For example, irinotecan is a cytotoxic drug for treatment of small cell lung cancer and NSCLC, and association between the toxicity and single-nucleotide polymorphism has been studied intensively about uridine diphosphate glucuronosyltransferase 1A1-glucuronosyltransferase 1A1. In fact, in comparative outcomes analysis of Japan Clinical Oncology Group 9511 and Southwest Oncology Group (SWOG) 0124 in extensive stage small cell lung cancer, significant differences in toxicity were observed in cisplatin plus irinotecan between the two populations.[6] Grade 3 or higher neutropenia was observed in 65% of Japanese patients, while 34% in the United States. It is suggested that carefully planned global clinical trials are vital to elucidate potential ethnic differences in adverse effects.

In this study, we report an ethnic difference in toxicity due to chemotherapy for patients with NSCLC through systematic review of the literature. Platinum-containing chemotherapy is still the cornerstone of treatment for patients,[7] and we chose treatment regimens that were globally used, including cisplatin plus gemcitabine (CG), cisplatin plus vinorelbine (CV), and carboplatin plus paclitaxel (CP) in NSCLC. The target ethnicity in this study was Asians and non-Asians who were mainly white.

METHODS

Literature Search and Data Extraction

Randomized trials with chemotherapy regimens of CP, CG, and CV in NSCLC published from January 1, 2000, to December 31, 2009, were identified from MEDLINE. We used keywords "nonsmall cell lung cancer," "cisplatin," "gemcitabine," "vinorelbine," "carboplatin," "paclitaxel," "phase II," "phase III," and "randomized trial." The trials with number of patients less than 50 were excluded to better ensure reliability. Search results were limited to reports written in the English language.

To evaluate the toxicity of chemotherapy, we excluded reports of postoperative chemotherapy, preoperative chemotherapy, and chemotherapy for elderly or poor performance status and chose the reports that used chemotherapy agents with doses and schedules close to those in Japan. We adopted the trials with cisplatin 75 to 80 mg/m^2 and gemcitabine 1000 to 1250 mg/m^2, every 3 weeks, and the trials with cisplatin 75 to 80 mg/m^2 and vinorelbine 25 to 30 mg/m^2. Similarly, we adopted the trials with carboplatin area under the curve 5 to 6 mg/ml/min and paclitaxel 200 to 225 mg/m^2. Trials involving radiation therapy were excluded. For each trial, data on sample size, characteristics of ethnicity, toxicity of neutropenia, anemia, and thrombocytopenia were collected. Each study has its own measure to evaluate side effects, which were World Health Organization criteria and the National Cancer Institute's common toxicity criteria version 1 to 4. Although there is a slight difference among them, the boundary of grades 2 and 3 is the same.

Literature search was performed independently by two investigators (Y.H. and T.T.) to assess the reliability of data extraction.

Statistical Analysis

To evaluate ethnic difference in toxicity due to chemotherapies, we calculated actual number of patients from published data.

Sample distributions for the patients with and without toxicities were tested with the χ^2 test and odds ratio (OR) with its 95% confidence interval. Because a certain number of reports did not include ethnicity information, we were uncertain of the ratio of Asian patients in study population in each clinical trial. We were also uncertain of the occurrence of

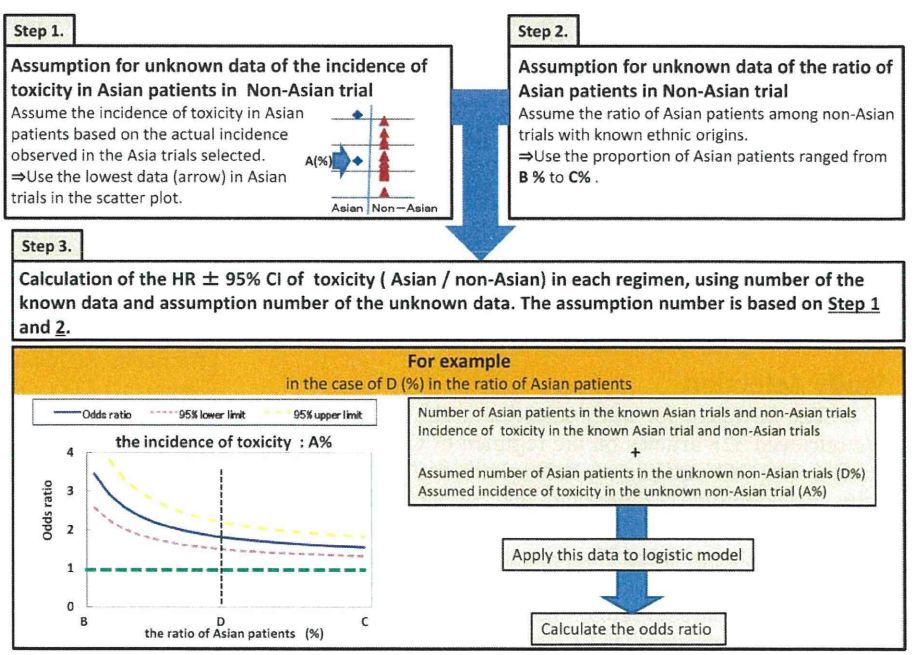
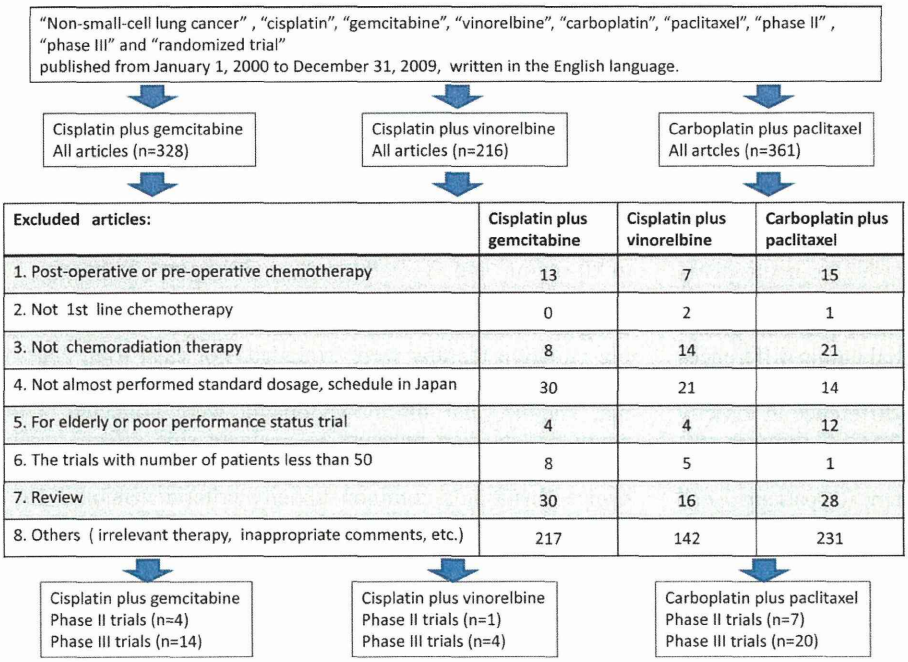


FIGURE 1. Schematic diagram of sensitivity analysis.



Excluded articles:	Cisplatin plus gemcitabine	Cisplatin plus vinorelbine	Carboplatin plus paclitaxel
1. Post-operative or pre-operative chemotherapy	13	7	15
2. Not 1st line chemotherapy	0	2	1
3. Not chemoradiation therapy	8	14	21
4. Not almost performed standard dosage, schedule in Japan	30	21	14
5. For elderly or poor performance status trial	4	4	12
6. The trials with number of patients less than 50	8	5	1
7. Review	30	16	28
8. Others (irrelevant therapy, inappropriate comments, etc.)	217	142	231

FIGURE 2. Flow chart showing the progress of trials through the review.

toxicity in the Asian patients. As shown in Figure 1, we used sensitivity analysis by systematically repeating the statistical analysis using different assumptions for the ratio of Asian patients in clinical trials performed in the United States and Europe. Sensitivity analysis can be used to determine how different values of an independent variable will impact a particular dependent variable under a given set of assumptions when the study involves uncertainty in data distributions.[8] Ratio of ethnicity is determined based on the trials that include ethnicity information. We applied the ranged ratios of Asian patients to the trials in which the racial ratio was not described. We assumed the incidence of hematological toxicity in Asian patients based on the actual incidence observed in the trials selected.

As for survival, we examined whether there are any differences in the distribution of median overall survival between Asian and US/Europe trials using Student's t test.

A p value less than 0.05 was considered statistically significant, and all reported p values were obtained with two-sided manner. All statistical analyses were performed using SPSS 16.0 for Windows (SPSS, Inc, Chicago, IL).

RESULTS

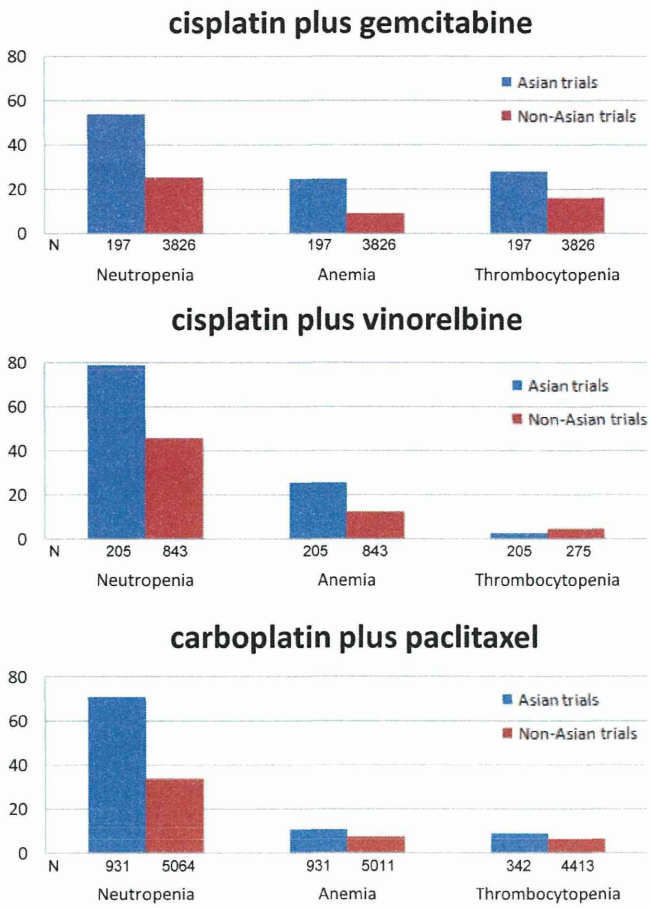
Study Selection

The flowchart for study selection is shown in Figure 2. We retrieved 328 articles on the regimen of CG, 216 articles on CV, and 361 articles on carboplatin and paclitaxel. Finally, we identified 12 phase II and 38 phase III trials of NSCLC with a total of 11,271 patients in three regimens.

All articles are listed in supplement 1 to 3B, Supplemental Digital Content 1 to 4; http://links.lww.com/JTO/A104, http://links.lww.com/JTO/A105, http://links.lww.com/JTO/A106, and http://links.lww.com/JTO/A108. In the regimen of CG, we identified four phase II trials and 14 phase III trials with a total of 4023 patients (supplement 1, Supplemental Digital Content 1, http://links.lww.com/JTO/A104). Two trials were performed in Japan and China (Asian studies), and 16 trials were performed in the United States and Europe (non-Asian studies). In CV, one phase II trial and four phase III trials were identified with a total of 1253 patients (supplement 2, Supplemental Digital Content 1, http://links.lww.com/JTO/A105). Two trials were performed in Japan, and three trials were performed in the United States and Europe. In CP, we selected seven phase II trials and 20 phase III trials with a total of 5995 patients (supplement 3A, 3B, Supplemental Digital Content 3 and 4, http://links.lww.com/JTO/A106 and http://links.lww.com/JTO/A108). Three trials were performed in Asian countries including Japan, and 24 trials were performed in the United States and Europe. With regard to patient characteristics, median age from 56.4 to 66 years, and good performance status ratio (Eastern Cooperative Oncology Group 0 to 1, World Health Organization 0 to 1, or Karnofsky ≥70) accounted for 99.8% as a median. In the comparison of age between Asian trials and non-Asian trials, there was no significant difference in the three regimens; CG ($p = 0.64$), CV ($p = 0.50$), and CP ($p = 0.82$), respectively. In the performance status, there were more patients with poor performance status included in non-Asian study in the two regimens: CG ($p < 0.001$) and CV ($p < 0.001$); there was no significant difference in CP ($p = 0.45$).

Hematological Toxicity in Asian and Non-Asian Studies

As shown in Figure 3, grade 3/4 toxicities were more frequently observed in the Asian studies, when the actual number of all the patients was combined. Distribution of the frequency of grade 3/4 toxicity is shown in Figure 4. In a regimen of CG, neutropenia, anemia, and thrombocytopenia



CDDP GEM	N	Neutropenia	Anemia	Thrombocytopenia
Asian trials	197	53.9%	24.7%	28.0%
Non-Asian trials	3826	25.3%	9.2%	16.0%
χ2test		P<0.001	P<0.001	P<0.001
OR		3.45	3.27	2.04
(95% CI)		(2.58-4.61)	(2.30-4.56)	(1.48-2.82)

CDDP VNR	N	Neutropenia	Anemia	Thrombocytopenia
Asian trials	205	78.8%	25.6%	2.6%
Non-Asian trials	843	45.6%	12.4%	4.5% (N=275)
χ2test		P<0.001	P<0.001	P=0.323
OR		4.43	2.43	0.57
(95% CI)		(3.09-6.36)	(1.67-3.54)	(0.57-1.59)

CBDCA PTX	N	Neutropenia	Anemia	Thrombocytopenia
Asian trials	931	70.9%	10.8%	8.8% (N=342)
Non-Asian trials	5064	33.7%	7.4% (N=5011)	6.5% (N=4413)
χ2test		P<0.001	P<0.001	P=0.115
OR		4.79	1.52	1.39
(95% CI)		(4.11-5.59)	(1.20-1.91)	(0.94-2.06)

FIGURE 3. Comparison of grades 3 and 4 hematological toxicity between combined Asian and non-Asian trials.

were more frequently observed in Asian studies than in non-Asian studies with a statistical significance ($p < 0.001$). In a regimen of CV, neutropenia and anemia were also significantly more frequently observed in Asian studies ($p < 0.001$) except thrombocytopenia ($p = 0.323$). In a regimen of CP, neutropenia and anemia occurred more frequently in Asian studies ($p < 0.001$), again excepting thrombocytopenia ($p = 0.115$).

Hematological Toxicity in Sensitivity Analysis

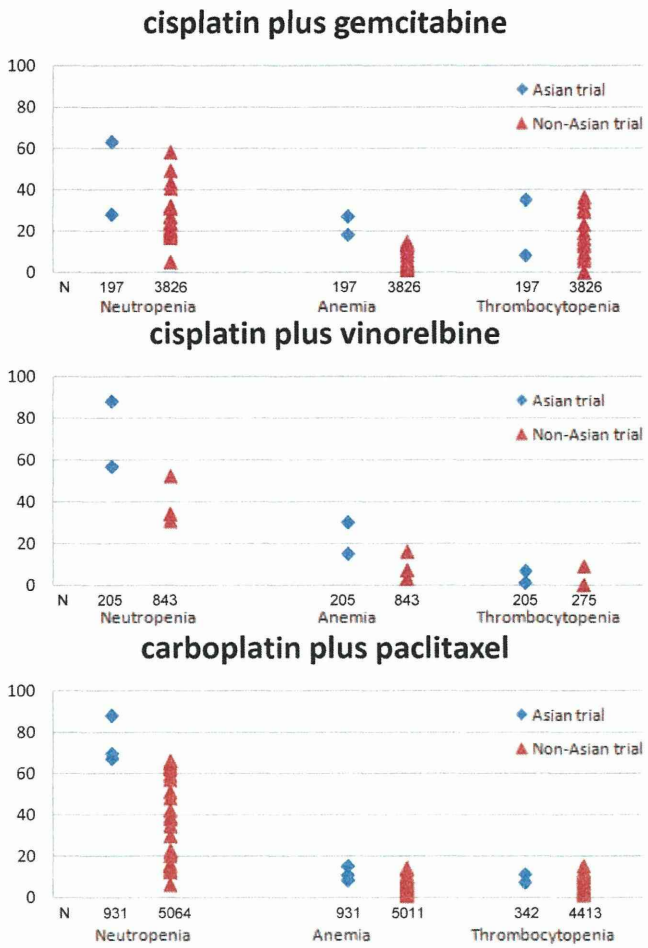
Asians in the Asian trial were Japanese and Chinese from East Asia, whereas non-Asians were large and heterogeneous populations without a specified origin. We extracted Asian and non-Asian patients from both separated Asian trials/non-Asian trials and trials enrolling Asian and non-Asian patients.

In a total of 50 trials of NSCLC that were identified, only 14 trials reported ethnic origins of the study participants. Among the 14 trials, 8 trials included Asian patients and they showed that the ratio of Asian and non-Asian patients is imbalanced, and the proportion of Asian patients ranged from 0.8 to 17.4% (median 6.0%).

We estimated the ratio of Asian patients in the 36 trials with unknown ethnic origins. We calculated the hazard ratio ± 95% confidence interval of hematological toxicity (Asian/non-Asian) in each regimen, varying a putative Asian population from 0 to 18%. We adopted the lowest frequency of each hematological toxicity observed in actual Asian trials, and we could reduce the margin of error to be more accurate, minimizing the possible differences in severity of hematological toxicities between Asians and non-Asians (Figure 1). On the basis of these assumptions, nine models were created as shown in Figure 5. x axis is the ratio of Asian, and y axis is the OR. We showed how an OR changed as we changed the Asian ratio. The green dotted line represents the OR of 1. As shown in Figure 5, even if we changed the Asian ratio, more frequent grade 3/4 neutropenia and anemia were observed significantly in Asian patients in the three regimens. However, there was no significant difference between Asian and non-Asian in frequency of severe thrombocytopenia.

Survival Analysis

We identified six phase II and 38 phase III trials of NSCLC in three regimens in survival analysis. We excluded the IRESSA Pan Asia Study trial because most of the patients in the study were never-smokers in East Asia who had outstanding



CDDP GEM	N	Neutropenia	Anemia	Thrombocytopenia
Asian trials	197	53.9% (28.0-63.0)	24.7% (18.0-27.0)	28.0% (8.0-35.0)
Non-Asian trials	3826	25.3% (4.8-58.0)	9.2% (1.0-14.6)	16.0% (0-36.3)

CDDP VNR	N	Neutropenia	Anemia	Thrombocytopenia
Asian trials	205	78.8% (56.7-88.0)	25.6% (15.0-30.0)	2.6% (1.0-6.7)
Non-Asian trials	843	45.6% (30.7-52.0)	12.4% (3.0-16.0)	4.5% (0-9.0) (N=275)

CBDCA PTX	N	Neutropenia	Anemia	Thrombocytopenia
Asian trials	931	70.9% (67.1-88.0)	10.8% (8.1-15.0)	8.8% (7.1-11.0) (N=342)
Non-Asian trials	5064	33.7% (5.9-66.0)	7.4% (0.6-14.1) (N=5011)	6.5% (1.0-15.1) (N=4413)

FIGURE 4. Comparison of grades 3 and 4 hematological toxicity between Asian and non-Asian trials; scatterplot.

good prognosis. In the comparison of overall survival by using Student's *t* test, median survival time was longer in the Asian studies than those in non-Asian studies as shown in Figure 6 (12.2 months versus 9.6 months, $p = 0.012$).

DISCUSSION

Global clinical trials including lung cancer have advanced year by year. Glickman et al.[9] reviewed 300 articles reporting the results of clinical trials in 1995–2005 and found that the number of countries serving as trial sites outside the United States more than doubled in 10 years, whereas the proportion of trials conducted in the United States and Western Europe decreased. Globalization of clinical trials may also shorten the timeline for clinical testing.

Although it is efficient to include patients globally for saving time and costs in completing large-scale clinical trials, ethnic difference in treatment benefit and toxicity is becoming a great concern. In IRESSA Survival Evaluation in Lung cancer study,[4] Asian patients lived longer compared with non-Asian patients treated with gefitinib (median 9.5 versus 5.2 months). EGFR mutation is a critical biomarker for EGFR-TKIs, and there is a higher rate of EGFR mutations in the Asian patients than whites, 19 to 61% versus 5 to 10%.[5] Ethnic difference in clinical benefit might be because of tumor biology among different ethnicities. However, instead of a large body of work focused on differences in clinical benefit, we find that those in hematological toxicity have not been fully studied. Given such a situation, we showed a significant difference of hematological toxicity due to cytotoxic chemotherapy between Asian and non-Asian in a pooled analysis on phase II and III clinical trials.

In this study, we showed that the degree of hematological toxicities of neutropenia and anemia was significantly different between Asian and the US/European studies. In sensitivity analysis, we demonstrated that Asian patients had a disadvantage in side effects compared with non-Asian patients who were mostly whites. Grade 3/4 neutropenia and anemia were more frequently observed in Asians in the common chemotherapy regimens of platinum doublets widely used in patients with NSCLC. Serious thrombocytopenia was also observed in CG, but not in CV and CP. It is suggested that dose setting be carefully conducted in global clinical trial and that dose modification according to ethnicity be considered.

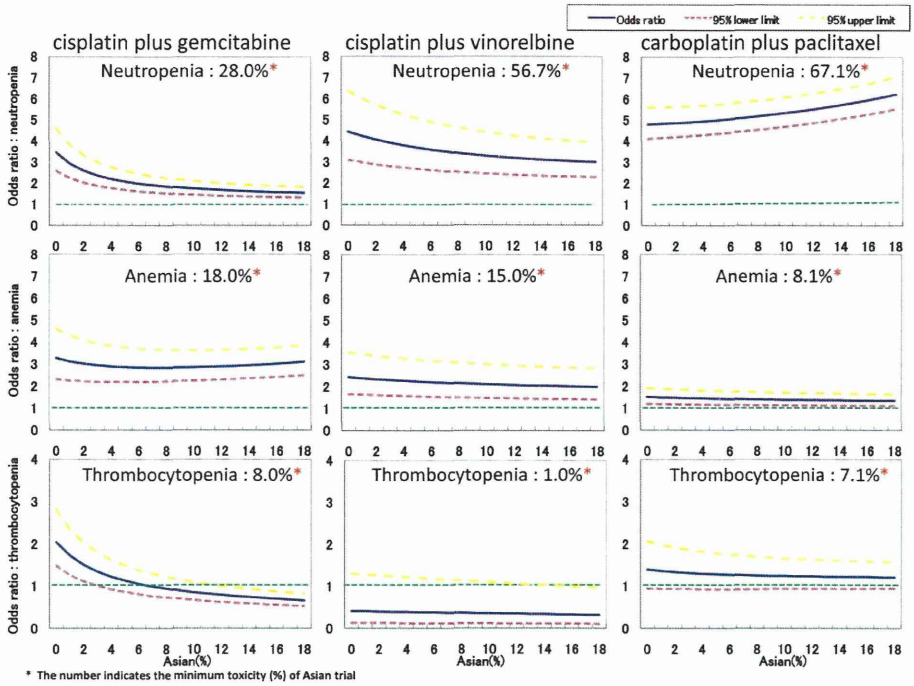


FIGURE 5. Sensitivity analysis: green dotted line represents the OR of 1. Red and yellow dotted lines represent probability with 95% confidence. Yellow dotted line is the highest probability. Red dotted line is the lowest probability. A difference is found when using the high figure in Figure 3. Because this is not representative of the entire group, it does not show a difference among all cases. If we use the lowest number, we reduce the margin of error to be more accurate. In this study, severe neutropenia and anemia were more frequently observed in Asians, excepting severe thrombocytopenia.

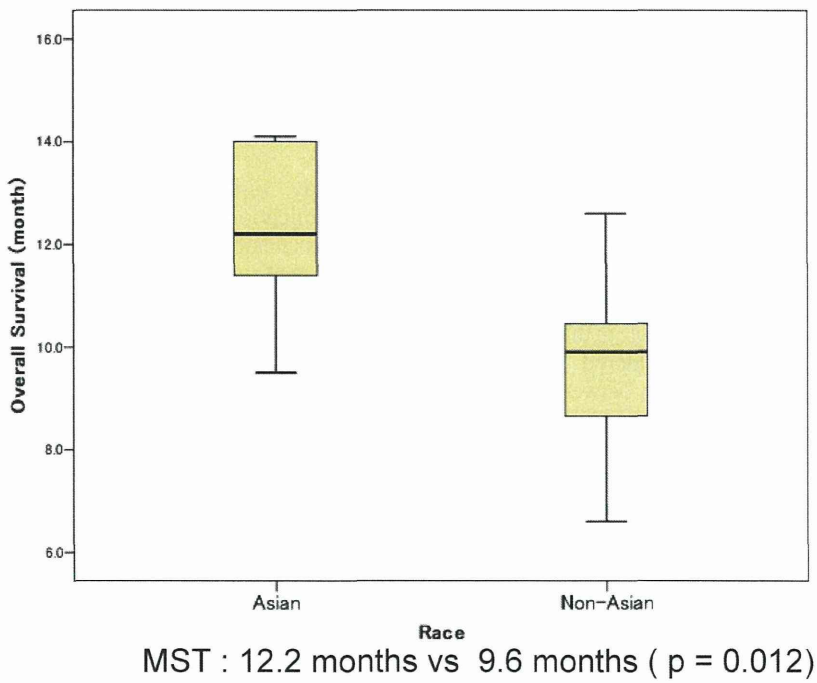


FIGURE 6. Survival and race: the comparison of overall survival time by using Student's *t* test.

There is substantial interindividual variability in drug metabolism.[10] Recent evidence suggests that there is even greater variability between individuals of different ethnicity. A number of genetic polymorphisms that reflect ethnic differences have been reported to affect pharmacokinetics and pharmacodynamics.[5] It is well known that pharmacokinetic factors that determine an individual's exposure to drugs and metabolites affect the potential for beneficial and toxic responses to that medicine, e.g., *CDA*3* for gemcitabine,[11] *ABCB1* for vinorelbine,[12] *CYP2C8* for paclitaxel,[13,14] and ERCC2, XPD for platinum compounds.[2,15]

Another explanation for ethnic difference is frequency of never-smokers in patients with NSCLC between Asians and whites. Epidemiological study showed that approximately 30% of patients with NSCLC were never-smokers in Asians, while 7 to 8% in whites.[16] In smokers, the dose-

normalized area under the plasma concentration–time curve of irinotecan was significantly lower compared with nonsmokers, and smokers experienced considerably less hematologic toxicity.[17] In addition, there was a significantly higher incidence of grades 3 and 4 neutropenia among patients treated with gemcitabine monotherapy without a history of smoking than among those with a history of smoking.[18] Therefore, smoking status may affect drug metabolism and toxicity, although this is still controversial.

Neutropenia during chemotherapy has been reported to be a predictor of longer survival in several studies.[19,20] In fact, among clinical trials analyzed in this study, we detected weak correlation between response rates and grade 3 and 4 hematological toxicities for neutropenia (data not shown). We showed that median survival time was better in the Asian studies than those in the non-Asian studies, although it is a statistical disadvantage without considering size of the study. Side effects of neutropenia shown in this study may affect the prognosis of Asians as well. In the international First-Line ErbituX in lung cancer study,[21] the 11% of patients who were Asian had a considerably better overall prognosis regardless of study treatment compared with whites (median survival, 19.5 months versus 9.6 months). Again, EGFR mutations were frequently observed in Asians, and survival benefit for Asians seemed to be because of EGFR mutations and EGFR-TKI treatment. Recently, Gandara et al.[22] reported that tumors with EGFR-activating mutations have lower expression level of genes associated with DNA repair, such as ERCC1 and suggested that low DNA repair capacity may be a more direct explanation for improved efficacy of platinum-based chemotherapy in Asian populations.

There are some limitations in our study. First, our unique methods may potentially influence publication bias. However, our primary focus of toxicity is not associated with end point originally designed in each clinical trial, and our results cannot be affected by published data selected. Other confounding factors that influence hematological toxicity also should be considered. There was no significant difference in age distribution between Asian trial and non-Asian trial, and there were poorer performance status patients included in non-Asian study, which likely relates with more side effects. More side effects occurred in Asian trials, which included more good performance status patients, and unbalanced performance status distribution between the two study groups cannot minimize our results. Second, the number of Asian trials was relatively small, and the chemotherapy regimen of cisplatin and pemetrexed was not included in this study, which is also commonly used globally. However, no phase II or phase III studies on cisplatin and pemetrexed conducted in Asian countries have been reported to date. Global clinical trials have increased, and more Asians will be enrolled in the near future. Third, the data of each trial were not based on individual data, and side effects are also affected by frequency of examination of blood count. In addition, each study has its own follow-up algorithm. There may be an argument that Asian doctors conducted the test more, which leads to more severe toxicity appearing in Asian study. However, SWOG and Japan Multi-National Organization common arm analysis used an identical protocol and they showed significant difference between them, and grades 3 and 4 neutropenia and febrile neutropenia were significantly greater than in SWOG trial.[2] This common arm analysis is a promising and reliable method to investigate toxicity and genetic backgrounds particularly for the study of ethnic differences. Another approach can be inclusion of the information of ethnicity in designing clinical trials, which will be collected individually for future meta-analysis.

In conclusion, we demonstrated that severe hematological toxicities were more frequently observed in Asian patients compared with non-Asians who were mainly white. This study suggests that global clinical trials should be carefully designed and conducted to account for potential genetic differences in the patient. Large-scale prospective studies focused on ethnic differences are warranted for global public benefit.

ACKNOWLEDGMENTS

Supported by the Osaka Medical Research Foundation for Incurable Diseases.

The authors thank Dr. Akihiro Tamiya, Kinki-chuo Chest Medical Center, for his discussion for the study.

REFERENCES

1. Sekine I, Nokihara H, Yamamoto N, et al. Common arm analysis: one approach to develop the basis for global standardization in clinical trials of non-small cell lung cancer. *Lung Cancer* 2006;53:157–164.
2. Gandara DR, Kawaguchi T, Crowley J, et al. Japanese-US common-arm analysis of paclitaxel plus carboplatin in advanced non-small-cell lung cancer: a model for assessing population-related pharmacogenomics. *J Clin Oncol* 2009;27:3540–3546.
3. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005;353: 123–132.
4. Thatcher N, Chang A, Parikh P, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet* 2005;366: 1527–1537.
5. Phan VH, Moore MM, McLachlan AJ, et al. Ethnic differences in drug metabolism and toxicity from chemotherapy. *Expert Opin Drug Metab Toxicol* 2009;5:243–257.
6. Lara PN Jr, Natale R, Crowley J, et al. Phase III trial of irinotecan/cisplatin compared with etoposide/cisplatin in extensive-stage small-cell lung cancer: clinical and pharmacogenomic results from SWOG S0124. *J Clin Oncol* 2009;27:2530–2535.
7. Azzoli CG, Baker S Jr, Temin S, et al. American Society of Clinical Oncology Clinical Practice Guideline update on chemotherapy for stage IV non-small-cell lung cancer. *J Clin Oncol* 2009;27:6251–6266.
8. Rothman KJ, Greenland S, Lash TL. Modern Epidemiology, 3rd Ed. Philadelphia, PA: Lippincott Williams & Wilkins, 2008.
9. Glickman SW, McHutchison JG, Peterson ED, et al. Ethical and scientific implications of the globalization of clinical research. *N Engl J Med* 2009;360:816–823.
10. Wilkinson GR. Drug metabolism and variability among patients in drug response. *N Engl J Med* 2005;352:2211–2221.
11. Sugiyama E, Kaniwa N, Kim SR, et al. Pharmacokinetics of gemcitabine in Japanese cancer patients: the impact of a cytidine deaminase polymorphism. *J Clin Oncol* 2007;25:32–42.
12. Wong M, Balleine RL, Blair EY, et al. Predictors of vinorelbine pharmacokinetics and pharmacodynamics in patients with cancer. *J Clin Oncol* 2006;24:2448–2455.
13. Harris JW, Rahman A, Kim BR, et al. Metabolism of taxol by human hepatic microsomes and liver slices: participation of cytochrome P450 3A4 and an unknown P450 enzyme. *Cancer Res* 1994;54:4026–4035.

14. Rahman A, Korzekwa KR, Grogan J, et al. Selective biotransformation of taxol to 6 alpha-hydroxytaxol by human cytochrome P450 2C8. *Cancer Res* 1994;54:5543–5546.
15. Gurubhagavatula S, Liu G, Park S, et al. XPD and XRCC1 genetic polymorphisms are prognostic factors in advanced non-small-cell lung cancer patients treated with platinum chemotherapy. *J Clin Oncol* 2004;22:2594–2601.
16. Kawaguchi T, Matsumura A, Fukai S, et al. Japanese ethnicity compared with Caucasian ethnicity and never-smoking status are independent favorable prognostic factors for overall survival in non-small cell lung cancer: a collaborative epidemiologic study of the National Hospital Organization Study Group for Lung Cancer (NHSGLC) in Japan and a Southern California Regional Cancer Registry Databases. *J Thorac Oncol* 2010;5:1001–1010.
17. van der Bol JM, Mathijssen RH, Loos WJ, et al. Cigarette smoking and irinotecan treatment: pharmacokinetic interaction and effects on neutropenia. *J Clin Oncol* 2007;25:2719–2726.
18. Kanai M, Morita S, Matsumoto S, et al. A history of smoking is inversely correlated with the incidence of gemcitabine-induced neutropenia. *Ann Oncol* 2009;20:1397–1401.
19. Di Maio M, Gridelli C, Gallo C, et al. Chemotherapy-induced neutropenia and treatment efficacy in advanced non-small-cell lung cancer: a pooled analysis of three randomised trials. *Lancet Oncol* 2005;6:669–677.
20. Kishida Y, Kawahara M, Teramukai S, et al. Chemotherapy-induced neutropenia as a prognostic factor in advanced non-small-cell lung cancer: results from Japan Multinational Trial Organization LC00–03. *Br J Cancer* 2009;101:1537–1542.
21. Pirker R, Pereira JR, Szczesna A, et al. Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial. *Lancet* 2009;373:1525–1531.
22. Gandara DR, Grimminger P, Mack PC, et al. Association of epidermal growth factor receptor activating mutations with low ERCC1 gene expression in non-small cell lung cancer. *J Thorac Oncol* 2010;5:1933–1938.

ANTICANCER RESEARCH *31*: 4561-4568 (2011)

Prospective Study Evaluating the Plasma Concentrations of Twenty-six Cytokines and Response to Morphine Treatment in Cancer Patients

CHIHIRO MAKIMURA[1], TOKUZO ARAO[2], HIROMICHI MATSUOKA[1], MASAYUKI TAKEDA[1], HIDEMI KIYOTA[1], JUNJI TSURUTANI[1], YOSHIHIKO FUJITA[2], KAZUKO MATSUMOTO[2], HIDEHARU KIMURA[2], MASATOMO OTSUKA[3], ATSUKO KOYAMA[3], CHIYO K. IMAMURA[4], TAKEHARU YAMANAKA[5], KYOKO TANAKA[6], KAZUTO NISHIO[2] and KAZUHIKO NAKAGAWA[1]

Departments of [1]Medical Oncology and [2]Genome Biology, Kinki University Faculty of Medicine, Osaka-Sayama, Osaka, Japan; [3]Department of Palliative Care, Sakai Hospital, Kinki University Faculty of Medicine, Sakai-minamiku, Osaka, Japan; [4]Department of Clinical Pharmacokinetics and Pharmacodynamics, Keio University, School of Medicine, Shinjuku-ku, Tokyo, Japan; [5]Institute for Clinical Research, National Kyushu Cancer Center, Minami-ku, Fukuoka, Japan; [6]Department of Nursing, School of Nursing, Osaka Prefecture University, Habikino-City, Osaka, Japan

Abstract. *Cytokine signaling is involved in pain and opioid-receptor signaling. In this prospective study, we studied the plasma cytokine levels in order to identify candidate biomarkers for predicting resistance to morphine treatment in a cohort of opioid-treatment-naïve cancer patients. We analyzed pain rating and the plasma concentrations of 26 cytokines at baseline and after morphine treatment using a multiplex immunoassay system for the following cytokines: eotaxin, colony stimulating factor, granulocyte (G-CSF), colony stimulating factor granulocyte-macrophage (GM-CSF), interferon α2 (IFN-α2), IFN-γ, interleukin 1α (IL-1α), IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17, IP-10, monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1α (MIP-1α), MIP-1β, tumor necrosis factor-α (TNF-α) and TNF-β. No correlation was observed between the clinical characteristics and the numerical rating scale for pain at baseline or among patients who developed resistance to morphine treatment. Interestingly, the plasma concentration of MIP-1α significantly decreased during morphine treatment (day 8 vs. baseline, p=0.03). Regarding the baseline plasma cytokine concentrations, none of the cytokine levels were correlated with the numerical rating scale for pain at baseline; however, the baseline plasma concentrations of eotaxin, IL-8, IL-12 (p40), IL-12 (p70), MIP-1α and MIP-1β were significantly lower in patients who required a high dose of morphine or who developed resistance to morphine treatment. In conclusion, this is the first report revealing that the plasma concentrations of several cytokines were significantly modulated during treatment and were correlated with treatment outcome of morphine. Our results suggest that plasma cytokine levels may be promising biomarkers for morphine treatment and that they warrant further study.*

Correspondence to: Kazuto Nishio, Department of Genome Biology, Kinki University School of Medicine, 377-2 Ohno-higashi, Osaka-Sayama, Osaka 589-8511, Japan. Tel: +81 723660221 (Ext 3150). Fax: +81 723676369, e-mail: knishio@med.kindai.ac.jp

Key Words: Cytokines, morphine, plasma, MIP-1α, IL-12.

Approximately 80% of advanced-stage cancer patients suffer from pain as a result of their disease, and more than 10 million cancer patients are thought to be treated with opioids worldwide (1). Therefore, controlling chronic, severe pain caused by cancer is considered a very important issue for improving the quality of life of cancer patients. Since the degree of pain sensation and the outcome of morphine treatment varies widely among individuals, pharmacogenetic, pharmacokinetic and pharmacodynamic biomarkers of opioid treatment, such as genetic determinants, have been investigated intensively to improve the effectiveness of morphine treatment (2). Several genetic variants associated with varying pain sensitivity have been identified in the general population, including of the genes for μ-opioid receptor (*OPRM1*); δ-opioid receptor (*OPRD1*); catecholamine-O-methyltransferase (*COMT*); guanosine

0250-7005/2011 $2.00+.40

triphosphate cyclohydrolase 1/DOPA-responsive dystonia (*GCH1*); melanocortin-1 receptor (*MC1R*); transient receptor potential cation channel, subfamily V, member 1 (*TRPV1*); and transient receptor potential cation channel, subfamily A, member 1 (*TRPA1*) (2, 3). With regard to morphine treatment, the most investigated genetic variant is *OPRM1* 118A>G. The *OPRM1* gene is the main target of morphine, and the 118A>G variant leads to a change in amino acids (asparagine to aspartic acid) at position 40 of the extracellular receptor region, affecting a putative glycosylation site of the receptor and suggesting that the different sensitizing to pain is biologically reasonable (2). Many studies have evaluated the correlation between *OPRM1* 118A>G and the outcome of morphine treatment; however, a recent meta-analysis showed no consistent associations between the *OPRM1* 118A>G genotype and most of the phenotypes in a heterogeneous set of eight clinical studies, except for weak evidence of an association with less nausea and increased opioid dosage requirements in homozygous carriers of the G allele (4). Other genetic variants (such as variations in *COMT*; *MC1R*; ATP-binding cassette, sub-family B, member 1 [*ABCB1*]; and UDP glucuronosyltransferase 2B7 [*UGT2B7*]) and combinations of such variants have been examined in several studies (2, 5). However, plasma cytokine levels have never been used as biomarkers for morphine treatment to date.

Meanwhile, emerging evidence has indicated that cytokine signaling is closely involved in pain and that bidirectional interactions exist between cytokine and opioid-receptor signaling (6, 7). In addition, the overexpression of cytokines and chemokines is frequently observed in many types of cancer (8, 9). However, few studies have evaluated the plasma concentrations of cytokines in association with pain scale ratings or the outcome of morphine treatment. Thus, we hypothesized that the plasma concentrations of some cytokines may be modulated or correlated with morphine treatment in cancer patients. In this prospective study, we examined the plasma concentrations of 26 cytokines to explore candidate biomarkers capable of predicting resistance to morphine treatment.

Materials and Methods

Patients. This prospective study started in July 2009 and enrollment was completed in March 2011 at the Kinki University Faculty of Medicine and Sakai Hospital, Kinki University Faculty of Medicine. Clinicopathological features including age, sex, ECOG performance status (PS), type of primary malignant neoplasm, metastatic sites, white blood cell count (WBC), hemoglobin (Hb) level, platelet count (PLT), and albumin (Alb) and C-reactive protein (CRP) levels were recorded. The numerical rating scale (NRS) for pain (10, 11) and the required doses of morphine were evaluated at baseline and on days 1 and 8 of morphine treatment. Morphine treatment was performed according to the standard method including titration (NCCN Guidelines™, Adult Cancer Pain) (12). Resistance to morphine treatment on day 1 (early phase) or on day 8 (stationary phase) was defined as the requirement of a high morphine dose (>30 mg) and the persistence of pain after morphine treatment (NRS ≥6) on days 1 or 8, respectively. The present study was approved by the Institutional Review Boards of both centers, and written informed consent was obtained from all the patients.

Preparation of plasma samples. Blood samples were collected before the initiation of morphine treatment (baseline) and on day 8. The separated sera were stocked at -80°C until further use.

Table I. *Clinical characteristics of study patients.*

Characteristic		Total n=44	
		No. of patients	%
Age, years	Median	69	
	Range	40-85	
Gender	Male	22	50
	Female	22	50
PS	0	0	0
	1	9	20
	2	24	55
	3	10	23
	4	1	2
Cancer type	Lung	19	43
	CRC	8	18
	Gastric	4	9
	CUP	4	9
	Pancreatic	2	5
	Breast	2	5
	GB	1	2
	RCC	1	2
	Lymphoma	1	2
	PCC	1	2
	Skin	1	2
Metastatic Sites (n)	0	4	9
	1	19	43
	2	13	29
	3≥	8	18
WBC (/μl)	<5000	8	18
	5000-9999	22	50
	≥10000	14	32
Hb (g/dl)	<8.5	3	7
	8.5-11.9	27	61
	≥12	14	32
PLT (104/μl)	<10	0	0
	10-29	24	55
	≥30	20	45
Alb (g/dl)	<2.5	4	9
	2.5-3.4	20	45
	≥3.5	20	45
CRP (mg/dl)	<1	12	27
	1.0-4.9	16	36
	≥5	16	36

CRC, colorectal; CUP, cancer of unknown primary; GB, gallbladder; RCC, renal cell carcinoma; Lymphoma, malignant lymphoma; PCC, malignant pheochromocytoma.

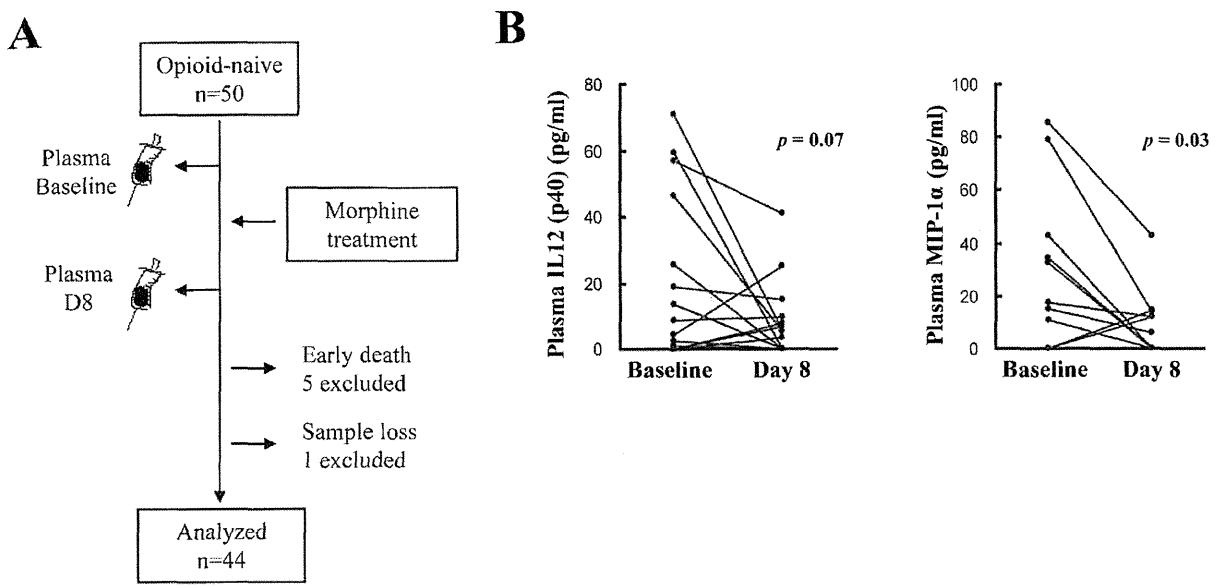


Figure 1. *A: Flow diagram of analyzed patients. B: Plasma concentrations of MIP-1α (left panel) and IL-12 (p40, right panel) at baseline and after morphine treatment (day 8).*

Antibody suspension bead array system. The plasma concentrations of 26 cytokines were determined using commercially available antibody suspension bead arrays (MILLIPLEX™ Human Panel 1 Pre-mixed 26 Plex #MPXHCYTO60KPMX26; Millipore, Billerica, MA, USA). The markers used in this panel were as follows: eotaxin, colony stimulating factor, granulocyte (G-CSF), colony stimulating factor, granulocyte-macrophage (GM-CSF), interferon, α2 (IFN-α2), IFN-γ, interleukin 1α (IL-1α), IL-1-β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17, IP-10, monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1α (MIP-1α), MIP-1β, tumor necrosis factor-α (TNF-α) and TNF-β. Data was obtained using a Bio-Plex suspension array system® (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. The method has been previously described (13-14).

Statistical analysis. Statistical analyses were performed to test for differences between groups, using Student's *t*-test or Fisher's exact test. A *p*-value of <0.05 was considered statistically significant. All analyses were performed by JMP (SAS Institute, Cary, NC, USA).

Results

Patient results. A total of 50 patients with opioid-treatment-naïve and histologically confirmed malignant neoplasms, who were scheduled to undergo opioid treatment were eligible for enrollment in this study. Five patients were excluded from the analysis because of early cancer death within two weeks, and one patient was excluded because of absence of a plasma sample. Thus, 44 patients were included in the final analysis (Figure 1A). Of the 44 patients, 75% had a PS of 0–2 and 43% had advanced lung cancer (Table I). Forty-seven percent of the patients had metastatic lesions in two or more organs. The laboratory data for WBC, Hb, PLT, Alb and CRP are also shown (Table I).

Clinical characteristics and outcome of morphine treatment. We evaluated whether the clinical characteristics were associated with the NRS for pain at baseline or among patients who had developed resistance to morphine treatment on days 1 or 8. Age, sex, PS, tumor type, metastatic sites, WBC, Hb, PLT, Alb and CRP were examined (Table II). Twenty-five patients (57%) had severe pain (NRS ≥6) at baseline. Resistance to morphine treatment was observed in 11 patients (25%) on day 1 and 14 patients (32%) on day 8. None of the examined clinical characteristics were associated with the NRS for pain or the outcome of morphine treatment. These results suggest that predicting the outcome of morphine treatment based on clinical parameters may be difficult.

Plasma concentrations of cytokines at baseline and changes after morphine treatment. We examined the changes in the plasma concentrations of 26 cytokines at baseline and after morphine treatment (Table III). The baseline plasma concentrations seemed to vary widely among individuals; for example, the plasma concentrations of G-CSF and IL-6 varied from 0 to 2332 pg/ml and 0 to 1879 pg/ml, respectively. During morphine treatment, the plasma MIP-1α level decreased significantly (baseline: 7.2±19.3 pg/ml, day

Table II. *Relationship between clinical characteristics and resistance to morphine treatment.*

Characteristic		Pain scale* (Baseline)			Treatment outcome (day 1)			Treatment outcome (day 8)		
		Mild	Severe	*p*-value	Well controlled	Resistant**	*p*-value	Well controlled	Resistant**	*p*-value
Age (years)	65<	5	8	0.75	9	4	0.71	9	4	1.00
	65≥	14	17		24	7		21	10	
Gender	Male	11	11	0.54	16	6	1.00	14	8	0.75
	Female	8	14		17	5		16	6	
PS	0-2	12	21	0.16	24	9	0.70	21	12	0.46
	3-4	7	4		9	2		9	2	
Tumor types	Lung ca.	10	9	0.36	15	4	0.73	11	8	0.33
	Others	9	16		18	7		19	6	
Metastatic sites (n)	0-1	10	13	1.00	17	6	1.00	13	10	0.11
	2≥	9	12		16	5		17	4	
WBC (/μl)	<10000	14	16	0.53	24	6	0.29	21	9	0.74
	≥10000	5	9		9	5		9	5	
Hb (g/dl)	<10	6	13	0.23	15	4	0.73	14	5	0.53
	≥10	13	12		18	7		16	9	
PLT (104/μl)	<30	11	13	0.78	16	8	0.29	16	8	1.00
	≥30	8	12		17	3		14	6	
Alb (g/dl)	<3.5	10	14	1.00	21	3	0.08	16	8	1.00
	≥3.5	9	11		12	8		14	6	
CRP (mg/dl)	<5	13	15	0.75	19	9	0.28	20	8	0.74
	≥5	6	10		14	2		10	6	

Comparisons are between mild *vs.* severe pain groups and well-controlled *vs.* resistant to morphine treatment groups. *Pain was evaluated using the numerical rating scale for pain (NRS). Severe pain was defined as NRS ≥6. **Resistance group was defined as the requirement of a high dose of morphine (>30 mg) and persistent pain (NRS ≥6) after morphine treatment. The *p*-values were calculated using the Fisher's exact test.

8: 2.3±7.4 pg/ml, $p=0.03$). Although the difference was not significant, the plasma IL-12 (p40) level also decreased (baseline: 7.0±17.4 pg/ml, day 8: 2.7±7.6 pg/ml, $p=0.07$) (Figure 1B). Since the results were obtained from paired samples of the same individuals at baseline and after treatment, morphine treatment was thought to reduce these plasma concentrations. MIP-1α and IL-12 (p40) could be novel biomarkers for monitoring the effects of morphine treatment, although further studies are needed.

Baseline plasma cytokine concentrations and required dose of morphine. We analyzed whether the baseline plasma cytokine levels were associated with the required dose of morphine. IL-8, IL-12 (p40) and MIP-1α were significantly lower in patients who required a high dose (>30 mg) of morphine on day 1 after titration ($p=0.03$, $p=0.01$ and $p=0.02$, Table IV). Meanwhile, the concentration of eotaxin was significantly lower in patients who required a high dose of morphine on day 8 ($p=0.00026$).

Baseline plasma cytokines and outcome of morphine treatment. Finally, we analyzed whether the baseline plasma cytokine levels were associated with the outcome of morphine treatment. None of the cytokine levels were correlated with the NRS for pain at baseline (Table V). However, several cytokines, including IL-12 (p40), IL-12 (p70), MIP-1α and MIP-1β, were significantly lower in patients who developed resistance to morphine treatment on day 1 compared with the levels in patients whose pain was well controlled after morphine treatment ($p=0.03$, $p=0.03$, $p=0.02$ and $p=0.01$, respectively). Interestingly, the plasma concentrations of IL-12 (p40) and MIP-1α were identified by both changes and outcome of morphine treatment, suggesting that these cytokines may be closely involved in opioid signaling. Meanwhile, the concentration of eotaxin was significantly lower in patients with resistance to morphine treatment on day 8 (baseline: 53.8±26.0 pg/ml, day 8: 34.6±9.0 pg/ml, $p=0.0009$). Collectively, the baseline plasma concentrations of several cytokines were significantly associated with the outcome of morphine treatment.

Discussion

MIP-1α was identified as a macrophage inflammatory protein that has inflammatory and neutrophil chemokinetic properties (15). MIP-1α plays various roles in inflammatory responses by binding to receptors, including chemokine (C-C motif) receptor 1 (CCR1) and CCR5 (16). Cellular sources

Table III. *Plasma concentrations of cytokines at baseline and changes after morphine treatment. The plasma cytokine concentrations at baseline (before) and after (day 8) morphine treatment are shown as the minimum, maximum, and mean±SD. Comparisons between baseline and after treatment concentrations were evaluated using the t-test.*

Cytokine (pg/ml)	Baseline			After treatment*			Baseline *vs.* After
	Range		Mean±SD	Range		Mean±SD	
	Min	Max		Min	Max		*p*-value
Eotaxin	7.8	115.6	47.7±23.7	2.7	114.5	42.7±23.5	0.10
G-CSF	0.0	2331.9	265.1±363.4	12.0	4452.1	329.5±653.2	0.57
GM-CSF	0.0	13.5	2.6±2.5	0.0	17.7	2.7±2.9	0.75
IFN-α2	1.4	76.3	16.5±15.6	0.0	37.2	16.5±8.0	1.00
IFN-γ	0.0	86.6	7.7±16.9	0.0	68.2	6.4±12.2	0.62
IL-1α	0.0	581.9	82.8±118.5	0.0	594.8	88.4±113.8	0.63
IL-1β	0.0	4.9	0.5±1.3	0.0	4.5	0.3±1.0	0.40
IL-2	0.0	4.7	0.2±0.8	0.0	17.1	0.5±2.6	0.41
IL-3	0.0	2.1	0.1±0.3	0.0	0.0	0.0±0.0	0.28
IL-4	0.0	67.4	2.5±11.2	0.0	10.4	0.3±1.6	0.21
IL-5	0.0	1.4	0.1±0.2	0.0	0.8	0.1±0.2	0.89
IL-6	0.0	1878.7	49.2±282.4	0.0	75.2	13.4±20.1	0.41
IL-7	0.0	17.7	0.7±3.2	0.0	3.4	0.1±0.5	0.14
IL-8	0.0	280.7	24.7±52.5	0.0	247.5	29.8±53.9	0.48
IL-10	0.0	1880.7	50.5±284.4	0.0	751.7	22.1±112.9	0.28
IL-12 (p40)	0.0	70.7	7.0±17.4	0.0	40.8	2.7±7.6	0.07
IL-12 (p70)	0.0	31.3	3.1±6.9	0.0	33.9	2.7±6.9	0.55
IL-13	0.0	7.8	0.3±1.4	0.0	7.4	0.2±1.1	0.58
IL-15	0.0	10.5	0.8±2.1	0.0	15.1	1.2±3.1	0.34
IL-17	0.0	18.8	2.2±4.1	0.0	16.3	2.5±4.2	0.55
IP-10	199.9	18071.6	1303.0±2727.9	158.4	20000.0	1377.7±2965.7	0.53
MCP-1	92.3	2346.5	316.0±357.0	84.1	1772.6	346.7±346.2	0.67
MIP-1α	0.0	85.6	7.2±19.3	0.0	42.9	2.3±7.4	0.03
MIP-1β	0.0	90.4	16.7±20.3	0.0	56.5	13.8±13.9	0.44
TNF-α	0.4	175.2	12.3±29.3	0.2	98.5	9.5±14.9	0.37
TNF-β	0.0	9.9	0.7±1.7	0.0	3.0	0.3±0.7	0.15

and regulators of human MIP-1 are inducible in most mature hematopoietic cells and osteoblasts, astrocytes, microglia (fetal), epithelial cells, mesangial cells, fibroblasts, and vascular smooth muscle cells (16). We found that the plasma MIP-1α concentration decreased significantly during morphine treatment. In line with our findings, several studies have demonstrated that morphine directly down-regulates the expression of MIP-1β in leukocytes, astrocytes and astroglial cells *in vitro* (17-19). This effect was thought to be mediated through the opioid mu (μ) receptor (19). We hypothesized that morphine down-regulates the secretion of MIP-1α from mature hematopoietic cells, resulting in a decrease in the plasma concentrations of MIP-1α during morphine treatment. Thus, the concentration of MIP-1α may be useful as a pharmaco-dynamic biomarker of morphine treatment.

On the other hand, we found that the plasma concentrations of cytokines, including eotaxin, IL-8, IL-12 (p40), IL-12 (p70), MIP-1α and MIP-1β, are significantly correlated with the outcome of morphine treatment. The underlying mechanism explaining why these plasma concentrations of cytokines were lower in patients with resistance to morphine treatment remains unclear. Two possible hypotheses can be considered. Firstly, crosstalk between cytokine-signaling and opioid receptor-signaling may be involved. Accumulating evidence has indicated that stimulation of MIP-1α to its receptor CCR1 induced the internalization of μ-opioid receptors and severely impaired the μ-opioid receptor-mediated inhibition of cAMP accumulation in μ-opioid receptor/HEK293 cells (20). In addition, the prolonged activation of opioid receptors inhibited the function of chemokine receptors on leukocytes *via* a calcium-independent protein kinase C pathway (21). These studies indicate a direct link between these signaling pathways. Secondly, many leucocyte subpopulations in the peripheral blood, including lymphocytes, monocytes, and granulocytes, produce opioid peptides, such as met-enkephalin, β-endorphin, dynorphin, and endomorphins, in inflammatory peripheral tissue (22). Opioid peptides can bind to opioid receptors on sensory neurons and

Table IV. *Relationship between baseline plasma cytokine concentrations and required dose of morphine. The baseline plasma cytokine concentrations were analyzed between the groups according to required dose of morphine (>30 mg vs. 30 mg) using the t-test.*

Charasteristics	Base line plasma concentration			Base line plasma concentration		
	Required dose of morphine (after titration, day 1)			Required dose of morphine (day 8)		
(pg/ml)	>30 mg	30 mg	*p*-value	>30 mg	30 mg	*p*-value
Eotaxin	43.1±36.0	48.4±21.8	0.74	32.6±8.1	52.7±25.2	0.00026
G-CSF	243.7±244.2	268.5±381.2	0.84	294.8±254.9	255.2±395.9	0.70
GM-CSF	2.4±2.4	2.6±2.6	0.90	3.0±3.6	2.4±2.1	0.62
IFN-α2	11.5±8.6	17.3±16.4	0.21	17.1±21.1	16.3±13.7	0.90
IFN-γ	6.6±8.6	7.8±17.9	0.78	6.0±7.0	8.2±19.2	0.58
IL-1α	57.1±106.1	86.8±121.2	0.55	112.5±176.5	72.8±93.5	0.49
IL-1β	0.6±1.4	0.5±1.3	0.92	0.8±1.7	0.4±1.2	0.56
IL-2	0.0±0.0	0.2±0.8	0.10	0.4±1.4	0.1±0.3	0.46
IL-3	0.0±0.0	0.1±0.3	0.28	0.2±0.6	0.0±0.0	0.36
IL-4	0.0±0.0	2.8±12.1	0.16	0.0±0.0	3.3±12.9	0.16
IL-5	0.1±0.2	0.1±0.2	0.89	0.1±0.1	0.1±0.2	0.52
IL-6	1.5±3.6	56.8±303.7	0.27	5.7±9.6	63.7±326.0	0.31
IL-7	0.0±0.0	0.9±3.4	0.13	0.0±0.0	1.0±3.7	0.13
IL-8	6.6±5.0	27.5±56.0	0.03	41.8±84.5	18.9±36.5	0.40
IL-10	4.6±8.8	57.7±305.9	0.29	3.1±6.6	66.3±328.1	0.28
IL-12 (p40)	0.2±0.5	8.1±18.5	0.01	6.5±21.3	7.1±16.3	0.93
IL-12 (p70)	1.0±1.8	3.4±7.3	0.09	3.9±7.5	2.8±6.7	0.67
IL-13	0.0±0.0	0.4±1.5	0.13	0.1±0.3	0.4±1.6	0.32
IL-15	0.2±0.4	0.9±2.2	0.09	0.6±1.2	0.9±2.3	0.64
IL-17	2.2±3.2	2.2±4.2	0.99	1.9±2.5	2.3±4.5	0.70
IP-10	873.6±1167.8	1370.8±2903.3	0.47	2434.1±5265.6	926.0±865.0	0.37
MCP-1	209.0±102.7	332.9±380.2	0.11	215.2±99.6	349.6±404.4	0.09
MIP-1α	0.0±0.0	8.4±20.6	0.02	7.2±23.9	7.2±18.0	1.00
MIP-1β	7.7±9.8	18.1±21.3	0.07	16.0±21.4	17.0±20.3	0.90
TNF-α	5.8±4.6	13.3±31.4	0.18	6.0±3.8	14.4±33.6	0.17
TNF-β	0.6±1.5	0.7±1.8	0.95	0.7±1.5	0.6±1.8	0.82

elicit potent exogenous or endogenous analgesia in inflammatory tissue (23). Since chemokines regulate the migration of opioid peptide-containing leucocytes (23), the antinociceptive effects of chemokines may be involved in the outcome of morphine treatment.

Taken together, these results suggest that the plasma concentrations of several cytokines were correlated with resistance to morphine treatment. Our results provide novel insight into the relation between plasma cytokine levels and morphine treatment, which warrants for further study.

Disclosure Statement

All Authors declare they have no financial support or relationship that may pose conflict of interest.

Acknowledgements

This study was supported by the Third-Term Comprehensive 10-Year Strategy for Cancer Control and a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare (H22-037). We thank Mrs. Tomoko Kitayama, Mrs. Erina Hatashita, Mrs. Kiyoko Kuwata, and Mrs. Haruka Yamaguchi for their technical assistance.

References

1 Somogyi AA, Barratt DT and Coller JK: Pharmacogenetics of opioids. Clin Pharmacol Ther *81*: 429-444, 2007.

2 Lötsch J, Geisslinger G and Tegeder I: Genetic modulation of the pharmacological treatment of pain. Pharmacol Ther *124*: 168-184, 2009.

3 Tremblay J and Hamet P: Genetics of pain, opioids, and opioid responsiveness. Metabolism *59(Suppl 1)*: S5-8, 2010.

4 Walter C and Lötsch J: Meta-analysis of the relevance of the *OPRM1* 118A>G genetic variant for pain treatment. Pain *146*: 270-275, 2009.

5 Fujita K, Ando Y, Yamamoto W, Miya T, Endo H, Sunakawa Y, Araki K, Kodama K, Nagashima F, Ichikawa W, Narabayashi M, Akiyama Y, Kawara K, Shiomi M, Ogata H, Iwasa H, Okazaki Y, Hirose T and Sasaki Y: Association of UGT2B7 and ABCB1 genotypes with morphine-induced adverse drug reactions in Japanese patients with cancer. Cancer Chemother Pharmacol *65*: 251-258, 2010.

Table V. *Relationship between baseline plasma cytokine concentrations and resistance to morphine treatment.*

Cytokine	Base line plasma concentration								
	Pain scale*			Treatment outcome (day 1)			Treatment outcome (day 8)		
(pg/ml)	Mild	Severe	*p*-value	Well controlled	Resistant**	*p*-value	Well controlled	Resistant**	*p*-value
Eotaxin	49.2±23.2	46.5±24.6	0.71	49.6±22.3	41.9±28.1	0.42	53.8±26.0	34.6±9.0	0.0009
G-CSF	186.8±191.7	324.6±447.8	0.18	280.4±408.3	219.3±177.1	0.50	253.4±415.5	290.0±225.2	0.71
GM-CSF	2.9±3.4	2.3±1.6	0.45	2.7±2.7	2.2±1.9	0.56	2.6±2.1	2.6±3.3	0.98
IFN-α2	19.1±22.1	14.5±7.7	0.39	17.8±17.2	12.5±8.8	0.19	14.4±9.2	21.0±24.1	0.33
IFN-γ	10.6±24.3	5.4±7.7	0.38	8.6±19.1	4.9±7.0	0.35	8.3±20.1	6.2±6.7	0.61
IL-1α	78.7±102.8	85.9±131.2	0.84	71.6±91.5	116.1±178.9	0.44	76.3±97.5	96.6±158.0	0.66
IL-1β	0.5±1.3	0.5±1.3	0.85	0.6±1.4	0.3±1.0	0.51	0.4±1.2	0.7±1.5	0.53
IL-2	0.3±1.1	0.1±0.4	0.57	0.2±0.9	0.0±0.1	0.20	0.1±0.3	0.4±1.3	0.33
IL-3	0.1±0.5	0.0±0.0	0.37	0.1±0.4	0.0±0.0	0.28	0.0±0.0	0.2±0.6	0.36
IL-4	1.8±7.6	3.0±13.5	0.70	3.3±12.9	0.0±0.0	0.16	3.6±13.5	0.0±0.0	0.16
IL-5	0.1±0.3	0.1±0.1	0.76	0.1±0.3	0.1±0.1	0.58	0.1±0.3	0.1±0.1	0.75
IL-6	8.7±12.0	80.1±374.8	0.35	64.4±325.8	3.7±9.1	0.29	69.8±341.8	5.1±8.6	0.31
IL-7	1.6±4.7	0.1±0.7	0.21	1.0±3.7	0.0±0.0	0.13	1.1±3.8	0.0±0.0	0.13
IL-8	16.0±24.0	31.2±66.3	0.30	22.7±39.1	30.4±83.1	0.77	19.3±38.2	36.1±75.1	0.44
IL-10	100.5±431.1	12.5±45.2	0.39	66.3±328.1	3.2±6.5	0.28	72.8±343.9	2.6±5.8	0.27
IL-12 (p40)	10.6±22.2	4.2±12.4	0.27	9.0±19.7	0.9±2.6	0.03	6.0±14.2	9.2±23.3	0.64
IL-12 (p70)	4.3±8.8	2.1±4.9	0.34	3.8±7.8	0.7±1.3	0.03	3.0±7.1	3.3±6.7	0.89
IL-13	0.3±1.2	0.3±1.5	1.00	0.4±1.6	0.0±0.0	0.13	0.4±1.7	0.1±0.2	0.27
IL-15	1.0±2.8	0.6±1.3	0.53	0.9±2.3	0.5±1.2	0.50	0.9±2.4	0.5±1.1	0.39
IL-17	1.4±2.8	2.8±4.8	0.24	2.4±4.5	1.6±2.5	0.43	2.0±4.4	2.6±3.2	0.63
IP-10	950.7±893.8	1570.7±3544.0	0.41	902.9±845.3	2503.3±5253.4	0.34	948.8±904.7	2062.0±4677.3	0.39
MCP-1	333.7±229.2	302.5±434.2	0.76	346.5±405.8	224.3±93.8	0.12	366.6±420.8	207.5±89.7	0.06
MIP-1α	11.8±26.2	3.8±11.1	0.23	9.6±21.8	0.0±0.0	0.02	8.0±18.7	5.7±21.2	0.73
MIP-1β	19.8±22.0	14.3±19.1	0.39	19.8±22.1	7.5±9.3	0.01	16.8±21.1	16.6±19.3	0.97
TNF-α	15.2±39.0	10.0±19.7	0.60	14.2±33.7	6.3±4.0	0.19	15.3±35.2	5.8±3.4	0.16
TNF-β	0.3±0.6	0.9±2.2	0.19	0.7±1.9	0.4±1.1	0.50	0.6±1.9	0.8±1.4	0.73

The baseline plasma cytokine concentrations were analyzed between mild *vs.* severe pain groups and well-controlled *vs.* resistant to morphine treatment groups using the *t*-test. *Pain was evaluated using the numerical rating scale for pain (NRS). Severe pain was defined as NRS ≥6. **Resistance was defined as the requirement for a high dose of morphine (>30 mg) and persistent pain (NRS ≥6) after morphine treatment. The *p*-values were calculated using the *t*-test.

6 Rogers TJ, Steele AD, Howard OM and Oppenheim JJ: Bidirectional heterologous desensitization of opioid and chemokine receptors.Ann NY Acad Sci *917*: 19-28, 2000.

7 Finley MJ, Happel CM, Kaminsky DE and Rogers TJ: Opioid and nociceptin receptors regulate cytokine and cytokine receptor expression. Cell Immunol *252*: 146-154, 2008.

8 de la Cruz-Merino L, Grande-Pulido E, Albero-Tamarit A and Codes-Manuel de Villena ME: Cancer and immune response: old and new evidence for future challenges.Oncologist *13*: 1246-1254, 2008.

9 Balakin KV, Ivanenkov YA, Tkachenko SE, Kiselyov AS and Ivachtchenko AV: Regulators of chemokine receptor activity as promising anticancer therapeutics.Curr Cancer Drug Targets *8*: 299-340, 2008.

10 Lara-Muñoz C, De Leon SP, Feinstein AR, Puente A and Wells CK: Comparison of three rating scales for measuring subjective phenomena in clinical research. I. Use of experimentally controlled auditory stimuli. Arch Med Res *35*: 43-48, 2004.

11 Williamson A and Hoggart B: Pain: a review of three commonly used pain rating scales. J Clin Nurs *14*: 798-804, 2005.

12 Swarm R, Abernethy AP, Anghelescu DL, Benedetti C, Blinderman CD, Boston B, Cleeland C, Coyle N, Deleon-Casasola OA, Eilers JG, Ferrell B, Janjan NA, Karver SB, Levy MH, Lynch M, Moryl N, Murphy BA, Nesbit SA, Oakes L, Obbens EA, Paice JA, Rabow MW, Syrjala KL, Urba S and Weinstein SM: NCCN Adult Cancer Pain. Adult cancer pain. J Natl Compr Canc Netw *8*: 1046-1086, 2010.

13 Kasahara K, Arao T, Sakai K, Matsumoto K, Sakai A, Kimura H, Sone T, Horiike A, Nishio M, Ohira T, Ikeda N, Yamanaka T, Saijo N and Nishio K: Impact of serum hepatocyte growth factor on treatment response to epidermal growth factor receptor tyrosine kinase inhibitors in patients with non-small cell lung adenocarcinoma. Clin Cancer Res *16*: 4616-4624, 2010.

14 Yamada Y, Arao T, Matsumoto K, Gupta V, Tan W, Fedynyshyn J, Nakajima TE, Shimada Y, Hamaguchi T, Kato K, Taniguchi H, Saito Y, Matsuda T, Moriya Y, Akasu T, Fujita S, Yamamoto S and Nishio K: Plasma concentrations of VCAM-1 and PAI-1: a predictive biomarker for post-operative recurrence in colorectal cancer. Cancer Sci *101*: 1886-1890, 2010.

15 Wolpe SD, Davatelis G, Sherry B, Beutler B, Hesse DG, Nguyen HT, Moldawer LL, Nathan CF, Lowry SF and Cerami A: Macrophages secrete a novel heparin-binding protein with inflammatory and neutrophil chemokinetic properties. J Exp Med *167*: 570-581, 1988.

16 Menten P, Wuyts A and Van Damme J: Macrophage inflammatory protein-1. Cytokine Growth Factor Rev *13*: 455-481, 2002.

17 Mahajan SD, Schwartz SA, Aalinkeel R, Chawda RP, Sykes DE and Nair MP: Morphine modulates chemokine gene regulation in normal human astrocytes. Clin Immunol *115*: 323-332, 2005.

18 Choi Y, Chuang LF, Lam KM, Kung HF, Wang JM, Osburn BI and Chuang RY: Inhibition of chemokine-induced chemotaxis of monkey leukocytes by mu-opioid receptor agonists. In Vivo *13*: 389-396, 1999.

19 Mahajan SD, Schwartz SA, Shanahan TC, Chawda RP and Nair MP: Morphine regulates gene expression of alpha- and beta-chemokines and their receptors on astroglial cells *via* the opioid mu receptor. J Immunol *169*: 3589-3599, 2002.

20 Zhang N, Rogers TJ, Caterina M and Oppenheim JJ: Proinflammatory chemokines, such as C-C chemokine ligand 3, desensitize mu-opioid receptors on dorsal root ganglia neurons. J Immunol *173*: 594-599, 2004.

21 Zhang N, Hodge D, Rogers TJ and Oppenheim JJ: Ca^{2+}-independent protein kinase Cs mediate heterologous desensitization of leukocyte chemokine receptors by opioid receptors. J Biol Chem *278*: 12729-12736, 2003.

22 Labuz D, Berger S, Mousa SA, Zöllner C, Rittner HL, Shaqura MA, Segovia-Silvestre T, Przewlocka B, Stein C and Machelska H: Peripheral antinociceptive effects of exogenous and immune cell-derived endomorphins in prolonged inflammatory pain. J Neurosci *26*: 4350-4358, 2006.

23 Rittner HL, Brack A and Stein C: Pain and the immune system. Br J Anaesth *101*: 40-44, 2008.

Received August 29, 2011
Revised November 3, 2011
Accepted November 4, 2011

Acta Med Kinki Univ Vol. 36, No. 2 63 71, 2011

The significance of a specific psycho-oncology outpatient service for cancer patients run by psychosomatic medical doctors

Atsuko Koyama, Minoru Niki, Hiromichi Matsuoka, Ryo Sakamoto, Kiyohiro Sakai, Rikako Jinnai, Kanae Yasuda and Masatomo Otsuka[1]

Department of Psychosomatic Medicine,
[1]Department of Palliative Care Medicine, Sakai Hospital, Kinki University Faculty of Medicine

Abstract

Background: The field of psycho-oncology has been developed by psychiatrists in consultation-liaison fields, and the Department of Psychosomatic Medicine, Sakai Hospital, Kinki University Faculty of Medicine set up a new outpatient service for cancer patients. The primary aim of this paper is to clarify the role of psychosomatic medical doctors in cancer treatment and clinical practice, and the secondary aim is to clarify the significance of this special outpatient service for cancer patients from the viewpoint of psycho-oncology.

Methods: Multiple factors, such as age, sex, cancer site, clinical symptoms, the reason for consultation, psychiatric diagnosis, in which department patients were having physical treatment, whether they were hospitalized and whether patients were taking palliative care therapy, were analyzed.

Results: The data of fifty-eight cancer patients, including two family members of cancer patients, were analyzed. The most common psychiatric disorders were major depressive disorder, anxiety disorders and adjustment disorders. The reasons for consultation ranged from psychological support after receiving bad news, before/after surgery and chemotherapy to control delirium. Our psycho-oncological intervention involved a combination of psychotherapy and pharmacotherapy.

Conclusions: Psychosomatic medical doctors can play an important role in the field of cancer treatment through psycho-oncological activities. The advantages of a specific outpatient service for psycho-oncology are that it can open the door to patients and their families who belong to other departments/hospitals and it can support cancer patients intensively and efficiently. However, improvements are needed, particularly relating to financing and understaffing.

Key words: psycho-oncology, psychosomatic medicine, psychosomatic disorders, cancer, mental distress, multidisciplinary team approach

Introduction

Recently, the prevalence of cancer has been increasing in Japan. Approximately half of Japan's population may suffer from cancer during their lives and one third will die of cancer. Cancer has been the commonest cause of death of Japanese people since 1981.[1] The Cancer Control Bill[2] was enacted in 2007 and states the importance of mental care, as well as physical care for cancer patients.

Psycho-oncology is a broad approach to the emotional, social, and spiritual distress of cancer patients.[3] Psycho-oncology began in the USA and The International Psycho-Oncology Society (IPOS) was established in 1984.[4] Since then, much research and clinical practice have been conducted to identify the importance of psycho-

Received April 1, 2011; Accepted June 20, 2011

logical intervention for both cancer patients and their families ; however, these reports were mainly published by psychiatrists in consultation-liaison fields.[5–8]

Psychosomatic medicine (PSM) was established in 1996 as a specific medical field in which "psychosomatic disorders" are dealt with. Recently, PSM doctors, who mainly deal with stress-related physical symptoms, have been increasingly taking care of cancer patients in the psycho-oncology field, however, few reports have been published on the clinical practice of PSM doctors.[9]

The doctors in the Department of Psychosomatic Medicine, Sakai Hospital, Kinki University Faculty of Medicine mainly attend to psychosomatic disorder patients but have recently also been taking care of the mental distress of cancer patients. There is also a Department of Palliative Care in the hospital and the doctor attends to the physical distress of cancer patients, such as cancer-related pain. Both departments work in cooperation. In April 2010, the Department of Psychosomatic Medicine set up a new weekly outpatient service for psycho-oncology. The primary aim of this paper is to clarify the role of PSM doctors in cancer treatment and clinical practice, and the secondary aim is to clarify the significance of this special outpatient service for cancer patients from the viewpoint of psycho-oncology.

Methods

Study Sample

The study period was from April 2010 to January 2011. The data of patients who had symptoms related to cancer and had visited the specific outpatient service for psycho-oncology in the Department of Psychosomatic Medicine, Sakai Hospital, Kinki University Faculty of Medicine were collected. All patients were aged 16 years or over.

This study was conducted according to the ethics rules of our hospital. Since all the data assessed in this study were obtained as part of routine clinical assessments from the patients' medical charts, written consent was not obtained from the patients, in accordance with the guidelines of the Japanese Ministry of Health, Labor and Welfare.

Demographic and Clinical Variables

During the study period, all the items assessed during routine clinical practices were extracted from the patients' medical charts, including age, sex, primary cancer site, clinical symptoms, the reason for consultation, psychiatric diagnosis, which department patients were having physical treatment, whether they were hospitalized, and whether patients were undergoing palliative care therapy.

Psychiatric Diagnosis and Psychological Measurement

Each patient's status was evaluated via a formal medical interview, leading to a Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR)[10] diagnosis.

The Self-rating Depression Scale (SDS)[11] and the State-Trait Anxiety Inventory (STAI)[12] were used to evaluate emotional distress in terms of depression and anxiety. In SDS, a cut-off score of 50 was adopted in this study to determine that patients were considered to be in a depressive state. In STAI, cut-off scores of 42/45 (STAI-S/T for female) and 41/44 (STAI-S/T for male) were adopted in this study to determine that patients possessed a tendency toward anxiety.

Psycho-oncological intervention

Our psycho-oncological intervention involves a combination of psychotherapy and pharmacotherapy. Regarding psychotherapy, most of the patients received brief individual supportive sessions and autogenic training in relaxation. Counseling by clinical psychologists was added, if necessary.

As for pharmacotherapy, anxiolytics, antidepressants and hypnotic drugs were mainly used depending on the patient's condition. Antipsychotics were used to suppress delirium. These intervention principles are in agreement with those of the Japan Psycho-oncology Society (JPOS)[13].

Results

Demographic and Clinical Characteristics

Fifty-eight cancer patients, including two family members of cancer patients, visited the specific outpatient service for psycho-oncology in the Department of Psychosomatic Medicine for the first time during the examination period. Detailed demographic characteristics of the patients are listed in Table 1. This table demonstrated that there were more female patients than male patients. One of the reasons for this feature

Table 1 Characteristics of patients.

Clinical Characteristics	N	(%)
Total patients	58	100
Age (mean±SD), years	60.9±12.3	(range 35-83)
Sex		
Male	17	29.3
Female	41	70.7
Primary Cancer site		
Breast	26	44.8
Lung	7	12.1
Stomach	6	10.3
Colon	3	5.1
Pancreas	3	5.1
Bladder	3	5.1
Kidney	2	3.4
Ureter	1	1.7
Prostate	1	1.7
Uterus	1	1.7
Ovary	1	1.7
Leukemia (ATL+CML)	2	3.4
(Patient's family)	2	3.4
Department of physical treatment		
Surgery	33	56.9
Oncology	10	17.2
Urology	7	12.1
Gastroenterology	4	6.9
Gynecology	2	3.4
Hematology	2	3.4
Main hospital		
Sakai Hospital	23	39.7
Kinki University Hospital	14	24.1
Others	21	36.2
Hospitalized		
Yes/No	12/46	20.7/79.3
Palliative care		
Yes/No	10/48	17.2/82.8

SD=standard deviation
ATL=adult T-cell leukemia
CML=chronic myelogenous leukemia

seems to be that breast cancer patients make up about 45% of total cancer patients. Breast cancer patients have a longer survival period than others and may have increased prevalence of both physical and psychological problems and the need for psychological intervention.[14] Both inpatient and outpatient cancer patients from various departments in our hospital visited our special outpatient service. In addition, patients belonging to Sakai Hospital, the Main Kinki University Hospital in Osakasayama-city, and other hospitals made up one third of the study sample. The total ratio of hospitalized patients to all patients was 20.7%. Overall, 17.2% of patients were consulting the Department of Palliative Care at the same time, mainly in order to control cancer pain.

Symptoms present in the patients are listed in Table 2. Overall, 43.1% of patients complained of physical symptoms, mainly appetite loss, general fatigue and pain. Main psychiatric symptoms were depressive mood, anxiety and insomnia.

Reasons for consultation consisted of two

Table 2 Present symptoms.

Present symptoms	N	(%)	Present symptoms	N	(%)
Physical symptoms			Psychiatric symptoms		
appetite loss/body weight loss	22	37.9	depressive mood	30	51.7
general fatigue	18	31.0	anxiety	33	56.9
pain	10	17.2	insomnia	26	44.9
palpitations	8	13.8	irritation	6	10.3
feeling of difficulty breathing	3	5.2	panic attack	3	5.2
diarrhea/constipation	8	13.8	others	8	13.8
nausea/vomiting	6	10.3			
dizziness/faintness	5	8.6	None	10	17.2
tinnitus	3	5.2			
numbness	2	3.4			
urinary problems	2	3.4			
others	6	10.3			
None	33	56.9			

* multiple answers given

Table 3 Reasons for consultation.

Reasons for consultation	N	(%)	Reasons for consultation	N	(%)
Patient's own accord	22	37.9	Referred by main doctor	36	62.1
Control of physical symptoms	13	22.4	Control of physical symptoms	12	20.7
psychiatric symptoms	16	27.6	psychiatric symptoms	32	55.2
no symptoms	2	3.4	no symptoms	0	0
Psychological problems			Psychological support		
conflict between family members	5	8.6	receiving bad news	4	6.9
grievance against main doctor	4	6.9	chemotherapy	13	22.4
medical staff	2	3.4	surgery	5	8.6
other patients	1	1.7	control of delirium	2	3.4
work-related social problems	5	8.6			
spiritual problem	3	5.2	spiritual care for dying patient	1	1.7
			patient's family	2	3.4
Requesting information					
palliative care	2	3.4			
hospice/home care	3	5.2			
side effects of chemotherapy	3	5.2			
results of tumor markers	2	3.4			

* multiple answers given

components and are shown in Table 3. The first was that patients came to our department of their own accord, recognizing their physical or psychiatric symptoms. The aim of their consultation was mainly the control of physical and psychiatric symptoms. Other issues ranged widely from psychological problems, such as conflict between family members, grievances against their main doctors, medical staff and/or other patients, and work-related social problems, to spiritual problems related to the meaning of life. Some patients came requesting information regarding palliative care, hospice and home medical care services. Other patients sought information regarding the side effects of chemotherapy and the results of tumor markers, which they had

Table 4 Psychiatric diagnosis of patients.

Psychiatric Diagnosis	N		(%)	
Major Depressive Disorder	11		19.0	
Anxiety Disorders	9		15.5	
Panic Disorder		3		5.2
Obsessive-Compulsive Disorder		1		1.7
Generalized Anxiety Disorder		5		8.6
Adjustment Disorders	30		51.7	
Mixed anxiety and depressive mood		11		19.0
With anxiety		8		13.8
With depressive mood		6		10.3
Mixed disturbance of emotions and conduct		1		1.7
Unspecified		4		6.9
Delirium	2		3.4	
Dementia	1		1.7	
Personality change due to brain metastasis	1		1.7	
Other Psychotic Disorders	2		3.4	
None	2		3.4	

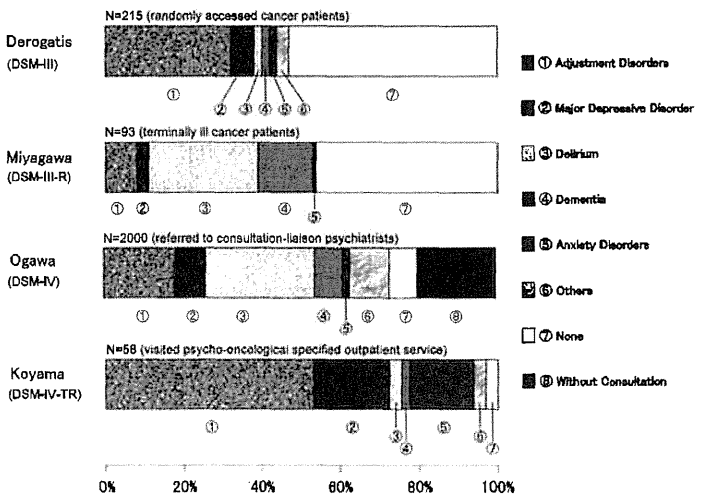


Fig. 1 Comparison of psychiatric diagnosis of cancer patients.
Compared with three previous studies, the patients who came to our specific outpatient service for psycho-oncology had a higher prevalence of major depressive disorder and anxiety disorders in particular.

hesitated to ask their main physical doctors. The other component was that the patient's main physical doctor recognized physical symptoms and/or psychological problems and referred them to our department. The doctor's aims for consultation included psychological support for surgery and chemotherapy, control of delirium, and spiritual care for dying patients and family care.

The psychiatric diagnosis is shown in Table 4. The SDS scores of 54 patients were 54.1±10.8 and 42 patients had higher scores than cut-off scores of 50. The STAI-S/T scores of 50 patients were 52.9±14.9/53.2±15.0. 24 female patients had higher scores than cut-off scores of 42/45 for females and 9 male patients had higher scores than cut-off scores of 41/44 for males. Adjustment disorders were 51.7% and the subtypes were determined by reference to SDS/STAI scores and patients' symptoms. Major depressive disorder was 19.0%, anxiety disorders including panic disorders, were 15.5%, delirium was 3.4%, and others, including dementia and personality change, were 6.8%. A comparison with the