor mutations.

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The clinical efficacy of epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs), such as gefitinib and erlotinib, has been demonstrated in non–small-cell lung cancer (NSCLC) patients in whom standard chemotherapy has failed.[1,2] Further studies have revealed that the presence of activating mutations in the EGFR kinase domain is strongly associated with the therapeutic efficacy of EGFR-TKIs.[3,4]

Randomized phase 3 trials have demonstrated that EGFR-TKIs significantly improve median progression-free survival (PFS) compared with platinum-doublet therapy in EGFR-mutated patients.[5–8] However, not all mutations in the EGFR kinase domain are responsive to EGFR-TKI treatment. These phase 3 trials have shown that EGFR-TKIs are effective for patients with common EGFR mutations, such as an exon 19 deletion or the L858R point mutation, which account for more than 90% of EGFR mutations. Retrospective studies and case reports suggest that some uncommon mutations are associated with sensitivity to EGFR-TKIs.[9–20] These mutations

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include G719X in exon 18, which accounts for approximately 3% of EGFR mutations, and L861Q in exon 21, which represents approximately 2% of EGFR mutations. However, these uncommon EGFR mutations have not been clearly shown to be predictive markers for the efficacy of EGFR-TKIs because of their low frequency.

To investigate the efficacy of gefitinib in patients with uncommon mutations, we conducted a post-hoc analysis of the NEJ002, which compared gefitinib and carboplatin-paclitaxel as first-line therapies for advanced NSCLC with activating EGFR mutations.

PATIENTS AND METHODS

Patient Population

We retrospectively analyzed the data of 225 patients who received gefitinib treatment at any point in the NEJ002 study.[6] The eligibility criteria of the NEJ002 study included the presence of advanced NSCLC harboring an EGFR mutation (exon 19 deletion or L858R, G719X, or L861Q point mutation) without the resistant EGFR mutation T790M (identified using the peptide nucleic acid–locked nucleic acid polymerase chain reaction clamp method), no history of chemotherapy, an age of 75 years or younger, a performance status of 0 to 1, and appropriate organ function.[21,22] Patients provided a written informed consent. The study was conducted in accordance with the Helsinki Declaration of the World Medical Association. The protocol was approved by the institutional review board of each participating institution.

Treatment

Eligible patients were randomly assigned to receive either gefitinib (250 mg/day) or paclitaxel (200 mg/m^2)/carboplatin (area under the curve, 6.0) on day 1 every 3 weeks. Chemotherapy was continued for at least three cycles. Gefitinib was administered until the disease progressed, intolerable toxicities developed, or consent was withdrawn. The protocol recommended that the crossover regimen be used as a second-line treatment.

Clinical Assessments

The antitumor response to treatment was assessed using computed tomography every 2 months. Unidirectional measurements were adopted on the basis of the Response Evaluation Criteria in Solid Tumors (version 1.0).[23] PFS was evaluated from the date of randomization to the date when disease progression was first observed or death occurred. The treatment response and PFS were determined by an external review of computed tomography scans by experts who were not aware of the treatment assignments. Overall survival (OS) was evaluated from the date of randomization to the date of death.

Statistical Analysis

To assess prognostic factors for OS, we used univariate and multivariate Cox proportional hazards models. Kaplan–Meier survival curves were constructed for PFS and OS, and differences between groups were identified using the log-rank test. Differences in response rates were identified using Fisher's exact test. Each analysis was two sided, with a 5% significance level and a 95% confidence interval. All analyses were performed using SAS for Windows software (release 9.1; SAS Institute, Cary, NC).

RESULTS

Patient Population

A total of 230 chemonaive patients were enrolled in the NEJ002 study: 115 patients were assigned to receive gefitinib and 115 were assigned to receive carboplatin-paclitaxel (Fig. 1). To evaluate the efficacy of gefitinib in NSCLC patients with uncommon EGFR mutations, we analyzed the data of 114 patients in the gefitinib group and 111 patients in the carboplatin-paclitaxel group. We identified five patients who had uncommon EGFR mutations in each group. Two patients, who had common mutations and were treated with first-line chemotherapy consisting of carboplatin-paclitaxel, were excluded from the PFS analysis in the NEJ002 study. However, both were treated with gefitinib and were included in this post-hoc analysis. The demographic and disease characteristics of the patients with uncommon EGFR mutations were similar to those of patients with common EGFR mutations (Table 1). The characteristics of each patient with uncommon EGFR mutations are shown in supplementary Table S1 (Supplemental Digital Content 1, http://links.lww.com/JTO/A494).

Survival Factors

In the univariate analysis of 225 patients who received gefitinib at any point, uncommon EGFR mutations had a significant detrimental effect on survival (Table 2). We also identified performance statuses 1 and 2, distant metastasis, brain metastasis, stable disease, and progressive disease as significant predictors of worse prognosis for standard chemotherapy and stable disease and progressive disease as significant predictors of worse prognosis for gefitinib. When these variables were included in the Cox proportional hazards model, we found that uncommon EGFR mutations, performance statuses 1 and 2, stable disease and progressive disease for standard chemotherapy, and stable disease and progressive disease for gefitinib had significant hazard ratios (Table 2).

Uncommon EGFR Mutations and Survival

The Kaplan–Meier curve for OS for uncommon versus common EGFR mutations is shown in Figure 2*A*. The OS was significantly shorter among patients with uncommon EGFR mutations compared with OS of those with common EGFR mutations in the overall population (12 versus 28.4 months; $p = 0.002$). A significantly shorter survival time was observed in patients with uncommon EGFR mutations compared with survival time in those with common EGFR mutations in the gefitinib group (11.9 versus 29.3 months; $p < 0.001$) (Fig. 2*B*). However, a similar survival time was observed between the subgroups of uncommon and common EGFR mutations in the carboplatin-paclitaxel group (22.8 versus 28 months; $p = 0.358$) (Fig. 2*C*).

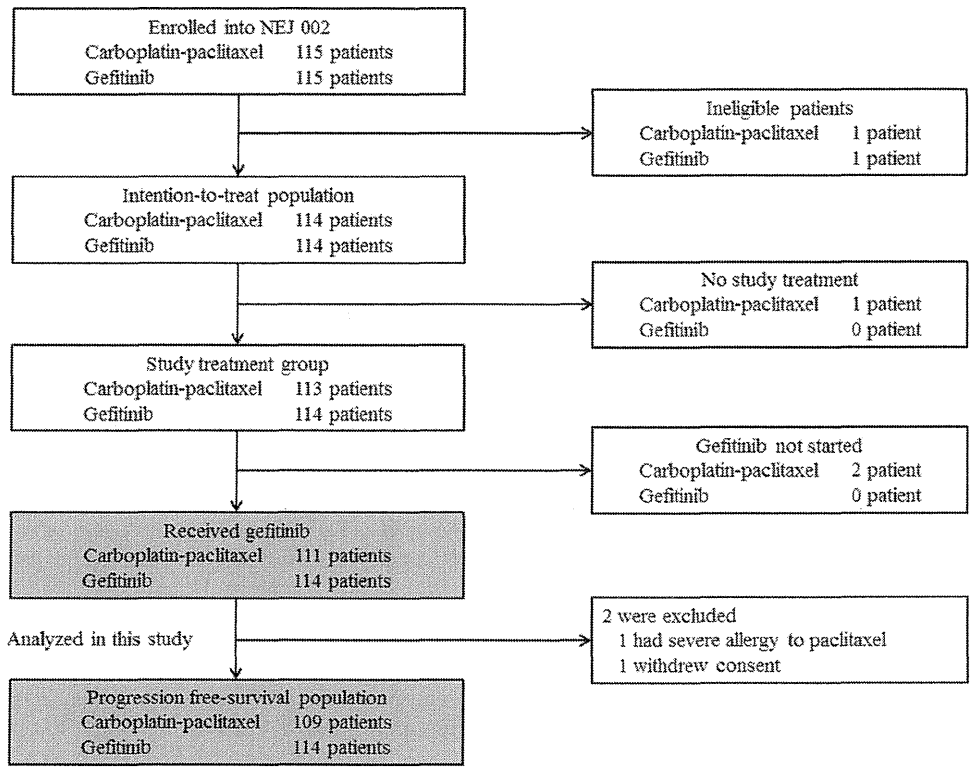

FIGURE 1. Enrollment, randomization, and follow-up of the study patients.

To examine whether the sequence of platinum doublet and gefitinib affected OS, we performed a further subgroup analysis. The survival time tended to be shorter among patients receiving first-line gefitinib compared with the survival time among those receiving first-line carboplatin-paclitaxel in the uncommon EGFR mutation group (11.9 versus 22.8 months; $p = 0.102$). Consistent with previous publications, a similar survival time was observed between patients receiving first-line gefitinib and those receiving first-line carboplatin-paclitaxel in the common EGFR mutation group (29.3 versus 28 months; $p = 0.378$).

Uncommon EGFR Mutations, PFS, and Response

In the gefitinib group, the median PFS was significantly shorter for patients with uncommon EGFR mutations compared with median PFS of those with common EGFR mutations (2.2 versus 11.4 months; $p < 0.001$) (Fig. 3*A*). By contrast, the median PFS did not differ significantly between patients with uncommon EGFR mutations and those with common EGFR mutations in the carboplatin-paclitaxel group (5.9 versus 5.4 months; $p = 0.847$) (Fig. 3*B*). The objective response rate was significantly lower in patients with uncommon EGFR mutations compared with the objective response rate in those with common EGFR mutations when treated with gefitinib (20% versus 76%; $p = 0.017$) (supplementary Table S2, Supplemental Digital Content 1, http://links.lww.com/JTO/A494). By contrast, similar objective response rates were observed for patients with uncommon EGFR mutations and those with common EGFR mutations in the carboplatin-paclitaxel group (20% versus 32%; $p = 0.336$) (supplementary Table S2, Supplemental Digital Content 1, http://links.lww.com/JTO/A494).

DISCUSSION

Recent studies suggest that NSCLC patients with uncommon EGFR mutations are less responsive to EGFR-TKIs compared with patients with L858R and exon 19 deletions.[9–20] However, the efficacy of EGFR-TKIs in NSCLC patients with uncommon mutations has not been fully elucidated.

We conducted a post-hoc analysis of the NEJ002 study to evaluate the effectiveness of gefitinib against NSCLC with G719X or L861Q. The NEJ002 study, comparing gefitinib and standard carboplatin-paclitaxel chemotherapy as the first-line treatment for patients with EGFR mutations, demonstrated no significant difference in OS between gefitinib and carboplatin-paclitaxel.[6] In contrast to other phase 3 trials investigating EGFR-TKIs for patients with common EGFR mutations of exon 19 deletion and L858R, the NEJ002 is the only study that included uncommon EGFR mutations of G719X and L861Q.

The current study clearly demonstrated that NSCLC patients with the uncommon EGFR mutations G719X and L861Q had shorter survival than the survival of those with an exon 19 deletion or L858R mutation (Fig. 2). Our results are consistent with other clinical studies on EGFR-TKIs in

TABLE 1. Patient Characteristics

Number of Patients	Uncommon Mutation 10	Common Mutation 215
Sex		
Female	4	139
Male	6	76
Age (yr)		
Median	63	65
Range	42–75	35–75
Smoking status		
Never smoked	5	134
Smoker	5	81
Performance status		
0/1/2	5/5/0	105/107/3
Histology		
Adenocarcinoma	9	202
Others	1	13
Clinical stage		
Stage IIIB	3	32
Stage IV	6	165
Postoperative	1	18
Type of EGFR mutation		
G719X	7	
L861Q	3	
Exon 19 deletion		115
L858R		97
19 deletion + L858R		3

EGFR, epidermal growth factor receptor.

patients with uncommon EGFR mutations (supplementary Table S3, Supplemental Digital Content 1, http://links.lww.com/JTO/A494). The overall response rate to EGFR-TKIs in patients with uncommon EGFR mutations was 41%, which is lower than the response rate to TKIs (62%–83%) of patients with common EGFR mutations.[7,8,24] In the NEJ002 study, G719X included G719C and G719S. No patients harbored G719A. To investigate the effectiveness of gefitinib on each uncommon EGFR mutations, we evaluated the difference in OS between patients with uncommon EGFR mutations (G719C versus G719S and G719X versus L861Q). There was no significant difference between these subgroups (data not shown).

This study showed that the PFS and OS tended to be shorter among patients treated with first-line gefitinib compared with PFS and OS among those treated with first-line carboplatin-paclitaxel in the uncommon EGFR mutation group (supplementary Table S2, Supplemental Digital Content 1, http://links.lww.com/JTO/A494). We also found poor disease control rate with gefitinib in patients with uncommon mutations. Three of five patients with uncommon mutations in the gefitinib group had progressive disease. By contrast, no patients with uncommon mutations had progressive disease in the carboplatin-paclitaxel group. Although the number of patients with uncommon mutations in each treatment group was small, platinum-doublet therapy might be a better choice than gefitinib for first-line therapy in patients with uncommon EGFR mutations. Because some of patients with uncommon mutations showed good clinical response to gefitinib in this study and they seemed to be heterogeneous in terms of response to gefitinib, administration of gefitinib should be considered for patients with uncommon mutations when disease progression was observed after first-line chemotherapy.

In vitro studies have indicated that the affinity of gefitinib for EGFR proteins with uncommon EGFR mutations is lower than the affinity of gefitinib for EGFR proteins with common EGFR mutations.[25] A sixfold or 14-fold higher concentration of gefitinib was required to inhibit the growth of cells expressing G719X or L861Q, respectively, compared with cells expressing L858R.[26] These results may explain the lack of response to gefitinib in patients with uncommon EGFR mutations. The authors also examined the sensitivity of G719X and L861Q mutations to erlotinib and irreversible TKIs.[27] Cells expressing G719X were less resistant to erlotinib than gefitinib in vitro; however, L861Q was resistant to both erlotinib and gefitinib. In contrast to erlotinib, irreversible TKIs inhibited the growth of cells with G719X or L861Q at a

TABLE 2. Univariate and Multivariate Analysis by Cox Proportional Hazards Model

	Univariate			Multivariate		
	HR	95% CI	*p*	HR	95% CI	*p*
Age (≥70/<70)	1.047	0.719–1.525	0.81			
Sex (female/male)	0.73	0.51–1.045	0.86			
Smoking status (+/−)	1.376	0.967–1.958	0.076			
Performance status (1, 2/0)	1.792	1.263–2.541	0.001	1.85	1.297–2.639	0.001
Histology (nonadeno/adeno)	0.647	0.302–1.387	0.263			
Types of EGFR-m (uncommon/common)	2.967	1.501–5.868	0.018	2.445	1.177–5.079	0.017
Distant metastasis (+/−)	4.914	1.113–5.741	0.027	2.849	1.241–6.54	0.135
Brain metastasis (+/−)	1.781	1.248–2.542	0.002	1.311	0.897–1.915	0.162
Response to Cb/TXL (SD, PD/CR, PR)	1.742	1.113–2.728	0.015	1.748	1.11–2.754	0.016
Response to G (SD, PD/CR, PR)	2.878	2.012–4.117	0.002	2.601	1.794–3.771	<0.001

HR, hazard ratio; CI, confidential interval; EGFR-m, epidermal growth factor receptor mutation; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; Cb/TXL, carboplatin plus paclitaxel; G, gefitinib.

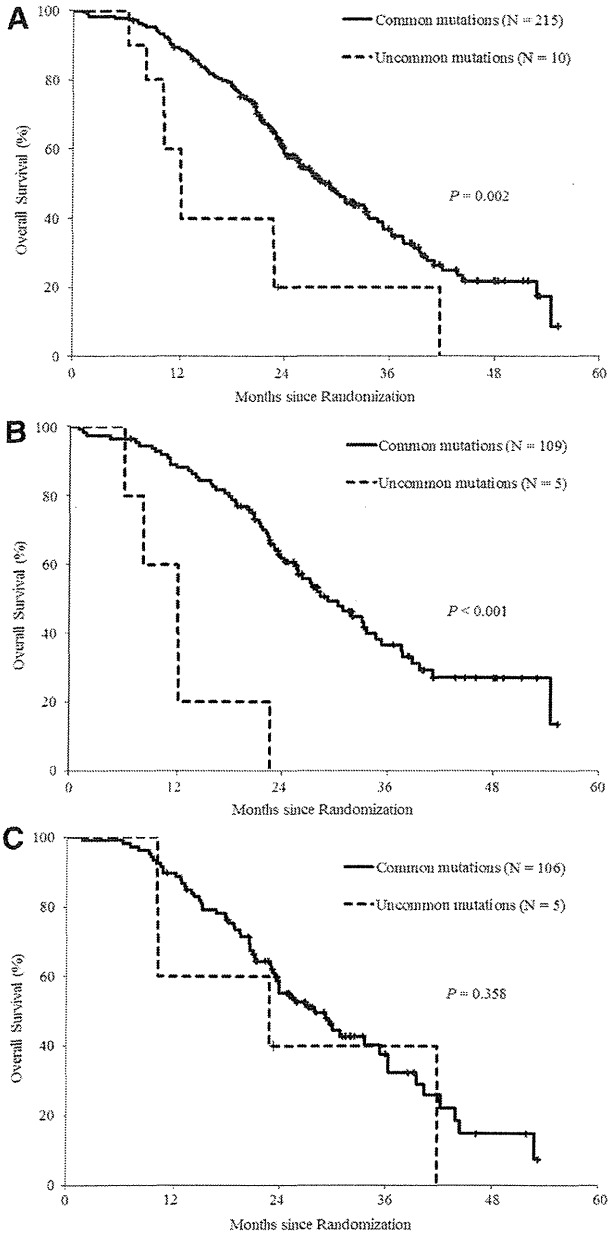

FIGURE 2. The overall survival curves of patients with common mutations and uncommon mutations in the entire population (*A*), the gefitinib group (*B*), and the carboplatin-paclitaxel group (*C*).

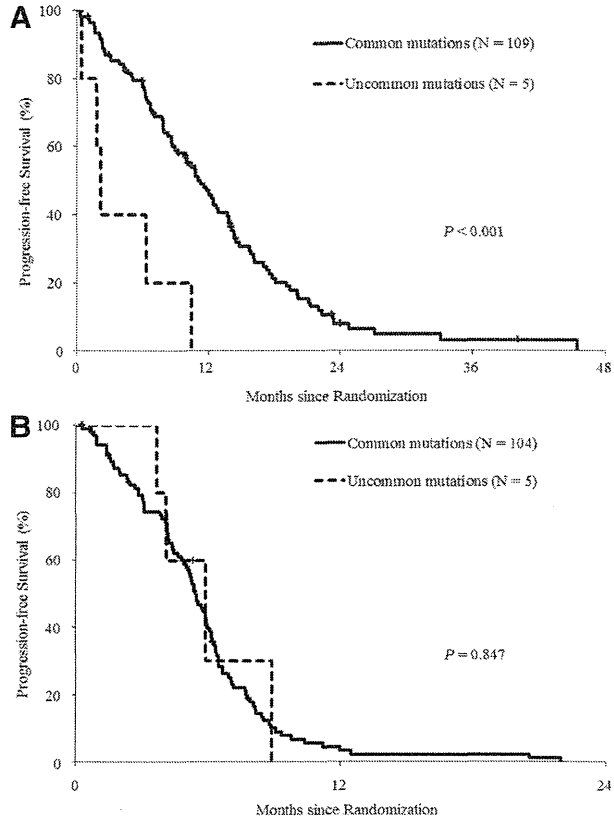

FIGURE 3. Progression-free survival curves in the gefitinib group (*A*) and the carboplatin-paclitaxel group (*B*) according to the type of epidermal growth factor receptor mutation.

lower concentration than those with wild-type EGFR. Indeed, Sequist et al.[28] reported that the effectiveness of an irreversible pan-ErbB receptor TKI, neratinib, on NSCLC patients with G719X. Niratinib induced partial responses in three of four patients with G719X and the fourth had durable stable disease for 40 weeks. It may be beneficial to evaluate erlotinib as a treatment for NSCLCs with G719X and irreversible EGFR-TKIs as treatments for NSCLCs with G719X and L861Q. Because previous phase 3 trials that investigated erlotinib or irreversible TKIs for NSCLC with EGFR mutations did not include uncommon EGFR mutations, further clinical studies may need to be performed.[7,8,29]

Another possible strategy for the treatment of uncommon EGFR mutations is the combination of EGFR-TKIs and cytotoxic agents. Our group has undertaken a randomized phase 3 trial to compare gefitinib plus carboplatin plus pemetrexed with gefitinib monotherapy for patients with NSCLC with an exon 19 deletion or an L858R, G719X, or L861Q EGFR mutation (NEJ009; University Hospital Medical Information Network Clinical Trials Registry [UMIN-CTR] number, UMIN000006340). The data from this study will advance the treatment of NSCLC with uncommon EGFR mutations.

In conclusion, our post-hoc analysis clearly demonstrated shorter survival of TKI-treated patients with uncommon EGFR mutations compared with survival of those with common EGFR mutations. Furthermore, the data suggest that the first-line chemotherapy may be relatively effective for NSCLC with uncommon EGFR mutations.

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ORIGINAL REPORT

LUX-Lung 4: A Phase II Trial of Afatinib in Patients With Advanced Non–Small-Cell Lung Cancer Who Progressed During Prior Treatment With Erlotinib, Gefitinib, or Both

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See accompanying editorial on page 3303 and articles on pages 3327 and 3342

A B S T R A C T

Purpose

New molecular targeted agents are needed for patients with non–small-cell lung cancer (NSCLC) who progress while receiving erlotinib, gefitinib, or both. Afatinib, an oral irreversible ErbB family blocker, has preclinical activity in epidermal growth factor receptor (EGFR [ErbB1]) mutant models with EGFR-activating mutations, including T790M.

Patients and Methods

This was a Japanese single-arm phase II trial conducted in patients with stage IIIB to IV pulmonary adenocarcinoma who progressed after ≥ 12 weeks of prior erlotinib and/or gefitinib. Patients received afatinib 50 mg per day. The primary end point was objective response rate (complete response or partial response) by independent review. Secondary end points included progression-free survival (PFS), overall survival (OS), and safety.

Results

Of 62 treated patients, 45 (72.6%) were *EGFR* mutation positive in their primary tumor according to local and/or central laboratory analyses. Fifty-one patients (82.3%) fulfilled the criteria of acquired resistance to erlotinib and/or gefitinib. Of 61 evaluable patients, five (8.2%; 95% CI, 2.7% to 18.1%) had a confirmed objective response rate (partial response). Median PFS was 4.4 months (95% CI, 2.8 to 4.6 months), and median OS was 19.0 months (95% CI, 14.9 months to not achieved). Two patients had acquired T790M mutations: L858R + T790M, and deletion in exon 19 + T790M; they had stable disease for 9 months and 1 month, respectively. The most common afatinib-related adverse events (AEs) were diarrhea (100%) and rash/acne (91.9%). Treatment-related AEs leading to afatinib discontinuation were experienced by 18 patients (29%), of whom four also had progressive disease.

Conclusion

Afatinib demonstrated modest but noteworthy efficacy in patients with NSCLC who had received third- or fourth-line treatment and who progressed while receiving erlotinib and/or gefitinib, including those with acquired resistance to erlotinib, gefitinib, or both.

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INTRODUCTION

Epidermal growth factor receptor (*EGFR* [ErbB1]) somatic mutations occur in 30% of patients with non–small-cell lung cancer (NSCLC) who are of East Asian ethnicity (eg, from Japan or Taiwan) compared with 8% of patients of other ethnicities (eg, from the United States or Australia).[1] The predictive significance of these mutations in NSCLC and the association with a considerable improvement in response and progression-free survival (PFS) with currently available tyrosine kinase inhibitor (TKI) therapy have been shown in several phase III trials.[2-7] Despite promising results, patients with NSCLC who harbor *EGFR* mutations will eventually experience disease progression as a result of the inevitable development of resistance mechanisms, in particular, the T790M mutation in exon 20, which is found in more than 50% of patients who received an EGFR TKI.[8,9] Currently, there are no treatments with proven efficacy for these patients; thus, there is an increased demand to develop novel molecular targeted agents.